

# **Veterinary Handbook for Herd Management in the bovine TB eradication programme**

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**June 2010**



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The assistance of all who have helped compile this manual is acknowledged and much appreciated.

## 1. Introduction

The Bovine TB Eradication Scheme commenced in 1954 using the Single Intradermal Comparative Tuberculin Test (SICTT). Before the scheme started it was common to find, ‘piner’ animals, cows with TB mastitis excreting *M. bovis* in milk as well as carcasses condemned with generalised TB. Many families had members suffering from TB and in 1952, the recorded rate of TB notifications in Ireland was 230/100,000 population. Initially, the incidence of TB in cattle was about 17% overall (and in cows as high as 22%). In the first 10 years of the scheme, from a cattle population of circa 5 million head some 800,000 TB reactors were slaughtered. By 1964 the position had improved significantly and that year, just 0.75% of the national herd of 5,659,344, or some 40,433 animals were TB reactors, which represented a 23-fold reduction in reactor numbers. In October 1965, Charles Haughey T.D. the then Minister for Agriculture announced “To mark this memorable occasion of the formal declaration of the whole state as cleared of bovine tuberculosis, I thought it well to publish a complete account of how the job was done”. In hindsight we know that this declaration was somewhat premature!

The objective of the current Irish bovine TB eradication programme is the eradication of TB from the cattle population. The programme is built on a twin-track approach to tackling the disease – systematically addressing both bovine-to-bovine spread as well as wildlife to bovine cycle. It is built upon conventional disease control strategies appropriate to disease control within a domesticated animal species test, slaughter and restrict movement and novel approaches to dealing with a disease that moves between domestic and wild animal populations namely reducing the badger population in areas where they are seen to be contributing to bovine TB prevalence while a vaccine for TB in badgers is in development and under trial. In summary, the measures in the programme include identification of individual herds as the epidemiological unit of relevance, an annual round screening test of all herds, individual animal identification, controls on movement of animals, restriction of holdings, removal and slaughter of reactors and specific targeted testing, including the use of blood tests, with appropriate follow-up testing, compensation for farmers whose herds are affected by disease, a focused badger population control where they have been implicated as a probable cause of TB and continued work towards the development and introduction of a vaccine to prevent TB in badgers.

While Bovine TB has been eradicated in Australia and to a very significant degree in New Zealand, both countries experienced a problem with wildlife infection that was dealt with by the widespread and systematic destruction of the wildlife reservoir. While it is acknowledged that eliminating badgers in Ireland would probably result in a more successful cattle tuberculosis eradication scheme, such a policy would be unacceptable at a number of levels. At the societal level, the destruction of one of our important native large species of mammal would be completely unthinkable. In addition, the EU is a signatory of the 1989 Convention on the Conservation of European Wildlife and Natural Habitats (Berne Convention), and the Irish government ratified this treaty in 1982. Under Irish Law (The Wildlife Act, 1976), the Eurasian badger (*M. meles*) is a protected species including protection of the underground burrows (setts) where badgers live and breed their young. Very considerable progress was made in the early years of the Tuberculosis eradication programmes and there has also been a progressive reduction, with some annual variations, in the level of the disease since 1998

particularly regarding reactor numbers which fell from c.45,000 to 23,805 in 2009. The average number of reactor removed in the 5-year period 2005-09 was, at c.26,295, 14% lower than in the preceding 5-year period (2000-2004), despite additional use of blood tests removing increasing numbers of animals disclosed as reactor. The herd incidence continues to fall and, during the same period, has fallen from 7.7% in 1999 to 5.09% in 2009. Ireland complies fully with EU Directive 64/432/EEC in order to facilitate trade in bovine animals from Ireland.

The tuberculin used in the initial years of the eradication programme was prepared from strains of *M. tuberculosis* (i.e. the human strain of TB) and in 1978 this was changed to tuberculin prepared from *M. bovis*. In the early 1980s the current supplier won the contract to supply tuberculin and has continued to do so for each subsequent tender. The potency of the tuberculin supplied is regularly checked, at Central Veterinary Research Laboratory, against EU and Irish standards using cattle naturally infected with *M. bovis*.

In the autumn of 1988 the first version of the 'Categorisation document' was circulated. This set out a strategic response to the management of herd restrictions. It defined herds in terms of the severity of the disease outbreak and provided for additional measures, to be employed where it was felt that these were warranted. This approach provided for a more efficient use of resources. At around the same period it was recognised that significant constraints existed in relation to TB eradication. Many of these constraints have been identified subsequently. The most significant is the presence of bovine Tuberculosis in wildlife reservoir hosts namely the badger *Meles meles* and to a lesser extent the wild deer population. (Bovine TB was first reported in badgers in Ireland in 1975 in West Cork.) The identification and alleviation of these constraints has been, and continues to be, the focus of considerable research funded by DAFF. The 'Handbook for the Management of herds under restriction for Tuberculosis' replaced the initial 'Categorisation Document' and is revised periodically in order to reflect the scientifically informed changes in this Department's policy with regard to the management of tuberculosis-restricted herds. It is intended to maintain this manual in line with current policy by means of regular reviews and consequent updates.

There have been major changes to the eradication programme over the years. The concept of cost sharing was introduced in 1979 with the introduction of the disease levies. The disease levy arrangements were amended in 1996 following the introduction of farmer pay testing, whereby the farmer pays the private veterinary practitioner directly for conducting one full herd test annually. The relevant Annexes to Directive 64/432/EEC have been amended and the current Annex A and Annex B – are at Appendix 1 of this manual. The resources available to the ERAD Division and our understanding of the underlying epidemiology of the disease have changed as scientific information and data analysis have become available, often through the auspices of the TB Investigation Unit now the Centre for Veterinary Epidemiology and Risk Analysis (CVERA). Much of the programme has been computerised since 1986 and an integrated system – The Animal Health Computer System (AHCS) has been functional in all DVOs since 2005.

Bovine TB is no longer the zoonotic threat that it was in the past and, in 2008, the most recent year for which even provisional figures are available, 470 human cases of TB were notified giving a national crude incidence rate of 11.1/100,000 population. Almost 40% of these TB cases were born outside Ireland. Of the 209 (44.5%) culture-confirmed cases, 177 (84.7%) were *M. tuberculosis* and ten (4.8%) were *M. bovis*.

## 2. Herdnumbers

Since 1957 and the inception of the Bovine TB eradication programme in Ireland each single unique epidemiologically distinct herd is allocated a herdnumber for the purpose of disease control. A comprehensive circular, ER 18 of 2004, dealing with all issues relating to herdnumbers, was issued in December 2004 and outlines procedures to be followed in all matters relating to herdnumbers.

([http://ezone/erad/circulars2004/er18\\_04/ER18\\_04\\_Herdnumbers.doc](http://ezone/erad/circulars2004/er18_04/ER18_04_Herdnumbers.doc))

A herdnumber is an administrative device issued under the bovine disease eradication schemes for the purpose of individual herd identification, the management of bovine animal identification and disease status certification under the various bovine disease testing programmes. **It is a pre-requisite that each distinct epidemiological unit must be represented by one and ONLY one herdnumber.**

Questions as to ownership of particular lands or animals are of secondary importance, as a herdnumber does not denote ownership of stock or land and functions solely as a method of herd identification and control from a disease viewpoint. Thus the person in whose name the herdnumber is registered, being the (nominated) keeper of the herd and thus the animals located therein, may or may not be the legal owner of the animals held under that herdnumber. An applicant's position *viz. a viz.* eligibility for support payments or other schemes is **not** to be considered in assessing eligibility for a herdnumber.

In assessing eligibility for a herdnumber, it must be remembered that the interests of disease control are paramount and thus it must be established that as a minimum

- A. the herd is managed at all times as a separate epidemiological unit without intermixing of bovine animals from other herds,
- B. the herd has an identifiable keeper (i.e. one person) responsible for the health and welfare of the animals and compliance with all animal health regulations,
- C. adequate handling facilities, not shared with or used by any other herd, are available to carry out satisfactory testing,
- D. the herd should, as far as possible, have separate farm equipment, housing and fodder i.e. tractors, trailers, fodder or other equipment should not routinely move between the applicant herd and any other establishment and
- E. satisfactory arrangements are in place to prevent disease spread due to waste disposal.

With regard to "A", please note that "separation" means a complete division of operations such that:

- a) there are separate entrances,
- b) entry points onto other adjoining lands, not part of the application, should be permanently blocked,
- c) perimeter fencing should be stock-proof at a minimum and should also prevent direct contact with stock on contiguous land,
- d) independent and separate facilities exist (i.e. separate crush, separate feeding and watering facilities),
- e) adequate facilities exist for the purpose of inspection, isolation, loading, unloading, marshalling, watering, feeding, housing as appropriate and treatment of animals and

- f) adequate provision has been made for animal bedding and the collection of manure and wastewater

Where a herdnumber is already in existence, but where the conditions/criteria required to qualify for a herdnumber are no longer being adhered to (e.g. where inter-mixing of animals occurs) then that number should be withdrawn in accordance with Circular 18 of 2004. All animals under common management should be amalgamated under a single herdnumber (preferably a new number unless all parties are in agreement as to which number they wish to retain). The various persons with an interest in the herd should be registered as herdowners, of the single epidemiological unit, and they must nominate a single keeper in whose name the herdnumber should be registered. The necessary changes should also be made to AHCS records. The withdrawal of herdnumbers should not be delayed pending the nomination of a keeper.

### **3. Test procedures**

Instructions for performing the single intradermal comparative tuberculin test (SICTT), in accordance with Annex B of Directive 64/432/EEC (Appendix 1), are contained in ERAD document ER4 (Appendix 2).

The ER4 document is reviewed annually and revised as necessary before sending a copy to each veterinary practitioner engaged in tuberculin testing at the start of each year. In order to maintain official approval to conduct tuberculin testing, the veterinary practitioner is required to acknowledge receipt of the ER4 document and confirm that the contents have been read and noted. In summary, this document details the equipment required, the animal identification notation, the injection site preparation, the injection technique, the measurement of skin thickness on both days, the method involved in reading the results of the tuberculin test and the requirement to identify reactors for segregation, assembly and security purposes. There is no category of PVP who is exempted from inspection and selected PVPs should be the subject of an unannounced supervision, while conducting a TB/Br test, at least once annually (ER13 – supervision report. See appendix 2). The performance of the veterinary practitioner in the operation of the eradication programme is also reviewed regularly, test reports are interpreted by V.I.s and using computer records of test results, the accuracy of supplied data and the timely submission of data etc. is assessed. Those whose performance is out of line with their peers are selected on the basis of these checks for targeted supervision.

The Standard Operating Procedures (SOP) for the management of TB Reactor Herds in DVOs is to be found in Circular 06/09.



## 4. Test Types (TTY)

The Single Intradermal Comparative Tuberculin Test (SICTT) is conducted under the following test types.

- |                                                 |                                               |
|-------------------------------------------------|-----------------------------------------------|
| (1) Round test                                  | (5g) Forward trace S.C.T. (animal)            |
| (2) New Herd Special Check Test (S.C.T.)        | (5h) Miscellaneous (animal) S.C.T.            |
| (3) Inconclusive re-test                        | (6) Private test                              |
| (4) Reactor re-test                             | (7a) Post depopulation C.T.                   |
| (5a) Classification related S.C.T.              | (7b) Post de-restriction C.T.                 |
| (5b) Associated to a reactor herd S.C.T.        | (8) Contiguous herd test                      |
| (5c) High incidence area/DED/Miscellaneous C.T. | (9a) Factory lesion test                      |
| (5d) Factory return S.C.T. (herd)               | (10a) Balance of herd test (factory lesion)   |
| (5d) Factory return S.C.T. (animal)             | (10b) Balance of herd test (private test)     |
| (5e) OTF regain status S.C.T.                   | (10c) Balance of herd test (inc retest)       |
| (5f) Backward trace S.C.T. (herd)               | (10d) Balance of herd test (fwd trace S.C.T.) |

Other tests: -ELISA Test, Anamnestic ELISA and Interferon Gamma Assay – see Section 8

## 5. Test Type Ranking

**Restricted Herds** (trading status suspended or withdrawn):

The **trading status** of all herds restricted due to tuberculosis is set at S (Suspended) or W (Withdrawn) on AHCS signifying that such herds cannot trade in cattle on the open market.

In herds currently with status withdrawn due to tuberculosis, only Test Types 3, 4, 9, and 10 apply. These test types are automatically prompted at follow up by AHCS as they have priority over all other test types. The first herd test post de-restriction must be a post-depopulation CT Test (7a) or a Post-derestriction CT Test (7b), except in the case of herds where the suspicion of tuberculosis has been resolved under the singleton protocol, which revert to Test Type 1 and will be prompted as such by the computer. See Section 13 ‘Singleton’ Protocol.

Where trading status has been suspended for administrative reasons and set to Status S (Suspended) on AHCS, e.g. failure to conduct a test within a given timeframe, breach of an official notice or Article of the TB Order, or where out of test animals have been sent for slaughter, then the listing will require test types other than 4 and 9.

The Activity status on AHCS may also be used to suspend a herd status for other reasons.

Object Status Add page - Microsoft Internet Explorer

Department of Agriculture, Fisheries and Food  
An Roinn Talmhaíochta, Iascaigh agus Bia

# AHCS - ANIMAL HEALTH COMPUTER SYSTEM

User agr2126-Anthony Duignan Current DVO ROSCOMMON D.V.O. Printer Location ROSCOMMON D.V.O. [Index](#) [Change](#) [Exit](#)

<b>Objects</b>	<b>Testing</b>	<b>Tracing</b>	<b>Permits</b>	<b>Payments</b>	<b>Valuations</b>	<b>Reports</b>	<b>Class A/B</b>
Animals	Herds	Farm Enterprises	Business Cust.	Company			
Main	Animal List	Tests	Associated Businesses	Valuations	Permits	Programmes/Projects	Grant Payments
Associated Herds	Breakdown	Extended	Add Animal	Documents	Traces		Contiguous Herds

Status Group\*

Status Code\* **Status Code Description**

ACTIVE	HERD IS ACTIVE
DEAD	HERD HAS CEASED ACTIVITY
DORMANT	HERD IS DORMANT
SUSPENSE	HERD IS SUSPENDED

Reason\*

Effective Date

Auto Expiry On

BSE SUSPECTED IN HERD  
BSE TRACE  
CMMS DISCREPANCIES AT TEST  
DEALER/AGENT UNDER INVESTIGATION  
DESTOCKED BY ORDER OF THE COURT  
FAILURE OF ID CHECK  
FEED INVESTIGATION  
ILLEGAL ANIMAL REMEDIES  
NON COMPLIANCE WITH CMMS  
REMEDIES WRONGLY ADMINISTERED  
UNDER INVESTIGATION  
USE OF HORMONES

start 3 Microsoft Of... TB Manual 2 Microsoft Of... Object Status A... Local Intranet 10:04

## Clear Herds:

Where a 'clear' herd is scheduled for a test, which has not yet commenced, and a test of higher priority is required at an earlier date than that scheduled, then the prioritising of test types for the new listing should follow the rules below:-

Test type 7 has priority over 8, 5 and 1.

Similarly test type 8 has priority over types 5 and 1 and finally test types 5 have priority over types 1, 2 and 3.

Where appropriate such as when a herd is being put on a contiguous programme and the next test due is a TT7, then TT7 should be re-scheduled to an earlier date if required.

## Test Type Priorities

Priority	TTY DESCRIPTION	TTY NO	Trading Status
<b>1</b>	REACTOR RE-TEST	4	Withdrawn/Suspended
<b>2</b>	FACTORY LESION RETEST	9a	Withdrawn
<b>3</b>	BALANCE OF HERD TEST (PRIVATE/MISC TEST)	10b	Withdrawn/Suspended
<b>4</b>	BALANCE OF HERD TEST (FORWARD TRACE C.T)	10d	Withdrawn/Suspended
<b>5</b>	BALANCE OF HERD TEST (INC RETEST)	10c	Withdrawn/Suspended
<b>6</b>	BALANCE OF HERD TEST (FACTORY LESION)	10a	Withdrawn
<b>7</b>	POST DE-RESTRICTION C.T.	7b	Free H or L herd
<b>8</b>	POST DEPOPULATION C.T.	7a	Free H herd
<b>9</b>	CONTIGUOUS HERD TEST	8	Free/Suspended
<b>10</b>	ASSOCIATED TO A REACTOR HERD S.C.T	5b	Suspended
<b>11</b>	CLASSIFICATION RELATED S.C.T	5a	Free
<b>12</b>	BACKWARD TRACE S.C.T. (HERD)	5f	Suspended
<b>13</b>	OTF REGAIN STATUS S.C.T.	5e	Suspended
<b>14</b>	HIGH INCIDENCE AREA / DED / MISC C.T.	5c	Free
<b>15</b>	INCONCLUSIVE RE-TEST	3	Suspended +/-Derogated
<b>16</b>	FACTORY slaughter out of date S.C.T. (Animals)	5d	Suspended
<b>17</b>	FORWARD TRACE S.C.T. (ANIMAL)	5g	Suspended
<b>18</b>	FACTORY slaughter out of date S.C.T. (HERD)	5d	Suspended
<b>19</b>	MISCELLANEOUS ANIMAL TEST	5h	Free/Suspended
<b>20</b>	ROUND TEST	1	Free
<b>21</b>	NEW HERD S.C.T	2	Suspended
<b>22</b>	PRIVATE TEST	6	Free

## 6. Herd Classification System

The classification system categorises herds based on the following three parameters:

### 6.1: Trading Status

TB Status Group / Code	Code	Meaning	Reasons
<b>Trading Status</b>	<b>F</b>	Trading Status Free	Normal OTF herd
	<b>S</b>	Trading Status Suspended	Not from OTF herd, Test not completed, Positive test, Pm suspect, Unresolved status, Failure to test, Singleton, Trace, animal out of test slaughtered, Epidemiological enquiry
	<b>W</b>	Trading Status Withdrawn	Positive reactor (defined below), Clinical TB (confirmed), Animal not from OTF herd, Test not carried out, Trace, Failure to test or C & D required.

### 6.2: TB risk status of herd

<b>TB Risk</b>	<b>D</b>	Default	Least risk (including Singleton Protocol herd)
	<b>H</b>	At Risk Higher	2 or more infected animals likely to have acquired infection in this herd over the course of the breakdown
	<b>L</b>	At Risk Lower	Other breakdowns

### 6.3: Disease Status of the herd

<b>TB Disease Status</b>	<b>0</b>	Clear	Clear
	<b>1</b>	Restricted Disease Status with Reactors in Last Test	Reactors at or infected animal detected since last test
	<b>2</b>	One clear test completed.	Awaiting Clearance test
	<b>3</b>	Scheduled for Inconclusive retest	Inconclusive Retest scheduled

Range:

Clear (**0**) to

Restricted **1**; **2** (**1** Reactors on last test; **2** One 'clear' test completed)

Inconclusive **3**.

A herd restricted under Irish legislation is, for the purpose of the TB eradication programme, equivalent to OTF status withdrawn and status suspended in Directive 64/432/EEC. Irish legislation authorises the restriction of a herd and does not dictate the duration of the restriction period. However, to ensure that trading certification is not compromised in any way V.I.s are mindful of the requirements of the Directive when derestricting herds

The Directive **requires** that **Status** be **withdrawn** when the presence of tuberculosis is confirmed by the isolation of *M. bovis*.

**Status** may also be **withdrawn** if:

- a. the conditions to retain OTF status are no longer fulfilled, or
- b. classical lesions of tuberculosis are seen at post-mortem examination, or
- c. an epidemiological enquiry establishes the likelihood of infection, or
- d. for any other reasons considered necessary for the purpose of controlling bovine tuberculosis.

Where status is withdrawn, tracing and checking of any herd considered as epidemiologically related must be undertaken. The status must remain withdrawn until cleansing and disinfection have been completed and all eligible animals have had two consecutive clear tests, the first conducted a minimum of 60 days and the second a minimum of 4 months after the removal of the last positive reactor.

For this purpose, then a 'positive reactor' results in herd status withdrawn and is an animal

- in which the presence of tuberculosis is confirmed by the isolation of *M. bovis* on laboratory examination, or
- in which classical lesions of tuberculosis are seen *post-mortem*, or
- where epidemiological enquiry has established the likelihood of infection, or
- where it is considered necessary for the purpose of controlling bovine tuberculosis to consider the animal 'positive' (e.g. test reactors or a singleton animal with an avian/bovine ratio of 12mm or more).

In summary, a withdrawn herd status requires two consecutive clear tests to restore status and applies to the majority of restrictions for disease reasons.

A suspended herd status is basically an unresolved or as yet undetermined status applied and remaining in place pending a decision as to the true TB infection status of that herd.

#### **Disease Risk Range -**

The **Risk** of the herd being positive at the next herd test:

**H** (Higher)

to

**L** (Lower)

to

**D** (Default or least risk of a positive test and includes singleton herds)

This classification system results in a matrix comprising nine classes of herds.

Risk of a positive next test	Current Status Restricted		Current Status Clear
	1	2	0
H	<b>H1</b>	<b>H2</b>	<b>H0</b>
L	<b>L1</b>	<b>L2</b>	<b>L0</b>
D	<b>D1 (singleton awaiting tissue test)</b>	<b>D2 (singleton awaiting test)</b>	<b>D0</b>



Herds are initially classified H or L following a restriction and may be updated from L to H on the basis of the cumulative severity over the entire TB breakdown.

#### **Risk Classification H:**

Herds with two or more TB infected animals over the course of the episode (duration of restriction under TB Order), as evidenced by the detection of lesions or by animals failing the SICTT at standard interpretation, are ordinarily classed H (Infection with concurrent spread → Higher risk of positives at next herd test i.e. a ‘H’ breakdown episode). See Appendix 14 Paper 3.

On epidemiological grounds and with the agreement of the SVI, a Veterinary Inspector may, where lesioned animals or other reactors have been traced back to a herd, classify a herd as H even though lesions/reactors have not been identified/confirmed in that herd.

On epidemiological grounds and with the agreement of the SVI, a Veterinary Inspector may determine that a herd is unlikely to be infected with *M. bovis* or that the infected animals are unlikely to have been infected in the herd and that therefore H classification is inappropriate (e.g. where the infected animals are all introduced and they were infected in their herd of origin or in NSI/Atypical herd situations) and such herds should be classified L (below).

#### **Risk Classification L:**

All breakdown episodes not classified as H are classed L (Lower risk of positives - i.e. TB infected- at next herd test) except in the case of Singletons which are classed D (they are classified as not TB - See Section 13 ‘Singleton’ Policy). A herd already classified H from a previous breakdown may experience a breakdown that would otherwise be classified as L (e.g. one reactor or one lesion but no concurrent spread) but such a repeat breakdown will not alter the prior H classification risk of the herd. However, if it occurs late in the existing H clearance cycle (e.g. at final TT5 stage), it may prolong the H classification while the herd undergoes the tests to clear the breakdown (i.e. 2xTT4+1xTT7b).

Herds classified H1 generally revert to H0 following a minimum of two consecutive clear tests, the first conducted a minimum of 60 days and the second a minimum of 4 months after the removal of the last positive reactor.

H0 herds will rejoin the default class, D0, on completing (and passing) a Type 7b test and two Type 5A tests carried out at six month intervals (7b; 5a; 5a six monthly testing cycle i.e. one and a half years clear from time of de-restriction).

<b>Herd Classification</b>	<b>Testing</b>	<b>Time</b>
<b>H1</b>	2 x Test type (TT) 4	4 months
<b>H0</b>	1 x TT7b + 2 x TT5a	6 months + 12 months
<b>D0</b>	Routine testing	

**H1** ⇒ 2 x TT4 (4months - clear) ⇒ **H0** ⇒ 1 x TT7b (6months) + 2 x TT5A (at six month intervals) ⇒ **D0**

i.e. to go from H1 to D0 the herd must complete and pass 5 tests over a 22 month period.

**L1** ⇒ 2 x TT4(4months - clear) ⇒ **L0** ⇒ 1 x TT7b (6months) ⇒ **D0** Herds classified L1 revert to L0 following typically two clear reactor retests carried out at 60 day intervals (**N.B.** see also Section 12 Singleton Policy). L0 herds will rejoin the default class, D0, on completing a Type 7b test.

Depopulated herds will rejoin the default class, D0, on completing a clear Type 7a test. When bought-in reactors occur on a TT7a, the herd should be classified as L pending resolution of this breakdown (see Section 16).

The default class D0, comprising herds with the least risk of having a TB reactor on the next test, would normally be scheduled for a Type 1 test, unless scheduled as part of the Contiguous or Area Special Check Test Programmes. L0 and H0 herds will be scheduled for either TT7A or 7B but TT5A may be replaced if part of a Contiguous or Special programme.

D0, LO or HO herds not tested during a defined period, depending on test type listed, should have their trading status suspended (or withdrawn at the discretion of the SVI depending on the seriousness and nature of the individual case) by virtue of regulations. These herds will then ordinarily retain their original risk status on completing one clear herd test e.g. FH0⇒SH0⇒FH0.

## 7. Interpretation of SICTT

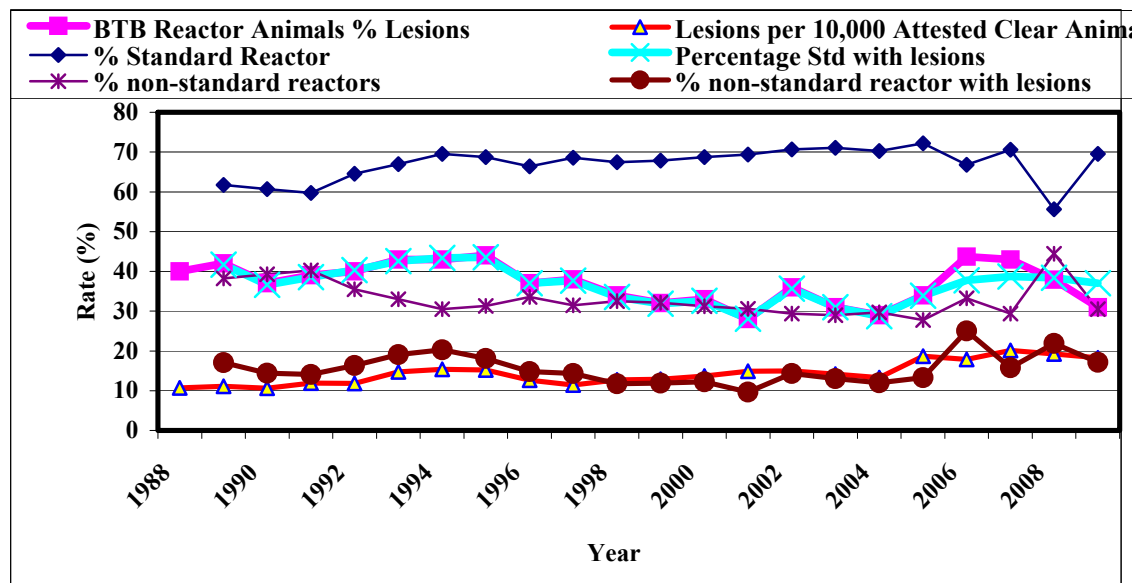
V.I. interpretation of tests carried out on herds in a breakdown should be based on the level of interpretation on the severity of the breakdown and epidemiological findings. Only a V.I. may make the final decision as to the status of an animal or herd and accordingly the provisional interpretation suggested by the PVP may be over-ruled by the V.I.

ER 4 instructions to state “When testing a ‘clear’ herd (i.e. clear status before the test) where 2 or more standard interpretation positives are found, all standard interpretation inconclusive reactors must be punched and tagged as reactors and recorded on the test report - unless instructed by the DVO to the contrary. Where the herd is undergoing a contiguous test, all standard interpretation inconclusive reactor animals must be identified for removal as reactors unless specific instructions in respect of the herd and interpretation have been received from the VI.”

*Note: Regarding the reaction to bovine tuberculin, the presence of diffuse or extensive oedema, necrosis, heat, pain at the injection site or swelling, heat or pain of the related pre-scapular lymph node is always indicative of likely infection with Mycobacterium bovis. Animals showing such reactions to bovine tuberculin are always reactors, irrespective of the measurements recorded.*

A VI may determine that a reaction is not a normal response to tuberculin or that the test performed was not the comparative intradermal test (no avian injection) in which

case the gIFN result would be a guide to determine the animal's status and such animals should not be removed as if they were reactors to the SICTT. Normal Regression of skin fold increases post Tuberculin injection (See Appendix 5E).



The chart above shows % standard interpretation and % severe interpretation reactors annually over the last 30 years – reasonably steady at 70/30. The dip in standard reactors and corresponding rise in severe reactors in 2008 is due to the trial involving the use of the interferon- $\gamma$  Assay conducted that year in high risk herds which resulted in the removal of animals as reactor that were not reactors to the SICTT. Since the lesion rate in severe reactors that year was 22%, it is probable that the removal of these animals was a matter of timing rather than being additional animals removed. The lesion rate in standard and severe reactors has also been relatively constant at between 30 and 40% (range 28-43%) for standard reactors and around 20% (range 10-25%) for severe reactors.

**Standard Interpretation** should normally be used in Lower risk herd breakdowns.

Where infection has not been confirmed in animals already removed as reactor and/or NSI is considered probable, such as when *M. bovis* infection has not been confirmed in the herd over the course of two tests, then interpretation must revert to standard and glands should be collected from any subsequent animal removed as reactor in an effort to detect if *M. bovis* is present in the herd. **Note:** *when a herd is actively infected with M. bovis, even with sensitivities of 50% for gross post-mortem, one should expect lesions in at least one reactor animal from every 5 reactors removed.*

**Severe interpretation** may be used in higher risk breakdowns following the epidemiological assessment by the interpreting VI.

When scheduling a herd for test, AHCS will prompt as Standard or Severe interpretation and the VI should select the level of interpretation appropriate to the disease risk over-ruling the AHCS prompt when appropriate.

The screenshot displays the 'TB Followup' web application. At the top, there's a status bar showing 'PVP TOTAL' as 0, 0, 69. Below this, a 'Last confirmed TB Date on' is noted as 28/03/2008, which was 630 days ago. The 'MESSAGES' section is empty. The 'ACTIONS' section contains a table with columns: Type, Date, Scheduled Vet, Interpretation Level, and Part. A 'New' button is visible. The 'ANIMALS' section has a 'Restrict' checkbox. The 'POS.' section has an 'ER26 Permit' checkbox. The 'NEG.' section has a 'De-Restrict any Restricted Animals' checkbox. The 'DOCUMENTS' section lists 'ER20', 'ER24', 'ER64', and 'ER24B'. At the bottom, there are 'Submit', 'Cancel', and 'Record Comment' buttons. The browser's address bar shows 'Local intranet' and the system clock indicates 16:48.

As part of the epidemiological assessment of a breakdown, a review should be made of the herd/area test history and the animals within or originating from the infected group. Consideration should be given to removal of additional animals on the basis of current or previous test measurements, IFN- $\gamma$  assay, ELISA and/or epidemiological considerations (see Section 8 following). Where an infected group of cattle can be targeted, the appropriate action may be the removal of the entire group, 'identified', as reactors and 'in-contacts'.

The minimum level of interpretation at subsequent reactor retests in higher risk breakdowns where infection is considered to be present should be:

- the removal, as reactor, of standard inconclusive animals within or originating from an infected group(s) of cattle, and
- depending on the extent of the infection, consideration of removal, as reactor, of animals 'inconclusive' to the severe interpretation chart or positive at the bovine site (straight bovine) within or originating from the infected group(s).

Animals, which have been identified and marked as standard inconclusive at interpretation, will on their next test have "I/C" on the interpretation screen under 'prev inc' opposite the animal's tag number. Animals which were deemed standard inconclusive at some test in their past but not at their most recent test will have "I/C\*" on the interpretation screen under 'prev inc', opposite the animal's tag number. Animals so marked should, at breakdown tests or reactor retest, have their full test

history reviewed by the Area VI, and their immediate removal should be considered if presenting with bovine readings and/or if present in a new or continued 'reactor' grouping.

Where disclosure of standard interpretation reactors continues into the 3rd reactor retest, the herd test history, ER76B report, post-mortem findings, results of supplementary tests and any other relevant data should be discussed between the area VI, SVI and the RSSVI to identify the most appropriate course of action for the herd. The SVI/SSVI at HQ may also be consulted at this point.

Where a repeat breakdown occurs at the post-de-restriction test (7b), or Classification related Check Test (5A), a full assessment of the herd should be conducted concentrating on the source of the residual or resurgent infection.

## **8. Supplementary Testing (Blood testing)**

To enable detection of the maximum number of infected and diseased animals in a herd or in a region, Commission Regulation 1226/2002, fully updating annex B of Directive 64/432/EEC, provides for the use of the gamma-interferon assay as an adjunct to the tuberculin test. The TB-sub-group of the Task Force (DG SANCO) has, furthermore, recommended extended use of the assay in infected herds. The Interferon Gamma Assay (IFN $\gamma$ ) received a statutory status nationally in 2005 (appendix 7) thereby facilitating the removal of animals positive to the assay.

### **When to use the Interferon- $\gamma$ assay**

The Interferon- $\gamma$  (IFN- $\gamma$ ) assay uses heparinised blood samples taken in vacutainers (green top). However, the Interferon- $\gamma$  samples require initial processing within 8 hours of collection. Blood samples must therefore, be delivered promptly to the laboratory. Please see Appendix 7 for the Protocol for the submission of Blood Samples for the IFN- $\gamma$  assay and the strategy for use in various situations. Prior approval for use of ancillary blood testing for bovine Tb must be obtained from HQ and should be made on the approved standard form.

The IFN- $\gamma$  assay and the SICTT both measure the cell-mediated T-cell response and thus there is to be expected a considerable overlap (~80%) between the animals that respond to these tests. The assay has a sensitivity of 84% that is comparable to, but lower than, the observed sensitivity of the SICTT (90%). However, there are sub-populations of *M. bovis*-infected cattle which give a positive reaction to the IFN- $\gamma$  assay and not to the tuberculin test and *vice versa*. Used in parallel with i.e. at the same time as the tuberculin test, the combined tests give a sensitivity of over 90%. The specificity of the IFN- $\gamma$  assay (97%) is lower than that of the SICTT (99.9%). This precludes the use of this assay as an alternative to the SICTT for screening purposes.

The application of the Interferon- $\gamma$  assay is therefore confined to test situations where there is a high probability that *M. bovis* infected cattle remain in the herd and is used in order to identify animals which pose a potential risk either within the current herd or if subsequently moved to another herd. This is the case in heavily infected herds where a regime of combined tuberculin test and IFN- $\gamma$  assay can be used effectively to remove



additional infected animals and thereby shorten the length of time taken to clear the herd. It is particularly useful, and thus should always be used, in herds that are considered for depopulation. It follows that when the assay has been used in these circumstances, positive animals should be removed as reactors. The definition of a reactor in Irish law allows the compulsory removal of these animals.

The obvious primary target herds therefore are those experiencing a Higher risk status (H) breakdown (see section 6 risk classification), which currently constitute approximately 30% of breakdowns. These herds are normally subject to an epidemiological investigation, which for the majority will also include a field visit, by the attendant V.I. who may be able to refine the targeted animals to particular groups or those associated with particular land fragments with regard to grazing/housing with the reactor animals during the relevant period prior to detection/removal. Where it is not possible to categorise particular epidemiological groups e.g. in smaller herds, all animals aged over 1 year should be tested.

While sampling at 10 days immediately following initial reactor disclosure such that all infected animals may be removed at the same time is desirable, the most practical time to carry out IFN $\gamma$ -assay on herds where SICTT reactors have already been removed may be in conjunction with the first reactor re-test, which should generally be carried out by the VI/WTVI. Positive IFN $\gamma$ -assay animals in the absence of concurrent Skin test reactors, i.e. within the same general timeframe, should be removed and the Singleton protocol may then be followed i.e. glands harvested and cultured, allowing de-restriction of those herds with negative results following a subsequent clear SICTT in 60 days.

Chronically infected herds, risk classification H, of which there are 253 (June 2010) are those that have maintained an H status continuously for at least 5-years and have therefore experienced repeat breakdowns over this timeframe. Such herds are at high risk of repeated breakdown and therefore H herds, which experience a H breakdown within 2 years of previous H-type breakdown, should also be targeted for sampling in an effort to remove all infected animals before restoring herd status (appendix 7). All adult animals in these herds should be targeted in an effort to reduce residual infection or possible spread to other herds. These herds could be IFN $\gamma$ -assayed at the time of the first Reactor retest by the VI/WTVI.

IFN- $\gamma$  assay should also be considered on all Inconclusive animals in non-derogated herds either 10 days immediately post disclosure or at the time of retest, provided that samples from such animals can be submitted together with other samples such that there are minimal submission costs. A VI may also use the IFN- $\gamma$  assay in herds being considered for derogation where there is any doubt as to eligibility or additional security is desirable. A VI must use the IFN- $\gamma$  assay in herds being considered for derogation where more than one inconclusive reactor animal has been disclosed in the herd. Inconclusive SICTT retests should, as far as possible, be carried out by a V.I.

Assays should be arranged in advance with the laboratory to streamline throughput. For maximum efficiency and reliability samples for assay should be taken on the day of injection for SICTT (Appendix 7).

The Interferon- $\gamma$  assay is also of particular assistance in determining the true tuberculosis status of an animal where skin measurements are likely to be compromised, for whatever reason and/or where it is suspected that there was ineffective injection of avian tuberculin (i.e. the test is not the SICTT but a positive bovine response is evident).

### **When to use ELISA test**

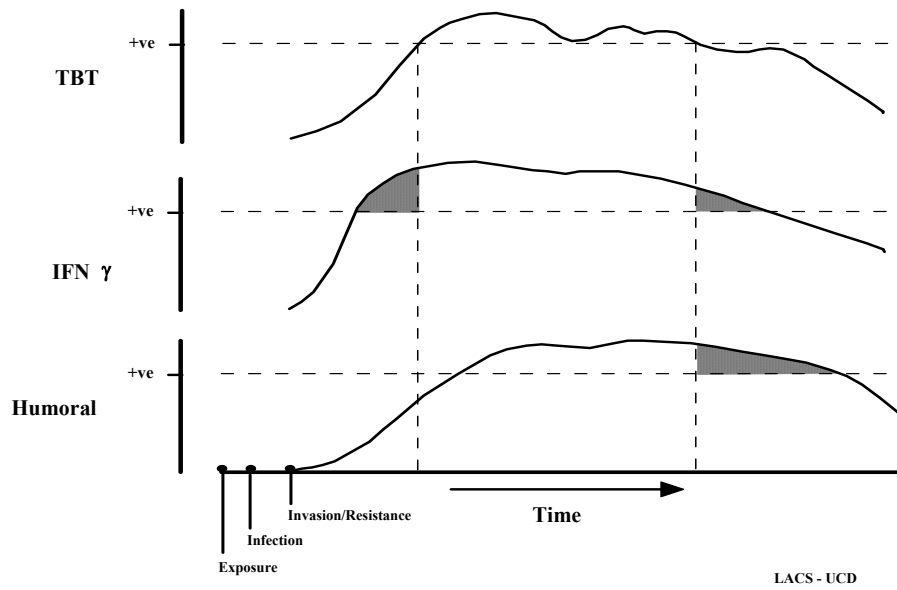
The ELISA and Anamnestic ELISA test uses serum from clotted blood samples taken in a similar manner to brucellosis samples. In contrast to the IFN- $\gamma$  assay and the SICTT, it measures the humeral response to infection with *M. bovis*. This response normally emerges later and lasts longer than the cell-mediated response (see illustration below).

The Anamnestic ELISA test is used to detect infected cattle that are anergic to the SICTT. For the purpose of this test blood samples are taken (from non-tuberculin test reactors) two weeks after injection for the SICTT. Work done in the CVRL indicates that the Anamnestic ELISA test has sensitivity in the region of 80%. However the specificity of this test may be as low as 50% in some situations. The Anamnestic ELISA test measures a serological response that may be useful in situations where the cell-mediated response has failed or has been overloaded. The application of the Anamnestic ELISA test is confined to known infected herds in which the presence of one or more anergic animals is suspected, and particularly, in cases in which infection is confined to particular sections of the herd – usually suspected where disclosure of test reactors (standard interpretation and/or lesioned animals) continues into or past the 3<sup>rd</sup> reactor retest despite severe interpretation etc. Its use is appropriate in an effort to shorten the length of time taken to clear such herds of infected cattle or particularly in herds which might otherwise be considered as candidates for depopulation and where the use of SICTT alone or in conjunction with the Interferon- $\gamma$  assay has failed to resolve the problem. Tests should be arranged in advance with the laboratory (CVRL).

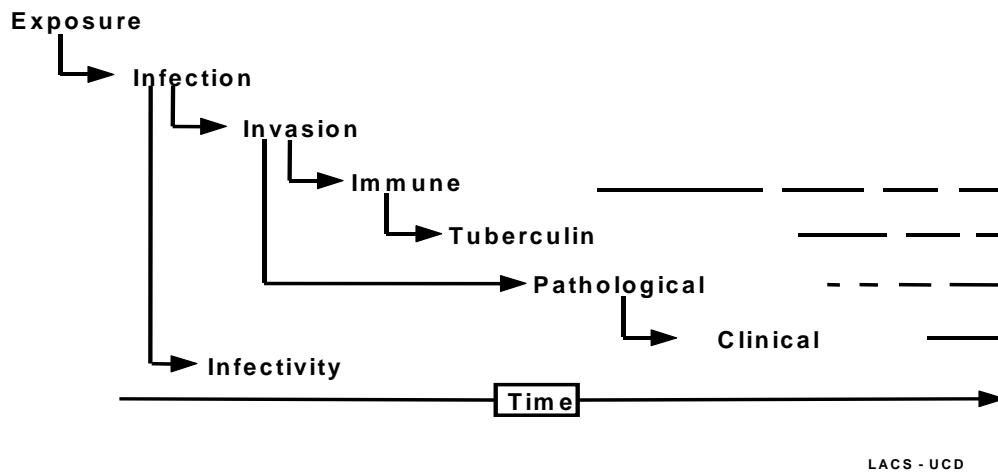
### **Enfer Multiplex assay for TB**

Research is underway in Ireland in conjunction with Enfer Scientific into a multiplex antibody assay for bovine TB. This assay is showing promise as a further means of detecting TB infected herds/animals. Staff will be kept apprised of developments in this area and the possibilities to avail of this test as part of the armoury to detect infection.

*Proposed relationships of immunological responsiveness of cattle following exposure to *M. bovis**



**Tuberculosis in Cattle: Sequence of**



## 9. Contiguous Testing Policy

Contiguous herd lists are compiled dynamically using data from the Departments iMap system. The AHCS system is populated from Herdfinder with a preliminary list from this data and this is validated prior to the initiation of a contiguous programme.

- (i) The preliminary ER 35 gives a herd profile of TB and Brucellosis eligible animals and a phone number where known, of each of the contiguous herds that have submitted a map for the Single Farm Payment (SFP). This information is updated daily from the iMap system. It does **not** have information on herdnumbers that do **not** apply for Single Farm Payment. The list is generated at 150m and those herds >25m from the index herd are marked.
- (ii) The 'Herd Finder' software also gives each DVO the capacity to print a map of the index herd and its contiguous herds in colour. This will allow the contiguous herds to be viewed more clearly. The user can determine the 'buffer' however the buffers used should ordinarily be 25m for TB and 150m for Brucellosis.

*If an index herd's number does not appear to be mapped, please check CCS for associated herdnumbers to determine the herdnumber used for SFP claims and review if the total holding claimed (and mapped) under that herdnumber includes the land relevant to the index herd being investigated.*

This preliminary list should then be checked and modified as necessary, during the course of visits by DVO staff to the restricted holding. Any additions or deletions to the ER35 list of herds contiguous at 25 meters should be recorded on the contiguous herd module of the AHCS. When the ER35 list has been finalised notification of contiguity to a breakdown should be sent to each keeper on the list and herds identified by the VI as requiring test then put on an AHCS programme.

The contiguous testing policy is dependent on the risk classification (H or L) of the index herd. Where herds are contiguous to more than one index herd, they should be put on a programme for each as appropriate. A programme will then continue for the contiguous herd until the last breakdown in the last index herd ends.

### **Testing policy in relation to herds contiguous to breakdown episodes classed as H (Higher risk)**

Herds contiguous to infected fragments of herds experiencing a higher-risk breakdown (H1; H2) should be restricted, pending test, if not tested in the previous six-months. Those subsequently determined during the ER76B visit as not associated or relevant to the breakdown e.g. not contiguous to or having had no animals contiguous to fragments associated with the reactor animals should be de-restricted. The contiguous herds deemed 'relevant' to the breakdown should, for the purpose of test listing, be recorded as a contiguous herd on AHCS and a High risk contiguous testing programme set up (see appendix 9). The programme on AHCS lists contiguous tests according to the following rules:

- (i) where the contiguous herd **was not** tested during the period 90 days prior to or subsequent to the breakdown in the index herd, an immediate test will issue or

- (ii) otherwise the contiguous herd is scheduled at a 120-day interval from the previous test carried out in that period and
- (iii) a contiguous test will be prioritised on the basis of the herd's existing scheduled test (Section 5).

Herds contiguous to a herd experiencing a higher-risk breakdown should remain on a 120-day-roll-over test schedule while the index herd remains restricted. The end date of the contiguous programme is extended where the index herd discloses further reactors at subsequent reactor tests so that programme is continued; it is automatically pushed out 180 days when the PM is followed up. The programme should not cease until a clear contiguous test (or a test type of higher priority) carried out **more than 60 days** after the last positive reactor was removed from the index herd. Herds on a contiguous programme to one index herd should be included on other programmes if further **relevant** index herds are identified. AHCS will maintain the contiguous herd on a testing programme until the next scheduled date for any contiguous test related to that herd passes the end date of the programme for the final relevant index herd.

The V.I. investigating the breakdown in the index herd decides which herds are relevant to the breakdown and therefore decides which herds should go on the programme. If he/she is of the opinion that a herd is not contiguous or not at risk (e.g. animals tested post housing and therefore no subsequent exposure) then it should not be included in or else removed from the higher risk contiguous programme. Where an index herd is considered to be no longer infected i.e. is being regarded as an Atypical herd the contiguous programme should be ended.

**V.I.s should actively monitor and manage** the contiguous programmes in their areas of responsibility and regularly review the herds to be listed for contiguous tests including any that should be added to a programme. In cases where there is deviation from policy there should be a record on the herd file as to the decision of the V.I. so it will stand up to potential future audit.

Standard inconclusive animals should be removed as reactors when identified in herds contiguous to herds whose current episode is classed H. If a VI wishes to deviate from this interpretation a IFN- $\gamma$  assay must be conducted and the agreement of the SVI sought and recorded on file before the decision is finalised.

When badgers have been implicated in a breakdown if, following survey under the wildlife policy, it is clear that badgers paths extend from the holding of the index herd to one or more other farms outside the 25m contiguity list then such farms should be added to the contiguous testing programme.

### **Testing policy in relation to herds contiguous to herds classed as L (Lower risk) or classed as H but experiencing a lower-risk breakdown episode.**

In general there is no requirement to specifically list herds for test if contiguous to a lower risk index herd (L1; 2) or to a herd classified H but experiencing an L breakdown episode. These herds, however, require assessment, from an epidemiological perspective, as to the necessity to test contiguous herds. When the SVI undertakes to have contiguous testing in herds contiguous to a herd classified L the SVI should authorise and record, on the index herd file, the reason for the input of these herds as contiguous herd into AHCS and their placement on a L contiguous programme.



## 10. Factory lesion policy

Suspect TB lesions found in clean cattle at routine *post-mortem* examination are reported to the DVO of the supplying herd, on AHCS by an automatic email generated following creation of the ER47 at the slaughter plant, on the day of slaughter. Following receipt of the report, the details should be input on a PM test created on AHCS. The herd trading status must then be **suspended** (restricted). Any other relevant herd should be identified and its status suspended with the appropriate reason recorded e.g. *PM suspect* if suspect animal left herd in previous six weeks and *Trace* if greater than six weeks. The laboratory process can be tracked on the lab test queue on AHCS. When the laboratory examinations are completed, details are returned to the factory and the DVO via AHCS.

1. A lesion found at slaughter and submitted to the laboratory must be 'seen' in the laboratory. If there is no visible lesion in the 'lesion' submitted to the laboratory, then sample substitution or some other error must be considered and the finding at slaughter must be regarded as confirmed.
2. Where TB is not confirmed and an alternative diagnosis is made (i.e. reason for the suspect lesion is stated, such as *R. equi* or an *actino* spp.), the herd(s) status should be restored (no test required).
3. Where TB is not confirmed but no alternative diagnosis is made (i.e. no reason is stated for the suspect lesion), then this is a slaughter check lab test with an inconclusive result. The herd status remains suspended as PM suspect pending a clear TT 5E (OTF Regain Status S.C.T.) a minimum of 42 days after the animal left the herd before the herd status may be restored. Both index and trace-back herd, if relevant, should be tested. (*Note: failure to confirm TB in this case is not proof of absence of M. bovis*).
4. Where TB is confirmed, the herd disease status of the index herd is **withdrawn** and the herd may not be derestricted until cleansing and disinfection is completed and two consecutive clear tests conducted, the first (TT9a) at a minimum of 60 days and the second (TT4) at a minimum of 4 months after the removal of the last positive reactor/infected animal.  
In cases where the lesioned animal was physically present in **more than one herd** if
  - a) the lab test positive animal left the previous herd in the six weeks prior to slaughter (*PM suspect*), the herd status should remain **suspended** until cleansing and disinfection is completed and two consecutive clear tests conducted, the first (TT5f) at a minimum of 42 days and the second (TT5e) at a minimum of 4 months after the removal of the last positive reactor/infected animal.
  - b) the lab test positive animal left the previous herd more than six weeks prior to slaughter (*Trace*), the herd may not be derestricted until cleansing and disinfection is completed and one clear herd test (TT5f) is completed.
  - c) the animal was transported directly from the mart (or elsewhere) to the slaughter plant, the slaughter plant may record the animal as from the herd of the person presenting it for slaughter. If the animal was not physically present

in this herd, then the DVO should assign the breakdown to the last herd in which the animal was physically present.

**N.B.:** The V.I. should, in all cases, carry out an epidemiological assessment of the probability of disease being present and apply status, testing, and classification accordingly.

The decision, following the disclosure of a suspect factory lesion to conduct an immediate 'balance of the herd' (type 10a) test, is left to the discretion of the VI/SVI who will base the decision on an assessment of the risk factors (e.g. more than one suspect lesion/contig herd should be regarded as higher risk) involved and the likelihood that there is an active breakdown in progress in the herd. However, it **must** be explained to the keeper that, if this immediate test happens to be clear, it will not qualify as part of the status restoration procedure if conducted inside the 42 or 60-day timeframe from removal of the infected animal as specified above which is a mandatory requirement under the Directive such that no exemption may be permitted. See also circulars ER/37/99, M6/99/GV in Appendix 11.

**The Lab test queue on AHCS should be managed on a regular basis so as to ensure timely and appropriate follow up action in each case.**

## **11. Tracing Policy**

### **Trace Onward:**

Animals that have been identified as at a **high-risk** of infection with TB and that have moved out of recently restricted herds currently experiencing an ER76 qualifying (Higher risk) breakdown should be traced forward to their current location using AHCS and other records. The Directive also requires tracing of **all** animals that have moved out of a herd which was 'derogated' after an inconclusive reactor animal had been detected and which was subsequently confirmed with disease (See Section 14).

The V.I. should refer, for information, to the computer generated AHCS Forward Trace Awaiting Notification list of animals that have moved out of the index herd when conducting the epidemiological investigation. These animals should be individually assessed to risk score their probability of failing their next TB test as a consequence of infection acquired in the index herd.

Animals identified as being at higher risk of failing their next TB test, i.e. at significant risk of actually being infected over and above the normal risk of any animal in a reactor herd, and which have not been tested more than 42 days post leaving the herd, should be marked as H on AHCS and notified. These animals should be tested in their new herd to ascertain their disease status.

*When an animal is entered onto the higher risk forward trace, the V.I. so designating the animal higher risk should be satisfied, on the basis of an epidemiological investigation, that the animal should immediately be listed for a test in the herd in which it is now located as it is probable given the pattern of the outbreak that it was infected in the herd from which it moved and is therefore likely to fail its next test (e.g. (a) if the progeny of a clinically infected dam or if other calves that remained in the herd became reactor so a source of infection amongst the young calves, such as a*

*TB mastitis, is suspected and thus either all calves or calves that were sold after an identified TB mastitis case calved must be forward traced or (b) if none or very few calves that remained in the herd became reactor therefore there is no reason to suspect that calves that left the herd were particularly or any more likely to be infected before leaving than those that remained, so there should be no need to forward trace calves that left the herd).*

The V.I., in the DVO area to which the animal has been traced, in assessing the data provided about the index herd will decide whether to test just the individual animal(s) (see Section 4) or the full herd in which they are now located. The herd to which they moved should be restricted – trading status suspended - because of the high probability, as assessed by the V.I. of the index herd, of having moved in a TB infected animal. The keeper now in possession of the animal(s) should be informed accordingly.

The relevant SVI in Animal Health and Welfare Division should be notified and provided with details of the export certificate of animals assessed as higher risk, which may have been exported without a test more than 42 days, after leaving the index herd so that the CVO in the importing country can be notified that they have imported an animal designated as highly probable of being infected with TB such that the animal requires attention.

The VI assessing the data provided about the index herd will decide whether it is appropriate to require the keeper to isolate the animal(s) pending test. *Article 16 of the TB Order states: (1) An authorised officer may, by notice in writing served on the owner, occupier or person in charge of any holding or other land, require such owner, occupier or person for the period of time specified in the notice to keep any cattle, goats or swine specified in the notice on the holding or other land, being cattle, goats or swine affected, suspected by him of being affected or capable of infecting cattle, with bovine tuberculosis, under such control as may be so specified or to confine such cattle, goats or swine to such part, as may be so specified, of the holding or other land.*

#### **Trace Back:**

Trace back should be completed in respect of introduced animals that fail the tuberculin test or confirm with tuberculosis on slaughter. The automation of tracing back of newly identified diseased animals is catered for by AHCS. The notification from the index DVO will be transmitted electronically via the AHCS network. On receipt of Trace-back notification, the V.I. should make a decision as regards listing the herd of origin for test having regard to the current status of the herd, the date of movement of the animal(s) that are the subject of the report and the date of herd tests subsequent to such movement.

In cases where a herd test is being listed and

- a) the back traced animal left the previous herd in the six weeks prior to disclosure and no further reactors are disclosed, the herd status should remain **suspended** until cleansing and disinfection is completed and two consecutive clear tests conducted, the first (TT5F) at a minimum of 42 days and the second (TT5E) at a minimum of 4 months after the removal of the last positive reactor/infected animal.

- b) the backtraced animal left the previous herd more than six weeks prior to disclosure, the herd should remain **suspended** until cleansing and disinfection is completed and one clear herd test (TT5F) is completed.

Where one or more reactors are disclosed on a backtrace test, the herd should be treated as any normal herd with test reactors.

**N.B.:** The V.I. should, in all cases, carry out an epidemiological assessment of the probability of disease being present and apply status, testing and classification accordingly.

## 12. Epidemiology

Where herd status is withdrawn (see Section 6), tracing and checking of any herds and animals considered to be epidemiologically related is to be undertaken in accordance with Directive 64/432/EEC Annex A I 3B.

All herds experiencing a breakdown that would result in an H classification (see Section 6) warrant a full (ER76B) investigation and report.

### Objectives

The ER 76B provides for a detailed epidemiological investigation of a disease episode on a holding (i.e. not merely a farm visit) to

1. Identify possible and probable sources of *M. bovis* infection in the herd
2. Advise the keeper on the zoonotic implications for the family and on the best course for management of the outbreak
3. Identify and risk categorise animals for forward tracing
4. Identify herds for inclusion in the contiguous programme
5. Check that DAF requirements re herd register are being adhered to (so that tracing is accurate) and
6. Provide data for the analysis required for policy formulation.

The investigation commences with an office-based examination of all the relevant herd-file and computer records. A farm visit, preferably by appointment, is then undertaken to collect further information and discuss management aspects with the keeper, opportunities for spread from the herd and any zoonotic risks. Data from this visit should be recorded on an ER76B report form.

Additional and more detailed epidemiological investigations must be conducted on herds that experience repeat breakdowns in order to determine if current practices in terms of restricted herds are optimal.

## 13. Singleton Protocol

Following disclosure of tuberculin reactors, a herd is classified D1 or re-classified as H1 or L1. Those herds with a single tuberculin reactor and classified as D1 may be considered as candidates for the 'singleton protocol' as described in circulars ER/12(12a,12b)/96 First time single inconclusive animals where the herd owner decides

to send the animal for immediate slaughter are not eligible (Appendix 10). See also Appendix 14 for details of analysis conducted on 2005 - 2008 singleton herds.

Candidate herds must meet the following criteria:

- 1) There must be only one reactor disclosed at the index test*
- 2) The bovine increase over the avian increase must be less than 12 millimetres*
- 3) There should be no oedema present at the bovine site.*
- 4) The herd must not have had its trading status withdrawn during the 3 years prior to this reactor and*
- 5) None of the contiguous herds are concurrently classified as H1 or H2.*

The SVI may refuse to enter a herd into the programme, or to allow it continue in the programme on the basis of a consideration of the disease situation in the area as a whole, or the occurrence of subsequent breakdown(s) in previously clear contiguous herds or where infected animals have been traced back to the herd.

The disease status of herds that enter this programme is 'suspended' rather than 'withdrawn'. Thus, under this policy, these herds will be de-restricted where

- the criteria for eligibility continue to be met and
- TB is not confirmed at post mortem and
- laboratory examination of glands is negative and
- the herd has been subjected to SICTT conducted at least 42 days after the removal of the reactor animal and
- the results of the herd level SICTT are negative.

The Post-derestriction Test type 7B is not applied to herds in which tuberculosis is not confirmed. The classification of these herds on de-restriction will immediately revert to FD0.



TB Followup - Microsoft Internet Explorer provided by Department of Agriculture

**AHCS** Department of Agriculture and Food Animal Health Computer System

User agr1268-Leonard Dolan Current DVO OFFALY D.V.O. Printer Location OFFALY D.V.O. [Index](#) [Change](#) [Exit](#)

**Objects** **Testing** **Permits** **Payments** **Valuations** **Badgers** **Reports**

**Search** **Create Test** **Private Tests** **Advance Itinerary** **Re-Schedule** **Maintenance** **Test Queues**

**32962030**

Test Date 11/02/2006 Test Result NEGATIVE  
 Test Description TB SKIN TEST Test Type REACTOR RE-TEST  
 Herd Number [S1790974](#) Herd Owner MARK LOUGHNANE  
 Actual Tester [KATHERINE HARRISON](#) Interpretation STANDARD  
 Part Test Flag This test is not a Part Test Re-Interpreted This is not a reinterpreted test

**Clinical Remarks**

Class	Pos	Inc	Neg	Total
BULLS	0	0	0	0
CALVES	0	0	0	0
COWS	0	0	0	0
HEIFERS	0	0	61	61
STEERS	0	0	3	3
TOTAL	0	0	64	64
PVP TOTAL	0	0	64	

Singleton ☒ ER76 ☐

MESSAGES

ACTIONS

HERD	Existing Test	Type	Date Scheduled	Vet	Interpretation Level	Part	Current Status	S	D	2
	Change <input type="checkbox"/>									
	New <input checked="" type="checkbox"/>	1	30/01/2007	P026746	STANDARD		Next Status	F	D	0
		1	30/01/2007	P026746	STANDARD			F	D	0

Local intranet

Start TB Followup - Mi... 16:51

Note;

- Care must be exercised by the VI when interpreting 'singleton' herds on AHCS in cases where further reactors are disclosed at part herd tests or balance of herd tests. In the follow-up screen, there are two check boxes opposite **singleton**, the lower of which must be un-ticked in order to record the removal of the herd from the singleton programme. Failure to do so ensures that the herd remains as **Unresolved** on the singleton report.
- In cases where further positive animals are disclosed in the index herd or where a contiguous herd becomes classified as H1 or H2, then it must be removed manually from the programme.
- Where TB is regarded as confirmed (lesions, further reactors or epidemiologically), then the herd must be removed manually from the programme and the breakdown must be re-categorised as L or H as appropriate.
- In cases where a reactor retest (TT4) has been conducted on a herd with a status D1 prior to entry of a lab test result, a warning will appear on screen "Awaiting lab results". Interpretation of the skin test should be deferred until after entry of lab test result otherwise a second TT4 will be scheduled and the herd will be classified SD2.

## 14. Inconclusive Policy

Directive 64/432/EEC, as amended, requires that where a herd contains an animal(s), which has shown an inconclusive reactor result to the SICTT, the status of the herd must be suspended until the animal(s) either

- (a) passes a further test after 42 days or
- (b) is negative *post mortem* and on laboratory examination.

If, at disclosure, such a herd is classified D and has not had its trading status withdrawn in the previous 3 years, then a **derogation** is provided in the Directive and the V.I. may decide not to suspend the herd trading status for local trade. In making the decision, to allow or disallow derogation, a V.I. will of course consider other factors such as contiguity or the tuberculosis profile of the herd of origin of the inconclusive animal(s) where bought in. See Circulars ER/6B/2000 and ER/6C/2000 (Appendix 10). The VI may thus allow animals to leave the 'derogated' herd; however, these animals are **not** eligible for intra-Community trade and where the presence of disease is subsequently confirmed (positive reactor – section 6), any animals that have moved onward since the last clear test traced and tested. See Circular ER08/2005, Appendix A Tuberculosis, (ii) Procedures in relation to status suspended herds, where infection is confirmed and which qualified for a derogation for national trade (Appendix 10 of this document).

Where there is more than one inconclusive reactor animal, the V.I. may not allow derogation to facilitate the movement of animals from the herd unless the inconclusive reactors have passed an IFN- $\gamma$  assay conducted on **all** the inconclusive reactor animals. The VI may also decide to make use of the IFN- $\gamma$  assay as part of the decision process for any particular single inconclusive reactor animal which otherwise meets the herd and animal eligibility criteria for derogation.

PVPs have been informed that an export certificate (i.e. that the animal has passed a test to export standard) may not issue for any animal from a herd with an inconclusive animal while awaiting the resolution of status of such inconclusive reactor animal(s) (see ER4). This is a legal obligation under The Bovine Tuberculosis (Attestation of the State and General Provisions) Order, 2000, S.I. No. 161 of 2000, Article 3 (o) which states "where there is an inconclusive animal in a holding, a veterinary surgeon shall not issue a veterinary certificate stating the animal has passed the test to the export standard in respect of any animal in that herd until the health status of the inconclusive reactor animal has been resolved as provided for in Council Directive 98/46/EC".

The deployment of AIM at marts and export locations has further ensured that no animals from ineligible herds are exported.

**If the inconclusive animal(s) is/are inconclusive or fail at the subsequent re-test, then herd classification as per section 6 above applies and all animals that have left the herd since the time of the last clear herd test must be traced and tested - see Appendix 10.**

When an inconclusive retest discloses a reactor, the necessity to carry out a Balance of herd test TT10c should be assessed by the V.I. based on the epidemiological information available.

## 15. Atypical Herds

The vast majority of TB reactor herds behave in a typical manner and are progressed through their restriction to clear status without raising further questions. However, within the restricted herd population there is a subset of herds that behave in an atypical manner in that:

1. They produce unusually large numbers of no visible lesion (NVL) reactors; and
2. They experience repeat 'reactor' episodes.

These restricted herds pose a challenging management problem. A serious doubt exists as to their true disease status that is not easily resolved.

In 2002 a special programme was commenced for such herds whereby as far as possible they are treated in a standard manner. Interpretation of SICTT is strictly in accordance with Directive 64/432/EEC Annex A I. 3A (b) but, given their history, a potential NSI problem must be suspect and severe interpretation, as a routine, is not applied so as to avoid unnecessary decimation of the herd. A full epidemiological investigation is required including, where applicable, use of IFN $\gamma$  assay, Anamnestic ELISA testing, laboratory examination, gland culture, and environmental mycobacteria check. Where TB infection has not been confirmed for some period of time and where all reactors at a 1st reactor retest are culture negative, as per the 'Singleton Protocol' (see Section 13), that test may be considered clear so that a second reactor retest can be scheduled and the herd derestricted should that test be clear.

The background to this programme and operational instructions are provided in Appendix 12. These herds are tracked by putting them on a 'special programme' on AHCS (see appendix 9).

Normal Regression of skin fold increases post Tuberculin injection (See Appendix 5E).

## 16. Depopulation

Depopulation of an infected herd is a well-recognized method to eradicate disease and thus is routinely practiced for diseases such as FMD and it also played a significant role in the success of the Irish Brucellosis eradication programme. The intention of the depopulation is that following restocking these farms would be capable of attaining and retaining a bTB free status. However, in the past analysis, by CVERA, of the subsequent history of herds which had undergone a depopulation due to TB showed that this policy was not effective in removing all sources of infection from the holding and that these herds had the same propensity to experience a repeat breakdown whether or not a depopulation was undertaken. The conclusion drawn from the analysis was that depopulation alone was insufficient to ensure that the re-stocked herd would attain or retain an officially free bTB status (OTF) as defined by Directive 64/432/EEC and recommended that the elimination of other on-farm sources of *M. bovis* was essential to protect the reconstituted herd. A TB depopulation that fails to meet its objective is ultimately of no benefit to the farmer, the neighbourhood or DAFF.

As is normal in the Irish programme, where science and epidemiological research is used to inform policy, the depopulation policy was adapted to take account of these findings and recommendations. The Interferon- $\gamma$  assay and/or the Anamnestic ELISA were required to help ensure that the possibility that the problem within the herd was

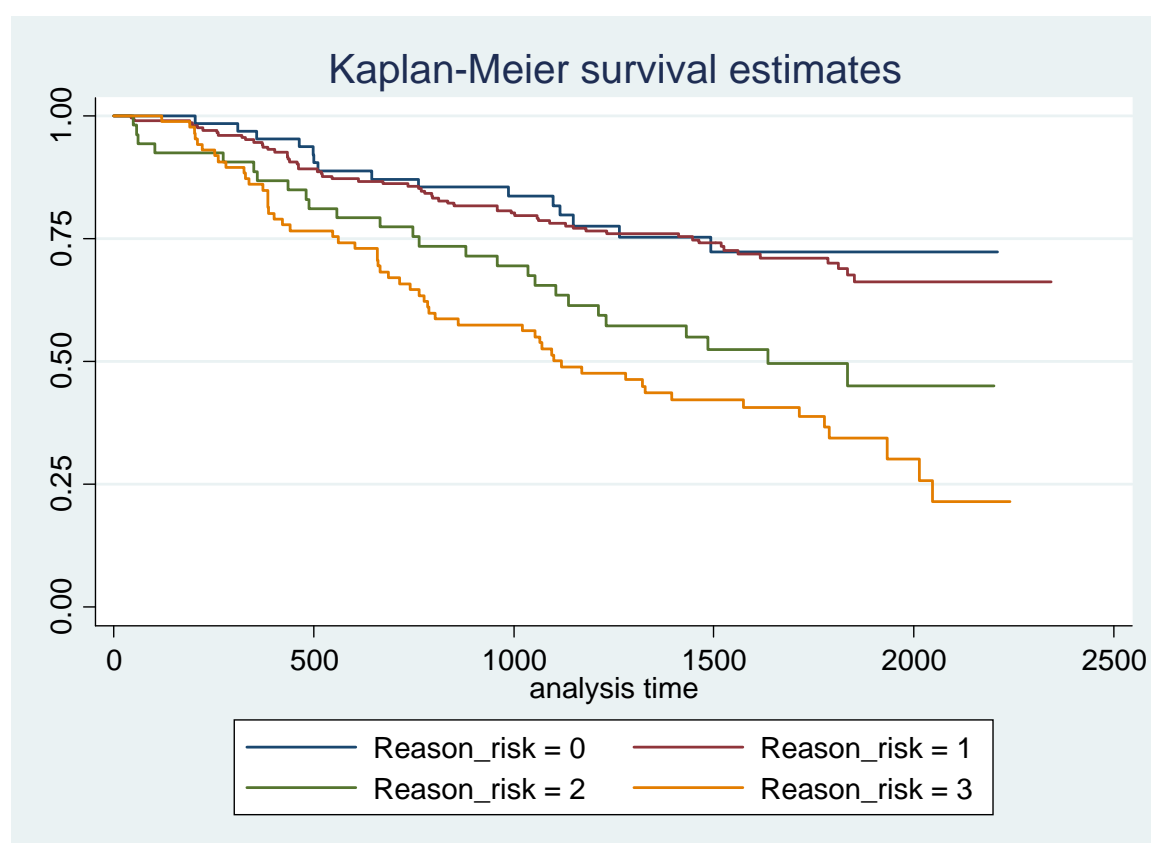
being driven by an infected bovine is significantly reduced. Herds depopulated are required, as are all TB infected herds, to undertake a programme of cleansing and disinfection as indicated by the investigating officer and the lands are kept free of bovine stock for a period not less than four-months. In addition, as applies for all TB infected herds, a programme of testing of herds contiguous to the depopulated herd is operated so that these herds are tested when the infection is active in the depopulated herd and also that they are tested at least 60-days after the last infected animal is removed from the depopulated herd. Furthermore, unless it is clear that the breakdown is not related to badgers a badger removal programme is operated for at least 18-months post-depopulation. The intention to remove other on-farm sources of bTB is therefore fulfilled.

A recent (2010) evaluation of the impact of herd depopulation on future bTB risk in the aftermath of the policy change was conducted on herds depopulated for either TB or BSE 2003-2005 inclusive and the outcome for these herds was tracked up until the end of 2009. This study concluded that the depopulation strategy employed in the Irish bTB eradication programme has succeeded in establishing herds that can attain and retain OTF status following restocking and thus the strategy should be maintained as part of the bTB eradication programme. However, this study indicates that when the other on-farm sources of bTB are removed there is no necessity to maintain the H risk classification for such herds post-restocking. In this study infection detected at the TT7a reflected animals that were infected in their herd of origin and therefore classified as bought-in. As is to be expected with bought-in infection there is little if any onward spread and the breakdown is transitory with ordinarily two clear tests followed by derestriction. It is therefore recommended that bTB depopulated herds revert to risk classification D post-restocking, with normal SICTT interpretation regimens applicable once the badger population in the vicinity has been reduced by culling (unless they have been eliminated as a probable source of the outbreak) and pending the availability of badger vaccination for TB. If reactors are detected at the TT7a, which should be conducted as soon as possible once restocking is substantially completed (~6-weeks) severe interpretation regimens should not apply as a routine but it should be regarded as an L breakdown and D classification applied post-derestriction.

As regards the criteria used to decide if a herd should be depopulated, it is policy that where the level of infection in the herd is such that, despite standard and repeated tuberculin testing, the application of the Interferon- $\gamma$  assay, and Anamnestic ELISA (if considered appropriate), epidemiological assessment and strategic removal of individual animals within the herd, disease continues to spread, serious consideration is given to depopulation. In the first instance, the herd or infected group must be subjected to the Interferon- $\gamma$  assay where it has not already been used, and then the suitability for removal of the entire infected group (partial depopulation or incontact removal) must be assessed. When the assay and/or incontact removal has failed to resolve the problem, then depopulation of the herd must be considered. As a more general rule, cases where more than 30% of the herd has tested positive may lead to depopulation being considered, whereas if 50% of the herd are reactors then depopulation must be considered. Depopulation must also be considered where the epidemiological assessment determines that control of TB in the herd or area will be otherwise compromised such as by an inability to implement satisfactory controls in the herd. Where herd depopulation has been deemed necessary the final decision will be made in consultation with the Regional SSVI. The SVI and RSSVI will determine the

appropriate rest period for the land usually a minimum of four months during which the keeper may not restock or have cattle on the land. Further, unless badgers have been excluded as a cause of the outbreak a badger capture programme must be conducted and a programme of testing undertaken in contiguous herds. See also section 18 Wildlife Policy.

Kaplan-Meier survival estimates for time to restriction following depopulation by reason-risk for depopulation and prior risk of bTb.



Reason-risk 0 = bTb, high risk, Reason-risk 1 = BSE, default risk, Reason-risk 2 = BSE, low risk, Reason-risk 3 = BSE, high risk.

*Note: Herds which have been depopulated may revert to 'D' classification going forward following restocking when at least one series of badger capture has been completed. A post-depopulation check test should be conducted as soon as practicable once restocking is substantially completed or 4-monhts post commencement whichever is first. Analysis has shown that this test is positive in ~5-7% of herds and ordinarily identifies animals that were infected in the herd of origin (i.e. bought-in) thus their prompt identification and removal is desirable.*

Where an infected group of cattle is present the appropriate response may be to confine the group to a specific location (see below) or possibly to remove the entire group, 'identified', as reactors. If the infected group is a discrete stand-alone entity (separately grazed/housed/tested) within the herd, with no contact with the remainder of the otherwise clear herd, then an application for a partial depopulation, with attendant grant eligibility may be made to the SVI. If the SVI considers that the application has merit it will be referred to the regional SSVI for decision. Please bear in mind that a partial

depopulation requires that the depopulated section of the holding remains free of cattle for the depopulation period in the same manner as if a full depopulation had occurred and under the TB Order, a formal notice of such should issue. *Article 16: (1) An authorised officer may, by notice in writing served on the owner, occupier or person in charge of any holding or other land, require such owner, occupier or person for the period of time specified in the notice to keep any cattle, goats or swine specified in the notice on the holding or other land, being cattle, goats or swine affected, suspected by him of being affected or capable of infecting cattle, with bovine tuberculosis, under such control as may be so specified or to confine such cattle, goats or swine to such part, as may be so specified, of the holding or other land.*

Further cleansing and disinfection, a contiguous herd testing programme and a badger removal programme should also operate as if a full herd depopulation had occurred.

## **17. Private Test & export-test certification.**

In the annual instructions to PVPs, they are informed, that “Any tuberculin test may only be conducted with the approval of a Veterinary Inspector”. This is provided for in the TB Order (Article 5(1) “A test shall not be carried out without the approval of a veterinary inspector”). Thus, a veterinary practitioner must have permission, before commencing a pre-movement test or a post-movement test for TB”. Private test permission will not be given for animals in herds, which have not had a herd test within the previous twelve months. In such cases, the herd test will be brought forward where requested. When assessing whether private test permission should issue for a particular herd, the Veterinary Inspector must assess whether there is epidemiological information available to establish a likelihood of exposure to infection. If such a likelihood of exposure to infection is identified, then permission to conduct a private tuberculin test must be refused and the listing or advancement of a herd test must be considered. Thus, the status of contiguous herds, any traceback information, presence of an inconclusive TB reactor, herd history, next test type scheduled and date that it is due have all to be considered. Private test permission should not issue when a herd test is already “Awaiting Itinerary”.

An ER9 must be submitted for Private tests arranged by the PVP in advance of the private TB test. The minimum test interval for a pre-movement tuberculin test for intracommunity trade in bovine animals is 42 days. Thus PVPs have been informed that export certification must not issue for an animal following a tuberculin test where the animal was also tuberculin tested within the previous 42 days. This applies equally to animals tested in the course of a herd test. (See ER4 appendix 2).

AIM checks will ensure that ineligible animals are not exported.

## **18. Wildlife Policy**

DAFF’s wildlife policy is primarily driven by the completion of an ER76B following a ‘H’-type breakdown (see section 6) and a request for a badger survey by the DVO SVI via AHCS. There will be occasions where SVIs will schedule herds other than ‘H’ type breakdowns for ER76B type investigations in response to an atypical breakdown, an

area problem or other local problems. The principle objective of the ER76B investigation is to establish firstly if the breakdown was due to TB and then if an introduced or residually infected animal was the likely source of the breakdown? If not, did the investigation detect badger signs in the local environment of the herd such that a local survey for badger habitats is warranted? Following this survey, the locations of any setts identified are computerised on DAFF's GIS databases and, where warranted, badgers are removed under a licence granted by the Parks and Wildlife division of the Department of Environment, Heritage & Local Government.

Once a sett is assigned to a treatment area for capturing, it will continue to be monitored for signs of re-population and where this occurs re-captures will be undertaken.

The long-term aim of DAFF's wildlife policy is to administer a BCG vaccine to badgers, thus rendering the species less susceptible to spreading and/or becoming infected with *M. bovis* and thus less likely to be a source of infection for cattle locally. The current Wildlife policy is designed to reduce the density of infected badgers in areas where TB has been identified in cattle herds, to record data on the location of setts and to increase the chance of success of a future vaccination program for badgers. Testing of a candidate vaccine is well advanced and results to date are promising. A large-scale field trial, which will be completed by 2013, commenced in 2009 to quantify the efficacy of this vaccine.

Depopulated herds: Unless the cause of the breakdown has been conclusively identified as non-badger related, an application for a capture block should normally be made. This block should be serviced every 3-4 months for 18 months after depopulation (full or partial).

Badgers reported dead or dying by farmers or other members of the public should normally be disposed of via the same route as badgers captured by the Department but not selected for *post-mortem* examination. However, where such badgers have been reported as dying in a field or shed where cattle are located then they should, if fresh, be submitted immediately for *post-mortem* examination. If positive for TB, the holding on which they were found should be restricted and a TB test conducted a minimum of 42-days post contact with the dying/dead TB-positive badger.

## **19. Survival of *M. bovis***

Depending on when, where and under what conditions the research has been conducted various survival times are reported in the literature. Thus the early work done suggested that *M. bovis* is a highly resistant organism surviving in cow faeces for at least 5 months in winter, 4 months in autumn, 2 months in summer and in soil for up to 2 years; 4 months in liquid manure stored underground and for 1-2 months in soil during the summer months (Williams and Hoy, 1930). Maddock (1933) reported that direct sunlight killed bacilli in cultures within a few hours whereas bacilli present in pus and morbid discharges may remain viable for several weeks. In summer months in England, *M. bovis* could not be recovered from grass contaminated with infected badger urine after 3 days or from naturally infected badger faeces after periods of 1 or 2 weeks. The activity of sunlight and of other bacteria, protozoa and fungi, which normally contribute to the breakdown of faeces, appears to destroy *M. bovis*. Similarly, the decomposition

of a carcass will destroy *M. bovis*. In a carcass left on pasture, the level of infection had dropped sharply after 2 weeks and after 4 weeks *M. bovis* could not be recovered. In 3 buried badger carcasses, *M. bovis* could not be recovered after 2, 3, and 6 weeks respectively. (Third Report MAFF, London, 1979). Other times quoted are summarised below.

*M. bovis* survival in a variety of conditions as reported in the literature.

Sample	Condition	Survival	Author
Cattle faeces	Summer - open jar	152-178 days	Maddock 1933
	Exposed grassland	56 days	Reuss 1955
	Shade in sealed flask 16-18°C	12 weeks	Zorawski <i>et al.</i> , 1978
	Sunlight 27.5°C	37 days	Vera & Volkovsky 1980
	Shade 27.5°C	71 days	
	Lab. 2-4°C	>135 days	Duffield & Young 1985
Solid manure		790 days	Turgenbaev 1989
Grass	Summer	49 days	Maddock 1933
	Autumn	63 days	Maddock 1934
Crop	Contaminated Sewage	35 days	Donsel & Larkin 1977
Dust		90-120 days	
Sputum	Cool, dark location	6-8 months	
Clothing		45 days	
Cheese – manufactured with raw milk containing <i>M.bovis</i>	The inoculation level is not given for most of the experiments in the reference	<i>M.bovis</i> detectable after	Collection of data from Spahr and Schaefroth 2001 (Appl. Env. Microbiol. 67, 4199-4205)
Camembert		180 days	
Cheddar		220 days	
Camembert		60 days	
Edam		60 days	
Bulgarian White		120 days	
Swiss Emmentaler	Both types of cheese are manufactured from naturally infected milk with 1-10 cfu/ml	5 days (not after 22 days)	
Gruyere		22 days(not after 31 days)	
Swiss Tilsiter		305 days	
Camembert		47 days	
Emmentaler	Infection of guinea pigs possible after 90-days	90 days	
Blue		90-120 days	



## 20. Desktop Resources

### Ezone

Access to ERAD Intranet. By clicking on “Business Areas” on the top bar of the screen, navigate to the “ERAD” area where the ERAD circulars and Forms may be accessed under “Circulars”. <http://ezone/intranet/businessareas/eraddivision/>

### Herdfinder

Contiguous Herd Enquiry or Herdfinder is accessed on the main screen of the Ezone-lower left hand side. Herdfinder can also be accessed by typing “HF” on the address bar of the Ezone. A new help section is now available on Herdfinder – see main Herdfinder page. The help section is a series of training videos that instruct users on how to do various tasks and can be viewed as many times as desired. The videos will be updated from time to time and as any new features and functions of Herdfinder become available. If you cannot ‘see’ the video on your PC because the correct software is not installed – click on the appropriate link and an e-mail will be sent to ISD who will in turn install the correct software to allow you to ‘view’ the video on your PC.

Please enter herd no. including check digit of herd and follow on screen instructions. Help videos should become available during the year (2010) when ISD have agreed on a common standard player. This Web programme is simple to use and additional training is available from time to time.

You can also search for named individuals and confine it to DVO areas using the name and address search. You can get X and Y co-ordinates and Latitude and Longitude values as well as mapping several chosen herds on the suite of programmes available on the Herdfinder site.

#### Library (also on main screen of Intranet)

- Legal resources
  - o Animal diseases consolidated legislation - TB / BR Orders
  - o OJ Online - don’t forget to logoff when finished
- Online journals and articles
- Many other sources of interest

#### Vetzone – This area is no available under “Business Areas” and “Veterinary Service” on the Ezone

- Other areas being updated

### Single Sign On System.

This is the Departments strategic platform and contains the area where AIM (Animal Identification and Movement), AHCS (Animal Health Computer System) and iMap are located. Log-Ins are arranged locally through the Corporate Customer System (CCS).

## Appendix 1 Directive 64/432/EEC

### ANNEX A

#### *ANNEX A (98/46/EC)*

##### I. Officially tuberculosis-free bovine herd

For the purposes of this section 'bovine animals' means all bovine animals with the exception of animals taking part in cultural or sporting events.

##### 1. A bovine herd is officially tuberculosis-free if:

- (a) all the animals are free from clinical signs of tuberculosis;
- (b) all the bovine animals over six weeks old have reacted negatively to at least two official intradermal tuberculin tests carried out in accordance with Annex B. the first six months after the elimination of any infection from the herd and the second six months later or. where the herd has been assembled solely from animals that originate in officially tuberculosis-free herds. the first test shall be carried out at least 60 days after assembly and the second shall not be required;
- (c) following the completion of the first test referred to in (b), no bovine animal over six weeks old has been introduced into the herd unless it has reacted negatively to an intradermal tuberculin test performed and assessed according to Annex B and carried out either in the 30 days prior to, or the 30 days after the date of its introduction into the herd; in the latter case the animal(s) must be isolated physically from the other animals of the herd in a way to avoid any direct or indirect contact with the other animals until proven negative.

However, the competent authority may not require this test to be carried out for movements of animals on its own territory if the animal is from an officially tuberculosis-free herd, except in a Member State where, on 1 January 1998 and until the status of officially tuberculosis -free region is obtained. the competent authority required such tests to be carried out for animals moving between herds participating in a network system as referred to in Article 14.

##### 2. A bovine herd will retain officially tuberculosis -free status if:

- (a) the conditions detailed in 1 (a) and (c) continue to apply;
- (b) all animals entering the holding come from herds of officially tuberculosis-free status;
- (c) all animals on the holding, with the exception of calves under six weeks old which were born in the holding. are subjected to routine tuberculin testing in accordance with Annex B at yearly intervals.

However the competent authority of a Member State may. for the Member State or part of the Member State where all the bovine herds are subject to an official programme to combat tuberculosis alter the frequency of the routine tests as follows:

- if the average -determined at 31 December of each year- of the annual percentages of bovine herds confirmed as infected with tuberculosis is not more than 1 % of all herds within the defined area during the two most recent annual supervisory periods the interval between routine herd tests may be increased to two years and male animals for fattening within an isolated epidemiological unit may be exempted from tuberculin testing provided that they come from officially tuberculosis-free herds and that the competent authority guarantees that the males for fattening will not be used for breeding and will go direct for slaughter.
- if the average -determined at 31 December of each year -of the annual percentages of bovine herds confirmed as infected with tuberculosis is not more than 0.2 % of all herds within the defined area during the two most recent biennial supervisory periods. the interval between routine tests may be increased to three years and/or the age at which animals have to undergo these tests may be increased to 24 months.

- if the average -determined at 31 December of each year- of the annual percentages of bovine herds confirmed as infected with tuberculosis is not more than 0.1 % of all herds within the defined area during the two most recent supervisory triennial periods the interval between routine tests may be increased to four years, or, providing the following conditions are met, the competent authority may dispense with tuberculin testing of the herds:

(1) before the introduction into the herd all the bovine animals are subjected to an intra- dermal tuberculin test with negative results; or

(2) all bovine animals slaughtered are examined for lesions of tuberculosis and any such lesions are submitted to histopathological and bacteriological examination for evidence of tuberculosis.

The competent authority may also in respect of the Member State or a part thereof, increase the frequency of tuberculin testing if the level of the disease has increased.

3A. The officially tuberculosis-free status of a herd is to be suspended if:

(a) the conditions detailed in paragraph 2 are no longer fulfilled; or

(b) one or more animals are deemed to have given a positive reaction to a tuberculin test, or a case of tuberculosis is suspected at post-mortem examination.

When an animal is considered to be a positive reactor it will be removed from the herd and slaughtered. Appropriate post-mortem, laboratory and epidemiological examinations shall be carried out on the positive reactor or the carcase of the suspect animal. The status of the herd will remain suspended until such time as all laboratory examinations have been completed. If the presence of tuberculosis is not confirmed, the suspension of the officially tuberculosis-free status may be lifted following a test of all animals over six weeks of age with negative results at least 42 days after the removal of the reactor animal(s); or

(c) the herd contains animals of unresolved status as described in Annex B. In this case, the status of the herd is to remain suspended until the animals' status has been clarified. Such animals must be isolated from the other animals of the herd until their status has been clarified, either by a further test after 42 days or by post-mortem and laboratory examination;

(d) however, by way of derogation from the requirements of paragraph (c), in a Member State where the competent authority carries out routine herd testing using the comparative tuberculin test described in Annex B, and in the case of a herd where no confirmed reactor animals have been disclosed for at least three years, the competent authority may decide not to restrict the movement of other animals in the herd, provided that the status of any inconclusive reactors is resolved by a further test after 42 days and that no animals from the holding are allowed to enter into intra-Community trade until the status of any inconclusive reactors has been resolved. If at this further test any animal either gives a positive reaction or continues to give an inconclusive reaction, then the conditions of paragraph (b) apply. If the presence of disease is subsequently confirmed, all animals leaving the holding since the time of the last clear herd test must be traced and tested.

3B. The officially tuberculosis-free status of the herd is to be withdrawn if the presence of tuberculosis is confirmed by the isolation of *M. bovis* on laboratory examination.

The competent authority may withdraw status if:

(a) the conditions detailed in point 2 are no longer fulfilled, or

(b) classical lesions of tuberculosis are seen at post-mortem examination, or

(c) an epidemiological enquiry establishes the likelihood of infection.

(d) or for any other reasons considered necessary for the purpose of controlling bovine tuberculosis.

Tracing and checking is to be undertaken by the competent authority of any herd considered to be epidemiologically related. The officially tuberculosis-free status of a herd is to remain withdrawn until cleansing and disinfection of the premises and utensils has been completed and all animals over six weeks of age have reacted negatively to at least two consecutive tuberculin tests, the first no less than 60 days and the second no less than four months and no more than 12 months after the removal of the last positive reactor.

4. On the basis of information supplied in accordance with Article 8, a Member State or part of a Member State may be declared officially tuberculosis-free according to the procedure laid down in Article 17 if it meets the following conditions:

- (a) the percentage of bovine herds confirmed as infected with tuberculosis has not exceeded 0.1% per year of all herds for six consecutive years and at least 99.9% of herds have achieved officially tuberculosis-free status each year for six consecutive years the calculation of this latter percentage to take place on 31 December each calendar year;
- (b) each bovine animal is identified in accordance with Community legislation, and
- (c) all bovine animals slaughtered are subjected to an official post-mortem examination;
- (d) the procedures for suspension and withdrawal of officially tuberculosis-free status are complied with.

5. The Member State or part of a Member State will retain officially tuberculosis-free status if the conditions 4(a) to (d) continue to be met. However if there is evidence of a significant change in the situation as regards tuberculosis in a Member State or part of a Member State which has been recognised as officially tuberculosis-free, the Commission may, in accordance with the procedure laid down in Article 17, take a Decision suspending or revoking the status until the requirements of the Decision have been fulfilled.

## **ANNEX B**

### **TUBERCULOSIS**

#### **1. IDENTIFICATION OF THE AGENT**

The presence of *Mycobacterium bovis* (*M. bovis*), agent of bovine tuberculosis, in clinical and post-mortem specimens may be demonstrated by examination of stained smears or immunoperoxidase techniques and confirmed by cultivation of the organism on primary isolation medium.

Pathological material for the confirmation of *M. bovis* should be taken from abnormal lymph nodes and parenchymatous organs such as lungs, liver, spleen, etc. In the cases where the animal does not present pathological lesions samples from the retropharyngeal, bronchial, mediastinal, supramammary, mandibular, and some mesenteric lymph nodes and liver should be collected for examination and culture.

Identification of isolates may be usually carried out by determining cultural and biochemical properties. The polymerase chain reaction (PCR) may also be employed for the detection of the *M. tuberculosis* complex. DNA analysis techniques may prove to be faster and more reliable than biochemical methods for the differentiation of *M. bovis* from other members of the *M. tuberculosis* complex. Genetic fingerprinting allows distinguishing between different strains of *M. bovis* and will enable patterns of origin, transmission and spread of *M. bovis* to be described.

The techniques and media used, their standardisation and the interpretation of results must conform to that specified in the OIE Manual of Standards for Diagnostic Tests and Vaccines. Fourth Edition. 2000. Chapter 2.3.3 (bovine tuberculosis}.

#### **2. THE TUBERCULIN SKIN TEST**

Tuberculin PPD (Purified Protein Derivatives} that fulfil the standards laid down in paragraph 2.1 shall be used for carrying out official tuberculin skin test following the procedures referred to in paragraph 2.2.

## 2.1. Standards for tuberculin (bovine and avian)

### 2.1.1. Definition

Tuberculin purified protein derivative (tuberculin PPD, bovine or avian) is a preparation obtained from the heat-treated products of growth and lysis of *Mycobacterium bovis* or *Mycobacterium avium* (as appropriate) capable of revealing a delayed hypersensitivity in an animal sensitised to micro-organisms of the same species.

### 2.1.2. Production

It is obtained from the water-soluble fractions prepared by heating in free-flowing steam and subsequently filtering cultures of *M. bovis* or *M. avium* (as appropriate) grown in a liquid synthetic medium. The active fraction of the filtrate consisting mainly of protein is isolated by precipitation, washed and re-dissolved. An antimicrobial preservative that does not give rise to false positive reactions, such as phenol may be added. The final sterile preparation free from mycobacterium is distributed aseptically into sterile tamper-proof glass containers which are then closed so as to prevent contamination. The preparation may be freeze-dried.

### 2.1.3. Identification the product

Inject a range of graded doses intradermally at different sites into suitably sensitised albino guinea-pigs, each weighing not less than 250 g. After 24 h to 28 h, reactions appear in the form of oedematous swellings with erythema with or without necrosis at the points of injection. The size and severity of the reactions vary according to the dose. Un-sensitised guinea-pigs show no reactions to similar injections.

### 2.1.4. Test

#### 2.1.4.1. PH: The pH is 6.5 to 7.5

#### 2.1.4.2. Phenol: If the preparation to be examined contains phenol, its concentration is not more than 5 g/l.

#### 2.1.4.3. Sensitising effect: Use a group of three guinea-pigs that have not been treated with any material which will interfere with the test. On 3 occasions at intervals of five days inject intradermally into each guinea-pig a dose of the preparation to be examined equivalent to 500 IU in 0.1 ml. 15 to 21 days after the third injection inject the same dose (500 IU) intradermally into these animals and into a control group of three guinea-pigs of the same mass and which have not previously received injections of tuberculin. 24 to 28 hours after the last injections, the reactions of the two groups are not significantly different.

#### 2.1.4.4. Toxicity: Use two guinea-pigs, each weighing not less than 250 g and which have not previously been treated with any material which will interfere with the test. Inject subcutaneously into each guinea-pig 0.5 ml of the preparation to be examined. Observe the animals for seven days. No abnormal effects occur during the observation period.

#### 2.1.4.5. Sterility: It complies with the test for sterility prescribed in the monograph on Vaccines for veterinary use 4th Edition 2002 of the European Pharmacopoeia.

### 2.1.5. Potency

The potency of tuberculin purified protein derivative (bovine and avian) is determined by comparing the reactions produced in sensitised guinea-pigs by the intradermal injection of a series of dilutions of the preparation to be examined with those produced by known concentrations of a reference preparation of tuberculin (bovine or avian, as appropriate) purified protein derivative calibrated in International Units.

To test the potency, sensitise not fewer than nine albino guinea-pigs, each weighing 400 g to 600 g, by the deep intramuscular injection of 0.0001 mg of wet mass of living *M. bovis* of strain AN5

suspended in 0.5 ml of a 9 g/l solution of sodium chloride R for bovine tuberculin, or a suitable dose of inactivated or live *M. avium* for avian tuberculin. Not less than four weeks after the sensitisation of the guinea-pigs, shave their flanks to provide space for not more than four injection sites on each side. Prepare dilutions of the preparation to be examined and of the reference preparation using isotonic phosphate-buffered saline (pH 6.5-7.5) containing 0.005 g/L of polysorbate 80 R. Use not fewer than three doses of the reference preparation and not fewer than three doses of the preparation to be examined. Choose the doses such that the lesions produced have a diameter of not less than 8 mm and not more than 25 mm. Allocate the dilutions randomly to the sites using a Latin square design. Inject each dose intradermally in a constant volume of 0, 1 ml or 0.2 ml. Measure the diameters of the lesions after 24 to 28 hours and calculate the result of the test using the usual statistical methods and assuming that the diameters of the lesions are directly proportional to the logarithm of the concentration of the tuberculins.

The test is not valid unless the fiducial limits of error ( $P = 0, 95$ ) are not less than 50% and not more than 200% of the estimated potency. The estimated potency is not less than 66% and not more than 150% of the stated potency for bovine tuberculin. The estimated potency is not less than 75% and not more than 133% of the stated potency for avian tuberculin. The stated potency is not less than 20000 IU/ml for both tuberculins (bovine and avian).

#### 2.1.6. *Storage*

Store protected from light, at a temperature of  $5 \pm 3^\circ\text{C}$ .

#### 2.1.7. *Labelling*

The label states:

- the potency in International Units per millilitre,
- the name and quantity of any added substance,
- for freeze-dried preparations.
  - the name and volume of the reconstituting liquid to be added.
  - that the product should be used immediately after reconstitution.

### 2.2. **Test procedures**

- 2.2.1. The following shall be recognised as official intradermal tuberculin tests:
- the single intradermal test: this test requires a single injection of bovine tuberculin,
  - the intradermal comparative test: this test requires one injection of bovine tuberculin and one injection of avian tuberculin given simultaneously.

- 2.2.2 The dose of tuberculin injected shall be:
- not less than 2 000 IU of bovine tuberculin.
  - not less than 2 000 IU of avian tuberculin.

- 2.2.3 The volume of each injection dose shall not exceed 0.2 ml.

- 2.2.4 Tuberculin tests shall be carried out by injecting tuberculin(s) into the skin of the neck. The injection sites shall be situated at the border of the anterior and middle thirds of the neck. When both avian and bovine tuberculins are injected in the same animal, the site for injection of avian tuberculins shall be about 10 cm from the crest of the neck and the site for the injection of bovine tuberculin about 12.5 cm lower on a line roughly parallel with the line of the shoulder or on different sides of the neck; in young animals in which there is not room to separate the sites sufficiently on one side of the neck, one injection shall be made on each side of the neck at identical sites in the centre of the middle third of the neck.

- 2.2.5 The technique of tuberculin testing and interpretation of reactions shall be as follows:

#### 2.2.5.1 *Technique:*

Injection sites shall be clipped and cleansed. A fold of skin within each clipped area shall be taken between the forefinger and thumb and measured with callipers and recorded. The dose of

tuberculin shall then be injected by a method that ensures that the tuberculin is delivered intradermally. A short sterile needle, bevel edge outwards, with graduated syringe charged with tuberculin, inserted obliquely into the deeper layers of the skin may be used. A correct injection shall be confirmed by palpating a small pea-like swelling at each site of injection. The skin-fold thickness of each injection site shall be remeasured 72 hours (+/- 4 hours) after injection and recorded.

#### 2.2.5.2 Interpretation of reactions

The interpretation of reactions shall be based on clinical observations and the recorded increase(s) in skin-fold thickness at the sites of injection 72 hours after injection of tuberculin(s).

- (a) Negative reaction: if only limited swelling is observed, with an increase of not more than 2 mm in the thickness of the fold of skin without clinical signs such as diffuse or extensive oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes.
- (b) Inconclusive reaction: if no clinical signs such as mentioned in a) are observed and if the increase in skin-fold thickness is more than 2 mm and less than 4 mm.
- (c) Positive reaction: if clinical signs such as mentioned in a) are observed or there is an increase of 4 mm or more in the thickness of the fold of skin at the injection site.

#### 2.2.5.3 The interpretation of official intradermal tuberculin tests shall be as follows:

##### 2.2.5.3.1. Single intradermal test:

- (a) positive: - a positive bovine reaction as defined in paragraph 2.2.5.2(c);
- (b) inconclusive: an inconclusive reaction as defined in paragraph 2.2.5.2(b);
- (c) negative: a negative bovine reaction as defined in paragraph 2.2.5.2(a).

Animals inconclusive to the single intradermal test shall be subjected to another test after a minimum of 42 days.

Animals which are not negative to this second test shall be deemed to be positive to the test.

Animals positive to the single intradermal test may be subjected to an intradermal comparative test if false positive reaction or interference reaction is suspected.

##### 2.2.5.3.2. Intradermal comparative test for the establishment and maintenance of officially tuberculosis-free herd status:

- (a) positive: - a positive bovine reaction which is more than 4 mm greater than the avian reaction, or the presence of clinical signs:
- (b) inconclusive: a positive or inconclusive bovine reaction which is from 1 to 4 mm greater than the avian reaction, and the absence of clinical signs:
- (c) negative: a negative bovine reaction, or a positive or inconclusive bovine reaction but which is equal to or less than a positive or inconclusive avian reaction and the absence of clinical signs in both cases.

Animals inconclusive to the intradermal comparative test shall be subjected to another test after a minimum of 42 days. Animals, which are not negative to this second test, shall be deemed to be positive to the test.

2.2.5.3.3. Officially tuberculosis-free herd status may be suspended and animals from the herd shall not be allowed to enter intra-Community trade until such time as the status of the following animals is resolved:

- (a) animals which have been deemed to be inconclusive to the single intradermal tuberculin test:
- (b) animals which have been deemed to be positive to the single intradermal tuberculin test but are awaiting retest with an intradermal comparative test:
- (c) animals which have been deemed to be inconclusive to the intradermal comparative test.

2.2.5.3.4. Where animals are required by Community legislation to be subjected to an intradermal test prior to movement, the test shall be interpreted so that no animal which shows an increase in skin-fold thickness greater than 2 mm or the presence of clinical signs is entered into intra-Community trade.

2.2.5.3.5. To enable detection of the maximum number of infected and diseased animals in a herd or in a region, Member States may modify the criteria for the interpretation of the test in order to achieve improved test sensitivity considering all inconclusive reactions referred in 2.2.5.3.1 (b) and 2.2.5.3.2(b) as positive reactions.

### 3. SUPPLEMENTARY TESTING

To enable detection of the maximum number of infected and diseased animals in a herd or in a region, Member States may authorise the employ of the gamma-interferon assay referred in the OIE Manual of Standards for Diagnostic Tests and Vaccines, 4th Edition, 2000, Chapter 2.3.3. (bovine tuberculosis), in addition to the tuberculin test.

### 4. STATE INSTITUTES AND NATIONAL REFERENCE LABORATORIES

#### 4.1 **Tasks and responsibilities**

The State Institutes and Reference Laboratories included in paragraph 3.2 shall be responsible for the official testing of tuberculins or reagents included in paragraph 2 and 3 in their respective States to ensure each of these tuberculins or reagents are adequate in relation to the standards above referred.

#### 4.2 **List of State institutes and national reference laboratories:**



## **Appendix 2 PVP Quality Control**

### **Appendix 2 (a)**

#### **ERAD Document No. ER4 (Revised 2010)**

ERAD Document No. ER4 (Revised 2010)

Now incorporating ER68 and ER71

**CONDITIONS AND INSTRUCTIONS FOR VETERINARY PRACTITIONERS INVOLVED IN TESTING AND SAMPLING UNDER THE BOVINE TUBERCULOSIS AND BRUCELLOSIS ERADICATION PROGRAMMES.**

**ACKNOWLEDGEMENT AND ACCEPTANCE OF THESE CONDITIONS/ INSTRUCTIONS IS REQUIRED FROM PVPs AND WTVIs AS THE APPLICATION FOR CONTINUED APPROVAL TO TEST/SAMPLE FOR THESE PROGRAMMES UNDER THE APPLICABLE LEGISLATION.**

**Please Note:** If there is any element of this document which is unclear or which you do not understand please contact the SVI<sup>1</sup> in your local DVO<sup>2</sup> to discuss the matter.

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<sup>1</sup> Superintending Veterinary Inspector

<sup>2</sup> District Veterinary Office

## 1. Conditions Common both to Tuberculin testing and Sampling for Brucellosis

### 1.1 General Approval of Private Veterinary Practitioners (PVPs) ( and conditions for Eligibility to Test/Sample )

To conduct the single intradermal comparative tuberculin test (SICTT) and sampling for Brucellosis under the Diseases of Animals Act, 1966, Orders made there under and related EU legislation, it is a requirement that a PVP:

- A be **entered** in the current Register of Practitioners for Ireland,
- B be **authorised** by the Minister under the Act, and,
- C be **approved** under the TB and/or Brucellosis Orders as appropriate.

Ministerial approval requires that a PVP must:

- i. apply for approval – New applicants must complete and submit form ER3 to the local DVO and complete the ER67 Contract in the presence of DVO SVI;
  - ii. attend at a training course, if the Minister so requires;
  - iii. commit formally and adhere strictly to the instructions, terms and conditions as laid down in this ER4 document; (*Approval to test takes place on an annual basis following receipt of the acknowledgement attached to the circular letter that accompanies the ER4*);
- and,
- iv. use and update the unique identity codes (user code and password) and access number (Personal Identification number (PIN)) issued for personal use in respect of the Animal Health Computer System and keep details of these confidential. A PVP must not allow any third party access to their user code and/or password as this would facilitate false certification of a test.

**The PVP, having been issued with an approved test(s) may, in exceptional circumstances, request the Superintending Veterinary Inspector (SVI) or the Veterinary Inspector (VI) in charge for permission not to carry out the approved test(s) issued.**

If the PVP does not complete approved test(s) within the allotted time, or on the planned date(s) submitted to the DVO, in the absence of a valid reason(s) given in writing, and/or does not submit test reports within the prescribed timeframes, further approved test(s) will not be issued other than in exceptional circumstances.

No liability shall attach to the Minister for compensation or damages in respect of any claims arising from the performance of testing/sampling.

Failure to comply with the instructions, terms and conditions, including those relating to equipment, performance of test, record keeping and other administrative procedures, may, depending on the nature of the infringement, result in sanction appropriate to the infringement up to and including the immediate withdrawal of approval to conduct the SICTT and Brucellosis sampling in accordance with the “**Protocol for the assessment and maintenance of quality control standards in the delivery of services by private veterinary practitioners (PVPs).**”

The approval to test/sample may be terminated by notice of either party or following a decision in the context of the Appeals Procedure referred to at (a) below and subject to (b) below:

- (a) Disputes arising and regarding the performance of testing by an approved veterinary practitioner (including withdrawal of authorisation to carry out TB testing and or

Brucellosis sampling) shall be subjected to the appeals procedure established by the Department in consultation with Veterinary Ireland.

- (b) Notwithstanding the above, the decision of the Minister in relation to all aspects of approval and termination thereof shall be final.

## 1.2 Specific Test Approval at herd/animal level

Notwithstanding the authorisation and approval to test outlined above, a SICTT or blood sampling for Brucellosis may not be conducted at animal (TB) or herd level (TB and Brucellosis) without the prior approval of a Veterinary Inspector. An approved test is therefore a test for which permission has been granted by the SVI or VI in charge at the local DVO.

**Approval may be subject to the conditions attached, such as date after which test is to be done, interpretation level, home-reared calves under 6-weeks of age to be tested etc.**

Further details on the conditions specific for TB testing are set out in paragraph 9 of this document.

Further details on the conditions specific for Brucellosis testing are set out in paragraph 10 of this document.

Fees will not be paid by the Department in respect of unapproved tests.

### 1.3 Requesting test approval at animal or herd level

- As stated in paragraph 1.2, for TB, a SICTT may not be conducted at animal or herd level, or in advance of the date scheduled for the test, or other than in accordance with the conditions attached to the test listing. This is vital so that herds where status is being restored comply with EU Directives to resume trading eligibility. All tests must have the prior approval of a Veterinary Inspector and it is illegal to carry out a test without such approval. An approved test is therefore a test for which permission has been granted by the SVI or VI in Charge at the Local DVO and subject to any conditions attached. Test approval, in every case, is subject to the receipt of an advance itinerary, within the instructed timeframe.
- Herd test itineraries may be forwarded to the DVO through AHCS up to the day prior to the commencement of the test and up to mid-day on the Thursday of the week prior to commencement of the test in the case of non-electronic submissions. In very *exceptional* circumstances, substitutions to the advance itinerary, cancellations, alterations or change of PVP, are permitted even on the date of the test if notified to the DVO for approval prior to the commencement of the test. Communication by telephone is permitted in such exceptional circumstances.
- Private test itineraries with the planned PVP must be submitted as part of private approval for online PVPs. Itineraries for private tests can be submitted on AHCS on or before the planned date. It should be noted that private tests carried out on dates not corresponding to the planned date or the planned PVP are not approved and therefore illegal. For PVPs working offline test approval is subject to the submission of ER9 form. Private test approval will lapse 14-days after it was originally granted and permission must be applied for again if the test has not been conducted within the submitted timeframe.
- All cancellations or alteration to the test itinerary must be forwarded to the DVO through AHCS (when interacting electronically) or ER9 if still operating offline (not interacting electronically with AHCS). A current pre-printed or downloaded herd profile, obtained from AHCS (i.e. drawn down no earlier than one week prior to test commencement), **must** be used for each test. Download of the herd profile constitutes the permission to test as per the conditions pertaining to the test listing and the advance itinerary submitted (appointed date and time). Tests carried out at dates and times other than as per the submitted advanced itinerary are therefore not approved and are illegal.

**Under no circumstance is tuberculin testing or blood sampling for Brucellosis permitted at or on a Mart premises.**

#### 1.4 A Herd Test.

The herd is all the bovine animals on the holding comprising an epidemiological unit. Whenever a full-herd SICTT is being conducted, the presence of animals on the holding that are not presented for testing must be reported to the D.V.O. In the case of a herd test, when all eligible bovine animals on the holding are not tested, then de-facto the herd status may not be certified. In the case of blood sampling for Brucellosis, only those required by the Department to be sampled must be presented.

**N.B. In the case of tuberculin testing, during the course of a full herd-test, with the exception of calves under 6 weeks old that were born in the holding, all animals must be tested regardless of ownership, date of previous test or plans to slaughter them.**

Where a test is conducted in sections, all parts must be completed within 14-days of commencement of the first part to be considered a full herd test. Furthermore, in the case of Department pay testing, the test will **not** be eligible for payment if not completed within the 14-days. The test must be conducted on all parts of the herd before the test is complete and the herd status certified and in particular before **any** passports, dated for the actual test date of the individual animal, are returned to the keeper. It is an offence on the part of the keeper not to present all animals on the holding for such a herd test (TB Order). Bovine animals kept on the same holding (epidemiological unit) cannot be tested and certified under different herdnumbers. Where the DVO has requested completion of the ER11 (Declaration of presentation of animals and surrender of passports), it must be presented to the keeper for signature and it is a legal offence if the keeper refuses to sign same (TB Order). Directive 64/432/EEC, as amended, requires that, when conducting a herd-test in order to establish, retain or restore officially TB-free status, all animals on the holding, with the exception of calves under six weeks old which were born in the holding, must be subjected to routine tuberculin testing.

The same Practitioner must conduct all parts of the test so as to be in a position to certify the status of the herd/animals. ONLY the DVO may sanction a deviation from this requirement.

#### 1.5 Herd Profile

- The herd profile provided from AHCS either pre-printed or downloaded electronically to the testing PVP by the Department is a list of all the animals present on the holding as notified to the Department by the keeper or his/her agent in compliance with the legal obligation to so notify.
- The keeper must account for animals listed on the herd profile but not presented for testing.
- It is possible to download the herd profile independent of the scheduling of a herd test. This is to provide a client with a herd profile so that he/she may ensure any necessary updates/corrections to CMMS are made and reflected in the herd profile or when permission has been given for a private test and you wish to use the herd profile to ensure accurate recording of tagnumbers etc.
- In order to safeguard the integrity of veterinary certification of the health status of the herd, the PVP must be satisfied that all animals in the herd have been presented for test. When conducting a full herd-test, if there are animals on the profile that have not been presented for test, the PVP must query the keeper as to the location of such animal(s) and/or the reason for non-presentation for test (See section F below and Appendix 1). The reason given by the keeper must be duly recorded by the PVP and reported to the DVO via the test report. When the certifying veterinary practitioner is satisfied on the basis of the keeper's information that all animals on the holding have been tested as required, then test certification and thus herd health status certification may proceed. Where 'missing' animals are not adequately accounted for, the certifier (PVP) should present the keeper with an ER11 for completion (i.e. do not delay submitting the test report if the keeper cannot provide an answer immediately).
- When a test has been certified as complete and submitted to the Department, it shall be understood by veterinary officers of the Department that the veterinary practitioner is satisfied that all animals on the holding have been tested as required.
- All surplus passports should be submitted to the DVO with an accompanying ER124 (PVP Passport submission).

**Note (1):** *Once a herd test has commenced, no animals may leave the holding (even for slaughter) until the test is complete and any animal on the holding intended for slaughter in the immediate future or animals tested in the recent past must also be tested for the herd test to be certified as completed.*

**Note (2):** *Information supplied by the PVP on a test report may not be used to update the animal identification and movement database (AIM) on behalf of the keeper.*

1.6 Administration of Veterinary Medicines  
(in the context of Testing/Sampling)

- PVPs should not treat any cattle with a veterinary medicine in the course of carrying out the SICTT/sampling unless (i) the medication is urgently required and (ii) the withdrawal period is likely to elapse before any reactor is required to be removed from the herd (unless there is no alternative treatment for the condition requiring urgent treatment). Furthermore, herd keepers should be advised by PVPs, in particular if a prescription has been requested to coincide with a test, not to carry out routine treatment of animals immediately prior to or for the duration of a test until the individual test result for the animal is known. Such routine treatments will delay removal of reactor animals from the holding and prolong the restriction period accordingly. No compensation shall be payable, by DAFF, for the additional testing or restriction period attributable to such treatments.
- If a PVP is aware that any reactors have been treated with a veterinary medicine and the withdrawal period will not elapse before the reactors are required to be removed from the holding, (s)he should notify the DVO.

2. **Number of tests**

2.1 The number of approved tests issued to an approved PVP will be determined, **at any particular time**, by:

- **the level of finances available to the Bovine TB and Brucellosis Eradication Schemes,**
- **farmer nominations and**
- **the requirements of the Eradication Schemes.**

2.2 **The volume of testing for TB performed by the PVP shall not, on a regular basis, exceed 750 animals per week in any month, defined as six full-days testing amounting to approximately 250 animals injected or read per day.**

3. **The Basis for Veterinary Certification and use of AHCS 1 amendment forms.**

- The legal basis for veterinary certification are the European Communities (Certification of Animals and Animal Products) Regulations 1999 (S.I. No. 380 of 1999), Regulation (EC) 882/2004, the TB and Brucellosis Orders where relevant and, otherwise, the principles of certification as defined by the Veterinary Council apply.
- The veterinary practitioner carrying out the SICTT or blood sampling is required to ensure no conflict of interest exists or may be inferred (see note 1 below).
- The veterinary practitioner carrying out the SICTT or blood sampling is solely responsible for the accuracy of technique and recording and is the only person who may certify any aspect of the test/sampling.
- The need for meticulous attention to detail cannot be too strongly emphasised. The injection of tuberculin in doses less than the prescribed amount is likely to lead to infected cattle not being detected (bovine tuberculin) or conversely non-infected animals being deemed reactor (avian tuberculin).
- The accuracy of testing/sampling technique and recording must, at all times, be clearly demonstrable as the basis for secure Veterinary Certification and so as to ensure continued approval to test/sample. The veterinary practitioner's certifying signature must be clearly

legible on all reports (where such are not submitted electronically) and on passports or other certifying documents.

- Where tests are submitted electronically through AHCS, the veterinary practitioner who performed the test must personally **certify and sign off the test on-line using the unique access codes and identity number** assigned for that purpose.
- SICTT results and/or Brucellosis sampling details submitted to the Department shall be considered as certified by the Veterinary practitioner and, as such, an accurate reflection of the facts as stated.
- **Requests for changes to original certification are a very serious matter** and will only be considered when made by the original certifier and where appropriate justification is provided. Form AHCS1 must be used to request a change where the test results are submitted electronically. The DVO will require explanation and/or evidence of the reason for the original error and/or details for validation of the change in certification. Animals will not be added to, deleted from or have readings changed on a certified test unless there is documentary evidence to verify that the change in certification is fully supported.
- A PVP must not provide to a third party or allow others access to details of his/her unique identity codes and access number issued for electronic certification purposes such that matters are purported to be certified by him/her. A PVP will at all times be responsible for the data entered, or edited under their code in the AHCS. In circumstances where this instruction has not been followed the PVP shall be held responsible for such false certification and **liable** for any consequences of such false certification. If a PVP believes that his/her AHCS password and/or PIN have become known to a third party s/he must immediately change the password and/or PIN.
- A “**herdnumber**” is allocated to the “**keeper**” of a “**herd**” on a “**holding**” for the purposes of administering the TB and Brucellosis Eradication schemes and the herdnumber so issued is also the registration number of the herd and the holding. Under no circumstances should a test/sampling of animals be conducted under a herdnumber where it is clear that the animals are not located in that herd, on the holding occupied by that herd, at the time of test e.g. where animals are in a B&B holding, holding of a contract ‘rearer’, pound or elsewhere, they should not be reported as tested/sampled in the herdnumber or on the holding of the keeper of the animals. The appropriate herdnumber in such cases is the herdnumber of the keeper in whose care and at which location the animals are held. In case of doubt, please contact the local DVO to discuss the particular situation before returning to read the test or submit samples for analysis.

***Please be aware:** A Veterinary Inspector in the DVO ultimately is responsible for test interpretation and is therefore required to counter-certify the status of each herd/animal and various other matters pertaining to the test and to conformity with EU trading requirements etc. on the basis of the certification made by the testing PVP. A Veterinary Inspector may avail of ancillary tests to support certification and decision making in this process.*

#### **Note 1: Conflict of interest**

**Please note:** The legislation cited above prohibits certification of animals or products owned or from a holding owned, in whole or in part by the **certifier, his/her spouse or their close relatives or by his veterinary partner/assistant**. The responsibility lies with the veterinary practitioner to ensure that no conflict of interest exists or may be inferred. Therefore, should a PVP become aware that he/she has been issued an approved test in contravention of SI 380 of 1999 European Communities (Certification of Animals and Animal Products) Regulations 1999, or otherwise where a conflict of interest might be inferred, the **onus is on the PVP** to bring this fact to the notice of the Superintending Veterinary Inspector or the Veterinary Inspector in charge. The PVP may not test the herd/animal(s) in question.

#### **4. Handling Facilities and Farmer Assistance.**

It is absolutely imperative that all animals to be tested must be **properly identified** in accordance with legislative requirements and assembled in a yard, shed or paddock, located convenient to the testing facilities which must be such that the PVP can **safely and effectively inject the tuberculin and conduct the reading**. The PVP should not proceed with the test unless satisfied that the on-farm facilities are suitable and animals can be adequately restrained for the accurate performance of the test or sampling. As stated in Section 25 of the Diseases of Animals Act 1966, (as amended), farmers are required to provide “such assistance or to carry

out such instructions as may be reasonably necessary” to ensure that the test/sampling can be performed accurately. Assistance in restraining cattle is, ordinarily, essential to proper testing/sampling. The farmer should already have all cattle properly identified by means of a pair of plastic ear tags (one in each ear).

## 5. **Quality Control of Testing**

As part of this Department’s routine quality control of the testing programme, test performance and results will be monitored and assessed as a matter of routine.

For each approved PVP conducting testing/sampling, the DVO will, amongst other things, arrange for the

- supervision/inspection of equipment, test methodology and procedure including recording of data, biosecurity, cleansing and disinfection protocols while actually performing a test. Supervisions will ordinarily be carried out unannounced at least once per year or subject to risk based assessments at a frequency as determined necessary by the DVO;
- carrying out of random check tests of herds;
- carrying out of random checks of animals after completion of a test;
- monitoring recently tested animals for the presence and location of SICTT clip marks and the presence and location of reactions or DNA match with sample submission; and
- carrying out any other monitoring considered to be appropriate.

In addition, items such as insufficient/haemolysed/unsuitable sample submission rate, reactor disclosure rates, lesion disclosure rate in reactors and/or clear cattle, trace-back reactors in recently tested cattle etc. will be subject to regular monitoring and comparison with the norm for the region.

Further, the Department will conduct regular audits on administrative procedures such as test permission requests, advance itinerary submission and accuracy, compliance with legal requirements *viz.* a *viz.* prompt sample and/or test report submission, rate of submission of amendments to test reports, submission of sorted passports and other computer based procedures.

A copy of the ER13A performance report will be sent to each PVP/WTVI on a quarterly basis and discrepancies identified therein will be followed up, as appropriate, by way of warning, retraining, suspension from testing or complaint to the Veterinary Council of Ireland.

## 6. **Charge on PVPs for additional costs incurred by the Department**

Article 28 of Regulation EC/882/2004 states “When the detection of non-compliance leads to official controls that exceed the competent authority's normal control activities, the competent authority shall charge the operators responsible for the non-compliance, or may charge the operator owning or keeping the goods at the time when the additional official controls are carried out, for the expenses arising from the additional official controls.” In light of this legal requirement, **the Department will in future recover any additional costs which it incurs arising from the need to conduct additional visits over and above the norm and which are necessitated by non-conformity by the PVP/practice with requirements set out in this document.** In particular, additional costs (based on salary, travel and subsistence) arising from a change of itinerary which renders it impossible to conduct a planned supervisory check on a PVP will be charged to the PVP/practice as appropriate. Where such charges are notified, an opportunity to appeal will be afforded to the PVP/practice, the details of which will be set out in the notification. It will be open to the PVP/practice to pay the charge directly but, where payment is not forthcoming, the charge will be deducted from fees normally payable to the PVP/practice for testing.

## 7. **Payment of Fees**

Fees calculated in accordance with the scale of fees applicable at that time shall be paid to the PVP/practice who has conducted the test in respect of tests satisfactorily completed, including reporting timeframe, and which were nominated to be paid for by this Department.

Where a PVP refuses to proceed with testing because of inadequate handling facilities and/or assistance and certifies this to the DVO, the Department will pay the appropriate visit fee to the PVP and follow-up with the keeper as necessary.

The Minister reserves the right to refuse or reduce payment to the PVP in respect of testing which was not carried out and/or not reported in accordance with this document and any other conditions of testing set down in legislation.

In accordance with Department of Finance Circular 43/2006, Tax Clearance Procedures Public Sector Contracts, in the case of all public sector contracts of a value of €10,000 (inclusive of VAT) or more **within any 12-month period, the contractor will be required to produce a valid tax clearance certificate.** Accordingly, all payments in excess of €10,000 will be withheld until such a certificate is provided.

## 8. Legislation

- Full details of the relevant national (TB/Brucellosis Orders) and EU legislation (EC Directive 64/432/EEC, as amended, Regulation (EC) 882/2004 on compliance with animal health and animal welfare rules) and of other matters pertinent to the SICTT and its application or sampling for Brucellosis in accordance with Irish legislation, are available from the DVO.
- Guidance in use of AHCS e.g. data entry, part herd test date recording etc. is available from the DVO.

Further sources of the legal framework and useful information websites are:

<http://eur-lex.europa.eu/en/legis/index.htm>

<http://www.irishstatutebook.ie>

[http://www.oie.int/eng/normes/en\\_mmanual.htm](http://www.oie.int/eng/normes/en_mmanual.htm)

<http://www.avinformatix.org/>

## 9. Instructions for the Single Intradermal Comparative Tuberculin Test.

### 9.1 Purpose of the Test.

The purpose of the SICTT is to identify those cattle that are affected with bovine tuberculosis or capable of infecting other animals with bovine tuberculosis and to distinguish such animals from those that are not infected but which have become sensitised to bovine tuberculin as a result of exposure to cross-reactive antigens.

### 9.2 Intradermal Injection.

The test, when carried out correctly, is highly reliable and has been assessed under Irish conditions as 90-98% sensitive and 99.95% specific.

This reliability, however, is dependent upon the proper intradermal injection of the tuberculin (Bovine/Avian PPD) together with recording the accurate clinical observations together with characterisation, measurement and comparison of the reactions 72 hours later. The reliability is also influenced by the volume of tuberculin administered and by the site of delivery of the tuberculin (both injections should be in the same plane in the middle third of the neck on a line parallel to the blade of the scapula – see Section 8.6.4.1A below).

The sub-cutaneous injection of tuberculin must be avoided as this will give rise to a false negative result in an infected animal and is also likely to lead to desensitisation of the site for a variable period.

### 9.3 Equipment.

For quality control purposes all equipment must be produced when requested and surrendered for inspection/examination.



### 9.3.1 *Syringes, Needles, Holsters*

The syringes and needles used in the SICTT must be reserved for this purpose alone. Syringes must be clearly marked to distinguish between those used for avian tuberculin (red) and those used for bovine tuberculin (blue).

Veterinary practitioners must have a minimum of 3, properly identified, working syringes in their possession and immediately available when conducting SICTTs. Spare needles, adaptors and identification thumb knobs must be carried at all times. Syringes must be emptied before commencing a test in a new herd. Syringes should be emptied at the end of each working day in order to prevent crystallisation of the tuberculin in the barrel, which could lead to syringe and/or tuberculin delivery problems.

In order to qualify for testing under the programme each PVP must submit certificates of purchase or service of at least two syringes within the previous 12 month period i.e. **every** calendar year each PVP must purchase or service **at least** two syringes.

Syringes used for testing must be individually identified such that certification is specific to a particular syringe. Hence ensure that any syringes in your possession at the performance of a SICTT conform to this requirement i.e. each syringe identified and either new or serviced no earlier than 30-months prior to the commencement date of the test being performed.

**At all times syringes must be clean and in perfect working order.**

**Before a test commences, it is essential to ensure that the loaded syringe is free of air and contains the correct tuberculin. The needle used should not protrude more than 3mm from the adaptor otherwise the tuberculin is likely to be injected subcutaneously. The needle should protrude at least 2 mm so that a successful intradermal injection is achieved.**

A fresh plug of cotton wool, soaked in methylated spirits, must be placed in each syringe holster at commencement of the tuberculin test in each herd such that the needle of the syringe will make contact with and rest in the methylated spirits between each injection.

### 9.3.2 *Callipers, Scissors.*

Two callipers must be carried and maintained in good working order. Both lugs, together with the thumb-piece, must be stable and both the millimetre measurements and the reference mark must be clearly legible. A suitable curved scissors, or other suitable clipping device, with a sharp cutting edge should be used and maintained in good working order.

### 9.3.3 *Other Equipment*

The following other items of equipment must be available at the time of the tuberculin test:

#### *A field-book –*

References will be made to the ‘field-book’ in this document and may be taken hereinafter as referring to any one of the following three approved recording methods:

- A hand-held computer operating one of the two Department approved software programmes with the current herd profile of the herd to be tested (i.e. drawn down no earlier than one week prior to test commencement). In the case of Comtag Software - Version 1.41 or greater and in the case of Qubos Software - Version 2.3 or greater, or,
- An official field book (ER14) **and**, the current herd profile of the herd to be tested (i.e. drawn down no earlier than one week prior to test commencement). *(Please note even when a hand-held computer is routinely used, in the event of a malfunction, a back-up manual recording system **must** be available when the test is being conducted i.e. Dept. supplied ER14 and wherewithal to write in it).*

All relevant details must be recorded contemporaneous to the conducting of the test and paper based records must be kept as the contemporaneous record for a period of not less than 7 years.

PVPs are also advised to keep back-up records of electronic data (print-out, CD, DVD or other), being the contemporaneous record, for a period of 7 years which advice, we believe, accords to the period specified under tax legislation.

***Thermometer and stethoscope.***

*A thermometer and stethoscope, appropriate for the clinical examination of cattle.*

***Metal Ear Tags.***

Scheme metal ear tags (marked TT for temporary ID purposes only) must be stored in a secure place until required. It is mandatory to have a supply immediately available together with a tagger (i.e. both physically present) when testing since performance of SICTT on un-identified animals is prohibited in law.

***Reactor Tags/Discs.***

Reactor Tags, Red discs and Taggers for applying the tags described and as supplied by the Department.

***Protective Clothing.***

Boots and protective clothing and a supply of a disinfectant officially approved and effective against *M. bovis*.

To minimise the risk of the spread of infection, proper biosecurity and hygienic procedures, including disinfection, must be carried out before entering and on leaving each farm.

**ALL EQUIPMENT SHOULD BE CLEAN AND MAINTAINED IN GOOD WORKING ORDER.**

9.4 Equipment checklist:

*(all clean and in good working order at commencement of test)*

- 1) Boots, protective clothing and approved disinfectant;
- 2) Avian & Bovine tuberculin ppd;
- 3) Syringes x 3;
  - a. 2 in use and 1 Spare syringe each certified no more than 30-months previously and provided certification has been submitted for 2 syringes, in the previous 12 months, in compliance with 8.3.1 above.
  - b. identification thumb knobs,
  - c. needles, adaptors and spanner to change needle;
- 4) Cotton wool & Methylated spirits;
- 5) Callipers x 2;
- 6) Curved scissors/clipping device;
- 7) Field book –(ER14) as supplied by the Department or the approved recording method for test;
- 8) ER 14 – a spare field book, as supplied by the Department, is required as back up even when electronic recording is the norm;
- 9) Thermometer & stethoscope;
- 10) Scheme metal ear tags and taggers – for identification of untagged animals – ***mandatory for both days***;
- 11) Reactor Tags, taggers, ear-punch & red discs – as supplied by the Department for the identification of reactors.

9.5. Tuberculin: –

**Bovine and Avian PPD supplied by the Department.**

Ensure that both tuberculins are within the expiry dates and record the batch number and expiry dates for each test (this may be relevant should there be a problem subsequently or legal challenge to the test). The tuberculin should be kept refrigerated between 2 to 8°C and protected from light until the date required. Not more than a single day's supply of tuberculin should be kept un-refrigerated at any time. Since each vial of tuberculin currently (2010) costs the State and therefore the Irish Taxpayer almost €3, you should ensure that you operate appropriate stock control procedures to avoid product going out of date or other wastage.

Tuberculin must only be used on the day on which the vial is opened. Used vials should be returned to the practice centre for safe disposal and should not be discarded on farms.

The dose of each reagent is as follows: -

Avian tuberculin PPD: -	50 micrograms (0.1ml)
Bovine tuberculin PPD: -	100 micrograms (0.1ml)

## 9.6 Testing Procedures

### 9.6.1 Export Testing.

For tests reported on-line no paper certification of the TB test result is required. To be eligible for export purposes following a herd-test, the animal must have been individually tested and the interval to the previous tuberculin test a minimum of 42 days. In all circumstances, other than a full herd-test, a tuberculin test may only be conducted if the interval to the previous tuberculin test (injection day to injection day) is a minimum of 42 days. For tests not reported on-line, paper based certification will not be considered in cases where the test was conducted and the test report not received within the legally stipulated 7-days.

**N.B. Where a standard inconclusive reactor animal is present in a herd, under no circumstances (legal prohibition) should an export test certificate be issued for any animals in or from the herd until the clear disease status of the herd is confirmed by the DVO.**

Where there is an inconclusive animal in a herd, the herd disease status is restored by way of the following:

- a clear test of the inconclusive reactor(s) after 42-days, or,
- slaughter and laboratory examination of the inconclusive reactor(s) with negative results, or
- slaughter of the inconclusive reactor followed by a clear herd test at a minimum of 42-days after removal of the inconclusive reactor(s).

In certain cases, the DVO may allow the herd to trade on the domestic market under a derogation provided for in Directive 64/432/EEC and will inform the PVP that passports may be returned to the keeper. Export certificates may not be issued for any animal from such derogated herds and animals from such herds will be precluded from export on the Animal Identification and Movement System (AIM).

## 9.6.2 Detailed TB Test procedure

### 9.6.2.1 Day 1 (Injection)

Before commencing a test the testing veterinarian must: -

- Check that the facilities and help provided are as specified previously,
- Ensure that a fresh plug of cotton wool and methylated spirit has been placed in each holster, and,
- Ensure that syringes, needles etc. are free of any material that might constitute contamination from a previous herd.

#### A. Animal Identification

Before commencing a test the testing veterinarian must request, and take possession of, **all** passports/cattle identity cards in the keeper's possession for the cattle on that holding.

The testing veterinary practitioner must be satisfied as to the identity of each animal being tested and also personally take the calliper readings. When not personally recording the details of animal identity, test measurements etc., it is the responsibility of the testing veterinary practitioner, who will be expected to be in a position to certify the test, to assure him/herself of the accuracy of such recording. It is essential to record or verify each eartag number in full.

**N.B.** Please check if details for animals not on the profile, which have been input as added animals correspond to the details on the animal's passport. Any deviation from the details on the passport and/or database or changes made to the details on the profile e.g. sex, will be considered as deliberate (certified by the testing veterinary practitioner) and an indication that the animal and

passport/database records do not match. Where the details on the passport require amending please return the passport to the DVO. Where tests are submitted electronically, discrepancies in Gender, DOB and Class will be highlighted prior to completion of certification.

All animals on the herd-profile must be accounted for at a herd test – animals left blank with no annotation as to why they were not tested will raise queries, in the DVO at test interpretation, as to whether the full test is completed or not. Processing of incomplete tests will not be finalised until such blanks are resolved. This will delay sign-off, test payment (where relevant) and eligibility to sell and export animals for your client.

Under **no** circumstances is the testing of unidentified animals permitted (TB Order). When unidentified animals are presented for test they **must, before testing**, be tagged in the left ear using temporary tags (brass and marked TT) as supplied by the Department. Testing or sampling of unidentified animals and certification of such tests is considered an extremely serious breach of testing and certification procedures, which may result in prosecution under the TB and/or Brucellosis Order. The presence of untagged/unregistered animals must be noted and procedures in Circular letters ER6A/2000 and ER16/2008 followed – copy attached at appendix 2.

It is the keeper's responsibility to correlate the temporary tagnumber inserted for test purposes with the tagnumber registered to the animal using the ER96 (Declaration in respect of bovines temporarily tagged). The keeper has been informed of this responsibility and that failure to have animals identified properly for test may result in test details, for the animal(s) involved, not being certified by the testing practitioner until such time as they have satisfied themselves as to the correlation between the animal brass-tagged and the plastic tag number on the passport. This ordinarily will require re-visiting the herd to check the animal(s) involved and **the herdowner/keeper will be responsible for any costs involved**. The current ER96 has two sections, namely:

**Section A:** signed declaration by the keeper regarding the correlation of temporary brass tags with the permanent identity contained on plastic tags.

**Section B:** signed declaration by the testing veterinary practitioner that the correlation is correct and that he/she can certify the TB and/or Br test.

If Section A is completed, the DVO will consider the 'discrepancy' for the registered tagnumber resolved but unless Section B is completed and signed by the testing veterinary practitioner it will not record a test date on AHCS against that tagnumber. In addition, it will not return the passport to the keeper (assuming the passport has been submitted to it by the testing practitioner as required).

**Keepers should be strongly encouraged to order and replace missing plastic tags before the reading of the TB test is completed as this simplifies subsequent difficulties with certification and identity and obviates the need for completion of the ER96 form.**

The sooner keepers experiencing problems with the bovine identification regulations are identified the quicker and easier it is to resolve those problems and prevent the potential destruction of unidentifiable animals, development of animal welfare problems or penalties under the Single Farm Payment. Thus, it is in the keeper's best interest to ensure that the DVO is made aware of the difficulties at the earliest opportunity. *If the PVP considers that a welfare problem may exist or could be anticipated the DVO should be notified and please consider if the Welfare 'Early Warning System' should be activated.*

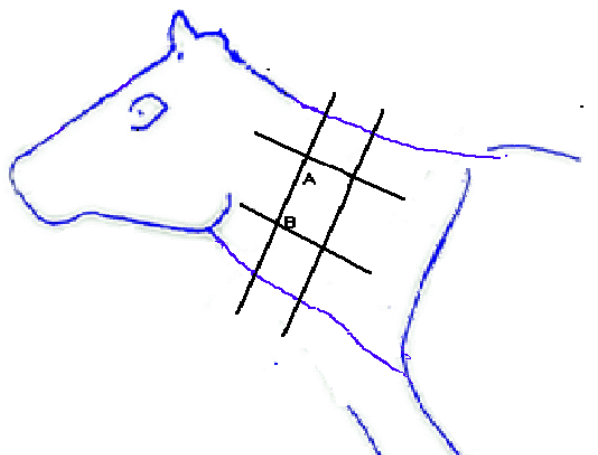
Each location where animals or groups of animals are tested and the date of part tests **must be recorded and reported** – this can provide considerable assistance in the event that an epidemiological investigation is required at any stage in the future. Clinical and other observations or other treatments likely to have a bearing on the results of the test must be recorded under Clinical Remarks on handheld devices or ER15B and, where appropriate, linked by tagnumber reference to the individual animal(s).

## **B Site of Injection**

For accurate and consistent testing the injection site is very important. The approved injection sites are situated at the border of the anterior and middle thirds of either side of the neck.

The upper site (for avian tuberculin) is about four inches (10cm) below the crest.

The lower site (for bovine tuberculin) should be about five inches (12.5cm) from the upper site, in the same plane along a line drawn parallel with the ridge on the scapula (the representational diagram below is a guideline only).



In calves under six weeks of age, or in animals where there is insufficient space to inject both tuberculins into the same side of the neck, the tuberculin should be injected, one on each side of the neck (avian on the left, bovine on the right), at corresponding sites in the centre of the middle third of the neck.

For animals, which have non-associated lumps or swellings adjacent to or obstructing the injection site(s) on the presenting side of the neck the tuberculin should be injected into the opposite side and recorded in the 'field book' linked by tagnumber reference to the individual animal.

**C. Site Preparation**

There is a legal requirement that the selected sites should be clipped (an area not less than 2.5cm in diameter) and cleansed i.e. any dirt/debris removed, prior to injection. The presence of any abnormalities at the injection site(s) should be recorded (not under Clinical remarks) in the 'field book' at the time and linked by tagnumber reference to the individual animal. The presence of skin tuberculosis should always be recorded as a clinical remark.

**D. Measurement of Skin Thickness**

Before injection, a fold of skin at each of the injection sites and within the clipped area must be taken between the forefinger and thumb and accurately measured to the nearest millimetre using a callipers; and the measurements recorded in the field book linked by tagnumber reference to the individual animal.

**E. Injection Technique**

The needle should be introduced, bevel outwards, into the skin in such a manner as to ensure the intradermal delivery of the tuberculin. This usually requires the insertion of the needle at a narrow angle to the skin. The insertion of the needle at a right angle to the skin will generally result in a subcutaneous injection being made. Such injections give rise to false negative results and must be avoided. Considerable pressure on the plunger of the syringe is usually necessary to make an intradermal injection. Absence of resistance to the flow of the tuberculin is an indication that it has not been injected intradermally, or that the syringe is leaking or improperly loaded.

Each injection must be confirmed; by palpating at the site a small pea-like swelling that is created by a properly administered intradermal injection.

If there is any doubt about either of the injections being delivered intradermally, a further injection should be made, preferably at a corresponding site on the other side of the neck. Such a

procedure must be recorded in the 'field book' linked by tagnumber reference to the individual animal (in the Non clinical field and not under Clinical Remarks).

#### **F Recording of remarks:**

Tested animals:

On handheld devices, two fields exist for recording remarks against tested animals:

1) Clinical Remarks (apparent to the VI at interpretation):

Entries under this heading, either in code abbreviation or text detail, should be confined to **any clinical detail** relating to an animal under test and which may have a bearing on the test result/interpretation and thus test certification (e.g. Skin TB, emaciation etc); any other detail i.e. export cert, reactor tag etc. should be recorded in the non-clinical field

2) Non clinical field:

This should be used to record non-clinical details or reminders relating to the tested animal e.g. abnormality at injection site, export cert, reactor tag etc. or to serve as a reminder to the practitioner e.g. item for billing.

The ER15B only provides for clinical remarks; other remarks can be recorded on ER14.

Untested animals (including missing on day 2):

See above under animal identification. Any keeper reply relating to animals on the herd profile, which is not presented for testing, should be recorded under *Clinical Remarks* (test certification issue). No annotation against untested animals will raise discrepancy queries. Please see appendix 1 instructions re checking handheld for untested animals.

#### **9.6.2.2 Day 2 (72 hours +/- 4 hours post-injection)**

A Reading of the Tuberculin Test.

The SICTT **must** be completed by the PVP who commenced the test, on the same holding, and using all the data recorded contemporaneously in the 'field book' on Day 1. Any departure from this **must** be for very exceptional reasons and have the advance permission of the SVI/VI in charge in the DVO responsible for the issue of the herdnumber under which the SICTT is being conducted.

Each animal must again have its eartag number verified in full and its measurements, reactions, clinical symptoms/signs and any other observations immediately recorded in the 'field book' and correlated to the measurements and remarks recorded on Day 1 linked by tagnumber reference to the individual animal.

Each site where tuberculin was injected must be examined, palpated and measured. Measurements must be taken carefully by placing the callipers across the broadest width of any response present, without applying undue pressure, and recording the findings in the 'field book' **N.B.** all measurements must be rounded **up** to the next whole millimetre. Any additional remarks must be recorded immediately and correlated to the measurements and remarks recorded on Day 1 linked by tagnumber reference to the individual animal.

Clinical signs directly associated with a reaction to the tuberculin must be recorded, contemporaneous with the time of reading, in the 'field book', linked by tagnumber reference to the individual animal. These signs include the presence of oedema, exudative necrosis, heat, pain or swelling at the individual injection site and/or heat, pain or swelling of the related prescapular lymph node.

The presence of diffuse or extensive oedema, necrosis, heat, pain at the bovine injection site and/or swelling of the lymphatic ducts in the region or the related pre-scapular lymph node are regarded as clinical signs and always indicative of likely tuberculosis infection. Animals showing such reactions to bovine tuberculin or with diffuse or extensive oedema, necrosis, heat or pain at the injection site must always be deemed as reactors, irrespective of the measurements recorded.

The purpose in observing clinical signs, characterising reactions to the tuberculins and considering herd and animal histories and the status of contiguous herds is to facilitate the

identification of animals, which may be infected but which have not been identified as reactors to the tuberculin i.e. False Negatives.

The interpretation of reactions:

- (a) **Positive:** clinical signs or an increase of 4mm or more in skin-fold thickness.
- (b) **Inconclusive:** no clinical signs and the increase in skin-fold thickness is more than 2mm but less than 4mm.
- (c) **Negative:** if only limited swelling, with an increase of not more than 2 mm without clinical signs.

If you form the impression that the reaction is not a normal tuberculin response, you must contact a Veterinary Inspector at the DVO at the earliest possible opportunity.

#### B Interpretation of the test.

Where 2 or more standard interpretation positives are found in a 'clear' herd (i.e. clear status before the test) all standard interpretation inconclusive reactors must be punched and tagged as reactors and recorded on the test report - unless instructed by the DVO to the contrary.

Where the herd is undergoing a contiguous test, all standard interpretation inconclusive reactor animals must be identified for removal as reactors unless specific instructions in respect of the herd and interpretation have been received from the VI.

##### (i) Standard Interpretation:

Standard interpretation should be applied when testing clear herds with a disease-free history or '**restricted**' herds where an instruction has been received to apply standard interpretation. Standard interpretation of the single intradermal comparative tuberculin is as follows:

**Positive:** - a positive bovine reaction that is more than 4mm greater than the avian reaction or the presence of clinical signs.

**Inconclusive:** - a positive or inconclusive bovine reaction, which is from 1 to 4mm greater than the avian reaction and the absence of clinical signs.

**Negative:** - a negative bovine reaction, or a positive or inconclusive reaction, which is equal to or less than a positive or inconclusive avian reaction and the absence of clinical signs in both cases.

##### (ii) Severe Interpretation:

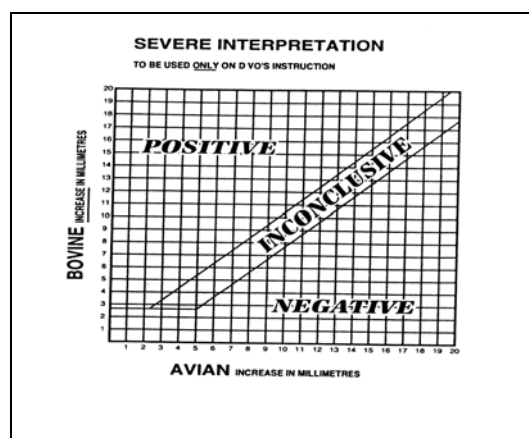
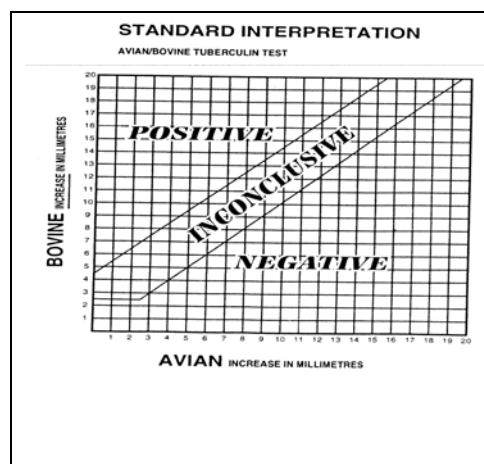
Severe interpretation of the SICCT, to be used only on specific instruction from the veterinary inspector, is as follows:

**Positive:** - a positive or inconclusive bovine reaction which is greater than the avian increase.

**Inconclusive:** - a positive or inconclusive bovine reaction, which is equal to, or 1 to 2mm less than, the avian reaction.

**Negative:** - a negative bovine reaction, or a positive or inconclusive bovine reaction, which is more than 2mm less than a positive or inconclusive avian reaction.

Animals displaying reactions to tuberculin, which cause them to be classified as reactors or inconclusive reactors must be identified and recorded as such in all cases.





### **C. Identification of Reactors.**

All animals classified as reactor must have a reactor tag, together with a red disc, inserted in the left ear for identification. An alternative method for securing the identification of reactors is under trial and thus individual Practitioners participating in the trial will receive alternative instructions, which will derogate them from the requirements laid down here.

### **D. Inconclusive Reactors**

When, as a result of the test, animal(s) are deemed as standard interpretation inconclusive reactor(s), their respective passport(s)/card(s) must be forwarded to the DVO and the keeper must be advised to isolate such inconclusive reactors from the herd pending re-test. Such inconclusive reactors should be kept separately from any reactors identified. Where no further reactors are identified, passports/cattle identity cards for the remaining 'clear' animals in the herd should be updated and held by the PVP until contacted by the DVO with further instructions.

Inconclusive reactors should be classified in accordance with the above instructions and the keeper advised immediately (see Advice to Keepers below).

**NB.** As referred to in paragraph 8.6.3, **where a standard inconclusive reactor animal is present in a herd, under no circumstances (legal prohibition) should an export test certificate be issued for any animals in the herd until the clear status of the herd is confirmed by the DVO.**

Severe inconclusive reactors are marked as such. They will not ordinarily require a retest at individual animal level or be removed if severe inconclusive again but, if considered epidemiologically appropriate, a Veterinary Inspector may remove them as reactor e.g. if part of an infected group.

The veterinary inspector in the DVO will make the final interpretation of any test.

### **E. Animals Missing from Day 2.**

An animal, recorded in the 'field-book' as injected with tuberculin on Day 1 and for which no Day 2 skin measurements or tuberculin response details is recorded, will be regarded as not 'read' and the test will therefore be treated as incomplete and thus ordinarily herd status may not be certified. In such cases, the herd status will, at a minimum, be suspended until it can be clarified by means of test or otherwise. If an animal recorded in the 'field book' on Day 1 is not presented for reading on Day 2, the keeper should in the first instance be queried as to the absence of the animal, the explanation recorded in the clinical remarks column and the DVO informed immediately (*if the animal died on farm the DVO may wish to arrange for examination of the injection site and/or a post-mortem*).

### **F. Advice to Keepers.**

When animal(s) are deemed reactor, the keepers must be advised:

- to isolate them, pending slaughter;
- that milk from reactor animals may not be used for any purpose even if heat-treated;
- that milk from healthy animals belonging to reactor herds may not be used for the manufacture of heat-treated milk or for the manufacture of milk-based products unless it is first heat-treated at an establishment authorised by the Department;
- that no animal on the holding may leave without a movement permit from the DVO; and
- that no animal may be moved into the holding without prior approval from the DVO.

### **G. Removal of Test Materials from Holdings.**

It is essential that the residue of all test materials employed in the test procedure including syringe parts, used tuberculin vials, needles, cartons and other items are gathered and removed at the time of leaving the holding.

The safe and proper disposal of such materials in compliance with relevant legislation is the responsibility of the testing veterinary practitioner.

### **H. Completion of the 'Field Book' and Test report.**

Specific details on the completion of the three types of 'field book' are to be found at the front of the field book (ER 14) or from the appropriate software manuals for the handheld devices.



Instructions for the completion of the pre-printed ER15B are attached at Appendix 3. If operating 'online' with AHCS, the test report must be completed, submitted and certified by uploading and interacting electronically with AHCS or if operating 'off-line' by completing submitting, certifying and returning the supplied pre-printed ER15b herd profile to the DVO.

**I. Timely submission of Test Reports.**

- The DVO should be informed on the day or at latest by the morning of the following working day, by telephone or otherwise, of all tests where reactors have been disclosed. Test reports must be submitted completed clearly and in full, including details of present/missing/surplus identity cards/passports.
- The test report on which a reactor or inconclusive reactor is disclosed must, by law, be submitted so as to reach the DVO not later than three (3) working days after the completion of the test. All the animal passports/identity cards, appropriately sorted (reactor/valid/surplus etc.), must also be submitted within the same 3-day period using ER124T (PVP submission of cards).
- Test reports on which no reactors are disclosed must, by law, be submitted to reach the DVO within seven (7) working days of test completion. Animals presented for sale at a mart may be rejected if the test report is not in the DVO and/or on AHCS within this timeframe.

***Please note:** under the proposed revision of the TB Order, most likely to be signed into law during 2010, and in keeping with electronic test report submission the submission dates for reports of completed TB tests will be 1 day for tests with reactors and 3 days for tests with no reactors. With the Department's computer systems now "live" at Marts, there is no real necessity to have test dates recorded on passports - for sale purposes. It is therefore the Department's intention to remove the requirement to record TB and Brucellosis test dates from passports during 2010 thus making the timely submission of reports all the more critical for your clients who wish to sell animals. It is also the Department's intention to abolish the Cattle passport in its current form as soon as possible.*

- Ensure that test reports for animals, which are to be moved or slaughtered, are submitted immediately. All test data and, therefore, export eligibility is relayed to marts and meat plants via the AHCS system. Failure to promptly upload and sign off on tests will result in rejection of your client's animals at sales, export points or slaughter plants.

Details of temporary tags etc. must be recorded on the report, against the relevant existing animal on the profile if possible, and all missing animal passports/identity cards recorded; surplus passports/identity cards must be submitted to the DVO within the timeframes detailed above. Where details for animals added to the profile do not correspond to the details on the animal's passport, this will be considered as deliberate certification and an indication that the animal and passport (AIM) do not match and will consequently raise a discrepancy against the animal.

Passports/cattle identity cards for 'clear' animals should be fully updated after each test where there are no reactors identified.

**NB.1.** Where a TB test is carried out in conjunction with a Brucellosis test, Passports/Identity cards for **all** animals must be retained until the result of the Brucellosis test is available.

The Veterinary inspector is legally responsible for test interpretation and determination of animal and herd status. The local DVO will notify the PVP in writing of any changes to the field interpretation of test results.

The post mortem results of animals from herds tested by the PVP and which were slaughtered as reactors are notified on AHCS.

**J. No Stock Reports.**

The DVO will check the database prior to issuing herds for test. Herds with no stock on record will not be issued to a PVP for test until such time as the database indicates that stock have moved into the herd. In cases where a keeper who has been listed for a herd test and has been notified by you to test has no bovine animals, a no stock report should also be submitted by the due date on the listing

to the DVO. This will avoid further queries from the DVO re untested herds. The DVO will then deal directly with the keeper as appropriate to regularise the position on the database.

10. Instructions for Blood Sampling for Brucellosis.

**10.1. Animals to be tested**

The Brucellosis Order defines an "eligible animal" as any animal aged 12 months or more except a castrate. Following Ireland's attainment of Official Brucellosis Free Status, dairy herds will only be tested every second year from 2010 and only eligible animals aged 24 months or more are required to be sampled at the annual round test. All animals under 24 months are exempt from this test. However, the Department reserves the right to request that any animal be presented for test where deemed appropriate on veterinary grounds.

Please also note the following:

- (a) **Annual Brucellosis herd test:** The Department has also decided that from 2010 every second year testing will be applied to dairy herds. Thus, in 2010, approximately 50% of dairy herds will be exempted from the requirement to carry out an annual brucellosis round test. Herds will be selected on the basis of herdnumber and keepers of all herds requiring test will be notified in writing. Annual testing is required in all non-dairy herds.
- (b) **Pre-movement test:** A pre- movement test remains a legal requirement for female animals aged 18 months or more and for bulls aged 24 or more if leaving the holding other than directly to slaughter. The validity period of the pre-movement test has been extended from 30 days to 60 days. Please note also that for maximum compensation purposes, the requirement to carry out a post -movement test within 30 days of movement into a holding remains applicable.
- (c) **One movement per test rule:** Female cattle aged 18 months or more and bulls aged 24 months or more may not be sold more than once, whether by public or private sale, on foot of a brucellosis test and such cattle being sold must be moved from the holding where tests are undertaken direct to either the purchaser's holding or direct to a mart and from there direct to the purchaser's holding.

A provisional list of animals to be tested on the annual round test will be available on the pre-printed/downloaded herd profile from AHCS following submission of an advance itinerary. Please note that an ER11 or ER12 is no longer required, unless specifically requested by the DVO. An ER12 may be requested by the PVP if not satisfied that the keeper has presented all animals for test.

Each animal presented at the test must be correctly identified according to its tag number, breed, sex, type, stage of pregnancy, age and abortion history. All these details must be recorded in the field book.

In the case of a post abortion test(s), the passport/identity card must be returned immediately to the DVO.

**10.2. Equipment required**

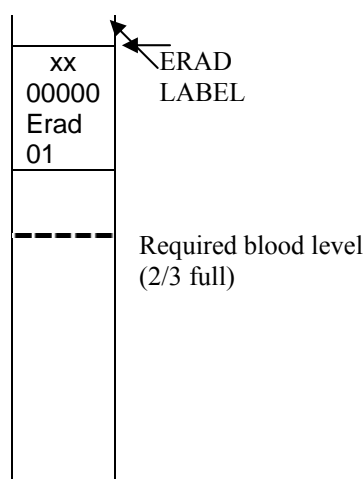
- A blood testing kit supplied by the Department.
- To record relevant details of the test, you may use a hand-held computer (with Department of Agriculture approved software), an official field book (ER14) or a computer printout. All relevant details must be recorded at the time of the test and the record kept for a period of not less than 7 years. (For those who are operating AHCS on-line, an electronic record is automatically generated and stored). Paper based records and back-up records, being the contemporaneous records, should be kept for a period of 7 years. ***Even when a hand-held computer is routinely used, you must have an ER14 manual recording system as a back-up.*** From now on, the term 'field-book' refers to any of these approved recording methods.
- Tagging equipment, which should be clean and in good working order.

- Scheme metal ear tags (for temporary use for test ID purposes only), stored in a secure place until required (please refer to instructions issued in regard to the Farmer Plastic Tagging System).
- Boots and protective clothing. To minimise the risk of the spread of infection, boots and protective clothing must be cleaned and disinfected on entering and leaving each farm.
- A supply of an officially approved disinfectant effective against brucellosis. A bucket and suitable hard brush should also be carried to facilitate cleaning and disinfection of boots.

### 10.3 Bleeding

A separate needle must be used for each animal to avoid cross contamination. Ensure that the tube fills to at least 2/3 of its capacity. Affix a pre-coded label to the upper 1/3 of the tube.

#### TEST TUBE



Only samples filled to the required mark (2/3 full) can be tested in the laboratory.

It is essential to correctly correlate the sample identification number (tube code) to the animal identification in the field book. To minimise the possibility of error, this correlation should be carried out at the time of sampling. The person taking the blood samples is solely responsible for the accuracy of this correlation. The accuracy of sample identification and sample correlation must, at all times, be demonstrable as the basis for Veterinary Certification.

### 10.4 Packing of Samples

- (i) Blood samples tubes should be placed in the correct order in the aeroboard mould in sequential order from left to right in each row. (See diagram below). As above the person who took the blood samples is solely responsible for ensuring the accuracy of the identification of the samples and the correct correlation between the samples and the animal sampled. Veterinary Certification is dependant on accurate sample identification and correlation.
- (ii) Tube codes should be entered on the ER16 in the order in which the samples are placed in the aeroboard mould.
- (iii) ER16 forms should be enclosed within the aeroboard unit in the document compartment.

## AEROBOARD MOULD

N.B Order in which samples must be placed in aeroboard mould.

1	2	3	4	5	Needle And Document Compartment
6	7	8	9	10	
11	12	13	14	15	
16	17	18	19	20	
21	22	23	24	25	

### 10.5 Description of herd and recording of data on animals sampled

It is important to record accurately the information requested on form ER16 in respect of the herd and of each animal from which a blood sample is collected. In particular, it is essential that the PREGNANCY STATUS and abortion history be recorded for each eligible female animal tested.

It is important that the location where the animals are blood sampled is recorded under “address of premises”.

### 10.6. Forwarding of Samples to Laboratory

- (1) Samples from more than one herd may be forwarded in the same box provided that the samples from the same herd are not divided among two or more boxes.
- (2) It is essential that care be exercised to prevent the box or its aeroboard mould or lid from becoming soiled with blood or other matter.
- (3) A separate ER16 form should be completed for each herd represented in a box. Where the number of samples from a herd exceeds 25, a separate ER16 form should be used for each box or part of a box, and each form should be noted “Part of herd of \_\_\_\_\_ animals” (inserting the total number of samples taken from the herd in question). Separate ER16s are produced on electronic submission.
- (4) Place the aeroboard box in the plastic bag provided before placing the whole lot in the cardboard outer box.
- (5) The procedure for sealing the box containing the samples is that adhesive security seal labels are provided and one label should be placed over each end of the box.
- (6) It is important that the county of the herd tested should be indicated in the space provided on the carton. This greatly simplifies sorting of samples in the laboratory. Filling in the practice name and address in the space provided on the package is also helpful to the laboratory staff.
- (7) The blood samples should be forwarded to “The Department of Agriculture, Fisheries and Food, Brucellosis Testing Laboratory, Model Farm Road, Cork” by regular post only. Samples incorrectly posted or packed may be destroyed without notification by the postal authorities. The postal authorities also have power to prosecute and recover damages should badly packed blood samples soil other post or personnel.

- (8) Samples should as far as possible be posted on the day of collection, but may be posted up to a day after collection. If blood samples must be held over for longer they should be placed in a refrigerator at 5 degrees centigrade. Blood samples should not be placed in the freezer compartment of a refrigerator.

**10.7 Unused Blood Tubes**

If there are unused tubes, please do not send these to the laboratory. Unused tubes sent to the laboratory cannot be reused, as there is no way of knowing whether or not they were punctured and hence whether they still have a vacuum or not. Please keep any unused tubes, order empty kits from the laboratory and refill these with the unused tubes. This will greatly reduce wastage and disposal costs.

**10.8 Disinfection**

**All personnel involved in the testing procedures are required to ensure that proper biosecurity precautions are taken and a thorough cleaning and disinfection of footwear and protective clothing is carried out before leaving the farm premises.**

**10.9 Removal of Test Materials from Holding**

It is essential that the residue of all materials employed in the test procedure including syringe covers, used cartons and all other items are gathered and removed at the time of leaving the premises. **Safe disposal of used needles is the responsibility of the testing Veterinary Practitioner/VI/Lay Sampler. Under no circumstances should used needles be included in the aeroboard container and sent to the laboratory.**

**10.10 Private Pre-Movement Testing**

In order to expedite tests in the above category it is important that the appropriate **Private Test** label is attached to the outside of the blood sampling kit. These labels should **not** be used for herd tests.

**10.11 General**

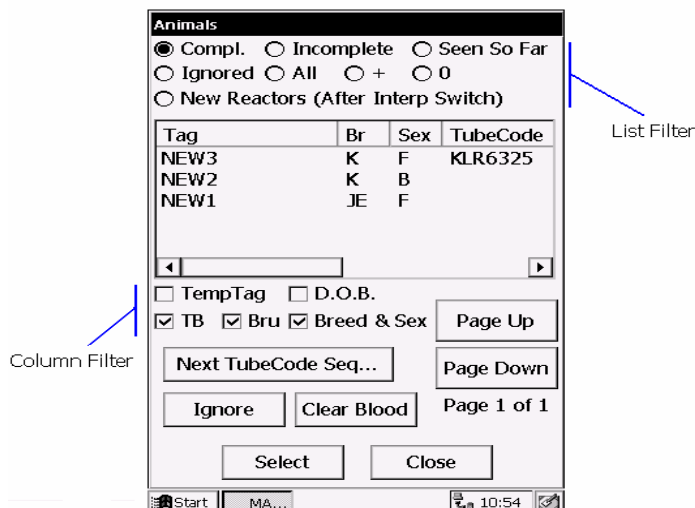
The signature of the testing veterinary practitioner/VI/Lay Sampler on the ER16 must be clearly legible. The signature, in full, of the Veterinary Practitioner, VI or Certifier on the passport/identity card must also be clearly legible.

### Appendix 3 To check handheld computer re untested animals.

#### Quoport MAHCS Handheld Manual - Page 32

##### 3.5 Viewing Filtered Lists of Animals

MAHCS provides the facility to see a list of animals selected according to a particular filter. By pressing the 'All...' button while in Search Mode (make sure keyboard is turned off so you can see the button), you will see a screen like the following:



#### List Filter

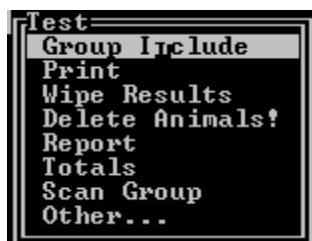
The type of list displayed is selected at the top. Tap on each option to change the list of animals displayed. The list options are:

- Completed – Shows the animals completed for the current day.
- Incomplete – Shows animals not complete for the current day.
- Seen So Far – Shows all the animals found and edited so far. (This is really only of use in BRU-only tests where this test will show all animals that have been seen – even those that were not blood tested)
- Ignored – Lists all ignored animals
- All – Lists all animals (except ignored animals) regardless of what their test status is
- + – Shows all reactor animals
- 0 – Shows all Inconclusive animals

\*\*\*\*\*  
\*\*\*\*\*

#### Manual for Husky Page 8.

On the Tag screen Press the F4 button to give the following drop down menu. You can use the arrow keys or



#### F4 Menu

type T to give the running Totals of the state of play of the test or R to give untested both for Days 1& 2.

**group include**  
**print**

**G** group include or exclude cards  
**P** There are a number of pint options. Plain paper/ certs etc  
wipes TB and blood bottle

**wipe**  
**report**

**W** readings e.g. for re-test situation.  
**R** full details of Testing position which is very useful to ensure no readings are missing

and other details available from other options displayed e.g. not in group may be used to show card missing.  
running totals & **batches\*\***

**total**

**T** (7secs.)

quick total & batch count of bloods & TB readings.

## Appendix 4 Relevant Circulars

### Circular Ref: ER 6A/2000

To the Veterinary Practice/Practitioner named in the address 6 March 2000

Dear Sir/Madam

#### **Bovine Animal identification and associated matters**

The following points cover areas where you have a direct involvement with bovine identification and also areas where you interact with your clients in this and other regards. A number of problems have arisen over the past year which have drawn attention to this area. As you are aware correct bovine identification is very important from the farmers perspective ensuring eligibility for various EU premia. It is also essential for disease control purposes and for maintenance of live cattle exports and beef markets. It is to be expected that various EU mission visits will concentrate more and more over the coming years on identification issues and our compliance with EU regulations in that regard. Your assistance in ensuring that the integrity of bovine identification is secure, in so far as you can, would be most appreciated.

#### **Correct animal identification and use of brass tags:**

For reasons of traceability and to promote consumer confidence in the wake of the BSE crisis, EU Regulations now require that all bovine animals born since 1.1.1996 are identified by means of two official plastic tags, inserted one in each ear. Up to the end of 1999 calves had to be identified by the keeper within 30 days of birth and registered within 7 days of identification. This interval was shortened to 20 days from 1.1.2000. Tags which become illegible or are lost require replacement by the keeper as soon as is practicable.

When notifying herdowners of your intention to carry out a test it is imperative, therefore, that you remind them that all animals 20 days of age and over must be identified before the test commences.

It is mandatory that the herdowner present all animals in the herd at the time of a herd test. However, home-bred animals under 6 weeks of age do not require testing for results to be valid. All animals should be entered on the test report giving the total number for animals presented without identification.

**Where <10% or 10 animals maximum, over 6 weeks of age, in the herd are not identified then the test should be completed and the unidentified animals provided with temporary identification by the use of a brass tag.** ID cards or passports should not be returned to the herdowner until all eligible animals are properly tagged.

**Where >10% or 10 animals maximum, over 6 weeks of age, in the herd are not identified** then a part herd test should be carried out on identified animals only and the remainder of the herd test completed following the identification of those animals in the herd over 20 days of age. The second visit fee involved will be borne by the farmer regardless of who was due to pay the original testing fee.

On the initial day of test you should remind the herdowner of his obligation to tag animals and that he



should immediately order tags for this purpose if necessary.

**Where animals over 6 weeks of age remain without plastic tags on the day of reading,** then the test report, partial or complete, and the passports/id cards for the herd, must be forwarded to the DVO and attention drawn to the presence of incorrectly identified animal(s). The DVO will arrange to serve a restriction notice for the animals(s) involved and/or the herd as is required by law and take whatever follow-up action is appropriate.

**If you have identified, for test purposes, an unidentified animal by means of a brass tag** then, of course, no passport (identity card) may subsequently have test details certified by you until such time as you have satisfied yourself as to the correlation between the animal brass-tagged and the plastic tag number on the passport. This ordinarily will require re-visiting the herd to check the animal(s) involved. **The herdowner/keeper will be responsible for any costs involved.**

Only those animals born prior to the introduction of the new plastic tag identification system may be permanently identified by means of a brass tag. It is the intention, therefore, that brass tags will, within a reasonable timeframe, cease to issue or be available except for the purpose of essential retagging of animals legally brass-tagged.

#### **Passports:**

Each animal born in Ireland, after 1.1.98 should have an official pre-printed passport. Obviously the description of an animal on its passport must match the animal. In addition the animal's keeper is required to sign the passport either on the front if the animal was born into the herd or on the back if purchased. Thus, when you are checking passports at time of test be aware that the herdowner's/keeper's signature should ordinarily be on each passport. It is the intention that each animal's passport will accompany it throughout its life to facilitate completion of a full traceback for consumer protection, fraud prevention, disease control etc. Great care must be taken therefore, to ensure that passports are not lost or mislaid while in your possession.

#### **Farm inspections under the Identification Regulations:**

The EU regulations mentioned above also require the competent authority to conduct inspections of a minimum of 10% of holdings, to monitor compliance with the regulations. Operational details for these inspections are currently being finalised. In

some instances the DVO may arrange inspections to coincide with TB or other tests being conducted on a herd. Registration records, passport details and entries on the herd register will be checked during these inspections in addition to individual animal tagging. Maintenance of national eligibility for export and other outlets for cattle and meat products and for various EU premia is dependent on compliance with all identification regulations. Therefore, you are advised as part of the general service to your clients to draw their attention to any deficiencies in this regard, that you may observe while on their holdings.

**ER Circular 16 /2008 Rev 1.**

**Issue date: 3<sup>rd</sup> November 2008.**

**Effective date: - Issue Date**

**To the SVI/HEO/DS at each DVO**

**Copy to each SSVI/RAP**

Subject: Bovine Identification and the use of Brass Tags

### **Purpose**

To standardise the manner in which DVOs correlate brass tags used to temporarily identify bovine animals at the time of testing with the animals' proper plastic tag identification.

### **Policy**

Circular ER6A/2000 that issues annually with the ER4 instructions to all testing PVPs and WTVIs sets out the procedures relating to the treatment of bovines that have been temporarily identified with brass tags. The basis of the veterinary certification contained in that Circular has not changed viz "if the testing vet has identified, for test purposes, an unidentified animal by means of a brass tag then, of course, no passport (identity card) may subsequently have test details certified by the testing vet until such time as they have satisfied themselves as to the correlation between the animal brass-tagged and the plastic tag number on the passport. This ordinarily will require re-visiting the herd to check the animal(s) involved. **The herdowner/keeper will be responsible for any costs involved**".

### **Legislation**

S.I. No. 276 of 1999 European Communities (Identification and Registration of Bovine Animals) Regulations 1999.

S.I. No. 277 of 1999 Bovine Tuberculosis (Attestation of the State and General Provisions) Order 1999.

### **Procedures**

**The AHCS discrepancy report highlights tests where brass tags have been used to temporarily identify animals for the purpose of the test. The ER96 (copy attached) has been revised and is now divided into two sections as follows:**

**Section A:** signed declaration by the keeper regarding the correlation of temporary brass tags with the permanent identity contained on plastic tags.

**Section B:** signed declaration by the testing veterinary surgeon that the correlation is correct and that he/she can certify the TB and/or Br test.

If the keeper submits the ER96 and **Section A only** is completed then the discrepancy flag on the AHCS can be lifted by using the facility on the AHCS Edit Animal screen to record the keeper declaration for the temporary tag number. Where relevant, the discrepancy flag should also be removed from the permanent tag number (in accordance with Council Regulation 1760/2000 the keeper is the person legally responsible for identifying the animal).

If the testing Practitioner has also completed **Section B**, then the test details may also be updated on AHCS using the Correlate Tags screen and the animal's passport, if in the DVO, can be returned to the PVP for updating the test details.

The paragraph in the discrepancy letter that refers to animals temporarily tagged has been modified to take account of the changes made to the ER96. A copy of the ER96 should always accompany the discrepancy letter.

**The revised ER96 form is now available on the Ezone and all copies of the old version should be withdrawn and destroyed immediately. Supplies of the revised version should also issue to PVPs so that they have copies available to give to the keeper at the time of the test.**

The following are the procedures for dealing with animals temporarily tagged at test or indeed animals withheld from test:

1. Details of un-correlated temporary tags (i.e. no ER96 submitted) can be identified by running discrepancy reports for the herd in question.
2. All surplus passports should be forwarded to DVO: this includes passports for animals temporarily tagged at test which are not the subject of an ER96 counter-signed by the testing VS and any animals not presented for test.
3. VS should **not** retain passports for animals not tested or not presented for test.
4. Passports should be held in the DVO until **Section B** on the ER96 has been completed by the testing VS.
5. For unregistered animals temporarily tagged at test that will be the subject of a late registration, check that all temporary tags used in the herd have been correlated and that the correlation has been certified by the testing VS, prior to authorizing the issue of a passport.

**Key Words: Brass tags, PVPs, Keepers and ER 96**

**Authorised by:** ERAD Management Committee

**Date:** November 2008

## Appendix 5(a) NOTES ON COMPLETION OF PRE PRINTED ER15B

1. In column headed '**Breed**' the following entries only should be used:

Angus	AA	Danish Red	RD	Kerry	KE	Romagnola	RM
Angler	AN	Dexter	DX	Limousin	LM	Rotbunte	RB
Aubrac	AU	Droimeann	IM	Maine Anjou	MA	Salers	SA
Ayshire	AY	Galloway	GA	Montbéliarde	MO	Shorthorn	SH
Belgian Blue	BB	Guernsey	GU	MRI/MRY	MY	Simmental	SI
Bison	BI	Hereford	HE	Murray Grey	GS	South Devon	SD
Blone D'Aquitane	BA	Highland	HI	Normande	NO	Swedish Red	SR
Brown Swiss	BS	Holstein/Friesian	FR	Norwegian Red	NR	Wagyu	WA
Charolais	CH	Irish Maol	IM	Parthenaise	PT		
Chianina	CI	Jersey	JE	Piemontese	PI		

In the case of a breed not listed please record the full name. Cross Breeds should be entered with an X after the dominant breed, e.g. HEX for a Hereford Cross.

2. All animals should be presented with two plastic ear tags for identification purposes. In the case of plastic tags containing an alpha-numeric identifier, the tag number should be written in the format: Letters: numbers: check digit e.g. BEA 19731-4 or Letters: numbers: check letter e.g. BCDF 0025Y. The tag number of plastic tags containing an all-numeric identifier should be written in the order in which the numbers appear on the tag. All zeros included in the number must be recorded. Normally the full space allocated should be used for recording an animal's tag number.

The tag space itself is partitioned into top and bottom halves to facilitate a double entry where a temporary tagging for test ID purposes takes place. The temporary tag number should be entered on the lower space and the permanent tag number (i.e. where the old tag or passport/animal ID card is available) is entered on the top line. In cases where the permanent identity of the animal is not known leave the top line blank. Where a temporary brass tag is inserted record TT in the Tag/Pass column. Brass tagging of animals for temporary identification for test purposes, is only allowed where <10% of herd require such. Otherwise return to complete test after farmer has regularised the identification of the animals. Correlation of temporary tag with permanent identification number, is required before any test certification may be completed on individual passport/animal I.D. card, export certificate etc. See letter Ref: 6A/2000 for details.

3. Tag/Pass - The absence of identification documentation (Passport/ID Card) should be indicated by:

(i) NC No Card ----- Or (ii) FC Full Card ----- Or (iii) WC Wrong Card  
Where a temporary tag was used to identify the animal TT should be inserted.

4. Age/DOB should be recorded in the following order – year/month or actual age.  
e.g. 1/0 one year old 1/6 one year and six months 0/10 ten months

5. The following codes should be used for recording the sex of the animal:

Cow	C	Bull	B
Heifer	H	Bullock/Steer	S

If an animal is pregnant state the length of pregnancy (e.g. C4 = cow pregnant 4 months).

6. The column headed '**Clinical Remarks**' is intended for recovering conditions relevant to both TB and Brucellosis testing. In the case of TB the following clinical conditions should be recorded if present:

Cough	CO	Emaciation	EM
Snoring	SN	Enlarged lymph glands	GL
Skin TB	ST	Mastitis	MA

In the case of Brucellosis blood testing any abortion history (i.e. date of abortion) should be entered.

7. In the column headed '**Reaction**' the following are the appropriate entries.

(i) Diffuse Oedema      D.O.    (ii) Extensive Oedema      E.O.    (iii) Circumscribed C.

8.      In the column headed '**Result**' you should indicate the result of both increases.  
In the column headed '**Test Result**' you should indicate the overall result of the test:  
(i) Positive +    Or (ii) Doubtful 0    Or (iii) Negative --

The column headed '**BR Tested**' must be completed where a prepared list of the animals to be tested is provided by the D.V.O. If an animal has been blood tested the letter 'Y' should be inserted in this column to indicate that the animal was blood tested.

## **Appendix 5(b) Protocol for PVP/WTVI Supervision.**

1. Preparation
  - Examination of recent ER13As
  - Print off ER 9.
2. On Farm Inspection ER13
  - Disinfection
  - Equipment
  - Testing technique.
3. Examination of Hand held Recording Device
  - Correct version of software(record version on ER13 currently 1.41 or greater)
  - Correct PVP Code. As on ER9 (record code on ER13).
  - Correct date
  - Using current profile
  - Tuberculin Batch recorded
  - Animal IDs /skin measurements and blood tube codes recorded
  - Number of animals tested and animals awaiting test data
  - Untested 72hrs
  - Examine data for previous herds
  - Profile discrepancies queried with keeper
  - Irregularities noted by VI on ER13 and copy given to PVP.

## Appendix 5(c) ER13 Field Inspection Report

ORIGINAL for retention by D.V.O. Serial No: 00000

### Department of Agriculture and Food

#### Report of on-farm supervision of Testing under Tuberculosis/Brucellosis Schemes

1.	Veterinary Surgeon _____ Vet. Register No. _____ I supervised the *commencement/completion of *tuberculin/brucellosis testing being carried out by the above named on _____ (Date) No. of herds supervised _____ Herd Nos. _____ Total No. of Animals _____	
	* Delete as appropriate	
2.	Equipment check Tuberculosis (if necessary please circle 'No' items if multiple options in question)	
	A Two (avian/bovine) properly functioning syringes	*Yes/No
	B Identification of A and B syringes	*Satisfactory/Unsatisfactory
	C Spare needles, needle adaptors etc.	*Yes/No
	D Appropriate supply of bovine/avian tuberculin	*Yes/No
	E Functioning callipers and sharp scissors	*Yes/No
	F Punch, Taggers, Reactor disks, Identification and Reactor tags	*Yes/No
	G Thermometer and stethoscope for clinical examinations	*Yes/No
	H Field book/ER15B as appropriate (necessary as back-up even if electronic recording routinely), blank ER11/12s.	*Yes/No
	♦ Electronic Recording used	*Yes/No
	(1) What version of software was used?	-----
	(2) Approved software	*Yes/No
	I Surgical spirits and cotton wool	*Yes/No
	J Disinfectant available and used	*Yes/No
	K Maintenance and condition of equipment	*Satisfactory/Unsatisfactory
	Brucellosis	
	L Supply of bottles and matching supply of needles and needle holders	*Yes/No
	M Supply of bottle labels or pre-labelled bottles	*Yes/No
3.	The following is the manner in which the testing was carried out: Tuberculosis	
	(a) Tag number and initial skin measurements recorded	*Yes/No
	(b) Callipers used in measuring all animals:	*Yes/No
	(c) Tag numbers and 72nd hour skin measurements recorded for each animal:	*Yes/No
	(d) Situation of injection sites:	*Satisfactory/Unsatisfactory
	(e) Clipping of injection sites:	*Satisfactory/Unsatisfactory
	(f) Was the 'pea' confirmed after injection?	*Yes/No
	(g) Was the nature of the 72nd hour reaction recorded?	*Yes/No
	(h) Presence of so-called S.T.B. or other swellings noted and recorded?	*Yes/No
	(i) Reactor(s) identified, punched and tagged.	*Yes/No
	Brucellosis	
	(j) Was the field book or other recording method properly completed?	*Yes/No
	(k) If samples were taken prior to inspection commencing, are an appropriate number of used needles held for safe disposal?	
	(l) Were all samples properly labelled and correlated to the ear-tag number of the animals sampled?	*Yes/No
		*Yes/No
	Field book examined, initialled and faults noted in respect of all above Herd No.:	*Yes/No
	Field book examined, initialled and faults noted in respect of all other Herds in the book	*Yes/No
	* Delete as appropriate	

**ORIGINAL for retention by D.V.O.**

4. Reference Signature

Sample signature of Veterinary Surgeon \_\_\_\_\_

Witnessed by (include V.I. Code) \_\_\_\_\_

Signature of Witness \_\_\_\_\_

Date \_\_\_\_\_

5. **Remarks**

(1) Was this inspection prearranged with V.S. or unannounced?	* Prearranged/Unannounced
(2) Punctuality	*Satisfactory/Unsatisfactory
(3) Completed ER11/12 signed by farmer and witnessed	*Yes/No
(4) Passports/Identity cards taken up at commencement of test:	*Satisfactory/Unsatisfactory
(5) Entry of Herd Number and V.S. signature on passports/identity cards (check ID documents that may be in V.S.'s possession for return to any herd):	*Yes/No
(6)	
(7) Protective clothing worn by V.S. – Rubber Boots	*Satisfactory/Unsatisfactory
(8) Was test completion within 72 +/- 4 hours of commencement?	*Yes/No
(9) Was cleaning and disinfection satisfactory on arrival and departure from farm?	*Yes/No
General observations.	

Signed: \_\_\_\_\_ (V.I.)

V.I Code: \_\_\_\_\_

Date: \_\_\_\_\_

*\*Delete as appropriate*

6. **Recommendations by SVI:**

Signed: \_\_\_\_\_ Date: \_\_\_\_\_

**Observations by SSVI:**

Signed: \_\_\_\_\_ Date: \_\_\_\_\_



## **Appendix 5(d) Recovery of costs for supervisions necessitated by non compliance Circular ER03/2010**

### **Policy**

The Department has decided that, in future, it will recover any additional costs which it incurs arising from the need to conduct additional visits over and above the norm on a PVP/Practice/Holding following the detection of non-compliance with the conditions set down in the ER4 contract or when a supervisory check is rendered impossible because a notice of changed itineraries has not been provided in accordance with the ER4 and it is necessary for the DVO Veterinary Inspector (VI) to revisit the testing PVP/Practice/Holding. The requirement to impose such a charge on non-compliant PVPs is set out in Section 6 of the 2010 version of the ER4.

### **Procedures**

#### **1. Calculation and recovery of additional costs for additional visits**

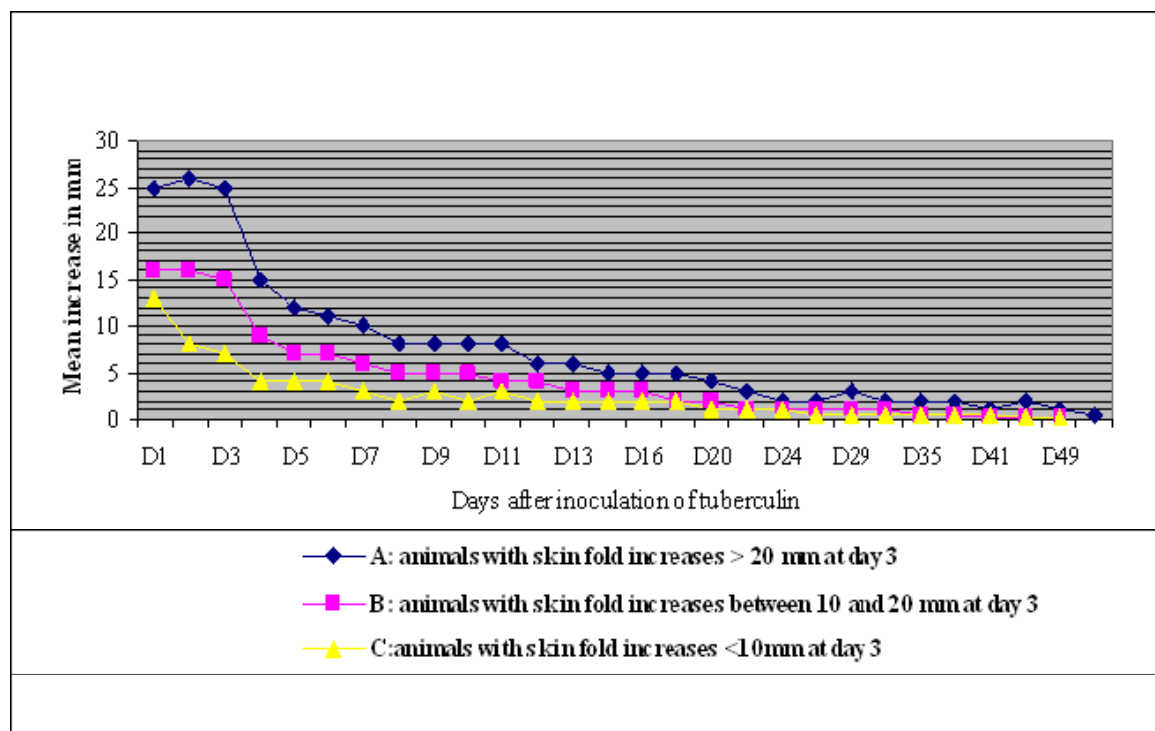
In instances where an additional inspection owing to non compliance is found to be necessary, the VI who conducted the additional inspection(s) should calculate the costs due having regard to the number of hours involved, the travel and subsistence costs incurred and using the hourly rate at the mid point of the VI salary scale applicable at the time of the additional inspection(s). The VI should then advise the PVP/Practice in writing of

- a)** the total amount due arising from the need for the additional inspection(s) and requesting payment within 21 days of the date of the letter and
- b)** the right to appeal the decision to the DVO SVI within 14 days.

If payment is submitted to the DVO by the PVP within the 21 day deadline, the payment cheque should be forwarded to Annette Dunne in the Cash Office in Cavan together with a fully completed Form OP1. If payment is not received within the 21 day deadline and no appeal is submitted, then the completed form OP1 should be sent to Mr. Paul Bates HEO Accounts Division Cavan who will arrange to deduct charge from TB testing fees due to the PVP.

This is also covered in similar terms under par 6 of the ER 4 so all PVPs have been warned and it has also been brought to the attention of Veterinary Ireland. If the ER9 notification from the PVP is not accurate and a VI has wasted time in trying to conduct an inspection on foot of such an inaccurate ER9 then the PVP should be informed that an unsuccessful attempt was made to supervise him/her on the date/time/herd in question and that accordingly the costs incurred in rescheduling the inspection will be charged. Then when you are satisfied that you have allowed sufficient time for the letter to arrive with him/her make a further attempt to conduct the supervision and charge for it.

# Appendix 5(e) Normal Regression of skin fold increases post Tuberculin injection.



**Appendix 5(f) TB Practice Inspection Report****TB ER13B**

Practice Name	
Address	
Date of Visit	

Number of PVPs Engaged in testing practice		Name of PVPs present at time of visit	

Number of clerical staff involved in TB programme	
---------------------------------------------------	--

**Tuberculin Storage** Delete as appropriate S/U or Y/N

Refrigerated at correct temperature (2-8C)	Y/N	All Tuberculin in date	Y/N
--------------------------------------------	-----	------------------------	-----

**Syringes**

Number examined		All syringes Individually Identified	Y/N
-----------------	--	--------------------------------------	-----

**Hand held devices**

Type Husky/Dapp/Quobos		Number of handhelds in use in practice	
Software Version		Frequency of upload of Data on Handheld	daily /weekly
Appropriate PVP code on Handheld	Y/N	Tuberculin Batch number Recorded	Y/N

**AHCS Procedures**

Demonstrated AHCS procedures	Y/N		
<b>Certification issues</b>		<b>Procedural issues</b>	
Correct use of Passwords and Codes	S/U	Profile download	S/U
Confirmation at Sign off	S/U	Management of Advanced Itineraries	S/U
Part testing	S/U	Cancellation/Alteration of itineraries	S/U
Private test management	S/U		S/U
Management of Upload errors	S/U	Missing animals	S/U

**Inspecting Official** **Name** **Comments** – Please print clearly

<b>Admin</b>		
<b>Regional AP</b>		
<b>VI</b>		
<b>SVI</b>	Follow up action	

## Guidelines for TB Inspection of Veterinary Practice.

The purpose of the TB inspection of a Veterinary Practice is to ensure compliance with ER4 testing instructions particularly in relation to certification, equipment and Tuberculin.

### 1. Preparation

- A **Practice Users Activity Report** for the month prior to the inspection and copies of recent **ER13A reports** for all PVPs in the practice should be brought by the inspecting officer.
- Inspections should be pre-arranged so as to ensure the presence of the appropriate clerical staff and at least one PVP.

### 2. Equipment

Examine any syringes available for working condition and identification. Syringes found to be defective should be sent for servicing.

### 3. Tuberculin

Ensure that Tuberculin is stored at the recommended temperatures. 2-8C .Out of date Tuberculin should be returned to the DO by the inspecting officer.

### 4. Hand held test recording devices

The make/model and software version should be recorded on the inspection sheet.

### 5. AHCS Procedures

The inspector should request demonstration by clerical staff and PVP of AHCS test processing procedures with particular attention to correct code usage by each party. Findings should be recorded on the form with comments where appropriate. The practice usage report should be discussed with the PVP and clerical staff. Anomalies should be followed up by way of written warning. Administrative and disease related anomalies found on the ER13A reports should also be discussed. Re-inspection should take place where certification is found to be compromised.

## Appendix 6 Tuberculosis Epidemiology: ER76 Investigations

### Guidelines for Veterinary Inspectors

#### Objectives

The ER 76 investigation is an epidemiological investigation of a disease episode on a holding to identify

- i. Possible and probable sources of *M. bovis* infection in the herd
- ii. Advise the keeper as to the best management of the outbreak and discuss the zoonotic implications for the family.
- iii. Identify and risk classify animals for forward tracing
- iv. Identify herds for inclusion in the contiguous programme
- v. Check that DAF requirements re herd register are being adhered to
- vi. Provide data for the analysis required for policy formulation

#### Preparation

When a VI receives a request to carry out an ER76 investigation on a herd he/she should ensure that the “Travel Pack” contains the following:

- i. Herd test history and animal test history as on AHCS ER76
- ii. ER 35
- iii. Herdfinder map
- iv. Wildlife map with setts (if available)
- v. ER128 Forward trace list
- vi. Back-trace report now part of ER76 on AHCS
- vii. Discrepancy report ER24 and AIM profile

The VI should acquaint him/herself with the information in the “Travel Pack”

The visit should be by appointment

The Keeper should be advised to have available the following:

- Herd register
- Area aid orthography or LPIS maps of the farm
- 60-90 minutes of time to spend on the consultation

The VI should check the current disease status of the contiguous herds (on ER 35 list) and previous herds, if there are introduced reactors. and animal test history for animals present during a previous episode in this or another herd and as far as possible endeavour to ascertain if any animals were previously part of an infected grouping.

#### On Farm Consultation

The VI should give a brief explanation of the purpose of the visit to the Keeper

The VI should use the ER 76 form as a framework for the consultation and to record the information gathered in the course of the consultation.

The VI should establish the group structure of the herd. Work through the grazing , housing pattern and animal groups with the keeper.

Establish the number of fragments on the farm and define the infected and non-infected fragments

Fill the badger section.

Determine the animals for “H” and “D” classification.

NB In designating a “H” status to an animal for onward tracing the VI should apply the following definition:

*An “H” category animal is an animal that, in the opinion of the investigating VI, is likely to fail a TB test in its **present** location*

Go through the ER 35 form and fill columns 5 & 6. Carry out a spot check of the boundary fence for overall quality and areas where nose-to-nose contact might occur.

Inspect yards, housing, feed stores and water troughs and advise re biosecurity

Record advice given to keeper and invite keeper to offer observations and to sign the relevant section of the ER 76 for

### **Follow up**

Report to SVI and request a badger survey if the investigation indicates that a badger survey is justified.

Completed VI ER76 checklist

Complete report on AHCS.

## Appendix 7(a) Ancillary Blood testing

### 4(a) Test approval

Ancillary blood testing for TB must receive prior approval from HQ . Please forward all requests to Margaret Good or Anthony Duignan on the Excel application form with all the relevant data.

<b>Application for Approval for Ancillary Blood testing for Bovine TB</b>								
DVO	Herd Number							
Application date								
Investigating VI								
	Date	Total animals	No. Reactors	No. In-contacts &/or co-exposed proposed for testing	No. Breeding animals in proposed group	Previous gIFN No. sampled	No. Positive	No. removed
Breakdown test								
1st reactor retest								
subsequent reactor retest								
subsequent reactor retest								
Herd description:								
SVI Comments								
Date ;								
HQ Decision								
Decision Date								

## **Appendix 7(b) Protocol for the submission of Blood samples for the IFN $\gamma$ -assay**

### **Notification and booking of samples to UCD**

The laboratory must be informed of any samples for submission **one full week's notice** in advance. Under no circumstances will samples be accepted at the laboratory without prior notification. The laboratory must be contacted on the day of taking the blood samples to confirm delivery. Please ensure that all calls relating to blood samples are made between 9:30am-5pm. The Herd status and the number of samples to be delivered should be clearly stated.

### **Time of testing**

It is advisable that the blood samples are taken on the same day as the skin test. Under circumstances where this cannot be carried out, it is preferable that samples are taken 10 or more days post skin test. This will normally be the case under the protocols for the atypical herd and in cases of depopulation.

### **Tubes**

The tubes used in the collection of the blood are the **10mls Green topped Lithium Heparin** tubes which should contain a minimum of 6mls of blood. **Any volume less than this is not sufficient to carry out the test.** Every effort should be made to ensure that each tube is full. Blood tubes can be obtained from the Gamma Interferon Lab in advance.

### **Packaging**

Pathopak boxes as currently used. Available from UCD lab.

### **Mode of Delivery**

The mode of delivery should be specified with estimated departure and arrival times. Samples should be transported to the Veterinary College at Belfield as soon as possible to ensure delivery within 10 hours of blood sampling. Samples should be stored at room temperature during transport.

By Car: Enter from Stillorgan Road side and turn right after the security hut at the entrance to the campus to the Veterinary College.

### **Sample Identification**

Samples must be clearly marked with Tube code labels as used for Brucellosis sampling should be used for identifying samples. These should be accompanied by a pre printed herd profile listing animals > 1y.o with the corresponding tube code inserted at the time of sampling.

Ensure this is legible and includes a contact (preferably mobile) number for the sampler Samples must be accompanied with the Herd Owner's name, full address and herd number. The onus will be on the Veterinary Inspector to match tube numbers and tag numbers.

### **Results**

Results will be sent by fax to the referring DVO upon completion of the test. Samples are assayed on a queued basis and every attempt is made to generate results as quickly as possible.



**Please note that the laboratory is now located on the main UCD campus in Belfield**

Mairead Doyle/Kevina McGill  
TB Diagnostics & Immunology Research Centre  
School of Agriculture, Food Science and Veterinary Medicine  
UCD, Belfield  
Dublin 4.

Contact Names: Mairead Doyle email; [mairiad.b.doyle@ucd.ie](mailto:mairiad.b.doyle@ucd.ie)

071-91-42191 (only if main no. out of order)

Sean MacFadden Senior Technician

Sligo laboratory will accept samples for GIF testing from Monday to Thursday, and will require them to be delivered to the laboratory here before 1pm on the day.

## 5B IFN- $\gamma$ ASSAY – Herds

The obvious primary target herds are those experiencing a Higher risk status (H) breakdown, which currently constitute approximately 30% of breakdowns. As these herds are currently subject to an epidemiological investigation by the attendant V.I. it should be possible to refine the targeted animals to particular groups or those associated with particular land fragments with regard to grazing/housing with the reactor animals during the relevant prior to detection/removal. Where it is not possible to categorise particular epidemiological groups e.g. in smaller herds, all animals aged over 1 year should be tested.

The most practical time to carry out IFN $\gamma$ -assay on these herds is either 10 days post SICTT so that all reactors may be removed at the one time or in conjunction with the first reactor re-test, which is generally carried out by the WTVI. Positive IFN $\gamma$ -assay animals in the absence of Skin test reactors could be removed following the Singleton protocol i.e. glands harvested and cultured allowing de-restriction of those herds with negative results following a subsequent clear skin test in 60 days.

Chronically infected herds of which there are 4,000-5,000 and which repeatedly breakdown should also be targeted for sampling i.e. H classified herds, which break down within 2 years of previous H-type breakdown. All adult animals in these herds should be targeted in an effort to reduce residual infection or possible spread to other herds. These herds could be IFN $\gamma$ -assayed at the time of the first Reactor retest by the WTVI.

For maximum efficiency and reliability samples for assay should be taken on the day of injection for SICTT.

IFN- $\gamma$  may also be used in atypical herds or to provide additional information where the SICCT may be compromised for whatever reason.

### Proposed Strategy

- 1) Use of IFN $\gamma$ -assay in Higher Risk Status breakdowns. The V.I. carrying out the epidemiological investigation should identify and flag the “higher-risk”, animals which remain in the herd, so that they can be traced either post movement to another group within the herd or to another herd subsequent to the breakdown. These animals should be subjected to IFN $\gamma$ -assay at the time of the first reactor re-test. Animals, which show positive reaction to the IFN $\gamma$ -assay, should be removed as reactors as they are part of the “higher-risk” group and subjected to singleton policy if there are no concurrent tuberculin skin test reactors in the herd.  
Where the investigating V.I. cannot identify “higher-risk” animals as in the case of small herds, all animals over 12 months old should be subjected to IFN $\gamma$ -assay at the time of the first reactor re-test. Animals, which show positive reaction to the IFN $\gamma$ -assay, should be removed as reactors and subjected to singleton protocol if there are no concurrent tuberculin skin test reactors in the herd.
- 2) High-risk categorised herds, which experience a further breakdown within two years, should have IFN $\gamma$ -assay carried out on all animals over 12 months old at the first reactor re-test. Animals, which show positive reaction to the IFN $\gamma$ -assay, should be removed as reactors and subjected to singleton protocol if there are no concurrent tuberculin skin test reactors in the herd.
- 3) IFN- $\gamma$  assay may also be considered on all Inconclusive animals in non-derogated herds either, 10 days immediately post disclosure or at the time of retest provided that samples from such animals can be submitted together with other samples such that there are minimal submission costs. A VI may also use the IFN- $\gamma$  assay in herds being considered for derogation where there is any doubt as to eligibility or additional security is desirable. A VI must use the IFN- $\gamma$  assay in herds being considered for derogation where more than one inconclusive reactor animal has been disclosed in the herd. Inconclusive SICTT retests should, where possible, be carried out by a VI.  
Animals, which show positive reaction to the IFN $\gamma$ -assay, should be removed as reactors and subjected to singleton protocol if there are no concurrent tuberculin skin test reactors in the herd.

### **Procedures for submission of samples for Gamma Interferon Testing to Sligo RVL.**

All samples for submission must be pre-arranged by phone to Sligo RVL by at the latest by the close of business on the **Wednesday prior** to sampling. The most suitable day for acceptance of samples for Gamma Interferon Assay at Sligo RVL is Monday, (*demand for Brucellosis testing is not usually high on Mondays*) but samples will also be accepted for other days by prior arrangement. Sample bookings will be accepted on a first come first served basis.

For advanced booking of samples please contact any of the following:

Madeline O Donoghue

Helga Keogh

Mary Kerrigan

at Sligo RVL      **071 91 42195** Or **071 91 42191**

The maximum number accepted for Assay will be 100 samples and it is expected that the normal submission in the North West Region will be 50-60 samples on any given day from an individual VI.

**Samples will not be accepted for testing after 2 PM** on any day this limitation will be **STRICTLY enforced** in order to allow for processing during normal working hours.

Please insure that all tubes submitted are full as more than 6 ml of whole blood is required for analysis. Please ensure tubes are labeled as near to the top as possible.

### **Supplies**

Supplies of Heparin Tubes and boxes are available from Mairead Doyle at UCD. Each DVO should keep sufficient supplies in stock to sample at least 100 animals. The herd profile for animals over 1 year old should be used when submitting samples. Tubes can either be labelled with Brucella tube code labels or pre printed tagnumber labels.

#### **4 (c) The following are the herds/animals to be targeted for sampling;**

1. Remainder of infected groups in Higher risk herds immediately following initial reactor disclosure such that all infected animals may be removed
2. Infected groups or entire Higher risk herds to be sampled in conjunction with the first Reactor Retest
3. Herds that might otherwise be considered for depopulation.
4. Reactors where confirmation of 'skin' response is desirable (e.g. 'volunteer' reactors suspected).
5. Where skin measurements are likely to be compromised - Atypical herds
6. Higher risk herds, which experience a further breakdown within two years of a previous H-type breakdown.
7. Inconclusive animals in all herds prior to allowing derogation.

**4 (f) Legal basis for removal of GIF positives**

**An Roinn Talmhaíochta agus Bia  
Department of Agriculture and Food**

**Bovine Tuberculosis (Attestation of the State and General Provisions) Order, 1989 (S.I. No 308 of 1989)**

**and**

**Bovine Tuberculosis (Attestation of the State and General Provisions) (Amendment) Order 2000 (S.I. No 161 of 2000)**

I, Mary Coughlan, Minister for Agriculture and Food, do hereby authorise the use of the Gamma Interferon Assay test for Bovine Tuberculosis under the Bovine Tuberculosis (Attestation of the State and General Provisions) (Amendment) Order 2000 (S.I. No 161 of 2000).

GIVEN under my Official Seal,  
this 26<sup>th</sup> day of April 2005

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Minister for Agriculture and Food

## Appendix 8 Singleton Reactors

### SINGLETON TUBERCULIN TEST REACTOR PROGRAMME

1. All index tests which reveal single reactors will be reviewed at the start of each week to assess the number of potential candidate herds/animals that will require separate identification for segregation and subsequent handling at the factory.
2. To facilitate this review it will be necessary to know the readings for these animals. Where reports are not yet to hand the testing veterinarians must be contacted.
3. An ER26S permit should be used in respect of candidate animals. The A.O. responsible for the reactor collection service must insert a further reactor tag with a green disk in the animals left right ear. This can be undertaken at the time of the farm visit.

#### **Procedure at the Factory**

1. Singleton reactors for special post-mortem examination will be identified by green coloured spray and highlighted on the singleton reactor movement permit (ER 26S)
2. These cattle should be kept separate and slaughtered as a group.
3. The line should be slowed down to allow adequate time for slicing of glands removal and identification of samples and sterilisation of knives between each animal.
4. If lesions typical of tuberculosis are detected samples need not be taken for laboratory examination.
5. If lesions are not detected then the retropharyngeal, submaxillary, parotid bronchial and mediastinal lymph nodes should be collected for laboratory examination. The head and thoracic glands should be put into separate plastic bags.
6. The Veterinary Inspector responsible for the factory should supervise the slicing and collection of lymph nodes to ensure that knives are sterilised between each sample. It is most important that cross contamination is avoided between samples. DVO support will be provided at the outset until the new procedures are operating effectively.
7. The pattern of isolates from NVL lymph nodes will be monitored as a quality control measure to check for cross contamination. VRL staff may also visit factories during sampling to assess quality control factors.

#### **Procedure for Packing and Identification of Samples from Singleton Tuberculin Test Reactors**

**Note; When creating the ER47 the drop down box on screen will prompt the permit type which should be input as ER26S Singleton and not Slaughter Check.**

1. The tag number should be written on the pathoseal bags containing the samples.
2. Samples from each animal should be placed in a separate pathoseal bag. Each bag must contain glands from only one animal and be accompanied by a copy of the ER47 placed in the pouch of the pathoseal bag.
3. A green label (to be issued) identifying the sample as from a singleton reactor should be put on the outside of bag.

4. A matching green label should be attached to the lower right hand side of the ER47 form accompanying the samples. A separate ER47 form should be completed for each animal and attached to the outside of the pathoseal bag.

#### **Laboratory Examination**

1. All samples will undergo further slicing in the laboratory to detect any lesions that may be missed at the factory. A sample of any lesions detected will be prepared for histopathological examination.
2. Where specific non tuberculous lesions are identified culturing of the remaining tissues will be undertaken.
3. Tuberculous lesions will be identified on histological examination where the presence of a granulomatous lymphadenitis (Langhans Giant cells and Epithelioid cells) associated with caseous necrosis are observed. No further culturing will be undertaken in these circumstances.
4. If visible lesions are not detected a sub-sample of the glands submitted will be prepared for cultural examination.
5. Cultural examination will consist of the BACTEC method plus the inoculation of two tubes of Lowenstein Jensen medium.
6. Presumptive identification of isolates will be based on growth characteristics, appearance of the isolate, detection of cording and sensitivity to para nitrobenzoic acid. A proportion of the isolates will be subjected to DNA probe and/or biochemical testing as a quality control measure.
7. Cultures will be deemed negative if growth is not detected after 7 weeks.
8. An email will be sent to the DVO via AHCS when final results (based on histopathological and or cultural examination) are available.
9. Progress of details of samples and results may be tracked on AHCS.

20th September 1996

Ref: ER 12B/96

To the SVI  
Each District Veterinary Office  
(Copy to HEO, SAO, SSVI, RHEO)

### **Protocol for Singleton Herds**

Please note that the eligibility criteria for animals for inclusion in this programme as set out in Circular 12/96, dated 4th April, is now amended in regard to inconclusive animals as follows-

Herds with single animals which test twice inconclusive (i.e. reactor) are eligible for this protocol.

First time single inconclusive animals where the herd owner decides to send the animal for immediate slaughter are not eligible.

---

John Ferris  
Deputy Chief Veterinary Officer

## Appendix 9 AHCS - Contiguous and Special Programmes

Contiguous and special testing programmes may be created on AHCS for either TB or Brucellosis. **A herd may be included in more than one testing programme for the same disease (e.g. if it is contiguous to two or more TB index herds) but it will only be tested in one programme at a time.**

If at the time of creation of a programme, the system finds that a herd is part of an existing programme, the herd can be added to the new programme but will continue to be tested in the existing programme. It will be flagged as “Not Tested in this Programme” in the new programme i.e. **test column will have an “N” present.**

When the herd’s current testing programme finishes, the herd will then be tested as part of whatever other programme(s) it is included in until such time as all of the programmes have ended. If it is part of more than one other programme, the system will make it active in whichever programme has started most recently and has the highest risk level. If the herd is not present in other programs, the herd will be listed for a Round Test twelve months from the date of last herd level test.

Similarly, in some cases, the equivalent test rules will mean that a new test will not be created in a programme if a herd is scheduled for a test with a higher priority. However, when the latter test is followed up the system will prompt for a test in the programme created (if the test is clear).

If a herd in a programme should itself breakdown, it will remain as part of the programme **but will go through the normal reactor re-test lifecycle.** When the herd goes clear and if the programme has not ended, it will be scheduled for a test in the programme if programme test has higher priority.

Each programme is allocated a Programme Number that allows it to be identified. Herds may be manually added to or removed from a programme as the need arises.



## Appendix 10 Inconclusive Reactors

### Inconclusive Reactor Circulars

Ref: ER/6B/2000

4th April 2000

#### IMPORTANT NOTICE (TB)

To the Veterinary Practitioner named in the address

#### **Re: Directive 64/432/EEC (as amended)**

You are hereby reminded that **no bovine animal** is eligible for export to another EU Member State if the herd of origin contains animals of unresolved status (i.e. TB inconclusive reactors to standard interpretation).

Accordingly please note that it is **not permissible** under any circumstances to issue a certificate stating that an animal from such a herd has passed the TB test to the export standard.

Your co-operation in ensuring the continued integrity of Veterinary Certification for the Irish live export cattle trade is much appreciated.

The EU directive above also requires suspension of the TB status (restriction) for herds in which inconclusive reactors are disclosed but does allow an exception to be made for internal trade only provided certain conditions are fulfilled.

In order to ensure that the conditions of this directive are met when an inconclusive reactor is disclosed in a herd please proceed as follows:

The test report and unsigned passport(s)/identity card(s) for the inconclusive reactor(s) should be sent to the DVO as heretofore,

if the inconclusive reactor(s) was disclosed on a part herd test, such as a private test, the passport(s)/identity card(s) for the **entire herd** must be returned to the DVO.

In the case of a part herd test the disclosure of an inconclusive reactor(s) casts doubt on the status of the remainder of the animals in the herd. Thus the herd will automatically be classed ineligible for return of passport(s)/identity card(s) until the status of either the inconclusive reactor or the 'rest of the herd' is resolved,

if the inconclusive reactor(s) was disclosed at a full herd test the passport(s)/identity card(s) for the 'rest of the tested herd' **must** be held pending further instructions from the relevant DVO,

On receipt of the test report in the DVO a Veterinary Inspector will assess the eligibility of the herd for return of passport(s)/identity card(s), based primarily on the disease history of the herd.

It is anticipated that where TB has not been confirmed in the herd in the previous three years (circa 85% of the cases) that, the DVO will notify you to date, sign and return the passport(s)/identity card(s) in your possession to the farmer. This will allow the farmer to trade internally (without an export

certificate) should he/she so wish. In the remaining, approximately 15% of cases, you will be requested to forward the passport(s)/identity card(s) to the DVO owing to the suspension of the herd status.

The inconclusive reactor(s) will be scheduled for retest in the normal way by the DVO.

You are further reminded that animals TB tested within the previous 42 days **may not be retested** unless presented for test during the course of a full herd test. If the DVO has issued a private test permission for the herd such animals are ineligible for private test.

If you have any queries on this matter please contact the SVI in your local DVO.

Thanking you for your co-operation in this matter,

---

Michael Sheridan DCVO,  
ERAD Division

June 2000

To each SVI/ HEO/ DS  
(copy to RSSVI/ RHEO)

**Re: Directive 64/432/EEC (as amended)**

**TB inconclusive Reactors**

Please see the attached Circular letter ER/6B/2000 sent to each testing PVP and relating to the management of herds in which a TB inconclusive reactor is disclosed on test.

Directive 64/432/EEC (as amended) requires the suspension of the TB status (restriction) for herds in which inconclusive reactors are disclosed. The purpose of this suspension is to prevent trade in animals from the same herd as an animal that may be infected with TB. It does however, allow an exception to be made, for non-intracommunity trade only, provided certain conditions are fulfilled.

When a test report, on which there is an inconclusive animal(s), is received in the DVO the interpreting VI must review the animal/herd history over the previous three (3) years to assess if these conditions are fulfilled. He/she must arrive at a decision as to whether the PVP may return the passports/identity cards for the remainder of the herd to the herdowner or whether the herd must be restricted and the passports/identity cards sent to the DVO. The PVP must be notified (promptly) in writing of that decision.

**In arriving at a decision the VI will be required to review herd test histories, viz.-**

1. Has the herd been restricted in the previous 3 years?

If it has, the herd must now have its disease status suspended, unless the restriction related to a 'singleton' episode where the cards were returned after one clear test (i.e. was previously "status suspended" rather than "status withdrawn").

If the animal was bought into the current herd then the herd-test history of the herd of origin and indeed any herds through which the animal passed are of relevance and should be treated as 1 at above.

If 'lesioned' animals have been traced back to the herd in the recent past this should be taken into consideration in arriving at a decision and normally the herd should be restricted.

The current status of contiguous herds should also be reviewed. Where one or more contiguous herds is experiencing an H class breakdown (see handbook) then normally the herd with the inconclusive should be restricted.

**A "DECISION CHART" which may of assistance to Vis is attached and should be displayed in the VI room.**

Administrative staff and the VI on office duty should give priority to the handling and interpretation of any test reports received on which an inconclusive animal is recorded. The attached form notifying the PVP of the requirement *vis-à-vis* the passports/identity cards should be completed and an ER22 signed if necessary. Such tests and associated documents should be processed within 48hrs of arrival in the DVO.

Where a herd is to be restricted due to an inconclusive it shall be sufficient to send a restriction notice, ER22, by post to the address at which the herd is registered. A copy of the attached letter outlining the different options for the herdowner should be sent with the ER22. Herds that are issued an ER22 should be 'flagged' using herd indicator 10 and entering I/R and date of ER22 issue. This 'flag' will result in the herd appearing in reverse video on herd enquiry etc. The herd status on computer should remain 0 so that DVO statistics on reactor herds

will remain unchanged. SWS, Bandon and the Beef Premium Unit, Portlaoise should be notified when the herd is restricted/derestricted. A derestriction notice should issue when the herd is being de-restricted and the 'flag' should then be removed.

### **DVO preparation for handling inconclusive herds:**

Pending the advent of the new Animal Health Computer System, which is being designed to prompt an appropriate course of action following the disclosure of an inconclusive animal, DVOs should immediately review all categorised herds.

Category A and B herds (new category H) are by definition subject to suspension in the event of an inconclusive animal being disclosed.

Other herds that have experienced a category C breakdown (new category L) in the past 3 years should be reviewed. Any such herds that have reverted to Category D should be re-categorised as C herds.

'Singleton' herds that had their status restored after one clear test should be re-categorised D on derestriction.

On completion of this review a list of all current category A, B and C herds not presently restricted together with the status dates should be generated for each PVP to identify those herds that had their status withdrawn/restored in the previous 3 years. The attached letter should be sent to the PVP together with the client-specific list so generated. A sample copy of the letter for herdowners who have had an inconclusive reactor(s) disclosed should also be sent to the PVP for information.

When an inconclusive is revealed in one of these herds, with a status date of less than 3 years ago the passport/identity card must be sent to the DVO, with the test report. Other herds in which inconclusive reactors are disclosed will require individual assessment regarding the requirement for status suspension. The list of categorised herds should be reviewed and updated periodically and a client-specific list issued to testing PVPs.

The completion of this review and its regular update will assist the VI in the rapid evaluation of many herds with inconclusive reactors. If an inconclusive is revealed in a category A, B or C herd a quick check of the status date (is the status date less than 3 years ago?) will determine if the herd is immediately ineligible for return of cards.

For historical review purposes categories A and B equate to the new category H ('highest risk'), category C herds equate to new category L ('lower risk') and category D ('default') remains unchanged.

### **Payment:**

Tests that reveal an inconclusive reactor(s) will be paid for by the party originally scheduled to pay for that test. If the inconclusive retest is clear the original test remains valid for subsequent movement and in the case of a round test is the 'annual' test for that year.

If the herdowner opts to slaughter the inconclusive and a SCT is completed then the Department will pay for the SCT if the herdowner has paid for a herd test in the calendar year or in the previous 10 months. If an inconclusive reactor becomes a reactor on retest or otherwise confirms with TB the Department will pay for the reactor re-tests. It will also pay for the clearance test if the farmer has paid for a herd test in the calendar year.

### **Inconclusive Reactor on a 'clearance' reactor re-test:**

As set out in the VI handbook (page 6) "the minimum level of interpretation at all subsequent reactor retests will be the removal of standard inconclusive reactors within or originating from the infected group(s) of cattle." Thus in normal circumstances such a 'standard' inconclusive reactor would be deemed reactor, with appropriate consequent compensatory payment, and unless qualifying as a 'singleton' (unlikely), needing 2 consecutive clear tests before de-restriction.

A VI may determine that the inconclusive reactor(s) need not be punched as a reactor(s) when not linked epidemiologically to the breakdown, or for some other reason. The herdowner then may decide to have the animal(s) slaughtered, without attracting any compensatory payment entitlement. In these circumstances if the inconclusive reactor(s) is NVL in the factory then before de-restriction, one further clear full herd reactor retest would be required 42 days post-slaughter.

Thus only exceptionally should a reactor retest, listed as the clearance test, be followed by an individual animal inconclusive retest where the inconclusive reactor was a 'standard' inconclusive. In such exceptional circumstances the restriction and any income supplement entitlement etc. continues and the Department will pay for the test.

If the inconclusive reactor is a 'severe' inconclusive that a Veterinary Inspector wishes to retest the herd may be de-restricted and passports/identity cards returned to the herdowner. In such circumstances this is the clearance test and payment should be made by the party originally scheduled to pay for that test.

#### **Private test:**

Where an inconclusive reactor(s) is disclosed on a part herd or private test the PVP is required to submit all passports/identity cards for the herd to the DVO. Such a herd will be restricted immediately. The DVO will then schedule the inconclusive retest at 42 days. However, in cases where the herdowner is anxious to sell some animals quickly e.g. 'springers' the DVO may decide, where the herd otherwise fulfils the criteria for return of passports/identity cards, to submit the remainder of the herd to test. In this way the status of the balance of herd can be established and an appropriate decision made.

Private test permission may only issue for a herd in which an inconclusive reactor is awaiting retest if the private test is conducted simultaneously with the inconclusive retest.

#### **Restoration of herd status (de-restriction):**

The Directive requires that an inconclusive reactor(s) is isolated from the other animals in the herd until its status has been clarified either by a further test after 42 days or by *post-mortem* and laboratory examination.

**An inconclusive reactor being sent to a factory should, therefore, have glands collected for submission to the laboratory in the same manner as for a 'singleton' animal.**

In some cases it will not be possible to resolve the animal's status by an inconclusive retest at 42 days because the animal dies on farm or because glands are not sent to the laboratory (by choice or accident). In these situations the herd status may be restored by a herd test carried out 42 days after the inconclusive reactor has left the herd.

An ER36 may issue as normal for an inconclusive reactor(s). The herdowner will be given the choice, as outlined in the sample letter attached to this Circular, to have glands collected for laboratory examination or to have a SCT on the herd. **Where glands are to be collected for examination in Abbotstown the VI must note this on the permit for the attention of the VI on duty in the export plant.** This notation should be drawn to the attention of the herdowner who should be aware that it is his/her responsibility to ensure that the factory is aware of this request when the animals are delivered. **This Department will not accept responsibility if glands are not collected at slaughter where the herdowner fails in this regard.** The herdowner should also be informed that negative laboratory results would not be expected earlier than 8 weeks from date of slaughter of the inconclusive. However, positive results may be available earlier. Alternatively, the herdowner, particularly in smaller herds, may opt to have the full herd listed for a SCT test 42 days after the slaughter date of an NVL inconclusive reactor instead of having glands collected for laboratory examination. If the glands of an NVL inconclusive animal(s) are under examination in the laboratory a tuberculin test should not be allowed in the herd of origin until full results are available. Herdowners who have had glands collected at the factory cannot subsequently change their minds and opt to have a 42-day special check test done instead. This rule is aimed at preventing a situation where a 42-day special check test is completed and clear, animals are sold (even exported) and the laboratory then reports at 8 weeks that the inconclusive reactor has confirmed positive for *M. bovis*.

Where an inconclusive reactor goes reactor on retest it may be assessed for eligibility for entry to the 'singleton' programme. If ineligible as a 'singleton' or if an inconclusive reactor otherwise confirms as infected with TB then 2 consecutive clear reactor retests are required for status restoration. The first reactor retest must be conducted after a minimum of 60 days and the second no less than 120 days after the last 'reactor' left the herd. In addition any animals that had moved out of the herd must be traced and tested.

**The following SAMPLE LETTERS are attached:**

- 1. to PVPs instructing them on return of passports/identity cards.**
  - 2. to PVPs to accompany the list of A, B and C herds is attached.**
- to herdowners accompanying the restriction notice ER 22. This letter outlines the options available to them when an inconclusive reactor(s) is disclosed. A Synopsis of Farmer Decision Chart which should be included with the letter to the herdowner is also attached.**

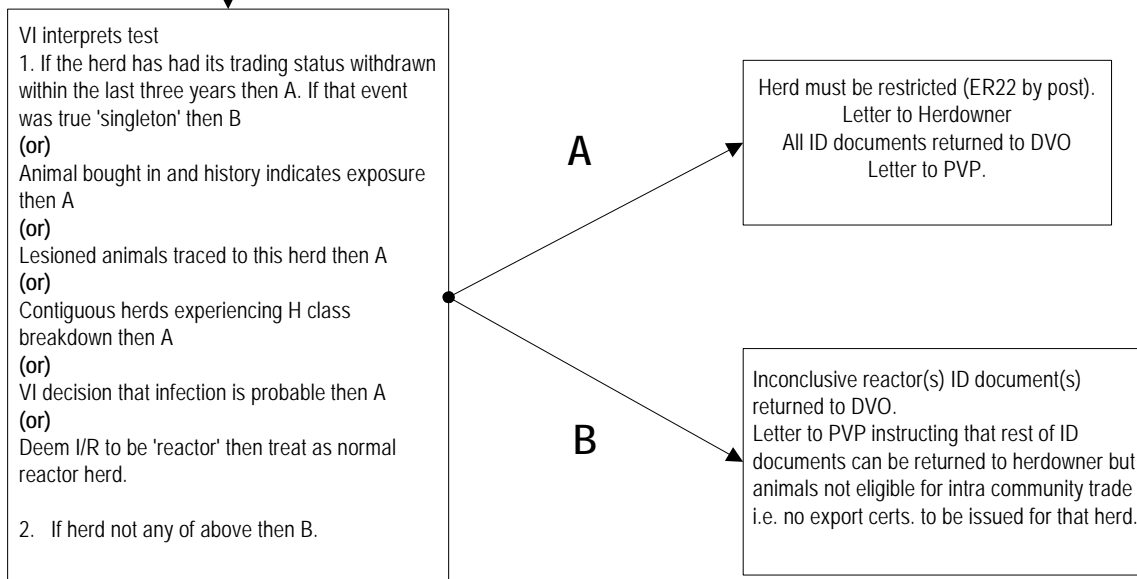
A copy of the implementing Statutory Instrument (S.I. No. 161 of 2000) is attached.

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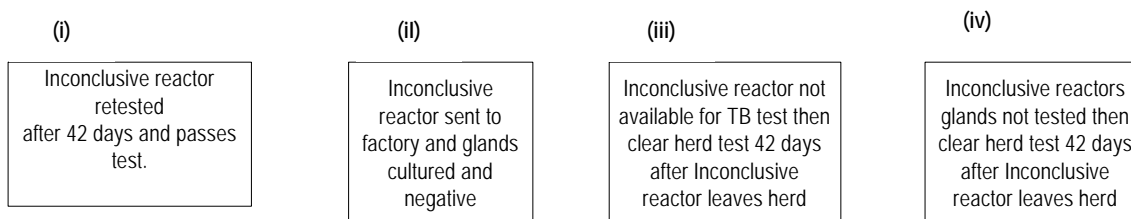
M. Sheridan  
DCVO

## Decision Chart for TB inconclusive reactors relating to amended 64/432/EEC

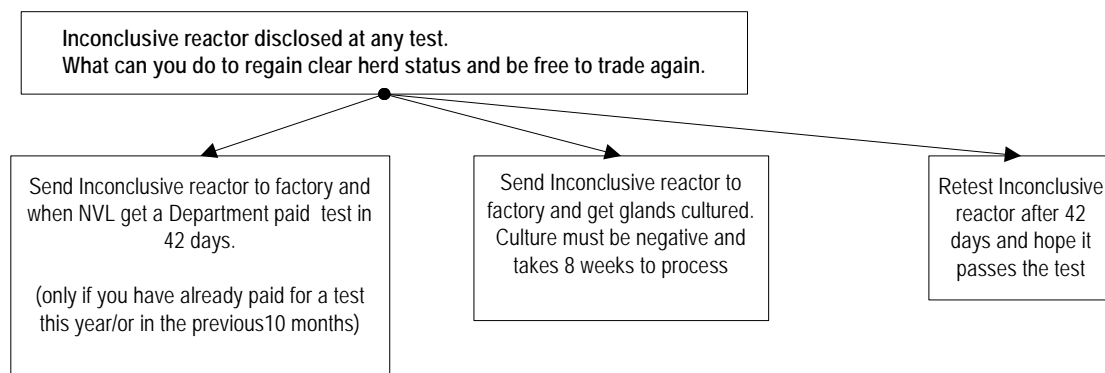
### Report with Inconclusive reactors received at DVO



### When A or B occurs restoration of herd's clear status can be by



### Synopsis of Farmer decision with TB Inconclusive reactors in his/her herd.



***Letter instructing PVP to return passports/cattle identity cards to herdowner or DVO as appropriate. This letter should be personalised to your particular DVO.***

Department of Agriculture, Food & Rural Development,  
DVO,

Co.

To

Co.

Re: **TB Inconclusive(s)** on date: \_\_\_\_\_  
in herd no. \_\_\_\_\_  
Name: \_\_\_\_\_  
Address: \_\_\_\_\_  
\_\_\_\_\_

Dear \_\_\_\_\_,

The TB test history and epidemiological profile of this herd has been examined and you are hereby

Authorised to endorse and return to the keeper the passports/identity cards for those animals that passed the test.

**Instructed to endorse and forward to the DVO the identity passports/identity cards for those animals that passed the test.**

*(delete as appropriate)*

Signed \_\_\_\_\_ Veterinary Inspector

Date \_\_\_\_\_



***Letter for PVP to accompany the list of A, B and C herds. This letter should be personalised to your particular DVO.***

Department of Agriculture, Food & Rural Development,  
DVO,

Co.

To

Co.

**Re: TB inconclusive Reactors in herds.**

Dear ,

Circular ER/6B/2000, sent to you in April last, on this issue reminded you that in accordance with Directive 64/432/EEC **no animal from a herd containing an inconclusive reactor is eligible for export certification**. Indeed, as you are aware many such herds are restricted by the DVO and thus prohibited from moving any stock, other than by permit, until the status of the inconclusive reactor is resolved. One of the areas that the Directive specifies must be examined in determining eligibility for the exception allowing sale of animals for internal trade from a herd with an inconclusive reactor is the herd's TB history for the previous 3 years.

It is the practice in DVOs to categorise each herd that experiences a TB breakdown, based on the severity of the breakdown and the likelihood of further reactors being disclosed in the herd. Please find enclosed a list of such categorised herds for which you are the nominated Veterinary Surgeon.

The status date recorded against each herd indicates the date that the Officially Tuberculosis Free status was restored following the most recent TB breakdown. In the event of an inconclusive reactor being disclosed in a herd that has experienced a TB breakdown in the previous 3 years that herd will **not** qualify for the exception mentioned in paragraph 1 above. The herd will be restricted and the passport(s)/identity card(s) will accordingly be required by the DVO (See Circular ER/6B/2000).

Herds not on this list or with a status date in excess of 3 years ago will require individual assessment by a Veterinary Inspector before a decision is made to return the passport(s)/identity card(s) or to restrict the herd. Matters such as recent events pertaining to the herd, status of contiguous herds and other epidemiological factors will form part of this individual herd assessment.

. Enclosed also for your information please find a sample letter such as will be sent to each herdowner in receipt of a restriction notice following disclosure of an inconclusive reactor. You may wish to advise him on the various options as outlined.

Tests that reveal an inconclusive reactor(s) will be paid for by the party originally scheduled to pay for that test. If the inconclusive reactor retest is clear the original test remains valid for subsequent movement and in the case of a round test is the 'annual' test for that year.

If the herdowner opts to slaughter the inconclusive reactor and a SCT is required before derestriction then the Department will pay for the SCT if the herdowner has paid for a herd test in the previous 10 months or in the calendar year.

If an inconclusive reactor becomes a reactor on retest or otherwise confirms with TB the Department will pay for the reactor retests. It will also pay for the clearance test if the herdowner has paid for a herd test in the calendar year.

Yours Sincerely,

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SVI

***Letter for herdowner to accompany the ER20 and ER22. This letter should be personalised to your particular DVO and the individual herdowner.***

Department of Agriculture, Food & Rural Development,  
DVO,

Co.

To

Co.

**Re: TB inconclusive Reactor(s) in your herd.**

Dear ,

A recent change in the EU Directive governing trade in bovine animals within the EU requires, in some circumstances, the suspension of the TB status (restriction) of herds in which inconclusive reactors are disclosed. The purpose of this suspension is to prevent trade in animals from the same herd as an animal that may be infected with TB. Your herd is being Restricted (notice attached) following the disclosure of a TB inconclusive Reactor(s) in your herd. The Directive requires that inconclusive reactors be isolated from the other animals in the herd until their status has been clarified either by a further test after 42 days or by *post-mortem* and laboratory examination.

The inconclusive reactor(s) will be due for retest in 42 days - on date . It is the intention that your Veterinary Surgeon (or the DVO) will conduct this test. You should make arrangements with him/her well ahead of this date to have the test completed on time. ***(DVO alter this paragraph to fit particular situation please)***. If this test is clear the herd will be de-restricted. If the animal(s) is inconclusive reactor again or fails the test it will be removed as a reactor and the restriction on your herd will continue.

Alternatively you may contact this office for a permit should you decide to have the animal(s) slaughtered before the inconclusive reactor retest is scheduled due.

In the event of you deciding to have the animal slaughtered you must choose **one** of the following options in order to resolve the status of your herd:

You may choose to have samples collected from the animal in the factory for examination for TB in the laboratory. A negative result from the laboratory will take a minimum of 8 weeks. A positive result may be available sooner, however. If you choose this option your herd will be de-restricted if a negative result is obtained when the laboratory examination is completed.

You may decide not to have samples collected but to have a further test carried out on your herd a minimum of 42 days after the inconclusive reactor is slaughtered. If you choose this option your herd will be derestricted if the inconclusive reactor shows no evidence of TB in the factory and a clear result is obtained when the herd is tested.

When requesting the permit from the DVO please inform the official that the permit request is for an inconclusive reactor and whether or not you wish to have samples collected in the factory for laboratory examination, the factory to which you intend delivering the animal and the proposed date of such delivery.

If you wish to have samples collected in the factory a note to this effect will be entered on the permit. You will need to ensure that this note is drawn to the attention of the Veterinary Officer at the factory.

If no samples are collected and sent for examination a further test on your herd will be required a minimum of 42 days after the inconclusive reactor is slaughtered.

If the inconclusive reactor shows evidence of TB on slaughter samples will be sent to the laboratory for confirmation and your herd cannot be derestricted until the laboratory examination is completed and the result proven negative.

If an inconclusive reactor is again inconclusive or reactor on retest, or if following slaughter TB is confirmed the restriction will continue until two consecutive clear tests have been conducted on your herd. The first test must be after a minimum of 60 days and the second a minimum of 120 days following the slaughter of the last reactor(s).

If the inconclusive reactor dies before retest or slaughter you should immediately inform the DVO and notify South Western Services, Bandon, Co Cork on the farm-to-farm movement form. A further test on your herd will be required a minimum of 42 days after the animal's death.

Private test permission may only issue for a herd in which an inconclusive reactor is awaiting retest if the private test is conducted simultaneously with the inconclusive retest.

A test that reveal an inconclusive reactor(s) will be paid for by the party originally scheduled to pay for that test. If the inconclusive retest is clear the original test remains valid for subsequent movement and in the case of a round test is the 'annual' test for that year.

If you opt to slaughter the inconclusive reactor or if it dies before retest and a Special Check Test is required before derestriction then the Department will pay for this test if you have paid for a herd test in the previous 10 months or in the calendar year. If an inconclusive animal becomes a reactor on retest or otherwise confirms with TB the Department will pay for the reactor retests. It will also pay for the clearance test if you have paid for a herd test in the calendar year.

If you have any questions in relation to this letter or if you wish to discuss the various options outlined above you may contact any of the Veterinary staff at this office.

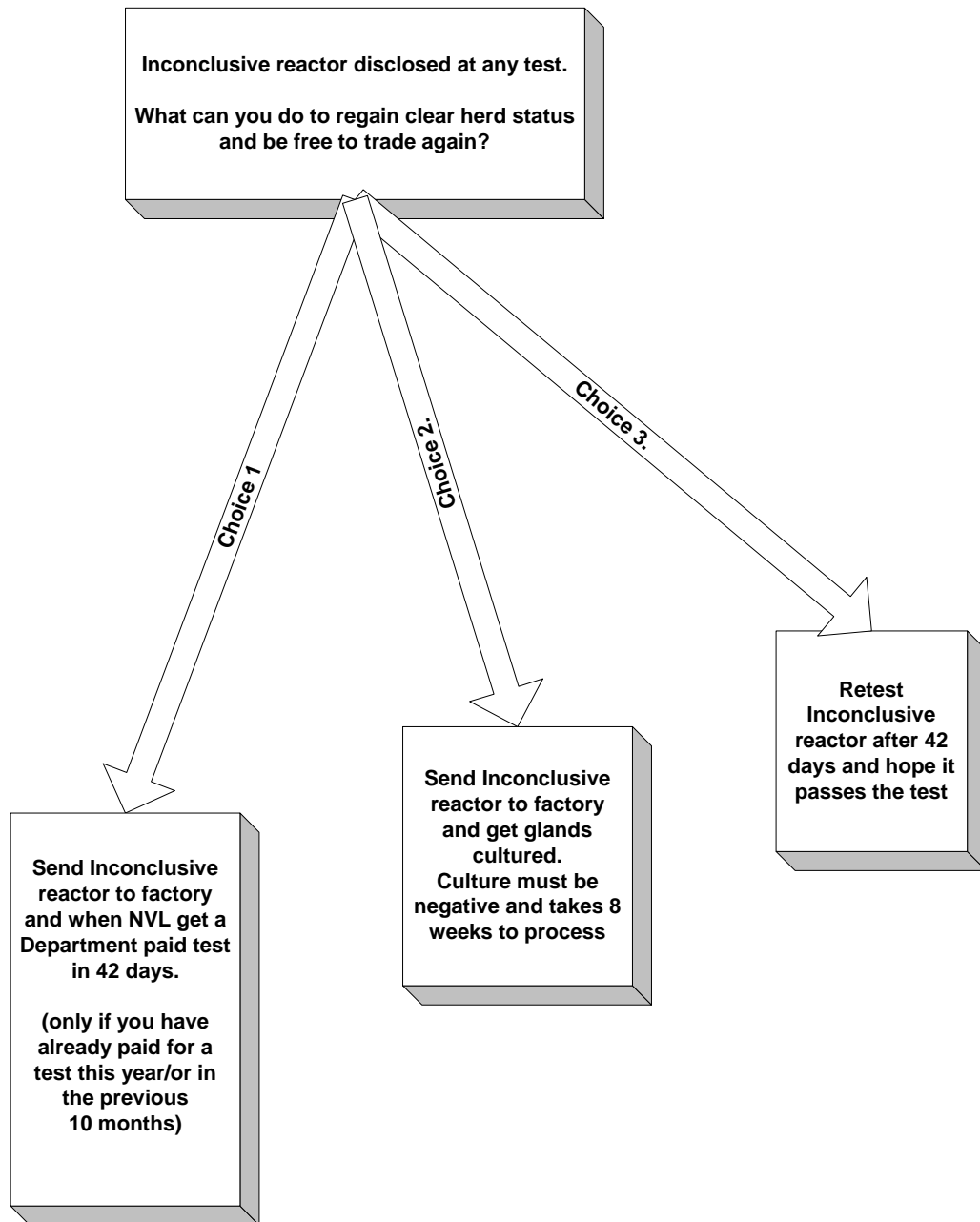
**A Farmer Decision Chart which may be of assistance to you is attached.**

Yours Sincerely,

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SVI

**Synopsis of Keeper decision with TB Inconclusive reactors in his/her herd.**



In the event of the death of the inconclusive reactor arrange for the proper disposal of the carcase and notify the National Movement Notification Agency (SWS Clonakilty) on an NBAS 31 D and notify the District Veterinary Office who will then arrange a herd test for you so that your herd can regain its status.

## **Appendix 11      Circulars on Factory Lesion Reactors**

Ref:    ER/37/99

13 October 1999

To Each SVI  
(Copy HEO, DS, SSVI, RHEO)

### Re: Suspect Factory Lesions in “Blue Card” cattle and Dir. 64/432/EEC

Please see attached circular which was recently sent to each meat factory.

To ensure compliance with Directive 64/432/EEC as amended (98/46/EC) please refer to circular ER/16/97 (attached).

The herd status must be suspended on receiving the report of a suspect TB lesion (items 1. and 2. on the circular).

Where TB is not confirmed and an alternative diagnosis is made (i.e. reason for the suspect lesion stated) the herd status should be restored (item 7 on the circular).

Where TB is not confirmed and no alternative diagnosis is made (i.e. no reason is stated for the suspect lesion) then a clear herd test is required a minimum of 42 days after the animal left the herd before the herd status is restored (derestriction)

Where TB is confirmed the herd status is withdrawn (items 4 and 6 on the circular) and the herd may not be derestricted until cleansing and disinfection is completed and two consecutive clear tests conducted 60 days and 120 days after the removal of the last positive reactor.

The decision, following the disclosure of a suspect factory lesion, to conduct an immediate ‘balance of the herd’ test (item 3 on the circular) will be left to the discretion of the SVI in the relevant DVO based on his assessment of the risk factors involved. However, if this test is clear it may not qualify as part of the status restoration procedure if conducted inside the 42 or 60 day timeframe as specified above.

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M. Sheridan DCVO

13.10.99

M6/99/GV

To Veterinary Inspector-in Charge  
Each Register Meat Export Factory  
(Copy Regional SVI)

Re: Lesion reporting in “Blue Card” cattle

I refer to circular dated 18th March 1993 signed S.P. O'Connor DDVS.

Suspect TB lesions found in clean cattle at routine postmortem examination are reported using form ER47. The original and two copies of this form accompany the sample sent to the laboratory in Abbotstown. The fourth copy is retained as the factory record. In due course when the laboratory examination(s) are completed part B of the ER47 is filled in and copies are returned to the meat factory and the DVO. The original top copy is retained by the laboratory.

Notification of the laboratory findings can take from one to six weeks depending on the examination procedures employed. The immediate notification to the DVO (of the owner/supplier, and also the DVO in which the animal was last tested if different) should be effected by means of a faxed copy of the ER47 on the day of slaughter.

Since this circular was originally issued fax numbers at many DVOs have been changed therefore a list of the up-to-date DVO fax numbers is attached for your information.

---

M. O'Sullivan SSVI

To the SVI  
Each District Veterinary Office  
(Copy to HEO, SAO, SSVI, RHEO)

### **MANAGEMENT OF FACTORY LESION HERDS**

The following are the procedures which should be carried out whenever a suspect TB lesion in a “Blue Card” animal is reported to a DVO

1. The file should be treated as a reactor file.
2. A restriction notice should be served and the cards taken up.
3. Permission to carry out a private test should not be given. Where permission has already been given it should be cancelled by telephone and the cancellation confirmed in writing.

Where, for other reasons a listing for a herd test has already been posted, the practitioner should be informed of the suspect lesion and asked to carry out the test immediately. However, the test report should not be processed until the laboratory result has been received.

4. On the computer
  - (a) The herd should be given a status “1” (restricted)
  - (b) The herd status date should be changed to the date of slaughter (and existing status date noted on file)
  - (c) The herd should be scheduled for a Factory Lesion Check Test (TT9) due twelve months forward from the date of slaughter.
  - (d) The herd should be flagged as a “suspect lesion” using the reverse video facility provided for the purpose.

(Some offices rely solely on the reverse video flag. This is an acceptable variation but demands regular and careful management of the suspect herds by way of the Report Generator facility on the computer.)

5. Control of all of the above procedures is exercised by producing a weekly report using the reactor herd register output format. A list of herds scheduled for a TT9 should be generated and reviewed. The laboratory should be contacted by phone if a report has not been received within three weeks of the slaughter date (status date)
6. Following a positive result from the laboratory the Factory Lesion check test should be brought forward from the national twelve month scheduled date already assigned and included on the next weekly listings (if not already listed as mentioned at 3 above).
7. Following a negative result a de-restriction notice should be served and the cards returned. The herd’s computer status should be returned to “O” (clear) and the original status date and next test type and date restored. The file should be returned to the clear section.



On occasions there are delays in getting results back from the laboratory. In situations where the herd owner is anxious to carry out the Factory Lesion Retest without awaiting laboratory culture procedures where these have been invoked he should be offered the chance to carry out an immediate herd test which would be at his own expense (if clear). In this way the period of restriction can be kept to a minimum. The test report should not be entered on the computer until the final result from the laboratory is known.

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John Ferris  
Deputy Chief Veterinary Officer

## Appendix 12 The Selection and management of NVL - Reactor herds.

### THE SELECTION AND MANAGEMENT OF A GROUP OF RECURRING NO VISIBLE LESIONS (NVL) REACTOR HERDS.

#### ONE DISTRICT VETERINARY OFFICE'S (DVO) EXPERIENCE.

John Murray and J McCarthy

##### Definition

The vast majority of Tb reactor herds behave in a typical manner and are progressed through their restriction to clear status without raising further questions. However, within the restricted herd population there is a subset of herds that behave in an atypical manner in that:

1. They produce unusually large numbers of no visible lesion (NVL) reactors.
2. They experience repeat 'reactor' episodes.

These restricted herds pose a challenging management problem. A serious doubt exists as to their true disease status that is not easily resolved.

##### Background

Table A lists 5 such herds in which investigations commenced in 1996 and proceeded onwards. Experience with these herds suggested that their reactors were not all due to infection with Tb. There was prima fascia evidence in one case of interference with the mammalian test site. The evidence was less obvious but no less convincing in other cases being based more on herd history, post mortem results and the type of reactor being disclosed. In these 5 herds the evidence was never sufficiently robust to define a prosecution for interference with a test under the animal health legislation and thus no case was ever taken before the courts.

From 1990 to 1999 these 5 herds produced large numbers of NVL tuberculin test reactors that were reported to the District Veterinary Office (DVO) already tagged and punched by the Private Veterinary Practitioner (PVP). From 1996 onwards the approach taken was to suspend the collection of the reactors pending further investigation. These investigations involved examining the reactors on farm, interviewing the clients, interviewing the PVP, taking blood samples, being present at the slaughter of the reactors, taking gland samples for culture while trying to maintain good relations with the unhappy client throughout the investigation. Ultimately, where the evidence was strong enough, grant payments were refused and this always led to protracted disputes.

This approach proved very difficult to sustain as herdowners wanted immediate explanations for their continuing restrictions. They wanted their reactors removed, grants paid and the herd derestricted. They were not impressed with the investigations. Representations were made through the Farmer representative organisations, Department of Agriculture headquarters, Legal representatives, Local Authority representatives, Politicians also made representations at every level and ultimately the ombudsman's opinion was sought.

It became clear that this approach was too difficult to sustain in a busy DVO. Legislation hampered the rational veterinary decision making process. The key obstruction was that once an animal was deemed to be a reactor by a veterinary surgeon and was tagged and punched, that its status could not be reversed.

##### Modified approach

The solution to the problems experienced was to devise a letter of instruction to the nominated PVP, advising him/her, amongst other things, of the herd history and instructing him/her to conduct the test as normal but **not** to tag and punch any animals with positive reactor measurements. The PVP was instructed to leave all interpretations to the DVO. He was also requested to advise the client of the change of procedure. A sample copy of this letter is attached (Appendix i). It was well received by the PVPs.

The effect of the letter was rationalised as follows:

If a herdowner was interfering with a test, clearly he would cease to do so having been advised by his PVP of the letters contents. The letter should therefore separate real problems, such as Tb and non-specific-infection (NSI), from contrived problems such as test interference. There was no basis for arguing against the letter or the policy behind it. It offered a very friendly management approach to the DVO, the herdowner and the PVP, for dealing with these difficult cases.

The methodology for dealing with any reactors arising was decided based on earlier experience gleaned from dealings with the herds in Table A. The approach was to be as follows:

3. Reactors were to be notified immediately by telephone, as per instructions, to the Superintending Veterinary Inspector (SVI). They were not to be tagged and punched as reactors.

4. An immediate appointment was to be made with the herdowner for the assembly of the reactors for inspection by the nominated Veterinary Inspector (V.I.).
5. The herd was to be restricted and the reactors segregated as normal pending resolution of the problem.
6. All reactors disclosed were to be examined. A lot of time and resources had been invested in setting up the exercise. Therefore, it was important that there was a clear intent to follow up every case.
7. Discussions with the herdowner were to be non confrontational and matter of fact.
8. Each reactor was to be examined to evaluate the sites of injection and to form a judgement as to whether the sites were normal.
9. Each reactor was to be examined to evaluate its status within the herd. Did the reactors represent a serious economic loss to the herdowner? Cases had come to light where over a number of years no culls were removed other than as reactors.
10. Blood samples were to be taken for ELISA Testing and/or Gamma Interferon Testing.
11. Based on observations a decision would be made whether to slaughter or hold the reactors.
12. If reactors were held they would be re-examined to see how the skin injection sites were progressing. It has been observed that where foreign substances are used for the manufacture of false reactors the skin lesions may progress in a manner quite unlike Tb type reactions.
13. Based on observations animals might be followed to the factory where skin and gland samples would be taken for analysis.
14. The opinion of the PVP who attended the herd would be sought.
15. In cases where reactors were to be held for long periods and where supporting tests indicated that the SICT had not provided an accurate assessment of the true disease status of the herd and the reactors, the SICT was to be repeated after an appropriate interval.
16. Because the animals would not have been presented tagged and punched as reactors a decision would ultimately have to be made by the SVI as to the disease status of the animals and of the herd. This would be done in consultation with H.Q.

From 1999 onwards this letter was sent to the PVP with all test notifications for the 5 herds in Table A. It seemed from an early stage that the letter had an effect on the disclosure of reactors in this particular group of herds. Up to the 31 Dec'2001 no further reactors were disclosed in these 5 herds. A very useful tool to deal with the Recurring NVL Reactor Herd had been discovered.

At the end of 1999 the apparent success of the exercise was so encouraging that the sample was expanded. Thus a list of 46 herds was drawn up at the beginning of 2000. The criteria used in selecting the sample were as follows:

1. All herds on the reactor register categorised at high risk of further Tb-breakdown were examined and those with recurring NVL reactors or other suspect features were selected.
2. Herds with substantial long-standing income supplement payments were likewise examined and selected if they fitted the criteria.

From the herds within the above 2 groups 41 herds were selected and were added to the original sample. The vast majority of these herds were selected purely from their records and were otherwise unknown. Once the selection was made no further herds were added to the list. Other herds that came to notice after the original selection were dealt with separately. It cannot therefore be argued that the outcome of this exercise was influenced by retrospective additions of information that fitted the emerging picture.

## Methodology

A special PVP code was created for these 46 herds so that when they fell due for a Tb test the test listing was given initially to the SVI. No targeted test scheduling of these herds took place. The only change of procedure was that the standard letter, adapted for each herd as it fell due for test, was sent by the SVI to the nominated PVP for the herd. It was intended to investigate all animals subsequently reported as having readings indicating failure of the tuberculin test. This task of examining, at short notice, all the reactors disclosed would stretch resources. As events unfolded however, the number of reactors fell from 522 in 1999 to 66 in 2000 and 26 in 2001 at 31/12/01 and therefore the problem was much less than expected.

From February 2000 every herd test undertaken on the herds within the selected group was conducted under this protocol. In excess of 150 letters were written by the SVI each one tailored to the particular case. All the reactors notified were brought to the SVI's attention. The farm was then visited and the reactors inspected. The outcome of this exercise is summarised, in its historical perspective, in the Tables below.

### Tables of Results

The results tables were constructed as follows:

1. Each herd was assigned a number, which is referenced to its herdnumber.
2. The average herd size is deduced for an 11-year period.
3. The review was conducted from 1990 to 2001 inclusive.
4. "Number of episodes" refers to the cumulative number of reactor tests with the disclosure of one or more reactors at each test since 1990.
5. The finding of single or multiple lesions per episode received a value of 1 and this total is expressed as "Episodes + Lesion".
6. The total number of reactors for the period 1990 to 2001 inclusive is given under "No. of Reactors to 2001".
7. The number of animals with a lesion is shown as "Reactors + Lesion".
8. The "Year of Last Reactors" gives the year in which reactors were last disclosed prior to 2000.
9. "Current status" should be interpreted as "Rnd'02"=clear and returned to round 2002; "SMCT,02"= SMCT due in 2002; "Ct'02"= Contiguous test due in 2002; "RR'02"= Reactor retest due in 2002.

### Findings:

No further reactors were disclosed in the 5 original herds (Table A) after the letter was first used in 1999.

**Table A.**

Initial 5 herds where investigation commenced in 1996 and letter issued from 1999

No	Herd Size	No. of Episodes	Episodes + Lesion	No. of Reactors to 2001	Reactors + Lesion	Year of last Rs	Current status
1	50	6	0	25	0	1996	Rnd'02
2	200	9	3	88	3	1999	Rnd'02
3	130	6	1	61	5	1999	2 <sup>nd</sup> FLR. Jan'02
4	80	13	3	68	3	1999	Rnd'02
5	180	10	3	139	3	1998	Rnd'02

No further reactors were disclosed in a group of 23 herds (Table B) after the letter was first used

from February 2000.

Reactors were disclosed in 14 herds after the letter was first used from February 2000, albeit in much reduced numbers (Table C). These animals were all investigated by the area V.I. and the SVI and decisions made as to their status. The reactors disclosed in these herds and their lesion status for 2000 and 2001 is summarised in the last four columns of Table C.

No. 33 is worthy of special comment. This herd disclosed a factory lesion and was manually listed for an immediate test to the nominated PVP. The factory lesion retest (FLR) did not alert the system of notification. No letter was ever sent. The test produced 14 reactors with 6 lesions. More reactors were disclosed at subsequent tests and the herd was depopulated. Clearly the failure to send the letter in this case had no bearing on the outcome.

No 45 also disclosed a factory lesion and escaped the letter for the test that recorded 6 reactor cows in 2000. Attention was not drawn to these animals and all were NVL.

In the course of this exercise two herdowners who were not part of the original selection but whose herds had fitted the same criteria, were suspected of interfering with their Tb tests (Table D). Their cases were investigated with the help of the Department of Agriculture's Special Investigation Unit and the Gardai. They were brought before the courts where they pleaded guilty to charges of fraud and cruelty by interfering with tuberculin injection sites for the manufacture of false reactors. The letter protocol was not put in place in the case of these herdowners until after they were prosecuted.

**Table B.**

23 herds from the selection of 46 where no reactors were disclosed following initial use of 'the letter' in February 2000.

No	Herd Size	No. of Episodes	Episodes + Lesion	No. of Reactors to 2001	Reactors + Lesion	Year of last Rs	Current status
6	65	4	1	25	0	1997	Rnd'02
7	120	6	5	62	9	1996	Rnd'02
8	90	10	3	61	5	1998	Rnd'02
9	60	3	0	21	0	1999	Rnd'02
10	100	6	2	42	3	1996	Rnd'02
11	80	2	2	29	3	1999	H. No. Cancel
12	100	5	2	32	2	1999	Rnd'02
13	120	7	3	75	5	1998	Rnd'02
14	80	4	0	20	0	1999	Rnd'02
15	300	11	3	69	4	1999	Rnd'02
16	100	5	2	34	5	1999	CT'01
17	85	5	0	18	1	1998	Rnd'02
18	50	7	1	39	1	1999	Rnd'02
19	70	10	4	49	9	1999	Rnd'02
20	55	6	2	71	2	1998	H. No. Cancel
21	150	4	0	20	0	1999	Rnd'02
22	150	3	0	47	0	1998	I/RT'01
23	350	7	3	64	24	1999	Rnd'02
24	150	5	1	43	2	1999	Rnd'02
25	15	3	2	25	4	1999	H. No. Cancel
26	120	6	1	29	1	1998	Rnd'02
27	240	3	1	24	1	1999	Rnd'02
28	120	5	1	32	5	1998	Rnd'02
29	160	6	1	47	1	1999	Rnd'02
30	110	4	3	27	3	1999	Rnd'02
31	100	9	5	41	10	1999	Rnd'02
32	100	4	1	39	1	1997	Rnd'01

The lesion rate in the reactors was uncomfortably low for '97, '98, and '99. It rose to 7% and 24% in 2000 and 2001 (Table E).

The reactor population in these herds was greatly reduced in 2000 and 2001 (Table F).

The visible lesion rate in 2000 remained very low (Table G). All the lesions disclosed in 2001 occurred in one herd i.e. Herd no 33.

Few reactors were disclosed, within the selected sample, during the course of the exercise. Therefore no significant number of reactors required investigation.

**Table C.**

14 herds from the selection of 46 where reduced numbers of Reactors were disclosed following initial use of 'the letter' in February 2000.

No	Herd Size	No. of Episodes	Episodes + Lesion	No. of Reactors to 2001	Reactors + Lesion	Year last reactor	Reactors 2000	Reactors 2000 + Lesion	Reactors 2001	Reactors 2001 + Lesion	Current Status
33	60	6	3	53	4	1999	0	0	20	6	SMC'02Dep
34	130	2	0	19	0	1998	8	0	0	0	Rnd'02
35	220	13	5	78	5	1999	12	0	0	0	Rnd'02
36	100	11	2	63	7	1999	5	0	0	0	Rnd'02
37	250	15	5	96	9	1998	6	2	3	0	SMC'02
38	100	5	1	28	1	1999	3	1	0	0	Rnd'02
39	190	12	0	42	0	1999	6	0	0	0	Rnd'02
40	160	15	3	82	3	1999	6	0	0	0	Rnd'02
41	200	8	4	50	8	1999	4	1	2	0	SMC'02
42	40	6	1	14	1	1999	3	0	0	0	Rnd'02
43	120	7	4	30	4	1999	5	2	0	0	SMC'01
44	165	7	3	46	5	1998	3	0	0	0	Rnd'02
45	130	4	1	33	3	1999	6	0	0	0	SMC'02
46	160	8	5	39	12	1999	1	1	0	0	Rnd'02

**Table D.**

Two herds not part of the original selection of 46 against whom legal proceedings were taken.

No	Herd Size	No. of Episodes	Episodes + Lesion	No. of Reactors to 2001	Reactors + Lesion	Year of last Rs	Current Status
47	160	5	1	43	2	1999	Rnd'02
48	155	8	2	46	3	1999	Rnd'02

**Table E.**

Lesion % Rate for Cows and Heifers

1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
12.5	26	11.5	7.5	5.4	2	8.7	6.2	4.3	5.8	7	24

**Table F.**

Reactor population in the 46 selected herds.

	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	Totals
cows	82	16	59	98	77	82	170	217	364	383	37	18	1603
heifers	22	6	10	35	34	22	35	37	71	82	11	4	369
steers	10	9	6	7	4	4	30	39	45	37	16	3	210
calves	1	1	2				1	1	4	12	1		23
bulls			1	3	2	3	4	4	8	8	1	1	35

**Table G.**

Visible Lesions in the 46 selected herds

	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	Totals
cows	12	3	6	7	4	2	13	8	12	25	2	5	99
heifers	1	2	2	3	2		5	8	7	2	2	1	35
steers	4	3	2	1			5	19	6	6	4		50
calves									2	1			3
bulls				1	1		1						3

## Discussion

The phenomenon of recurring NVL reactor herds, with few exceptions, was geographically confined to the mid-west of the DVO being confined to the hinterlands of 4 regional towns. This area has had a very high APT (Animals Reactor per

Thousand Animal Tests) for the last 5 years. Consequently the exercise was confined to a few veterinary practices, one practice having 15 of the herds listed.

Reference is made to the cow and heifer reactors only in the 2 graphs. This combination formed the majority of reactors and these reactors command the higher grant compensation rate especially pregnant heifers.

The downturn in reactor numbers, after the circulation of the letter was sudden and unexpected. This raises a number of questions.

1. Why did the reactor numbers decrease so dramatically? Was it co-incidentally a natural event in the cycle of the recurring NVL reactor phenomenon or was the letter substantially responsible for the massive drop in the reactor population in these herds after 1999? It is worthy of note that this group of herds produced more than 10% of all the reactors in the DVO in 1997, 1998 and 1999.
2. Would the same result have been seen with a greatly expanded sample or if the qualifying criteria had been less selective?

These questions can be answered only by enlarging the sample and repeating the exercise in other V.I. areas or even nationally. The approach taken was likely responsible for at least part of the decrease in reactor numbers. The cases of the two herdowners in Table D illustrate this point. It is certain that the disclosure of false reactors in these cases would have ceased had they been part of the protocol. The letter and its implied follow up procedures is therefore a very useful management tool where test interference is suspected. It could perhaps be further enhanced. It should not however be used to provide an escape route for people who have broken the law and against whom a case of fraud can be prosecuted.

### **Summary:**

This exercise was originally conducted for the purpose of formulating an approach to the vexatious problem of the persistent repeat NVL reactor herd. It had a surprising and unexpected outcome in that the phenomenon immediately disappeared both in the initially small and later expanded selected sample. This selection of problem reactor herds has been dealt with successfully in a cost effective manner. No short cuts were taken in any case. Few reactors were disclosed and all herds except No 3, which awaits its clearance test after the disclosure of a factory lesion, have clear status.

The entire operation was very time consuming. It was run on a day-to-day basis by the SVI and one designated V.I. Consideration should be given to the possibility of establishing such an exercise as part of the normal Tb eradication programme with administrative backup and with defined protocols to be followed in cases where reactors are disclosed.

**Appendix 13 (a) Sample letter to be adapted as required.**

Department of Ag, F and RD  
District Veterinary Office  
County  
31/12/01

Veterinary Surgeon  
Co.

Re: Round TB Test of John Farmer, XXXXXXXXXX, Co..  
Herd No. 123456x?  
Herd size. 50 animals . 20 cows.

Dear colleague,

I refer to the TB test currently listed to your practice for the above herd. As you are aware we have encountered great difficulty in resolving a reactor problem which has recurred in this herd since 1996.

Since the test of 17/10/'96 thirty nine reactors have been disclosed. 29 cows, 7 heifers, 1 bull and 2 calves have been removed as reactors. Only one of these showed lesions on post mortem and this was an unconfirmed prescapular lesion. This was as far back as 1998.

In order to progress this investigation further I require that you advise me in advance of the date and time of the injection of the herd with tuberculin. In the event of any animals with positive mammalian reactions arising at the reading of this test I require that this office be informed immediately by telephone of that fact and a copy of the test report forwarded to me at the DVO for interpretation. Please record all measurements and leave the interpretation of this test entirely to the DVO i.e, no animals are to be tagged and punched as reactors.

I would appreciate it if you would make your client aware of this investigation in advance of the test so that he will not be surprised by the changed procedures should reactors arise.

Your personal observations and recommendations will of course be appreciated.

Yours sincerely

\_\_\_\_\_ S.V.I.



Department of Ag and Food  
Cork Central  
District Veterinary Office  
Cork City  
Date

To: -  
XXXXXXXXX MRCVS  
XXXXXX,  
Co. Cork

Re: R/R Test XXXXXXXXXXXX, Co. Cork.  
Herd No. XXXXXX D  
Herd size. 100 animals . Test due: 24/4/'02

Dear colleague,

I refer to the R/R Tb test currently listed to your practice for the above herd. As you are aware we have encountered great difficulty in resolving a reactor problem, which has recurred in this herd since 1997. Furthermore, a review of this herd's Tb file suggests that severe interpretation is inappropriate to this herd at this time. The record is as follows since 1997:

Year	No. of episodes	No. of episodes with lesions	No. of reactors	No. of reactors with lesions.	Lesion rate %	Expected Lesion rate % in Standard reactors
1997	2	2	12	4	25	35
1998	2	1	14	1	7	35
1999	1	0	12	0	0	35
2000	3	0	25	0	0	35
2001	2	2	21	2	9	35
2002						

The department would like to investigate this type of case should reactors arise at the next test. In order to progress this investigation further I require that you advise me, in advance of the date and time of the injection of the herd with tuberculin. In the event of any animals with positive mammalian reactions arising at the reading of this test, I require that this office be informed immediately by telephone of that fact and a copy of the test report forwarded to me at the DVO for interpretation. Please record all measurements and leave the interpretation of this test entirely to the DVO i.e. no animals are to be tagged and punched as reactors.

The DVO will decide on the basis of post mortem findings or other supplementary tests what animals will be removed and we will advise you accordingly. I would appreciate it if you would make your client aware of this investigation in advance of the test so that he will not be surprised by the changed procedures should reactors arise.

Your personal observations and recommendations will of course be appreciated.

Yours sincerely

\_\_\_\_\_ S.V.I.

## Appendix 13 (b) ATYPICAL HERDS - DVO PROCEDURE

Mr. Martin Blake DCVO  
Ms. M. Good SSVI  
Mr. J. Murphy AP

RE: Management of herds designated as having a history of an Atypical TB breakdown(s).

As requested I have looked at the various possibilities of managing these herds as a group within the DVO. The various options for identifying and tracking herds included the following

- (i) a Herd Category other than A,B,C or D
- (ii) a Blackspot Letter not in use
- (iii) A distinct PVP code

In making the final decision, it must be kept in mind that when the test is listed and the listing is printed, it must ring a bell immediately that this listing is to be accompanied by the 'atypical' letter to the PVP.

If the means of identifying the herds was Herd Category or Blackspot, then it is entirely possible that a listing could issue to a PVP without anybody realising that it was an 'atypical' herd. Therefore from this perspective alone, **a unique PVP code seemed the best option.**

From the point of view of analysis of reports (stats and herd test histories) the unique PVP code offers the same flexibility as any other means of identification. It is possible to get a stat based on a PVP code. The herd test histories can be printed (six at a time) using the individual herdnumbers. It is also feasible to write a report in Report Generator giving summary information on herds with a certain PVP code.

From an ERAD management perspective, it would make more sense to use a specific PVP code for use in all DVO's (rather than using SVI codes). This, I presume, would enable ERAD HQ to print summary reports and the TIU to analyse data. I suggest a code such as 2020 this code has never been issued (relates to a qualification in 1920). I suggest we call this vet Murray McCarthy in honour of the men who pioneered the system.

The herdfiles should be identified/highlighted so that the interpreting VI is alerted to the fact that this herd is an 'atypical herd'. A sticker could be placed on the file with the following inscription ***Atypical Herd***

***Vet Code 2020.***

The listing of the herd/s would operate as follows:

The listing would print off in the normal way to Vet 2020.

The first page of the listing (containing the name of the vet) would be discarded and the second page (which is the actual listing) could be attached to the 'Murray' letter and sent to the PVP. *[It is possible to single-schedule the test to the PVP on the computer and then print off a listing but I don't think it is worth the trouble]*

Notes of caution

- 1 Make a record of the existing PVP on the file before changing it to 2020.
- 2 Each DVO should check the list of herds that were handed out at the last regional meeting before 'treating' them to this regime. In some cases the presence of lesions *post-mortem* may not have been accurately recorded on the computer and they do not in fact fit the high NVL rate criteria set.

Summary for DVO

- 1 Check the list supplied from HQ and eliminate those herds where the lesion rate is in fact normal (notify TIU of eliminations and details).
- 2 On computer, change the **attendant PVP and schedule vet** to 2020
- 3 Mark the herdfile with "Atypical Herd" Vet Code 2020. Original PVP Code ????
- 4 Prepare the 'Murray' letter to issue to the PVP.  
This can either be a standard template letter, which is filled-in appropriately at the time of the listing, or, a letter written as a Mail Merge Document and merged to a table of data.

Patrick Flanagan SSVI

## Appendix 13(c) ATYPICAL HERDS - ON-FARM PROCEDURE

### STEPS TO FOLLOW WHEN A HERD ON THE ATYPICAL LIST SHOWS 'TB REACTOR' SICCT READINGS.

As far as possible these herds should be treated in a standard manner. Given their history a potential NSI problem must be suspect and severe interpretation, as a routine, should be withdrawn to avoid unnecessary decimation of the herd.

At each stage any observations should be recorded (contemporaneous notes):

- (i) Visit the herds on the day of reading or as soon as possible thereafter;
- (ii) Check the animal(s) and record salient details such as body/udder condition, presence of scour (query Johne's disease), skin TB etc.;
- (iii) Query any pertinent husbandry details e.g. is poultry manure fed?;
- (iv) Examine the injection sites - these should be normal in appearance and texture with no abnormalities evident (*if an aromatic substance such as turpentine or diesel is used to interfere the smell may be detectable on fingers following palpation of the lump*) and if later than the reading day, lump measurement should show the normal post 72-hrs 'waning' response - record measurements and observations;
- (v) Examine both the tags **and the ears**. If a suspicion is formed that tags may have been switched then remove the tags and submit for examination maintaining chain of evidence procedures; *Please note that it appears that the latest way to switch a tag is using a sharp knife make a small slit out from the tag-hole, roll up the tag and feed it through the slit, the tag remains undamaged and the slit heals naturally. If this is suspected then a blood sample should be taken for DNA analysis and comparison with 'blood relatives' (dam/offspring/siblings).* The reactor in question may well be genuine and will have to be removed from the herd in any event in which case the ear should be taken for laboratory examination (*presence of scar/healing wound*).
- (vi) Return to farm day 10 (or later) following the injection of tuberculin to take blood samples and submit for standard interferon  $\gamma$  assay and additionally request ESAT6 interferon  $\gamma$  assay.

When results of Gamma interferon tests are to hand there are three possible directions.

1. The Gamma interferon test results correlate (~80% correlation is usual) and the decision is made to deem the animal(s) reactor and remove as normal. Reactors from these NSI herds should be treated as per the 'singleton' regime. *This will serve two purposes (i) allow data analysis to determine if there is a particular type of interferon- $\gamma$  assay NSI-type response and (ii) provide an adequate database on which to base future decisions on the herd in question.*
2. The Gamma interferon test results do not correlate.  
The herd should be re-visited and the injection sites re-examined and details including measurements of the waning injection site response noted.

- a. If test interference at the injection site is evident e.g. larger lump, abscess formation, adherence to underlying tissue etc. the Gardaí should be alerted to a suspect fraud, and the animals should remain for retest.
  - b. If test interference at the injection site is not evident or is unclear then it may be desirable to have skin sites taken at p.m. for analysis (*Abbotstown requests that both avian and bovine sites should be submitted for response comparison*) and glands should be taken for full laboratory examination. Full chain of evidence protocols should be rigorously followed. Reactor payment section should be notified that payments should not be made until the situation has been clarified.
3. The Gamma interferon test results show poor correlation.  
In such cases the procedure outlined in two above should be followed bearing in mind that there may be other factors superimposed on a genuine TB problem.

At any stage where the situation is unclear the Regional SSVI should be consulted for guidance.

## Appendix 14 Scientific Papers

### Paper 1. Incidence and Prevalence.

**Margaret Good**

Department of Agriculture, Food and Rural Development, Agriculture Hse., Kildare St., Dublin 2. Ireland.

The terms incidence and prevalence are often used incorrectly and sometimes even interchangeably in the same article. Hence, I have tried to provide a guideline as to their meanings below that I hope may prove helpful.

**Incidence** is a dynamic measure of disease occurrence. It describes the probability of a new case developing during a specified time interval. Thus by definition, incidence rates require a minimum of two sets of measurements or tests: – one at the start of the period of observation to ensure that the animals did not have the disease, condition or infestation and the second sometime later to investigate if the disease developed during the intervening period. The period must always be stated when reporting results because the rate may change with time, from season to season or year to year. A basic rule is that one herd can only experience the event once during the study period. Thus, if multiple observations are made (i.e. more than 2) the average number of herds at risk over the study period must either be calculated or estimated. An estimate of the approximate number of herds at risk (NAR) could be taken as the number at risk initially added to the number at risk at the end of the period divided by 2. Rates may be reported for time intervals that are sub-sections of the full study period as when individual monthly rates are calculated from a 12 month study. The general formula for incidence is the number of herds developing disease during the time period divided by the average herds at risk taking into account the time component being quoted. Thus the incidence of TB in herds over say a study period of one month would be:

$$\frac{\text{Number of new herd breakdowns during a month}}{\text{Number of herds tested during that month (NAR)}}$$

Herds which experience breakdowns during the month should not be included as at risk at the end of the month even if they are disease free. If detailed records are available the exact denominator could be calculated but generally such accuracy is not required and the NAR may be estimated as above. The easiest way therefore to handle multiple occurrences of the condition over a long study period is to shorten the time interval sufficient to make the constraints reasonable and give perhaps several rates e.g. one for each 30-day interval.

**Prevalence** is a static measure of disease frequency. It is the fraction of a population that is diseased at a point in time. It is a measure of existing cases based on one test or examination. For example a survey done, by examining once, a population of herds for the presence of TB results in a calculation of the prevalence of TB in the herd population under study at that time. The general formula for the calculation is:

$$\frac{\text{Number of herds restricted (at a point in time)}}{\text{Number of herds at risk at that point in time}}$$

#### Reference:

Martin, S.W., Meek, A. H. and Willeberg, P. (1987) Veterinary Epidemiology: Principles and methods. Ames, Iowa: Iowa State University Press. pp 48-56  
Irish Veterinary Journal; March 2006

## Paper 2. Bovine Tuberculosis Eradication in Ireland

Volume 59 (3) : March, 2006 Irish Veterinary Journal

**Margaret Good** Department of Agriculture & Food, Kildare St., Dublin 2

### Abstract

In Ireland the bovine tuberculosis (BTB) eradication programme commenced in 1950 and became compulsory throughout Ireland by 1962. The initial driving forces for the programme were production losses in cattle, human health problems and a desire to trade in live bovine animals primarily store cattle to the U.K. While the operation of the programme ensures that production losses in cattle and human health concerns are no longer an active issue, the programme remains necessary to ensure that trading conditions may be met which possibilities expanded post 1965, as Ireland met European trading conditions for live animals. Despite strict adherence to testing and control measures exceeding those of countries that had eradicated BTB, the eradication programme in Ireland has not achieved the target of final eradication although the prevalence of BTB has been considerably reduced.. Unlike in those countries, which have succeeded in eradicating BTB Ireland has a wildlife species (*Meles meles*) in which BTB is endemic sharing the same environment as bovine animals. Considerable research effort has been devoted to determining the contribution of wildlife to the BTB problem and in trying to develop a viable long-term solution to the wildlife issue. When the tools are finally developed to control the disease in wild animals, Ireland should at last achieve the target it set for itself in 1950.

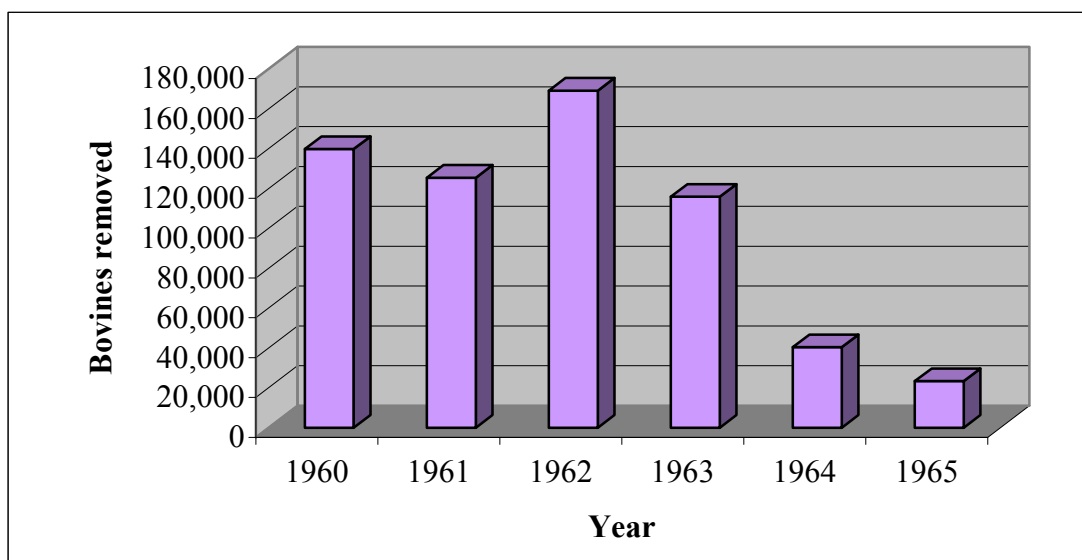
### Introduction

Some fifteen years after the commencement of the bovine tuberculosis (BTB) eradication programme in Ireland an account of the success of the programme was written (Watchorn, 1965). Now, some forty years after the country was declared TB attested it is appropriate to look again at progress towards the eradication of BTB in Ireland.

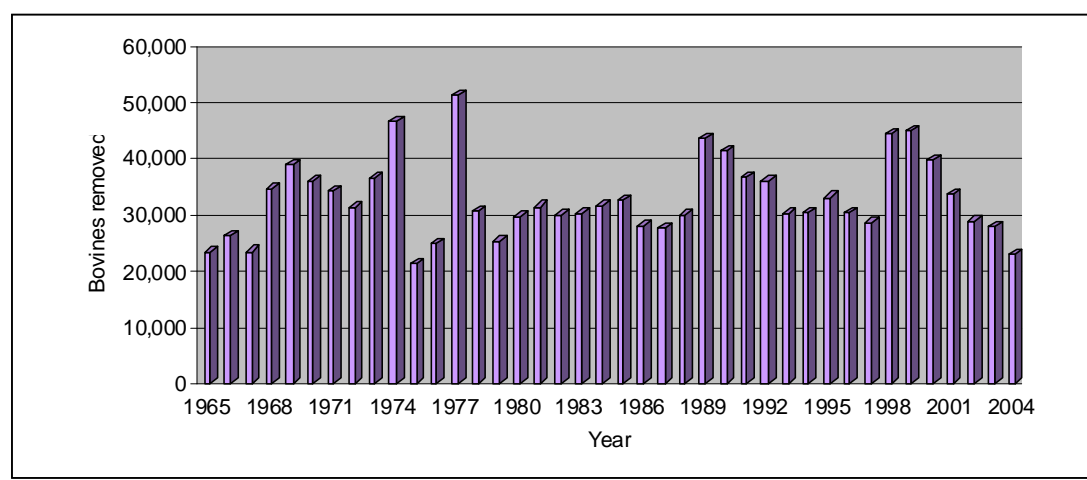
### Progress towards eradication

A number of factors led to the decision to commence an eradication programme for BTB in Ireland. These included losses due to overt disease in cattle, human health problems caused by *M. bovis* and trading requirements. The programme commenced in 1950, initially on a pilot basis, to assess levels of infection and methodologies. A voluntary eradication programme was introduced, on a phased basis, in September 1954. The BTB programme became compulsory starting, on an area basis, in 1957. The compulsory programme had been extended throughout the whole country by 1962, and in October 1965 the Government of the day optimistically and based on the observed trend (Figure 1) declared the country attested, i.e. virtually free of tuberculosis (Watchorn, 1965). The BTB scheme had commenced in Britain in 1935. Consequently, British research and experience in the conduct of the tuberculin test in Great Britain was an important contributor to the Irish eradication programme (Ritchie, 1942; Ministry of Agriculture and Fisheries, 1942a, b). Further, the British requirement for attested store cattle from Ireland was also a very significant driver in the Irish programme (Watchorn, 1965).

In 1964 Directive 64/432/EEC, the 'trading' Directive had been adopted by the then European Economic Community. All countries wishing to trade in live bovine animals with Member States of the Community would have to conform to this Directive. By 1965 there were no herds of unknown disease status in Ireland and on at least one occasion during the 11 years to 1965, all Irish herds either had individually achieved Officially Tuberculosis Free (OTF) status or been designated infected in accordance with this Directive thus fulfilling the primary conditions to allow Ireland to take advantage of possible trading opportunities opening up within Europe. Furthermore, at this time, the downward trend was expected to continue towards final eradication. Instead, the eradication programme stalled with circa 30,000 animals failing the tuberculin test and being removed annually (Figure 2, Table 1). The veterinary strike in 1975/76 and again in early 1985 curtailed the testing programme while they were ongoing, but seemed to have no lasting impact.



**FIGURE 1:** Bovines removed as reactors to the tuberculin test, 1960 to 1965.



**FIGURE 2:** Bovines removed as reactors to the tuberculin test, 1965 to 2004.

The trend in cattle population and disease incidence since 1960 is presented in Table 1 (TB Testing Programme, Comparative statistics. DAF). As can be seen from the table, considerable progress was made in the early years of the Tuberculosis eradication programmes. However, over the more recent years until 2002, it was difficult to breach the 30,000 reactors per annum floor. In 1954, when the eradication programme commenced, there were some 250,000 herds with 4.5 million cattle registered in Ireland with an animal test reactor incidence of 17% (cows 22%, other cattle 8%) (Watchorn, 1965). In 2003 there were 125,000 herds with approximately 7 million cattle and an animal test reactor incidence of 0.4% (DAF, statistics). Over the course of the programme to date, in excess of 250M individual animal tuberculin tests have been conducted on the Irish cattle population.

**TABLE 1:** Cattle Population and Tuberculin testing statistics over four decades; five-yearly from 1960-1985, then yearly from 1988.

Year	Cattle Tested	Number of Animal Tests	No. of Reactors	Disease Incidence (%)	APT **	RPT ***	Number Herds	% Herds Tested	New Herds Detected (%)
1960	4,683,700	*	139,881	2.99	-	29.9			
1965	5,359,300	*	23,378	0.44	-	4.4			
1970	5,956,500	*	35,982	0.60	-	6.0			
1975	7,168,100	*	21,339	0.30	-	3.0			
1980	6,908,900	8,878,924	29,827	0.43	3.6	4.3			8,772
1985	6,907,200	11,180,602	32,608	0.47	2.9	4.7			9,768
1988	6,604,100	11,125,500	29,994	0.45	2.7	4.7	176,019	98.4	10,596(6.0)
1989	6,800,100	12,436,982	43,580	0.64	3.5	6.5	172,976	97.9	13,964(8.1)
1990	6,899,929	12,427,144	41,419	0.60	3.3	6.0	172,765	95.6	13,489 (7.8)
1991	6,814,229	8,209,105	36,832	0.54	4.4	5.4	172,272	41.9	9,873 (5.7)
1992	7,084,441	10,887,513	35,997	0.51	3.3	5.2	172,260	93.7	11,196 (6.5)
1993	7,043,913	10,446,265	30,359	0.43	2.9	4.3	168,591	94.9	10,162 (6.0)
1994	7,137,696	10,435,076	30,439	0.43	2.9	4.3	159,818	97.1	9,453 (5.9)
1995	7,174,016	10,112,939	33,180	0.46	3.3	4.6	154,401	88.7	9,518 (6.2)
1996	7,412,933	10,073,859	30,400	0.41	3.0	4.1	149,128	98.2	8,867 (5.9)
1997	7,725,634	9,910,074	28,647	0.37	2.9	3.7	145,209	98.1	8,139 (5.6)
1998	7,946,989	10,677,291	44,498	0.56	4.2	5.6	142,302	97.8	10,055 (7.1)
1999	7,569,735	10,749,580	44,903	0.59	4.2	5.9	138,263	97.9	10,660 (7.7)
2000	7,032,407	10,304,162	39,847	0.57	3.9	5.7	133,542	98	10,785 (8.2)
2001	7,097,430	9,402,196	33,702	0.48	3.5	4.8	130,525	95.1	9,195 (7.4)
2002	7,025,096	9,400,065	28,930	0.41	3.1	4.1	127,711	97.2	8,338 (6.7)
2003	6,936,820	9,168,722	27,978	0.40	3.1	4.0	125,517	97.2	7,669 (6.3)
2004	6,992,264	8,825,720	22,967	0.33	2.6	3.3	124,414	96.7	6,882 (5.7)

\* Accurate figures for the total number of animal tests per year were not available until 1978.

\*\* The APT is used as a measure of the incidence of disease compared to the level of testing being carried out. The APT figures represent the number of reactor animals disclosed per 1,000 tests.

\*\*\* The RPT is used as a measure of the incidence of disease compared to the total population of animals. The RPT figures represent the number of reactor animals disclosed per 1,000 animals.

## The national programme

### a. The current programme - 2006

Ireland has as the basis of the BTB eradication programme:

- A mandatory registration system for herds as the relevant epidemiological entity,
- Individual bovine animal unique identification system,
- Animal passport or official permit mandatory to accompany each animal on movement
- A computerised movement monitoring system for bovine animals (CMMS)
- An animal health computer system (AHCS)
- A comprehensive programme of disease surveillance including:
  - Farm-based testing:
    - routine use of the single intradermal cervical comparative tuberculin<sup>3</sup> test, a mandatory annual test of all herds, testing of herds contiguous to or otherwise epidemiologically linked to infected herds, a check-test of herds in 'at-risk' areas, a herd test six-months following restoration of status and
    - Veterinary inspection of all bovine carcasses presented for human consumption.
- Disease controls
  - prompt removal of reactor animals;
  - more severe interpretation of farm-based tests following establishment of infection;
  - epidemiological investigation following confirmation of infection and spread;
  - tuberculin test at ~60-day intervals until two clear tests in succession achieved;
  - hygienic controls on infected holdings and of vehicles;
  - trace of TB infected or potentially infected animals back from and forward to other herds where appropriate;
  - use of the Interferon- $\gamma$  assay, the ELISA and the anamnestic ELISA test in problem herds as an adjunct to the tuberculin test; and

<sup>3</sup> Definition: Tuberculin purified protein derivative (tuberculin PPD, bovine or avian) is a preparation obtained from the heat-treated products of growth and lysis of *Mycobacterium bovis* or *Mycobacterium avium* (as appropriate) capable of revealing a delayed hypersensitivity in an animal sensitised to microorganisms of the same species.



- depopulation of infected herds where the level or duration of infection indicates that this is necessary to clear the herd and/or protect the neighbourhood.
- Compensation
  - market value for each animal removed but with a maximum allowable valuation.
- Quality control
  - tuberculin testing conducted by specifically authorised Veterinary Surgeons;
  - annual monitor on equipment, test performance and results for each testing Veterinary Surgeon; and
  - only animals tested within the previous 12-months permitted into slaughter plants.

#### b. *Programme changes*

The eradication programme as it currently exists has evolved over the years and while the basic principles laid down in the 'trading' Directive, as amended, continue to be met, the programme has been enhanced by other measures in an effort to achieve eradication. Various other controls such as extended status withdrawal for infected herds have been introduced and later abandoned as not providing any significant benefit towards achieving the goal of BTB Eradication. Up until 1996, a pre-movement test requirement had been a feature of the Irish eradication programme. This requirement was then abandoned as not being cost efficient and not contributing significantly to the BTB eradication programme, with only 0.8-6.9% of breakdowns being attributed to purchased animals (O'Keeffe and Driscoll, 1996). There was also little evidence of onward transmission of infection in the herd to which the animal moved (Flanagan, *et al.*, 1998). Accordingly since 1996 animals may move for up to 12-months from the date of their last tuberculin test. Breakdown severity, during a bovine tuberculosis episode, is a predictor for future herd breakdown (Olea-Popelka, 2004) therefore, high risk herds with a history of bovine tuberculosis are tested more frequently and thus the window of opportunity for movement from such herds is in fact less than 12-months. The Centre for Veterinary Epidemiology and Risk Analysis (CVERA) in 2005 specifically undertook an analysis to determine whether there is a subset of animals and/or a subset of herds where a pre-movement test could have a demonstrable cost benefit for TB Eradication. No such cost benefit was discernable for any group of animals or herds under present circumstances.

Leslie *et al.* (1975) highlighted that bovine tuberculin PPD had both sensitivity and specificity advantages over human PPD and thus in 1978, the tuberculin PPD used in the programme was changed from human to bovine. Bovine tuberculin PPD of two differing strengths was used in routine monitor testing (single strength) and in infected herds (double strength) (O'Reilly, 1983) but from April 1991 a decision was taken to use one strength tuberculin. In 1994 an Irish reference preparation for bovine tuberculin PPD was calibrated against the International Standard of which only limited stock remains (O'Reilly, *et al.*, 1997). Since that time Ireland has used a standardized bovine tuberculin PPD at or about 30,000 I.U./ml PPD (+/-10%) and avian tuberculin 25,000 I.U./ml PPD (+/-10%). The SICCT is the most specific of the tuberculin tests available and the greater the strength (tuberculo-protein concentration) of bovine tuberculin relative to the avian tuberculin the less the specificity and the greater the sensitivity of the SICCT and vice versa (O'Reilly, 1993). Thus to ensure optimum specificity and uniform performance potency of both tuberculins is matched within 500 I.U. and the bovine PPD is never less potent than the matched avian. Additionally periodic validation of tuberculin potency in naturally infected tuberculous cattle is conducted using the Irish reference preparation (Haagsma, *et al.*, 1989).

### **Programme support**

#### a. *The establishment of the Eradication of Animal Disease Board*

Over the years, the BTB eradication programme has been subject to many reviews by many persons and organisations in an attempt to develop strategies that would achieve final eradication (O'Connor, 1986 and 1989; Sheehy and Christiansen, 1991; Downey, 1991 and 1992a,b; More 2005). Professor Bob O'Connor of the Irish Economic and Social Research Institute conducted a major BTB review in 1986; he consulted widely and listed all the issues that the then current wisdom perceived as the reason for the stalled programme (O'Connor, 1986). In response to his recommendations, in April 1988, the Irish Government established a new initiative, ERAD, the Eradication of Animal Disease Board, as a specialised agency to implement a vigorous four-year eradication programme. ERAD was an executive agency of the Department of Agriculture and Food with a Board representative of the various interests, including farmers and veterinarians, involved in TB eradication. A strategic multi-annual plan was developed, a budget provided and an ambitious target set to reduce the reactor numbers by 50%. As well as screening testing, there was a very considerable strategic component. This involved additional special check testing of black spot areas, known high-risk herds, herds that were linked epidemiologically to infected herds and contiguous herds. Herds were categorised according to disease

incidence, with a specific strategy applied to each category. However, as can be seen from Figure 2, despite an increased reactor identification and extraction rate throughout the four years of the programme, the reactor numbers thereafter stubbornly remained at the ~30,000/annum level. During this phase, and indeed to date, the eradication programme comprised all the usual elements that had worked in countries that had succeeded in eradicating the disease. The eradication programme has, furthermore, ensured compliance with the terms of the 'trading' Directive 64/432/EEC, as amended, ensuring that Ireland met with trading conditions for bovine animals within Europe.

## **b. Supporting research**

### **The Centre for Veterinary Epidemiology and Risk Analysis (CVERA)**

During the last 15-20 years, in association with field- and laboratory-based operations, there has been an extensive programme of research (much of it epidemiological) to address gaps in knowledge of disease epidemiology, to objectively evaluate alternative strategy options, and to critically assess the implementation of disease control strategies. The Veterinary Epidemiology and Tuberculosis Investigation Unit, now CVERA, was established in 1989, based in the School of Agriculture, Food Science and Veterinary Medicine in University College Dublin. At establishment, the purpose of the unit was to investigate the factors that militate against the eradication of tuberculosis in cattle at the national or regional levels, and to identify means of improving the rate of eradication. Although the role of CVERA has now broadened considerably, it continues to manage and analyse retrospective and concurrent data relating to the occurrence of tuberculosis in cattle. In addition to data analysis, CVERA undertakes projects to answer specific questions and assess epidemiological elements, various components of infection and the role of wildlife (Costello *et al.*, 1999, and in press). An extensive research programme has also been undertaken looking at the elements of BTB diagnosis (Tuberculin skin test, Interferon- $\gamma$  assay, ELISA, anamnestic ELISA etc.) and developments therein (Collins, 1995; Monaghan, *et al.*, 1994 and 1997; Costello *et al.*, 1997a, b; Gormley, *et al.*, 2004). In addition, the role of environmental mycobacteria has been investigated (Cooney, *et al.*, 1997). DAF routinely conducts potency assays of tuberculin in naturally infected cattle (Haagsma, *et al.*, 1989). The effect of a recent tuberculin test (Doherty, *et al.*, 1995) and the nature of the response to tuberculin test (Basset, *et al.*, 2002) have been elucidated and strain typing of *M. bovis* (Costello *et al.*, 1999 and in press) undertaken. These research outputs have contributed to the development and ongoing assessment of national animal health policy.

### **Research findings**

Knowledge about disease epidemiology (including disease causation and, if infectious, also the transmission and maintenance of infection) is central to the development of disease control strategies (Thrusfield, 1995). In 1974 the first infected badger (*M. meles*) was detected in Ireland (Noonan, *et al.*, 1975) and by the mid 1980s infected badgers had been found throughout the whole country. Over the subsequent 30 years, evidence has been building of the potential role of wildlife in bovine tuberculosis (O'Boyle, 1998, 1999, 2001; O'Boyle *et al.*, 2003). The same strain types of *M. bovis* are detected in both badgers and cattle (Grange *et al.*, 1990). In a review of the Irish TB Eradication programme, commissioned by the ERAD Board, in 1990, Morris and Pfeiffer (unpublished) said that infection in the badger is the underlying driving factor causing special difficulties, that this has been present for at least 30 years, and that a strategy was required to develop a solution for this wildlife constraint. DAF commissioned a number of research projects to accurately estimate the contribution of the tuberculous badger population. First of these was the East Offaly study which removed badgers from a central area and used an area around this as a control with a buffer zone between the two. The central area was kept as clear of badgers as was possible, given that there were minimal barriers preventing badger immigration from the surrounding area. The results showed a reduction in the number of cattle being removed as reactor over the study period. This reduction was 40% greater in the project area compared to the control area (Table 2). The number of reactor animals per 1000 animal tests (APT) had also significantly reduced and this decrease was 50% greater in the project area. (Dolan, *et al.*, 1995). This work has been replicated, with greater scientific rigour, in four additional areas in Ireland and the findings of the East Offaly study have been validated in that there was a significant difference between the removal and reference areas in each county (Griffin, *et al.*, 2005).

**TABLE 2:** The outcome of the East Offaly Study over the years of the project 1988-1995 in terms of bovines removed as tuberculin test reactors in the removal and control areas () per cent change from 1988 figure.

Year	Removal		Control	
	No. of Reactors	APT	No. of Reactors	APT
1988	326	3.9	910	3.4
1989	362	3.5	982	3.3
1990	299	2.9	904	3.2
1991	194	2.7	979	4.5
1992	89	1.4	594	2.5
1993	54	0.8	404	1.9
1994	54	0.8	443	2.1
1995	30 (-91%)	0.5 (-88%)	430 (-53%)	2.1 (-38%)

One of the recognised criteria for the eradication of a pathogen is that there is a single host species with no external reservoir species. However, at present, the wild life reservoir is the major impediment to the eradication of tuberculosis in cattle in New Zealand, southwest Britain, and Ireland and to ignore this impediment would be tantamount to dismissing one of the basic tenets of eradication. Ireland has commenced a project to develop a vaccination strategy for badgers in an attempt to overcome this obstacle to eradication (Gormley and Collins, 2000). The initial phases of vaccine development have included the evaluation of the immune response of *M. bovis* BCG injected sub-cutaneously in badgers, the comparison of response in vaccinates and non-vaccinates; the measurement and comparison of the immune responses of vaccinated badgers against a panel of known T-cell antigens, the assessment of the heterogeneity of immune responses among individual badgers to vaccination (Southey, *et al.* 2001), and the development of an infection model in the badger. Initial challenge trials currently underway have shown promise and a field trial is due to commence in 2006. There is optimism that this research will bear fruit over the next 5-8 years.

## Conclusion

Ireland continues to comply with and even goes beyond the requirements of EU Directive 64/432/EEC, as amended, thereby ensuring that Irish farmers meet the conditions required to trade within Europe and further afield. The consistent application of the programme ensures that BTB is no longer the scourge it was when it caused economic losses because of overt disease in cattle. Bovine tuberculosis, is no longer a significantly important disease in humans. As a result of the lower incidence of the disease in bovines and also because of pasteurisation of milk and other veterinary and public health controls, which ensures minimal opportunities of exposure BTB is not considered a significant public health threat (FSAI, 2002; 2003; 2004). Eradication of BTB is as the ultimate objective of the national programme. Realistically, however, this can only be achieved by simultaneously tackling the disease in all the maintenance hosts in which the disease is endemic and which share the same environment as bovine animals. Ireland is not alone in experiencing problems with the occurrence of BTB in wildlife species and with spill-over from those species into bovines as was evidenced at the 4<sup>th</sup> International conference on *M. bovis*, held in Dublin during 2005 (More, *et al.*, 2006). Scientists and those that manage the occurrence and control of bovine tuberculosis in wild and domestic species are sharing knowledge and co-operating in developing methodologies to achieve this objective. When the tools are finally developed to control the disease in wild animals, Ireland should at last achieve the target it set for itself in 1950.

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### **Paper 3 Multiple Standard Reactors During a Bovine Tuberculosis Episode as a Predictor of Future Herd Breakdowns in Ireland.**

Preventive Veterinary Medicine Volume 63, Issues 3-4, 14 May 2004, Pages 163-172

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#### **Introduction**

This study focuses on a retrospective cohort study of bovine tuberculosis (BTB) at the herd level. The main objective was to compare the hazard ratios (i.e. incidence rate ratios) for becoming re-infected with BTB between herds with “multiple standard reactors” during the BTB episode that began in 1995, and free (clear) herds. All herds were followed to the end of year 2000. The major outcome was the rate of a new bovine tuberculosis breakdown (i.e. the detection of 1 or more reactors in the herd) after a herd was deemed “clear” of the disease. Potential confounders were also included.

#### **Materials and Methods**

Data were organized on 2876 herds that had a new BTB breakdown with “multiple standard reactors” in 1995 (“exposed” herds) and 10,926 herds (approximately 10% of the entire Irish cattle population) that were randomly selected from herds that were free of BTB during 1995 (“non-exposed” herds). SAS V 6.12 (Statistical Analytical System Institute Inc., Cary, NC), and STATA (Stata Statistical Software: Release 7.0. College Station, TX) were used for data manipulation, descriptive and the survival analysis.

#### **Exclusion Criteria**

All herds selected as exposed or unexposed herds with evidence of tuberculosis prior to 1995 were excluded. This included “exposed” herds that were identified on a “reactor re-test” before April 1995 and “exposed” herds detected before August 1995, as a result of a “six month test”. Herds that either never became “clear” of BTB, or were never tested subsequently also were deleted.

#### *Exposure Categories (Risk Factors)*

The herds that were free of BTB during 1995 were our reference group, while those herds that had a new “multiple standard reactor” (“exposed” herds) BTB were categorized in 3 different levels of severity; namely, 2-3 standard reactors, 4-8 standard reactors and more than 8 standard reactors during the BTB episode in 1995.

#### *Confounders*

The following factors based on 1995 data were included as potential confounders

- Herd size (the total number of cattle in the herd)
- Number of cows in the herd
- Prevalence of herd restriction in the herd’s District Electoral Division (DED)
- BTB occurrence in the 5 years before 1995 (treated as yes or no)
- Number of cattle with confirmed tuberculous lesions

#### **Outcome for Survival Analysis**

The hazard for “exposed” herds was contrasted to the hazard in the non-exposed group using a proportional hazards model (Proc PHREG in SAS). Life tables were created to display the crude survival risks for the four groups. “Exposed” herds were deemed to be free (clear) of BTB after they passed a “6 month check test” (essentially 3 negative herd tests at this point). For a non-exposed herd the first clear test in 1995 was used as the date of being free of BTB. As a censoring point we selected the last herd test date in the study period for those herds that did not become “restricted”. The herd test date at which a herd became “restricted” after the 1995 episode was used as the failure time.

#### **Results**

##### *Descriptive results*

Regarding the characteristics of the 1995 “exposed” and non-exposed herds (Table 1), the “non-exposed” group had had fewer herds with a previous history of tuberculosis, had fewer cattle, fewer cows and the prevalence of herd restriction in the same District Electoral Division (DED) was lower than in any level of the “exposed” herds. In addition, breakdown severity increased directly with the number of animals in a herd and to a lesser extent with the number of cows in the herd. The prevalence of herd restrictions in the same District Electoral division (DED) increased with the severity of exposure.

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The risk of a future BTB breakdown was higher for “exposed” herds ( $\Pi^2 = 364.2$ , (df=1),  $p < 0.0001$ ; odds ratio = 2.39) (see Table 1) and increased as the severity level increased. A total of 2269 “exposed” herds (78.9%) had animals with tuberculosis lesions confirmed on slaughter during 1995 episode, and these herds had a greater crude risk of having a future BTB breakdown ( $\Pi^2 = 5.77$ , (df=1),  $p = 0.016$ ).

### Survival analysis results

The survival curve for each breakdown severity level was compared to the “non-exposed” group as shown in Figure 1. The rate of failure was higher in the “exposed” herds compared to the “non-exposed” herds. When the additional covariates were added to the model the hazard ratio for each severity level decreased but remained statistically significant (Table 2). Compared to the non-exposed herds any of the “exposed” herds had a higher hazard ratio for any breakdown (or only a singleton or a future multiple breakdown) in the future. The hazard ratio increased with breakdown severity except for herds with more than 8 standard reactors in predicting future singleton breakdowns. Herds with a previous history of BTB, larger herds and herds located where the prevalence of herd restriction during 1995 was higher had an increased the hazard ratio. Time varying covariates were significant for the models with 4-8 and >8 standard reactors in the 1995 episode and indicated that their rates of future breakdown were initially high but declined somewhat over time (Table 2).

### Discussion

The major aim of this study was to assess the role of “severity” of tuberculosis breakdown as a predictor of a future tuberculosis breakdown after controlling for the effects of presumed confounders. The difference in prevalence of BTB across DVO’s was an important predictor; however, for our purposes, we included DVO as an indicator variable only to prevent confounding by DVO and the results are not shown. We are confident that the herd data are representative of the national situation as all “multiple standard reactor” BTB breakdowns occurring in 1995 were included and the “non-exposed” herds were obtained using the “check-digit” assigned to a 10% random sample of all clear herds, annually. The number of “standard reactors” to the SICTT was used to classify BTB breakdown severity as O’Keeffe, 1992 recommended.

#### *Descriptive analysis*

The prevalence of herd restriction in the local DED was lower in the “non-exposed” herds in year 1995 than in any of the categories for “exposed” herds. The prevalence in the local DED increased as the severity of the 1995 BTB breakdown increased (Table 1). This means that in areas where the herd prevalence of BTB is lower, the higher the probability of finding clear herds. Hammond *et al.*, 1999 concluded that: “by identifying and delineating those areas of the country in which *Mycobacterium bovis* has been and continues to be a constant hazard, acceleration of the eradication programme can be achieved”

In our study, a high percentage (78.9%) of all case herds had at least one animal that disclosed with tuberculous lesions at slaughter. We assumed that the presence of a lesion indicated some chronicity of BTB in the herd, and when we compared “exposed” herds with lesions against those without lesions (but not controlling for other variables) there was a significant difference in the risk of future BTB breakdown. Martin *et al.*, 2001 considered that the risk of an animal of having a lesion disclosed varied with the slaughterhouse (factory) at which the animals were processed and *post-mortem* examination done. Meanwhile, Corner *et al.*, 1994, concluded that the standard *post-mortem* examination of animals has a low sensitivity (47%) in detecting tuberculosis lesions.

## Survival analysis

The proportional hazard model assumes that the herd hazard of a BTB breakdown at any time for each of the “exposed” severity levels is proportional to the hazard for a herd in the baseline group (“non-exposed” herds)(i.e. that the ratio reflecting the difference in risk between categories is constant).

The use of survival methods adjusts for the time at risk before determining the rate (i.e. hazard) of BTB breakdowns in each exposure category. A hazard ratio  $> 1$  suggests a shorter time to a BTB breakdown for herds in that exposure category.

In our univariable survival model, each severity level in the “exposed” herds in the year 1995 episode was a significant predictor of the rate of future breakdowns. When we incorporated other covariates into the survival model, the effects of the breakdown severity lessened but remained significant (Table 2).

At any given time between the herds becoming de-restricted until the end of the study period (31 December 2000), the hazard ratio for herds with 2-3 standard reactors was 1.76. This means that these herds had a 76% increased probability over that of the non-exposed herds of undergoing a new BTB breakdown, after controlling for the other covariates in the model. This also indicates that the time that it takes for a herd to become infected again after being deemed “clear” is less for the 2-3 standard reactors than the clear herds. The hazard ratio for the two groups, that we denoted as having “severe” breakdowns during the 1995 episode (4-8 standard reactors and more than 8 standard reactors) varied with time as can be seen in Table 2. After one year at risk, these two categories had a hazard ratio of 2.24 and 2.55 for 4-8 standard reactors and more than 8 standard reactor categories respectively. After 3 years at risk, those hazard ratios declined to 1.81 and 1.60 for the same two categories.

Each of the following factors was associated with a herd having a multiple standard reactor breakdown in 1995: prior history of BTB, large herd size and level of BTB restriction in the area (DED). These factors were also predictive of future herd breakdowns. This confirms that these are significant risk factors for the occurrence of bovine tuberculosis.

A high percentage (78.9%) of all “exposed” herds had at least one animal that disclosed with tuberculous lesions at slaughter. We were expecting that animals with lesions were going to be an important predictor; however, the disclosure of tuberculous lesions at slaughter was not an important predictor of future tuberculosis breakdowns when controlling for other variables in our survival analysis. This lack of effect could be a reflection of the large variability in lesion detection rates that has been described in different factories in Ireland as well as the overall low sensitivity of the *post-mortem* examination.

In a similar manner, Martin *et al.*, 1999 reported, at the animal level, an elevated risk of a cow for becoming a “standard reactor” relative to other classes of cattle. Thus we included this factor in our models, expecting that number of cows was going to be a significant predictor at the herd level. However, it appears that herd size had a stronger effect on future breakdown rate than number of cows.

## Conclusion

Herds with a multiple standard reactor breakdown had a higher rate of becoming re-infected with BTB than “non-exposed” (i.e. clear) herds. This information together with knowledge of other risk factors, including herd size, prevalence of BTB in the area and history of prior BTB in the herd can be useful in predicting the occurrence of future BTB breakdowns.

**Table 1.** Herd characteristics during the 1995 episode and history of BTB prior and post 1995 episode.

Exposure Category	No. of Herds	BTB History <sup>1</sup> No. / %	Herd size Mean Std. Dev.		Mean No. Cows in herd	BTB Prevalence in DED <sup>2</sup>	Risk of Future BTB
No reactors	10705	1816 (17.0)	45.0	50.30	14.9	5.0%	18.1
2-3 Standard reactors	1463	509 (34.8)	84.0	77.30	26.6	12.0%	32.3
4-8 Standard reactors	910	316 (34.7)	88.3	69.25	28.2	13.4%	36.4
>8 Standard reactors	503	183 (36.4)	119.4	97.63	40.7	14.3%	38.6
Total herds	13581						

<sup>1</sup> BTB History = Previous herd BTB breakdown (percentage in each category)

<sup>2</sup> DED = District Electoral Division



**Table 2.** Hazard ratios from univariable and multivariable proportional hazards analysis, of rate of future breakdown, Ireland 1996-2000.

Breakdown Severity 1995	Univariable analysis	Multivariable analysis		
	All breakdowns	All breakdowns	Singleton breakdown	>= 2 standard reactors
Controls 1995 (referent)	1.00	1.00	1.00	1.00
2-3 Standard reactors	2.90	1.76	1.71	1.88
4-8 Standard reactors	3.52	2.24 <sup>a</sup> 1.81 <sup>b</sup>	1.81	2.46 <sup>a</sup> 1.93 <sup>b</sup>
> 8 Standard reactors	4.27	2.55 <sup>a</sup> 1.60 <sup>b</sup>	1.48	3.00 <sup>a</sup> 1.76 <sup>b</sup>
Covariates <sup>a</sup>		Hazard Ratio	Hazard Ratio	Hazard Ratio
Herd size	-	1.09	1.09	1.11
(Herd size) <sup>2</sup>	-	0.99	0.99	0.99
Number of cows	-	1.00*	0.93	1.04*
(Number of cows) <sup>2</sup>	-	1.00*	1.00	0.99*
DED Prevalence	-	1.05	1.04	1.07
(DED Prevalence) <sup>2</sup>	-	0.99	0.99	0.99
Previous history	-	1.26	1.28	1.27

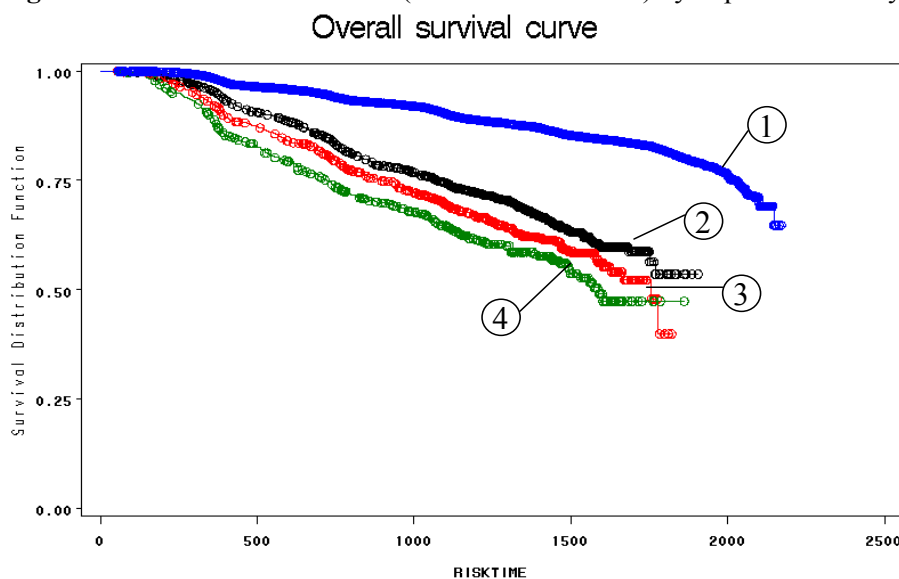
<sup>a</sup> See Material and Methods for definition.

<sup>a</sup> Hazard ratio after 1 year at risk

<sup>b</sup> Hazard ratio after 3 years at risk

Note: All the coefficients had a p-value < 0.03, except for \* p-value > 0.1

**Figure 1** Overall survival curves (no BTB breakdown) by exposure severity during the 1995 episode.



RISKTIME = Days at risk after de-restriction.

Non-exposed	1
2-3 Standard reactors	2
4-8 Standard reactors	3
> 8 Standard reactors	4

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**An evaluation of the Irish Single Reactor Breakdown Protocol for 2005 to 2008 and its use as a monitor of tuberculin test performance**

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**Abstract**

Under the Irish Bovine Tuberculosis (bTB) Eradication Programme all herds are subjected to at least one test per annum. The Single Intra-dermal Comparative Tuberculin Test (SICTT) is used in Ireland for the detection of cattle infected with *Mycobacterium bovis*. There have been concerns regarding the specificity of the SICTT, notably by farmers, and particularly in herds where the detection of a single positive animal in the absence of an obvious source of (bTB) infection could be perceived as a “false” positive. To address this issue the so-called ‘Singleton Protocol’ was established as part of the Irish bTB eradication programme. This protocol allows for the early restoration of free trading status to herds where a single positive animal was detected and where the herd was not confirmed as infected with *M. bovis* by epidemiological investigation, by *post mortem*, by laboratory examination, or by further test. This paper presents data from the 2005 to 2008 bTB programmes on the number of herds which were assessed and which qualified for inclusion under the ‘Singleton Protocol’ and the outcome for qualifying herds up to and including having status restored early as a consequence of inclusion in that programme. The outcome of this protocol reaffirms the reliability of the SICTT at current levels of infection. However as overall infection levels of bTB fall it is advocated that the ‘Singleton Protocol’ be continued as a monitor of herds in which a single positive animal is disclosed, to assess progress towards bTB eradication in Ireland.

*Keywords:* Bovine tuberculosis; *Mycobacterium bovis*; SICTT reactor; tuberculin test

**Introduction**

While all members of the closely related phylogenic grouping of *Mycobacteria* known collectively as the *M. tuberculosis* complex cause tuberculosis in cattle – bovine TB (bTB) - in Ireland the most important is *Mycobacterium bovis*. Tuberculin tests, which avail of a cell-mediated response, to *Mycobacteria*, have been used for the diagnosis of tuberculosis in man and animals for more than 100 years (Monaghan et al., 1994). However, other pathogenic mycobacteria such as *Mycobacterium paratuberculosis* subsp. *avium*, and non-pathogenic environmental *Mycobacteria* such as *M. hiberniae*, abundant in the Irish environment, do cause non-specific sensitisation (O'Reilly and Mac Clancy 1978; Cooney et al., 1997). The 1975 O'Reilly and Mac Clancy (1978) trial, in advance of the replacement of human with bovine tuberculin in the Irish bTB programme, showed that some 7% of cattle were positive to the single intradermal test but not to the SICTT. The SICTT uses bovine and avian tuberculin in combination to assess, measure and compare the response at 72+/-4hrs following intra-dermal injection so as to determine the infection status of the animal and herd (European Commission, 1964). *Mycobacterium caprae*, which has been reported to cause bovine tuberculosis elsewhere (Duarte et al., 2008; Prodinger et al., 2005) has never been detected in Ireland. It is now recognized that the pre-eminent constraint to eradication of TB in Ireland is the existence of a significant reservoir of infection in wildlife, notably *Meles Meles* the badger. The hope of developing an oral delivery system of BCG that will reduce the impact of tuberculosis in badgers is a realistic one and the expectation is that this in turn will reduce if not remove this factor as a constraint to TB eradication in cattle so that progress towards eradication can be accelerated.

Test ‘sensitivity’ Se, (the ability of a test to correctly identify infected animals) and ‘specificity’ Sp, (the ability of a test to correctly identify non-infected animals) is a function not just of the test itself and particularly the potency of the tuberculin used (Good et al., this publication) but also of the environment in which it is used. The Se and Sp of the SICTT has been assessed by O'Reilly (1992) under Irish conditions and slaughtering all 221 animals involved as 91 and 98% Se (standard and severe interpretation respectively) and by Costelloe et al., (1997) involving the examination of all 353

animals involved as 90.9% Se (89.6 and 91.2 – standard and severe interpretation respectively). Monaghan et al., (1994) acknowledge that experiments to establish test Se and Sp for a particular environment are expensive and labour intensive and that for this reason few studies involve slaughter of all the non-reacting cattle. In herds where infection has been established the use of the so-called ‘severe’ interpretation, which lowers the cut-off points for an animal to be declared a reactor, enhances the Se of the SICCT over the normal ‘standard’ interpretation. In a review of techniques for ante-mortem diagnosis of tuberculosis in bovines de la Rua-Domenech *et al* (2006) states that those studies, which used the same concentrations of bovine and avian tuberculin as in the current U.K. and indeed Irish bTB programmes (Bovine tuberculin PPD 30,000 I.U./ml; Avian tuberculin 25,000 I.U./ml as supplied by Lelystad Biologicals B.V.) and which interpreted the test in the same way, affirm that the Se of the SICCT test lay between 75% and 95.5% at standard interpretation. Herd level Se (HSe) is a function of the within-herd bTB prevalence and the number of animals tested. Since, in the bTB programme, the presence of a single test positive animal, regardless of herd size, determines the status of the herd the HSe will rapidly increase to its maximum level (100%) even when the within-herd bTB prevalence is low and the animal level Se is not perfect (Martin *et al.* 1992). It is not possible to determine test Sp with a high degree of accuracy except in a tuberculosis-free environment but Irish field experience indicates that the actual percentage of false positive reactors to the SICCT on a national basis is only a fraction of 1% (O’Reilly and Mac Clancy 1978). O’Reilly, (1992) calculated test specificity as 99.8-99.9% and O’Keeffe (1992), demonstrated mathematically that, since the Irish cattle population is not patently disease free, the Sp of the SICCT, as performed in Ireland, must be at least 99.95% otherwise far more positive animals would be identified by the Irish programme. However, when test Sp is less than 100%, as the number of animals tested increases, the probability of at least one false-positive animal increases and thus the herd level Sp decreases, as the number of cattle tested per herd increases – this is of particular importance and relevance to farmers when as stated above one test positive animal determines herd status (Martin et al., 1992). The predictive value of a positive test (PPV) is directly related to disease prevalence (Thoen and Steele 1995). The higher the population prevalence of disease, the more likely it is that a positive test is predictive of the disease. The shortfall in test specificity means that a percentage of positive SICCT responses are false positive (Martin et al., 1992). This is acknowledged in Directive 64/432/EEC (European Commission, 1964) where paragraph 3A of Annex AI allows for a rapid status-restoration possibility where disease is not confirmed following appropriate epidemiological, post mortem and laboratory examinations.

*Post mortem* and laboratory examination diagnostic limitations, are such that it is not possible to confirm all *M. bovis* infected animals even in heavily infected environments, or to use lack of confirmation as an absolute determinator of disease freedom or of a non-specific responder to the SICCT (O’Reilly and Mac Clancy 1978; de la Rua-Domenech et al., 2006). Moreover, in Ireland a detailed *post mortem* is not conducted on animals removed as a result of the SICCT (reactors). Instead, under Regulation 854/2004/EC, (European Commission 2004), a routine assessment of fitness for human consumption is conducted *post mortem* at the abattoir on products of animal origin intended for human consumption. While some of the measures laid down in this Regulation are aimed at the detection of bTB lesions they are not specifically designed to identify all bovine cases of tuberculosis and indeed lesion detection rate is highly variable (Corner et al., 1990; Whipple et al., 1996; Collins 1997; Frankena et al., 2007).

The national post mortem lesion detection rate, both in standard interpretation reactors slaughtered under the bTB eradication programme i.e., positive animals as per Directive 64/432/EEC (European Commission, 1964), and in non-reactor cattle routinely slaughtered, demonstrates significant annual fluctuation as shown from 1988 to 2008 in Figure 1. In 1995, 44% of standard interpretation tuberculin test reactors showed visible lesions, which was the highest detection rate over the 21-year period and, conversely, in 2001 only 28% of the same group showed lesions, reflecting the lowest detection rate over the period. The equivalent figure for 2008 was 38% (Figure 1). The lesion rate in all animals removed under the programme (not shown) exhibited the same fluctuation. Statistical analysis of DAF data (unpublished) carried out in conjunction with the Veterinary Epidemiology and Economic Research Unit (VEERU), at Reading University, has shown that if the annual variation in lesion detection were the product of random events, annual rates would vary by less than 1% (such are the numbers involved). However, the same analysis failed to find any significant correlation with any

component of the eradication programme itself, to explain the annual variation. The analysis went on to conclude that changes in the rate at which visible lesions are found *post mortem* cannot be used as a guide to changes in disease prevalence or the success or otherwise of the eradication programme. More significantly, visible lesion rate cannot be used directly as an indicator of tuberculin test reliability (O'Reilly 1992). Herd incidence is also included in Figure 1 for the same period and, clearly, since the incidence rate of new herd bTB breakdowns has fallen steadily between 2000 (8.2%) and 2008 (5.88%) and the average number of reactors removed in the 5-year period 2002-08 was, at 26,127, 26% lower than in the preceding 5-year period, despite additional reactors to the Interferon- $\gamma$  assay being included in this figure (DAF, unpublished), there is no consistency between lesion detection data in reactors, clear cattle, reactor numbers or herd incidence and thus measurements and analysis of these parameters is neither a satisfactory monitor of tuberculin test reliability or progress towards bTB eradication.

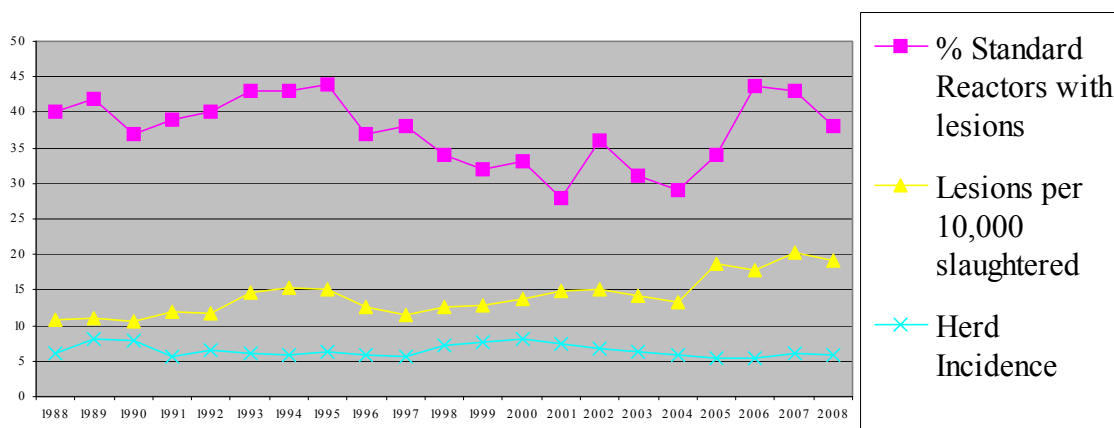


Figure 1: 1988 to 2008 inclusive % herd incidence bovine TB and national *post mortem* tuberculous lesion detection rate, % in Standard interpretation SICTT reactors, and per 10,000 non-reactor cattle slaughtered routinely.

To take the above issues into consideration the 'Singleton Protocol' was incorporated into the bTB eradication programme in 1996 (O'Sullivan 1997). The objective of this paper, is to reaffirm the reliability of the SICTT in Ireland at current levels of infection, and while accepting that this reliability will decrease as TB prevalence falls, to suggest the possibility of using the outcome of a continued Singleton Protocol as an alternative and more satisfactory monitor of progress towards bTB eradication than the other parameters discussed previously.

## Materials and methods

All herds in the Republic of Ireland are subjected to an annual test for bTB using the SICTT. Herds in which an animal responds positively to the SICTT i.e., are identified as a 'reactor', are said to be experiencing a TB breakdown. Herds in which only one 'reactor' has been identified are evaluated for inclusion under the 'Singleton Protocol'. To qualify the herd must meet certain epidemiological criteria designed to include only those herds with no specific indicators of probable infection with *M. bovis*. The qualifying criteria are as follows: the bovine:avian increase difference not greater than 12 mm, no oedema at the injection site and neither the herd itself, within the previous 3-years nor the neighbouring herds within the previous 2 years, have a history of tuberculosis. Herds, which qualify for the 'Singleton Protocol', are placed under movement control and may, subject to non-confirmation of infection at slaughter and laboratory followed by a clear SICTT at least 42-days after the removal of the reactor animal, in compliance with Directive 64/432/EEC (European Commission, 1964), then have their trading status restored earlier than TB infected herds.

All test results in the bTB programme are processed and recorded on the Animal Health Computer System (AHCS). The AHCS data was analysed for the years 2005 to 2008 for all bovine Tuberculosis breakdowns in the Republic of Ireland in order to determine the number of breakdowns that commenced with a single animal being detected as reactor to the SICTT. From this sub-set of breakdown herds, the number, which met the epidemiological qualifying conditions for the Singleton Protocol, was extracted and the subsequent outcome for these herds was assessed to determine how many confirmed as infected or had status restored early under the protocol.

## Results

The outcome of the bTB Eradication programme for the years 2005 to 2008, as numbers and percentages, is presented in Table 1. The reactor animals from herds that qualified for the Singleton Protocol were subjected to post-slaughter examination. If no visible lesions were detected, the head and thoracic lymph glands were submitted for histology and laboratory culture. Table 2 presents the outcome for these 'Singleton Protocol' participating herds during 2005 to 2008, as numbers and percentages.

Table 1: Herds tested under the bTB eradication programme in 2005 to 2008 number and percent with a bTB breakdown detected and the subset of these that commenced with a single reactor animal and the epidemiological outcome for these to determine eligibility for the 'Singleton Protocol'

	2005	2006	2007	2008
Herds tested during the calendar year (% total herds)	119,963 (97.3%)	118,929 (97.2%)	116,964 (96.9%)	116,184 (98.4%)
Herds with a TB breakdown detected in the calendar year (% incidence)	6,647 (5.5%)	6,386 (5.4%)	7,046 (6.02%)	6,837 (5.88%)
Herds with a Single Reactor at breakdown commencement (% total breakdowns)	2,267 (34.1%)	2,110 (33.0%)	2,471 (35.1%)	2,317 (33.9%)
Herds with single reactors assessed as probable infected because of:				
Test measurements (% Single reactor breakdowns)	578 (25.5%)	593 (28.1%)	698 (28.2%)	600 (25.9%)
Area/herd history (% Single reactor breakdowns)	702 (30.97%)	525 (24.88%)	615 (24.9%)	570 (24.6%)
Oedema (% Single reactor breakdowns)	34 (1.5%)	33 (1.6%)	36 (1.5%)	24 (1.0%)
<u>Total excluded</u> for epidemiological reasons (% single reactor breakdowns)	1,314 (57.96%)	1,151 (54.55%)	1,349 (54.59%)	1,194 (51.53%)
Herds qualifying for 'Singleton Protocol' (% single reactor breakdowns)	953 (42.04%)	959 (45.45%)	1,122 (45.41%)	1,123 (48.47%)

Table 2: Outcome for herds qualifying for 'Singleton Protocol' in 2005 to 2008; the reasons for they confirmed as bTB infected and numbers with status restored early

	2005			2006			2007			2008		
			% Single reactor bTB breakdowns			% Single reactor bTB breakdowns			% Single reactor bTB breakdowns			% Single reactor bTB breakdowns
Herds qualifying for 'Singleton Protocol'	953		42.04%	959		45.45%	1,122		45.41%	1,123		48.47%
Outcome for herds qualifying for 'Singleton Protocol'	<i>Number</i>	% qualifying singletons		<i>Number</i>	% qualifying singletons		<i>Number</i>	% qualifying singletons		<i>Number</i>	% qualifying singletons	
Lesions post mortem	256	26.86%	11.29%	323	33.68%	15.31%	306	27.27%	12.38%	306	27.25%	13.21%
Confirmed in laboratory	131	13.75%	5.79%	152	15.85%	7.20%	230	20.49%	9.31%	191	17.01%	8.24%
Other indicators of bTB in herd	143	15.11%	6.35%	130	13.55%	6.16%	151	14.43%	6.56%	182	16.21%	7.85%
Total of qualifying herds confirmed infected	530	55.72%	23.42%	605	63.09%	28.67%	687	62.30%	28.29%	679	60.46%	29.31%
Herds with status restored early	422	44.28%	18.62%	354	36.91%	16.77%	435	37.70%	17.12%	444	39.54%	19.16%
% Total breakdowns with status restored early	6.30%			5.50%			6.00%			6.50%		

## Discussion

There was little, if any change in the level of infection and a very similar distribution of bTB breakdown types between the years, 2005 to 2008 with 6% of the total breakdowns fulfilling all the Singleton Protocol criteria i.e. these herds did not confirm with bTB and had trading restrictions lifted earlier than those herds with confirmed TB.

Identifying the true health status of a herd with bovine tuberculosis is facilitated by the fact that tuberculosis is 'communicable' and therefore one can expect, though not rely on, spread – more than one infected animal – and a degree of persistence if the herd is actually diseased. In 2005, 27% (34% in 2006, 27% in 2007 and 27% in 2008) of the single reactor animals that qualified for inclusion in the 'Singleton Protocol' showed visible lesions at slaughter (Table 2). This lesion rate was somewhat lower but not fundamentally different from visible lesion rate in all standard reactors removed under the programme, which was 34% in 2005, 44%, in 2006, 43% in 2007 and 38% in 2008 (Department of Agriculture records unpublished). Further, it is accepted that the routine 'fitness for human consumption' examination (European Commission 2004) is not a bTB diagnostic instrument and that discrete tuberculous lesions may go undetected in between 47% (Corner *et al.*, 1990) and 53% (Corner 1994) of reactors with lesions and, in many cases, the only site of infection, may not be examined (Corner *et al.*, 1990; Whipple *et al.*, 1996). In addition the relative efficiency of factory surveillance in the disclosure of tuberculous lesions is variable (Frankena *et al.*, 2007). It follows that the 256 (323; 306; 306) lesions detected each successive year, as visible *post mortem* would only have represented approximately 50% of the expected number of 'Singleton Protocol' reactors that would have had visible lesions, 512 (643, 612, 612), had a detailed necropsy been undertaken. This level of *post mortem* diagnostic failure to detect lesions during abattoir inspection is most significant in an animal with a single lesion and during one study almost 70% of the reactors subjected to a detailed necropsy had only a single lesion (Corner 1994). While the collection, histological examination and/or culturing of glands attempts to reduce this diagnostic deficit the necessity to collect lymph nodes also contributes to the shortfall in confirmation. Lymph node collection can only be targeted at a small range of sample sites (head and thoracic nodes) under routine conditions at abattoir line speed as compared to the various sites where infection is possible. Furthermore, there is regularly a collection shortfall in the target number of nodes. Corner *et al.* (1990) reported that, in detailed necropsy findings, 9.8% of single lesions were found in the lung substance. The lungs are subject to palpation only and not submitted to any additional examination under the 'Singleton Protocol'. Thus, inevitably, some sites of infection are not collected and thus never submitted to the laboratory for examination. So, with only approximately 50% of the possible visible lesions detected at slaughter, and the additional possibility of culture isolation from lymph nodes that appear normal on gross pathological inspection (Whipple *et al.*, 1996; FSAI 2003) one might have expected to recover the 50% shortfall by detection and confirmation in the laboratory of 256, 323, 306 and 306 respectively for the years from 2005 to 2008. However, there is also the unavoidable affect of sample decontamination regimes in reducing the sensitivity of laboratory *in-vitro* culture, which in turn further contributes significantly to the inability to confirm infection in all the truly infected reactor animals. This is reflected in the fact that less than 50% of the expected numbers were actually confirmed (131 152, 230 and 191). Thus, unless other indicators of bTB are detected in the herd, it is inevitable that some herds which have status restored early are actually infected and not false positives due to the Sp shortfall of the SICTT. For these reasons, not confirming infection in the 6%, of total breakdown herds, that had trading restrictions lifted early cannot be taken as proof that the animal or herd in question was not actually infected. It is quite probable, at present levels of bTB, and taking the evidence and shortcomings cited above into consideration, that in some 50% of the herds where the status was restored 'early', the SICTT positive animal was infected with *M. bovis*. This probability has no detrimental effect on the programme's effectiveness (O'Keeffe and White 2005). It does however; confirm that the problem of 'NVL' reactors is not one of poor SICTT Sp but rather inadequate gold standards to determine true infection status (de la Rua-Domenech *et al.*, 2006).

Of the 137,763 breakdown episodes (defined as the time interval between restriction following detection of an infected animal and de-restriction allowing a return to trading) in Ireland during 1989-2002, some 52,868 (38.4%) episodes were associated with a single standard reactor i.e., the so-called singleton breakdowns, with or without a lesion at post mortem (O'Keeffe and White 2005). Detailed work has shown that such breakdowns are at lesser risk of a future herd breakdown than breakdowns with at least two standard reactors (with or without lesions) (Griffin *et al.*, 1993; O'Keeffe 1993; Olea-Popelka *et al.*, 2004). These studies have also shown that, having had a subsequent clear SICTT, herds with a single reactor animal only, regardless of visible lesion status, are very unlikely to cause problems into the

future and thus the early release of the herds which had an unconfirmed but actually infected animal will not have had a negative impact on bTB eradication.

Table 2 shows that only 6.3%, 5.5%, 6.0% and 6.5% of all breakdowns in the period from 2005 to 2008 respectively had their disease status restored 'early' under the 'Singleton Protocol' process. This is actually the maximum number of these breakdowns, which might reasonably be deemed to have been due to a non-specific response to tuberculin and a consequence of the less than 100% specificity of the SICTT. However, for the reasons outlined above this is likely to be at least a 50% overestimation of 'false-positive' breakdowns due to the inability to confirm infection in all the truly infected reactor animals. Therefore, it can be deduced that the outcome from the so-called 'Singleton Protocol' indicates that, at present levels of infection in Ireland, only circa 3% of total breakdowns were due to the shortfall in SICTT specificity below 100% and that the reliability (O'Reilly 1992) of the SICTT is currently in the region of 97%.

This estimate of 97% is broadly in agreement with test reliability calculated in the conventional manner as per O'Reilly (1992) who states that test reliability is a relatively crude index of the diagnostic ability of a test and is usually expressed as a percentage and which in turn using Irish figures for sensitivity and specificity (O'Keeffe, 1992; O'Reilly 1993; Costelloe et al., 1997) results in the equation

$$\begin{aligned}
 \% \text{ Test reliability} &= \frac{\% \text{ sensitivity} + \% \text{ specificity}}{2} \times 100 \\
 \% \text{ Test reliability} &= \frac{(91 \text{ to } 98) + (99.8 \text{ to } 99.95)}{2} \times 100 \\
 &= \frac{190.8 \text{ to } 199.75}{2} \times 100 \\
 &= 95.4 \text{ to } 99.88\% \text{ or} \\
 &\sim 97.64\%
 \end{aligned}$$

## Conclusion

The results of the Animal Health Computer System data for the Irish bTB eradication programme for the years 2005 to 2008 analysed and presented here are consistent with the published literature on the sensitivity, specificity and test reliability of the SICCT. The 'Singleton Protocol', including targeting the primary predilection lymph nodes for laboratory examination as heretofore, should therefore continue and be used as a monitor of the reliability of the SICCT in the Irish bTB eradication programme. Furthermore, as Ireland progresses towards bTB eradication the percentage of herds that fail to confirm as infected under the 'Singleton Protocol' should rise and therefore this may be a useful monitor of progress towards eradication.

## Acknowledgements

The authors wish to acknowledge the assistance of all who contributed to the writing of this paper and in particular Department of Agriculture staff involved in the Animal Health Computer System who helped with the compilation of the data, Veterinary Public Health staff who assisted in collection of samples from reactor animals and the Laboratory staff who analysed those samples. Thanks are also due to Dr Eamonn Gormley and Prof Simon More who provided considerable editorial support.

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**Paper 5 An outbreak of tuberculosis affecting cattle and people on an Irish dairy farm, following the consumption of raw milk from a cow with tuberculous mastitis**

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**Abstract**

Bovine tuberculosis is an ongoing problem in Ireland, and herd incidence has remained at approximately 5% for some years. Spillover of infection from cattle to people remains an ever-present possibility, given the ongoing pool of infection in the Irish cattle population. This paper describes an outbreak of tuberculosis affecting cattle and people on a dairy farm, following the consumption of raw milk from a cow with tuberculous mastitis. During 2005, a substantial number of calves and people became infected on a farm in southeastern Ireland following the consumption of milk from a 7 year old cow with tuberculous mastitis. 25 of 28 calves born during autumn 2004 and spring 2005 were subsequently as TB reactors, and 5 of 6 family members were positive on the Mantoux test. During 2005, milk from this cow had mainly been used to feed calves, and was added only occasionally to the bulk tank. Therefore, the calves each received infected milk on an almost continuous basis between birth and weaning. The family collected milk from the bulk milk tank, and consumed it without pasteurization. This case highlights the risks associated with the consumption of raw milk. In this family, TB has had a very significant impact on the health of two young children. These risks are well recognised, and relevant information for farmers is available. It is of concern, therefore, that raw milk consumption remains prevalent on Irish farms. New strategies are needed, in partnership with industry, to address this important issue.

**Key words:** bovine tuberculosis, TB, *Mycobacterium bovis*, Ireland, mastitis, zoonosis, milk, pasteurisation

**1. Introduction**

Bovine tuberculosis (TB; infection with *Mycobacterium bovis*) has been an ongoing problem in Ireland for many years. Prior to national control measures, disease in cattle was common. From

1929 to 1938, it was estimated that gross pathology consistent with tuberculosis was present in 31-33% of cattle slaughtered at the city abattoirs in Dublin (Cotter *et al.* 1996). A national eradication programme commenced in 1954, in part as a consequence of public health concerns, leading to a substantial reduction in disease prevalence by the mid 1960s (More and Good 2006). Although subsequent progress has slowed, herd incidence has remained at approximately 5% for some years. The eradication programme is complex (More and Good 2006), but includes annual testing of all Irish cattle with the single intradermal comparative cervical tuberculin (SICCT).

There have been substantial changes in the epidemiology of human tuberculosis in Europe, including Ireland. Throughout the 19<sup>th</sup> and early 20<sup>th</sup> century, a substantial proportion of human tuberculosis cases were caused by infection with *M. bovis* (Thoen *et al.* 2007), generally linked to the consumption of raw cows' milk (Quinn *et al.* 2006; de la Rua-Domenech 2006). In recent years, however, human infection with *M. bovis* has become very uncommon, accounting for between 1 and 4% of culture-confirmed tuberculosis cases (that is, cases attributed to infection with organisms of the *Mycobacterium tuberculosis* complex, of which *M. bovis* is a member) in Ireland between 2000 and 2004 (Jackson *et al.* 2007; Anon. 2008).

Spillover of infection from cattle to people remains an ever-present possibility, given the ongoing pool of infection in the Irish cattle population. This paper describes an outbreak of tuberculosis affecting cattle and people on a dairy farm in 2005, following the consumption of raw milk from a cow with tuberculous mastitis.

## **2. Case history**

### *2.1 The farm*

The 41 ha. farm ('the case farm') was located in southeastern Ireland. It was divided into four non-contiguous fragments, including three (Fragments 1, 2 and 4), which were grazed by cattle each spring and summer (Figure 1). A final fragment (Fragment 3), of 10 ha., was used exclusively for tillage. The farm was managed solely by the herdowner. Although the farm was an operating dairy, the farmer also fattened home-bred steers for sale. In March 2005, there were 100 cattle on the farm, including 38 cows, 32 steers, 8 heifers and 22 calves. The farmer had no other commercial livestock, such as goats or deer, and no cats. A dog had been present on the farm; however, in February 2005, this animal was treated for suspected paraquat poisoning and subsequently died after a road traffic accident.

Farm management was consistent from year to year (Figure 2). The milking cows remained on the home farm (Fragment 1) throughout the year, including a period of housing each winter (from November to March; adult winter housing). The cows calved in either spring or autumn, and the calves were held with their dam for approximately 1 week following birth. Autumn-born and early spring-born calves were housed (in designated calf housing in batches of 3-4

similar-aged calves, well-separated from other animals) from soon-following-birth until late April/early May, then turned out to pasture on Fragment 1 (May to September, designated pasture separate from the milking cows) and Fragment 4 (October to November, post-silage production). Late spring-born calves were added to this group during late spring and summer. During housing and prior to weaning, calves were fed hay, and approximately 1 kg of calf concentrate ration and 5 litres of 'discard' milk per day (from cows immediately post-calving, those with mastitis or high somatic cell count, and those with milk withheld due to medicine withhold requirements). Yearling animals were also held in adult cattle housing on Fragment 1, but grazed pasture on Fragment 2 during spring and summer. Older (2 year old) steers were housed for fattening. During winter housing, adult cattle are fed silage and rolled barley (each home-grown) and housed in a line of pens (in order: 2 yo steers, yearlings, dry cows, milking cows) under a common roof. There was ready contact between animals in adjacent pens, but not otherwise.

The farm was maintained as a closed unit; there had been no cattle introductions for six years prior to 2005, and all cows were artificially inseminated. The farm was well fenced and there was no history of contact with any animals from other herds. Animals were moved between Fragments 1 and 2, and between Fragments 1 and 4, on foot and via farm vehicle, respectively. Water was sourced from either a deep sealed well (the southern part of Fragment 1) or mains supply (all other areas), and supplied to water troughs (some less than 28 inches high, not all free-standing). No machinery, housing, crushes or other facilities were shared with other farms, and all slurry and dung spreading was conducted by the herdowner.

Liquid milk was stored in a bulk tank, before collection for processing by a local milk cooperative. Raw (unpasteurised) milk from the bulk tank was used routinely by the farm family. A total of 6 people lived on the farm, including the farmer and his wife, a grandparent, and three children (aged 4, 7 and 14).

## *2.2 Tuberculosis in animals*

The tuberculosis testing history on the case farm is presented in Figure 3. Between 1989 and 2004, the farm had been restricted during 1993 (with one reactor) 1995 (5) 2003 (1) and 2004 (1); in the latter three years with confirmed infection (that is, with TB-lesions in at least one reactor at *post mortem*). The herd experienced a significant TB breakdown, following initial detection on 12 March 2005, with 38 tuberculin reactors including cows (7), 2 yo animals (3 steers), 1 yo animals (2 steers, 1 heifer) and calves (13 autumn-born, 12 spring-born). At the tests in March and June 2005, TB-like lesions were detected on routine gross *post mortem* examination in 13 (48%) of these reactors, including 2 (67%) 2 yo animals and 8 (80%) autumn-born calves and 3 (75%) spring-born calves. The 11 reactors detected in September 2005 were removed alive as part of an unrelated tuberculin assay trial. They were subsequently slaughtered in July 2006; at *post-mortem*, TB-like lesions were detected in 8 of these animals.

Tuberculous mastitis was diagnosed in a 7 yo cow (no. 044) on 7 July 2005, following the isolation of *M. bovis* from a milk sample obtained from a mastitic quarter, after culture on Bactec 460 liquid culture system (Becton Dickinson Biosciences, Sparks, MD, USA) and isolate identification by AccuProbe (Gen-Probe Inc., San Diego, CA, USA) and GenoType MTBC (Hain Diagnostika, Nehren, Germany). *M. bovis* was not isolated from the three unaffected quarters. The 2005 lactation commenced, at calving, on 12 January 2005. Previously, the 2003/04 lactation extended from 18 August 2003 (calving) to 15 November 2004 (drying-off). As a result of chronic mastitis in one quarter (and an associated high somatic cell count; Figure 4), this cow's milk was mainly withheld from the bulk tank and fed to calves without treatment (that is, it was neither boiled nor pasteurized). The quarter was hard on palpation, with the consistency of the milk changing over time between thick and watery. However, on an intermittent basis (cumulatively for approximately 3 weeks) during the 2005 lactation, milk from cow no. 044 was added to the bulk tank. This cow had been negative to the SICCT on each of 7 occasions since 2003, but was positive to an antibody-based anamnestic ELISA (Costello *et al* 1997) using bovine purified protein derivative (PPD) antigen on 7 July 2005. The ELISA was done because it is a suitable test for the detection of anergic animals and there was a suspicion that cow no. 044 was such an animal. The test is used infrequently in the management of Irish reactor herds, usually when anergic cases are suspected. This cow was slaughtered on 12 August 2005. Although no detailed *post-mortem* was conducted, no lesions were detected in lymph nodes, including the supramammary lymph nodes, during routine abattoir inspection. The mammary gland was not examined. *M. bovis* was subsequently cultured from a supramammary lymph node.

The locality surrounding the case farm was not considered problematic for tuberculosis. A total of 6 cattle farms (Farms A-F) had land directly contiguous to Fragments 1, 2 and/or 4 of the case farm (Figure 1). Since 1998, there had been two TB breakdowns on Farm F (1 confirmed reactor in 1998/99; 1 unconfirmed reactor in 2003/04), one on Farm B (3 unconfirmed reactors in 2001/02) and none on the remainder.

Badgers were captured in this locality within a radius of approximately two kilometers of fragments 1, 2 and 4 on two occasions, in October 2005 and February 2006. A total of 41 badgers were removed from an area of 16.77 square kilometres (20 in 2005, 21 in 2006), and all were subjected to *post-mortem* examination. One of these animals had gross lesions consistent with TB. Of 22 badgers examined histopathologically, 5 (including 2 captured in 2005 and 3 in 2006) were positive. Of 18 badgers examined microbiologically, 8 (including 5 in 2005 and 3 in 2006) were positive. In total, based on results from histopathology and/or microbiology, 11 of 32 (34.4%; including 35.0% in 2005 and 33.3% in 2006) badgers had evidence of *M. bovis* infection.

### 2.3 Tuberculosis in people

Six family members (grandmother, father, mother, 14 year old girl, 2 boys aged 7 and 4) lived on the farm. The father, mother and 14 year old girl had previously been vaccinated using BCG. In June 2005, Mantoux testing was conducted on all family members, using 2 tuberculin units injected intradermally. The results of this test were as follows: grandmother (no induration), father (14mm diameter induration), mother (12mm), 14 year old (8mm), 7 year old (12mm) and 4 year old (14mm). Chest x-rays on all individuals showed no abnormalities.

The 7 year boy had no evidence of clinical TB and was treated as latent TB with prophylactic isoniazid initially, then rifampicin for a total of 6 months.

The 4 year old boy had two enlarged (approximately 5 mm diameter) lymph nodes in the right anterior triangle of the neck. Biopsy revealed acid-fast bacteria in these lymph nodes, but TB culture was negative. Pathological examination of the lymph nodes on microscopy showed partially opened cystic lesions measuring 1.5cm in maximum diameter, which contained creamy friable material. There were further features of caseating granulomas and some lymphoid tissue on microscopy, which were highly suspicious of tuberculosis. This child was considered an active case of TB. The initial drug choice (ethambutol, pyrazinamide, rifampicin and isoniazid) was modified to ethambutol and rifampicin, once antibiotic sensitivity results (highlighting partial resistance to isoniazid and pyrazinamide) became available from the culture of milk from cow no. 044. Treatment continued for 13 months. There were no liver or ocular complications from the treatment.

Four months after this boy had stopped treatment, a swelling recurred in the same site on the right side of his neck. On excision biopsy, caseating necrosis was seen in a lymph node with surrounding lymphohistiocytic granulomatous reaction. There was no evidence of acid-fast bacteria and TB culture was negative. Full immunological assessment was normal. A differential diagnosis of re-infection, relapse of either *M. bovis*, *M. tuberculosis*, atypical mycobacteria or delayed immune reconstitution syndrome was made. Atypical mycobacteria were essentially excluded by a positive blood quantiferon test (Mahomed *et al.* 2006), which would confirm the diagnosis of TB but would not discriminate between *M. bovis* or *M. tuberculosis*. Treatment was restarted with azithromycin, ethambutol, isoniazid, rifampicin and pyrazinamide and was continued for 6 months (with the exception of azithromycin and pyrazinamide, which were discontinued after 2 months). The swelling subsided and no further treatment is planned at the time of writing this article.

The grandmother, parents and 14 year old child had no overt signs of disease, and no treatment was undertaken.

### 2.4 VNTR profiling

VNTR typing was conducted on *M. bovis* isolated from the milk sample from cow no. 044 in July 2005 and from five badgers captured in October 2005, based on six VNTR loci: QUB11a,

QUB11b, ETRA, MIRU26, VNTR4052 and VNTR1895 and using primers and PCR conditions as described by Roring *et al.* (2004). Although *M. bovis* was isolated from the supramammary lymph node from cow no. 044 in August 2005 and a further three badgers in February 2006, VNTR typing is not available. Mycobacterial organisms were not cultured from the human cases.

Three different VNTR profiles were identified (*i*: 11 4 6 5 4 4 based on the earlier loci sequence, *ii*: 11 4 7 6 3 4, *iii*: 10 3 7 5 4 4). The isolates from the milk and from two badgers (captured at setts J and S, see Figure 1) were each VNTR profile *i*. Different VNTR profiles (*ii* and *iii*) were found in two badgers captured at sett N. VNTR profile *iii* was also identified in a badger captured at sett H.

### 3. Discussion

This paper reports an outbreak of bovine TB in cattle and people following the consumption of raw milk on an Irish dairy farm, which clearly demonstrates the infection risk for calves and people associated with a case of tuberculous mastitis. A wide range of measures is in place to minimise transmission between animals and people (see More and Good 2005), and infection is now very rare. However, these measures do not address practices such as on-farm pasteurisation, which remain the responsibility of individual farmers. The case highlights the importance of this on-farm measure.

During 2005, a substantial number of calves and people became infected following the consumption of milk from cow no. 044 (there is no evidence suggesting additional cases of tuberculous mastitis on the case farm). Of 16 and 12 calves born during autumn 2004 and spring 2005, 13 (81.3%) and 12 (100%), respectively, were subsequently identified as TB reactors between June and September 2005. Similarly, 5 of 6 family members were positive on the Mantoux test in June 2005. Although it is likely that some of these people were infected with *M. bovis*, bacteriology was negative, both on biopsy and sputum samples. The 2005 spring- and autumn-born calves each received infected milk on an almost continuous basis between birth and weaning. Due to milk quota restrictions, extended feeding of whole milk was undertaken in this herd. Generally, two cows (including cow no. 044) contributed to the discard milk, and calves were weaned at various ages between 5 and 8 months. On 10 June 2005, lesions were detected in 3 spring-born calves (at most, 4 months of age), which highlights the degree to which this milk was infectious. These findings are consistent with earlier work by Rentería Evangelista and Hemández De Anda (1996), who examined the role of colostrum and milk management in modifying *M. bovis* exposure risk in calves in TB-infected herds. They are also supported by information from the human cases. The family collected milk from the bulk milk tank, and consumed it without pasteurisation. During 2005, they will have had only very limited exposure to the milk from cow no. 044, because it was rarely added to the bulk tank, and will have been substantially diluted in any case. It is not possible to determine when cow no. 044

first became infected, although it should be noted that this cow had been consistently negative when tested using the SICCT on seven occasions since 2003. Although a substantial increase in the somatic cell count was observed during the latter third of the 2003/04 lactation (Figure 4), there is no indication that this cow was infectious until her 2005 lactation.

The consumption of raw milk does not fully explain the transmission of infection among cattle within the case herd. In addition to the 25 milk-fed calves, there were a further 13 SICCT reactors among the older animals, including milking cows (7 animals), steers (three 2 yo animals, two 1 yo animals) and a heifer (1 yo). Cow no. 044 may have been the case case during this outbreak. Pulmonary lesions were not detected, however, only a very limited examination is possible at abattoir post mortem inspection. The immunological profile of high antibody levels and an absence of skin reactivity to tuberculin has been associated with progressive disease and possible respiratory shedding of *M. bovis* (Welsh *et al.* 2005). This animal remained with each of the other milking cows (including 6 animals that subsequently reacted to the SICCT) throughout the year, but did not have direct contact with the younger stock, either whilst grazing or in housing. It is very unlikely that the younger animals had been infected during a previous lactation (for example, in 2004) since other cases would have been expected; further, extensive testing had been conducted during this period. Other alternatives are also feasible (for example, a number of animals infected independently from a common source). Although the initial transmission components remain uncertain, cow no. 044 played a key role in the later transmission of infection to the calves and the family. No other cows in the herd were screened for *M. bovis* in milk, nor were subjected to the anamnestic ELISA. No further cases occurred in calves in the herd after the animals that had been fed untreated milk from cow no. 044 were removed.

The TB outbreak during 2003 to 2005 in the case herd was the result of either residual infection or introduction from wildlife, although the latter seems more likely. The case farm had last experienced a confirmed TB outbreak in 1995, and it is possible that one or more animals, including the aged cow with lesions detected at slaughter in 2004, may have been infected (but remained anergic) during the intervening period. Note that cow no. 044 was born in 1998, three years after this earlier outbreak. Similarly, a diverse range of *M. bovis* strains, suggesting complex infection dynamics, are prevalent in local wildlife. Three different *M. bovis* strains were found in the locality, including strains with two different VNTR profiles in badgers from the same sett. The *M. bovis* isolate was identical from cow no. 044 and from badgers from two setts, including one adjacent to Fragment 1 (the home farm). This information provides no indication of the direction of transmission (badgers to cattle, cattle to badgers) nor can it distinguish whether both species were exposed to an independent common source. Nonetheless, earlier work has highlighted the importance of badgers in the epidemiology of bovine



tuberculosis in Ireland (Griffin *et al.* 2005). There is no evidence to support introduction of infection through purchased infection and as a result of spread from neighbouring farms. No cattle had been introduced onto the case farm for 6 years prior to 2005. Further, there was a high level of biosecurity on the case farm, and no opportunity for direct contact with cattle on neighbouring farms. The area was not a TB 'hot-spot'. This herd experienced a further TB breakdown in 2007, following a positive herd test on 16 August 2007. At this test, 4 SICCT reactors were identified, including one cow with miliary TB. It is currently unclear if the 2007 breakdown is due to residual recrudescence or further wildlife involvement.

Bovine infection with *M. bovis* has been eliminated or substantially reduced throughout Europe, and, as a consequence, is generally not considered a substantial zoonotic risk. Nonetheless, potential exposure to infected milk remains a hazard for the population, and particularly for some occupational groups. Public health concerns have recently been raised in the UK following the resurgence of *M. bovis* infection in cattle (de la Rua-Domenech 2006; Evans *et al.* 2007; Jalava *et al.* 2007), and it is recognised as an ongoing zoonotic risk in other industrialised countries, including Canada (Fanning *et al.* 1991; Liss *et al.* 1994) and New Zealand (Baker *et al.* 2006).

Although *M. bovis* remains prevalent in the Irish cattle population (More and Good 2006), many measures are in place to limit exposure to the human population. In particular, the majority of infected cattle are removed early in their clinical course as a consequence of annual tuberculin skin testing. Further, an administrative ban on the sale of raw milk was introduced in Ireland in 1997 and remained in place until the EU hygiene package was introduced in 2006. Under this package, the sale of raw cow's milk for direct human consumption in Ireland is now affected by herd TB-status. Regulation (EC) No 853/2004 of the European Parliament and of the Council (including subsequent corrigendum and amendments; Anon. 2004) prohibits milk of reactor animals (including inconclusive reactors and animals showing signs of tuberculosis) from entering the food chain from the time tuberculosis has been diagnosed (Anon. 2008). This milk can only be fed to calves if adequately heat-treated. Milk from non-reactor animals in these same herds can be used for human consumption and for the manufacture of dairy products on condition that it is first heat treated by an establishment authorized by the Department of Agriculture, Fisheries and Food. Nonetheless, herd-owners are strongly advised not to use or drink unpasteurised milk in the home, while there is disease in the herd. The widespread practice of feeding untreated surplus/discard cow's milk also presents a hazard, both for the animals and for the handlers. However, there is no legal requirement for heat-treatment of such milk, even if taken from mastitic cows, unless they are infected, or suspected of being infected, with TB. In summary, this legislation prohibits the use of raw milk from herds that are not officially TB free for the manufacture of milk or dairy products such as cheese. As an additional risk communication device, Regulation (EC) No 853/2004 also requires unpasteurised dairy

products to be labeled 'made with raw milk' to enable consumers to make informed choices (Anon. 2008). Therefore, producers may sell unpasteurised dairy products, (such as cheese, cream, ice-cream or yoghurt), directly from officially TB free farms, but only if registered and approved to do so by the Department of Agriculture, Fisheries and Food. The above-mentioned EU regulations also provide for member states to introduce national rules for prohibiting or restricting the sale of raw cow's milk for direct human consumption from officially TB free herds, and the Irish government has stated its intention to shortly introduce a ban on the sale of such milk.

This case highlights the risks associated with the consumption of raw milk. In this family, TB has had a very significant impact on the health of two young children. These risks are well recognised (Buckley *et al.* 1998; Anon. 2008), and relevant information for farmers is available (Anon. 2005). It is of concern, therefore, that raw milk consumption remains prevalent on Irish farms. In a survey of 230 liquid milk suppliers in 8 Irish counties, families on 84% of farms reported the consumption of unpasteurised milk (Buckley *et al.* 1998). There seems little doubt that new strategies are needed, in partnership with industry, to address this important issue.

### **Acknowledgements**

We gratefully acknowledge the help of the family throughout this investigation. We also thank Joanne McLernon and Daniel Collins, who completed the VNTR typing and mapping, respectively.

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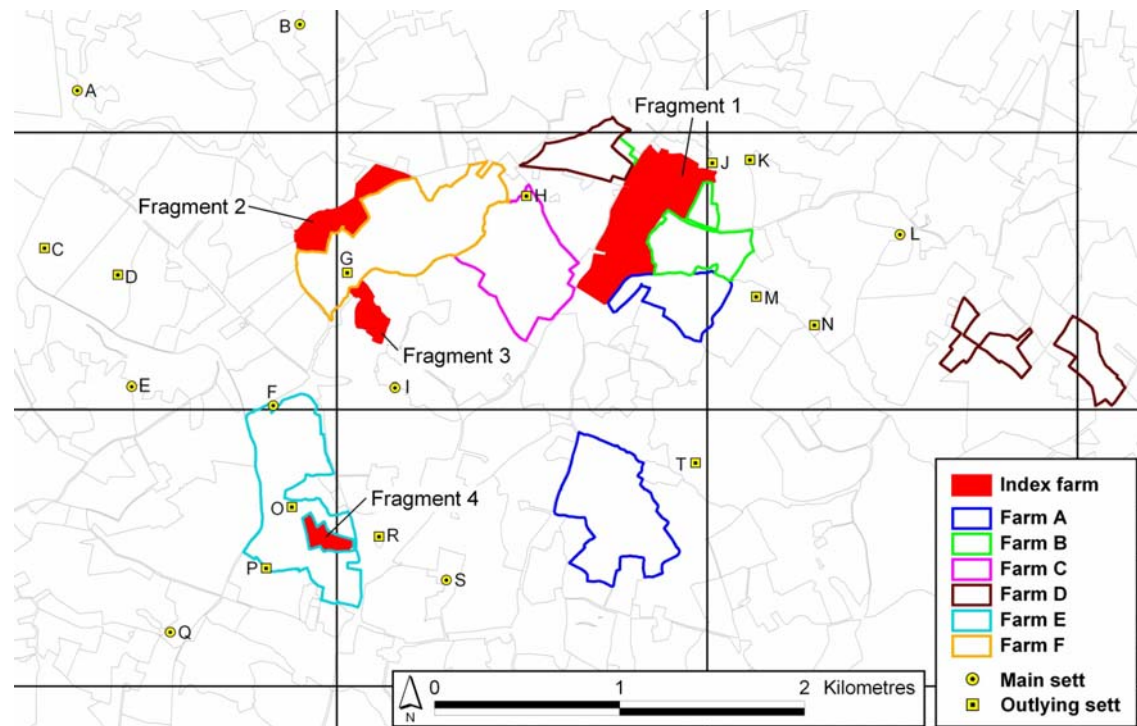


Figure 1. A map of the case farm, including Fragments 1 (the home farm), 2 (yearling summer grazing), 3 (tillage only) and 4 (silage production, autumn grazing for calves). The location of six neighbouring farms (A to F) and local badger setts (differentiated by sett type) are also indicated

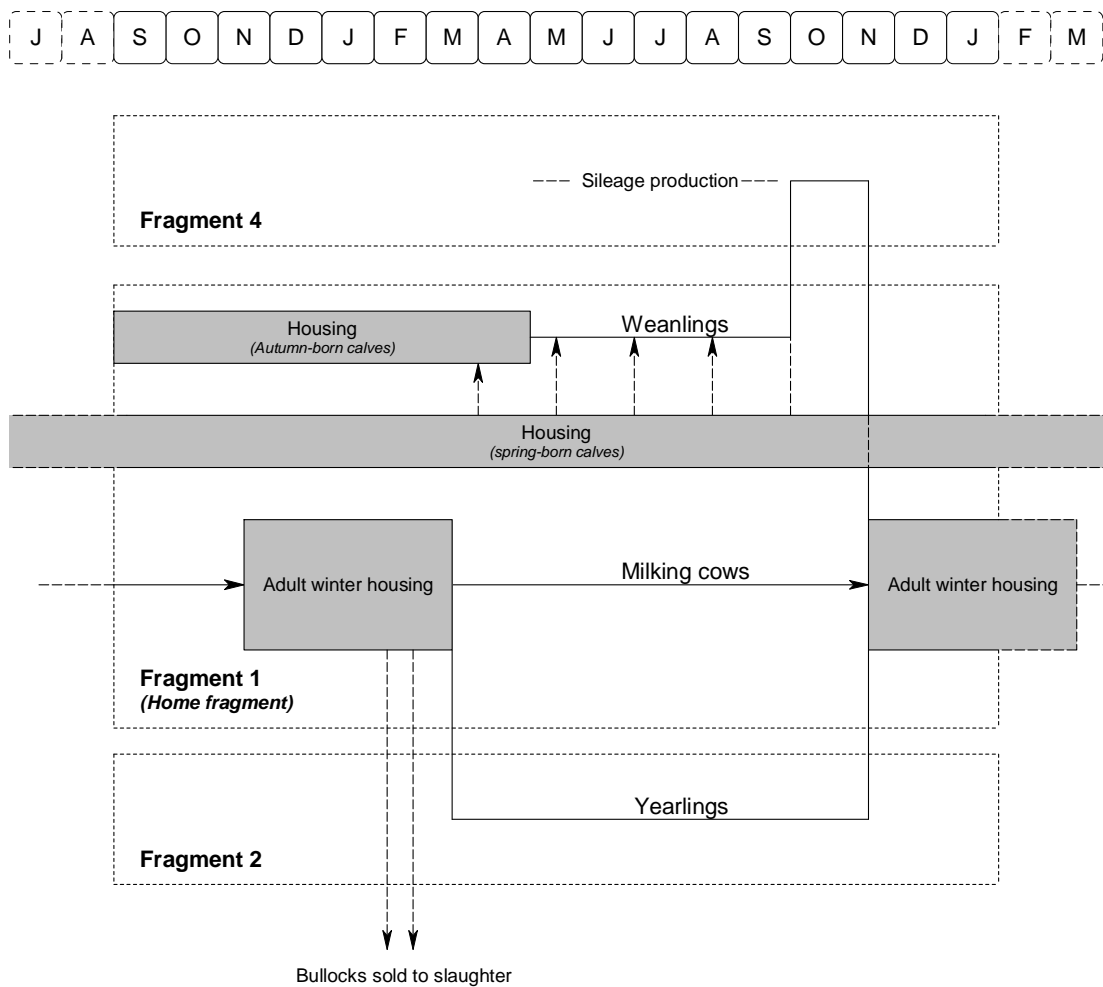


Figure 2. The annual management of cattle on the case farm

Figure 3. The testing history for tuberculosis on the case farm during 1989 to 2006. The test types include the round test (T1), the inconclusive re-test (T3), the reactor re-test (T4), the high incidence area test (T5C), the private test (T6), the post de-restriction test (T7B), the contiguous herd test (T8) and the factory lesion test (T9B). In this figure, the number of conclusive skin reactors (R), inconclusive skin reactors (I) and lesions detected (L) at each test are also presented. The herd was unable to trade, due to tuberculosis, during the periods encompassed by the red dashed line; further, the presence of unconfirmed and confirmed reactors is indicated by the dashed black and solid blue box, respectively.

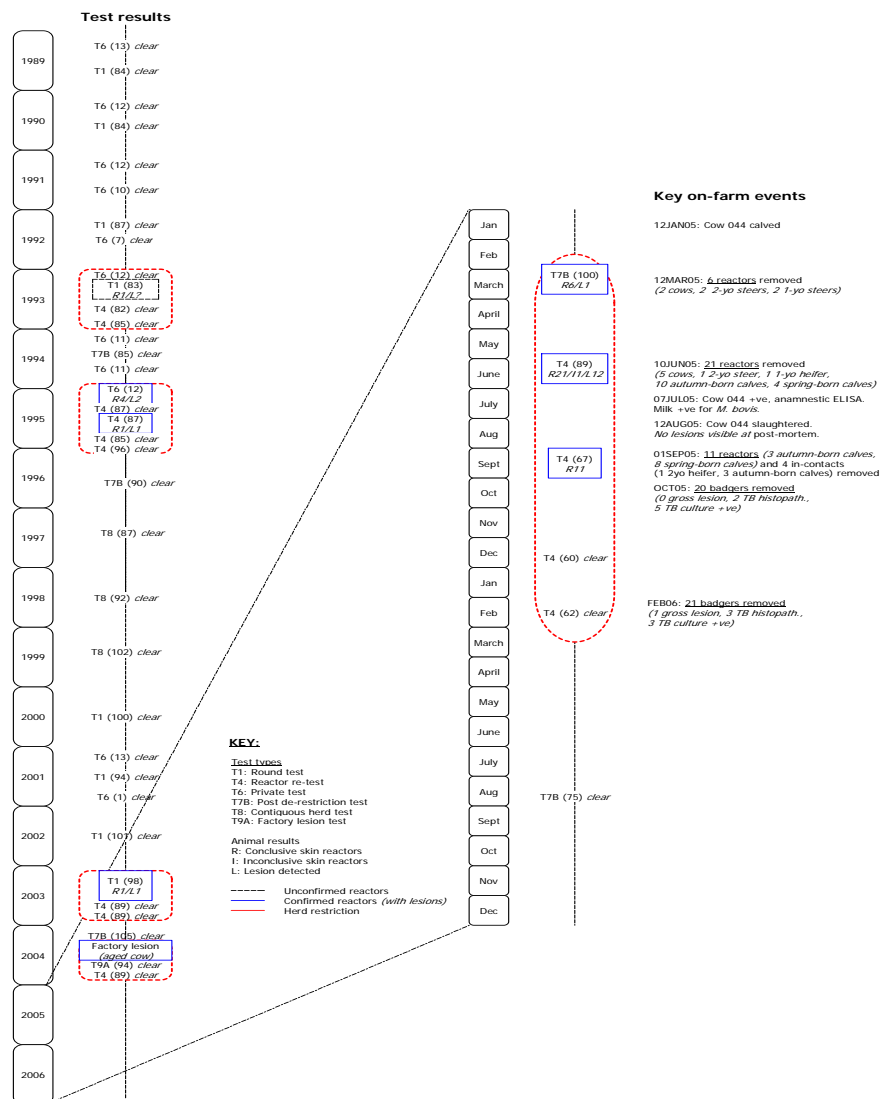
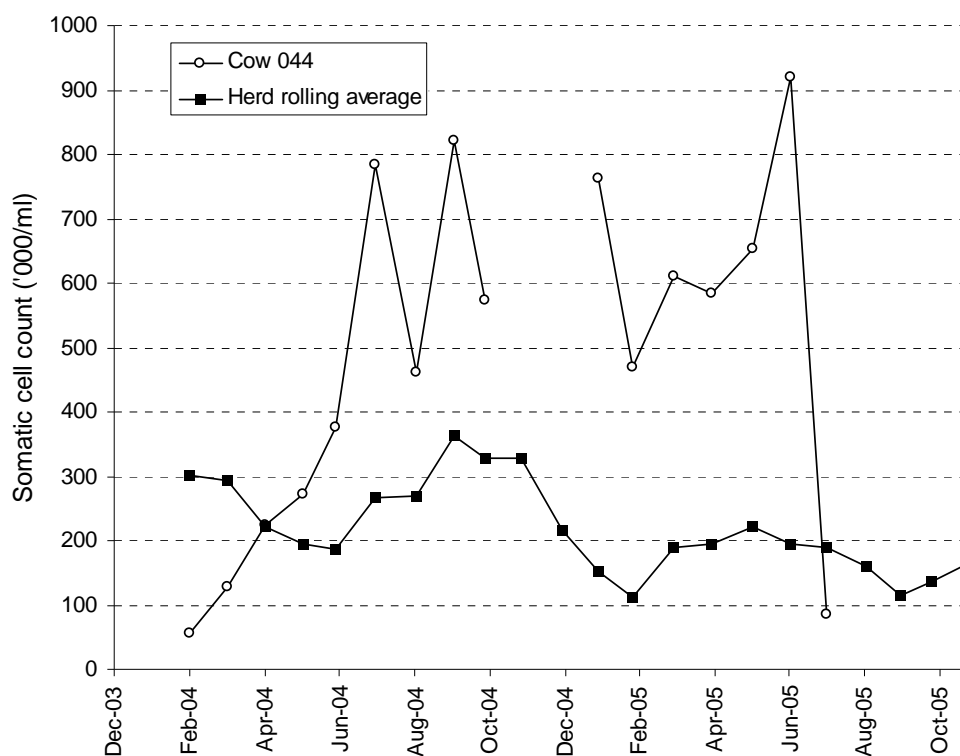


Figure 4. The somatic cell count (SCC) for cow no. 044 and all lactating animals on the case farm (3 month rolling average) during December 2003 to October 2005, based on individual milk recording conducted on a monthly basis. In July 2005, cow no. 044 was tested based on milk collected from the 3 non-mastitic quarters



## **Appendix 15 ERAD Circulars**

**ERAD Circulars can be accessed on Ezone using the link below**

Shortcut to ERAD Circulars Main Page

<http://ezone/intranet/businessareas/eraddivision/circulars/>

### **SUBJECT INDEX LIST FOR ERAD CIRCULARS**

An Aid to Locating Circulars related to a Particular Topic

#### **Feedlots / B & B**

**ER15/08** Movement from B&B/Contract Feeding Arrangements, Valuation and payment of Compensation

#### **Note 30/10/08 Subject ER 19/06 (Issued in 2008)**

Movement from B & B/Contract Feeding Arrangements – Valuation & Payment of Compensation Form V8

**ER19/06** Movement from B & B/Contract Feeding Arrangements – Valuation & Payment of Compensation

**ER14/05** Control of Feedlot Herds – TB Eradication Programme

**ER28/04** B&B Arrangements

**ER51/03** B&B Arrangements

#### **Herdnumbers**

**ER 21/07** Amendments to ERAD/AHCS herdnumber forms ER 1, ER1D and Information Note on herd/flock number of cattle, sheep or goats, and Introduction of new ER1E form regarding change of ownership/keeper details

**ER18/07** Trading and disease/ health status for newly established herds and revoking Circular ER 41/2003 and amending the provisions of Circular ER 18/2004, including the terms and conditions and application forms for herd numbers

**ER17/07** Clarification of Issuing of Herd Numbers for control of disease purposes

**ER18/04** Clarifications on the Issue of Herd Numbers

**ER41/03** Trading and Disease Status for newly established herds

**ER16/03** Reactivation of Dormancy Indicator on BTR/CMMS

**ER25/02** Milk Production Partnerships

**ER04/01** Approval and Registration of Dealers and Dealers' premises

**ER36/00** Renting holdings or Part of a Holding to Feed Cattle



**ER37/90** Herdnumbers

**ER06/88** Herdnumbers

### **Movement of Cattle**

**ER 15/09** Information Note on development of Animal Identification and Movement (AIM) System

**ER 03/09** Recording of Bovine Movements on AIM

**Note 30/10/08 Subject ER 19/06 (Issued in 2008)**

Movement from B & B/Contract Feeding Arrangements – Valuation & Payment of Compensation Form V8

**ER 19/08** National Beef Assurance Scheme Act 2000 (Animal Movement Regulations 2008 S.I. No. 400 of 2008

**ER 15/08** – Re 19/06 Movement from “B&B” /contract feeding arrangements – valuation and payment of compensation

**ER 05/08** Movement of Cattle into holdings restricted under the TB Order

**ER 19/06** Movement from “B&B” /contract feeding arrangements – valuation and payment of compensation

### **TB & Brucellosis Eradication Programme -Testing Programme**

**ER 02/10** 2010 TB and Brucellosis Testing Programmes - Round

**ER 01/10** 2010 Testing Programme: Approval of Private Veterinary Practitioners (PVPs) Annual Commitment /Acknowledgement of ER4 instructions  
(PVPs) Annual Commitment /Acknowledgement of ER4 instructions

**ER19/07** Brucellosis Annual Round Tests- Exemption of Bulls aged 24 months or less

**ER13/07** Supervision of Testing under TB/Brucellosis Programme

**ER11/06** Overdue Tests – Follow-up reminders and 14 day notices

**ER45/03** To clarify procedures to be followed on foot of suspicion of Brucellosis as a result of test, where only part herd test has been carried out

**ER18/03** Saturday Testing

## Appendix 16 Approved Disinfectants

Vet/Disinfec/doc/4z 1 **DEPARTMENT OF AGRICULTURE, FISHERIES AND FOOD**  
**Diseases of Animals (Disinfectants) Order, 1975 (Amendment) Order, 1978**  
**LIST OF APPROVED DISINFECTANTS (13/03/2008)\***

DISINFECTANT		Diseases in respect of which use is approved and dilution rates <sup>1</sup>			
	Foot and Mouth	Swine Vesicular Disease	Fowl Pest (Newcastle Disease, Fowl Plague(Avian Influenza)	Tuberculosis	Anthrax, Brucellosis, Contagious Bovine Pleuropneumonia, Glanders and other Scheduled diseases
Acidfoam-C				70	
Activ 8 Hard Surface Cleaner				Ready to use	
Agrisept MC Tabs	271	448*	271	---	450
Antec Ambicide	---	---	---	---	30
Antec Hyperox	150	50	375	100	179
Antec Virkon S	1300	200	280	----	120
Bio Dine	---	---	145	---	---
Bio Guard	---	---	80	---	---
Bio Kill	140	---	365	---	160
Bi-OO-Cyst	---	---	125	---	---
Bio Phen	---	175	190	---	---
Bio Phen Plus	400	160	210	---	---
Bio Shield	---	---	155	---	---
Bio VX	1200	135	285	---	100
Chlorasol	20	90	200	20	199
Citric acid <sup>2</sup>				0.2%	
Citrox	---	---	0.66	---	---
Clinidine	320	---	140	---	---
Clinidine 28	500	400	170	---	---
Deosan Iodel FD	215	234	130	26	147
Dermicidal Extra	50	150	125	13	61
Envirocare A	99	99	99	---	99
Enviroguard	60	300	150	10	60
Equisept	271	---	450	---	450
FAM (New Formulation)	525	400	150	15	110
FAM 30	550	600	125	30	180
GPC 8 (New	80	250	190	---	---