

VISI 194 (FIRST ISSUE)

SEB : EXAMINATION OF BADGERS FOR THE RANDOMISED BLOCK CULLING TRIAL (PROJECT SB4400)

TABLE OF CONTENTS

- 1 Introduction
- 2 Receipt of national random cull trial badgers at RLs.
- 3 Safety and security
- 4 Sample collection and data recording
- 5 Dispatch of samples from RLs
- 6 Contacts
- 7 Work recording
- 8 Further reading

APPENDICES

- 1 ISG SOP 11, 2.1
- 2 Safe working procedures for receipt, handling, post mortem and disposal of badgers
- 3 Thawing of badgers prior to necropsy examination

1 Introduction

- 1.1 Bovine tuberculosis (TB) is an important disease, largely because it has been a major zoonosis in this country and continues to be so in many areas of the developing world. It's increasing prevalence in British cattle also threatens international trade as well as posing a disease and welfare threat to affected animals. *Mycobacterium bovis* infection in badgers in the UK was first diagnosed in 1971 and since then there has been increasing but not conclusive evidence that they are an important source of infection for cattle. In 1997, the "Krebs" review of the subject recommended an epidemiological investigation of TB in badgers to determine their role in the bovine disease.

The Independent Scientific Group (ISG), under the chairmanship of Prof. John Bourne, is implementing this study, known as the Randomised Block Culling Trial (**RBCT**). It attempts to monitor the incidence of bovine TB in 10 areas of England, each of which is divided into three. These triplets are subjected to either total badger culling, no badger culling or badger culling only in response to the diagnosis of TB in a cattle herd (the former control option). The trial started in 1998 and will run for at least five years.

It is critically important to this study that the TB status of culled badgers is determined

as accurately as possible. It is the VLA's role to provide this diagnostic information through necropsy and subsequent laboratory examinations of culled badgers.

1.2 There are three types of RBCT badger culls:

- Proactive culls – for triplets subjected to total culling. Done twice, in two consecutive years. Followed by maintenance culls.
- Maintenance culls – also for triplets subjected to total culling, after two proactive culls. Done on a small scale and *ad hoc basis*.
- Reactive culls – for triplets where badgers are only culled on relevant farm and in response to diagnosis of TB in a herd.

The first usually results in large numbers of badgers for examination, the latter two in much smaller numbers.

1.3 Due to the involvement of the ISG, the means by which this work is commissioned and controlled differs from other VLA projects. For RL staff, the major difference is that they are required to perform necropsy examinations and sampling according to a standard operating procedure produced and owned by the ISG and DEFRA. SOP 11, contains the bulk of instructions for this project and it is enclosed as **Appendix 1**.

The remainder of this VISI provides additional guidance and instructions.

2 Receipt of RBCT badgers at RLs

2.1 Section 4 of SOP 11 provides relevant instructions.

2.2 In addition, there is a requirement for receiving RLs to enter preliminary information on the **Badger Database**. Instructions and guidance are provided in the VLA Badger Database 2002 User Manual issued to RLs on 9/9/02.

2.3 RLs should retain all badger carcase labels in case they are required for future audit. They may be kept on file with the appropriate case-control form. However, if they are soiled they should be placed in a sealed, labeled plastic bag and either this or a photocopy should attached to the copy of the RCT 9 form kept at the RL.

- 2.4 If an RL expects to receive several submissions of RBCT badgers in any one calendar month, it is advisable to make a single entry onto "Farmfile" at the end of the month. In this case, good written records or the Badger Database should be used to accurately determine the number of badger carcasses entered since the last Farmfile submission.

Where badgers are received intermittently and sporadically, it is acceptable to make the Farmfile entry soon after the examinations have been completed. The following codes are to be used for Farmfile entry:

Contract code:	SB4400	All RLs
Test codes:		
PME of badger	TC0537	All RLs
Histology – fixing and dispatch of tissues	TC0575	All RLs
Mycobacteria culture (routine method)	TC0540	[REDACTED] and [REDACTED] only
Histology for Mycobacteria	TC1109	[REDACTED] and [REDACTED] only

- 2.5 If badgers have been frozen, they should be thawed according to instructions in **Appendix 3**.

3 Safety and security

- 3.1 Staff are reminded that *Mycobacterium bovis* is a potentially very serious human pathogen. Category III containment facilities are required for laboratory examinations of this bacterium and *post mortem* room requirements are given in section 2 of SOP 11 and in:

- SOP AD 025.A
- Appendix 2: Safe working procedures for receipt, handling, post mortem and disposal of badgers

All staff working with badger carcasses must be familiar with these documents.

- 3.2 The culling of badgers is contentious and is sometimes subjected to sabotage, which may threaten the safety of DEFRA and VLA personnel. For this reason, staff should not inform people outwith DEFRA and VLA of their or their RL's involvement with this work. Suspicions of attempts to disrupt this work should be reported immediately to their line manager.

4 Sample collection and data recording

- 4.1 Samples should be collected as instructed in SOP 11.
- 4.2 Staff are reminded that the ratio of CPC preservative to tissues should be at least 5:1. If several lesions are collected, then more than one container of CPC may be necessary.
- 4.3 It is very important that RCT 9 and RCT 10 are fully and accurately completed - information from these forms is entered onto database by [REDACTED] staff and is used by a number of senior, independent scientists. Therefore, omissions and most errors will be readily apparent to non-VLA staff but may also lead to erroneous conclusions and advice on the role of badgers in TB and its control.
The examining (S)VIO should check each form carefully before (s)he signs it. It would be advisable to perform a second check at the time when the forms are dispatched.

4.4 Collection of ticks, fleas and lice

Although not part of the ISG trial, ticks, fleas and lice should be collected from badgers with visible lesions of tuberculosis.

It is only necessary to collect the ectoparasites when large, obvious numbers are present. As badgers should have been sprayed with insecticide between before bagging, dead arthropods from the interior of the bag should also be sampled. Ticks are most commonly found on the ears, belly, axillae and groin.

- Arthropods from badgers with visible lesions should be placed in a plastic disposable bijou along with a small wetted cotton wool plug in the top of the pot to prevent ticks drying out.
- Clearly label bijou with the unique badger number and dispatch on the same day if possible to VLA [REDACTED], where arthropods will be examined for the presence of *M. bovis*.
- There is no need to use fixatives or preservatives.

4.5 Photography of suspect TB lesions

This project provides a valuable, and possibly unique, opportunity to investigate tuberculosis in badgers. There is currently a dearth of high quality photographs of badger TB lesions and colleagues are requested to help address this deficiency in order to provide training and educational material in the future.

All suspect lesions should be photographed provided that this does not threaten staff health and safety and that it does not unduly delay examinations (staff time for this activity should be recorded as described in section 8).

Lesions should be photographed:

- with either a conventional or (preferably) a digital camera
- preferably with focusing rings or macro lens to allow for close up and adequate detail of small lesions
- preferably with natural, rather than flash, light to prevent reflective glare
- against a dark, preferably blue, background
- next to a piece of card with badger unique reference number and identity of tissue (with may be abbreviated if necessary, e.g. "R-ph LN").

It may be advisable to "experiment" and take several shots to allow for variables.

Successful photographs should be sent to the Project Manager. RLs may wish to keep copies for future local use.

The Project Manger will inform RLs when adequate numbers of photographs of a particular type have been collected.

4.6 **Collection of large volume samples for Freezing Study**

There is an important need to collect samples to determine the effect of freezing and thawing on the viability of *M.bovis* in lesions of tuberculosis in badgers. This study requires larger amounts of tissue than are routinely collected, because of the need to test a variety of different times and conditions of freezing and thawing on roughly equal aliquots of infected tissue.

Therefore, only large organs such as lung and liver with widespread, miliary tuberculosis are suitable.

If you encounter miliary tuberculosis:

- Take at least five, preferably six, one cm cubes of representative tissue. Ideally, the lesions should be of approximately the same number and nature in all the tissue samples.
- Put one cube into CPC, label with the badger reference number, date of collection and "Freezing trial" and send to [REDACTED] RL on the day of collection for next day delivery.
- Place each of the other cubes in a sterile Universal container, label with the badger reference number, date of collection and "Freezing trial" and place immediately in a freezer at -20°C .
- Contact [REDACTED] at [REDACTED] to arrange transport of the frozen samples, which must remain frozen throughout transit.

5 Dispatch of samples from RLs

- 5.1 Samples should be dispatched to the culture laboratory according to the instructions in SOP 11.
- 5.2 In addition, please inform the culture laboratory, by phone or e-mail on the day of dispatch, of the number of samples sent. This enables them to plan their work effectively and efficiently.
- 5.3 Samples for culture must **always** be placed directly into CPC. They should be dispatched on the day collection to enable culture on the following day. Culture laboratories are able to receive and process samples on Saturdays but not Sundays.

When samples are collected late on Friday and cannot be dispatched that day, they should be dispatched on Saturday to arrive at the culture laboratory on Monday morning. In such cases, please include ice packs to keep samples cool in transit.

6 Contacts

[REDACTED] is the Project Manager and [REDACTED] is the Deputy Project Manager. Both are at VLA [REDACTED], telephone: [REDACTED], fax: [REDACTED].

7 Work recording

[REDACTED] minute of 8/7/02 applies.

- 7.1 **Veterinary Surveillance Department** staff should record all *post mortem* and other relevant time to code SB4400, PS4400
- 7.2 **Laboratory Testing Department** staff should record their time as follows:

Test Description	WG	PACT Time	
		Parent Project	Work code
PME on badgers	All	TG0100	TG0100
Histology – Fixing & Despatching tissues	All	TG0060	TG0060
Mycobacteria culture (routine method)	LTST, LTTR	TG0070	TG0070
Histology for Mycobacteria	LTST, LTTR	TG0060	TG0060
Mycobacteria culture by Bactec (500)	LTST, LTTR	SB4400	CN4400
Archiving lymph nodes (3,000)	LTST, LTTR	SB4400	CN4400

8 Further reading

8.1 Reports and updates

- DEFRA website: Animal health & welfare/TB/Culling trial and also /ISG
- **An epidemiological investigation into bovine tuberculosis** (2001). Third report of the Independent Scientific Group on Cattle TB.
- Krebs J, Anderson R, Clutton-Brock T, Morrison I, Young D and Donnelly C (1997). **Bovine tuberculosis in cattle and badgers**. MAFF.

8.2 Scientific papers

- Gallagher J and Clifton-Hadley RS (2000). **Tuberculosis in badgers: a review of the disease**. *Research in Veterinary Science* **69** 203-217.

A comprehensive review, principally of John Gallagher's work in the 1970s and 1980s

- Gavier-Widen D, Chambers, MA, Palmer N, Newell DG and Hewinson RG (2001). **Pathology of natural *Mycobacterium bovis* infection in European badgers (*Meles meles*) and its relationship with bacterial excretion**. *Veterinary Record* **148** 299-304.

Records the findings of a more detailed examination of a small number of badgers with TB. Some conclusions are different from those of Gallagher and Clifton-Hadley.

- de Lisle GW, Bengis RG, Schmitt SM and O'Brien DJ (2002) **Tuberculosis in free-ranging wildlife: detection, diagnosis and management**. *Rev. sci. tech. Off. int. Epiz* **21** 317-334.

Interesting account of TB in a range of free-living animals in many parts of the world.

RANDOMISED BADGER CULLING TRIAL

STANDARD OPERATING PROCEDURE 11: POST MORTEM EXAMINATION OF BADGERS

VERSION 2.1

APPROVED BY	SIGNATORY	SIGNATURE	DATE
National Trial Manager	<div style="background-color: black; width: 100px; height: 15px; margin-bottom: 5px;"></div> <div style="background-color: black; width: 100px; height: 15px;"></div>		
Independent Scientific Group	Professor <div style="background-color: black; width: 80px; height: 15px; display: inline-block;"></div> (Delegate)		
Head of Animal Disease Control Division	<div style="background-color: black; width: 150px; height: 15px;"></div>		
Head of Veterinary Animal Endemic Diseases and Zoonoses Team	<div style="background-color: black; width: 180px; height: 15px;"></div>		

Version 2.1
11 July 2002

RANDOMISED BADGER CULLING TRIAL: STANDARD OPERATING PROCEDURE 11 (POST MORTEM EXAMINATION OF BADGERS)

1.0 INTRODUCTION

1.0.1 These instructions set out procedures for the post mortem examination of badgers to determine *Mycobacterium bovis* (*M. bovis*) infection at the time of death. This involves examination for the presence or absence of suspected tuberculous lesions and the collection of a standard selection of lymph nodes and, where applicable, collection of suspicious lesions. Infection with *M.bovis* is determined by bacteriological examination of the specimens collected at post mortem examination.

1.0.2 Other material may be collected for collateral research projects approved by the ISG.

2.0 SAFETY

2.0.1 General and specific safety information for these procedures is set out in VLA SOP AD.025.A. and VLA Badger DIN 001.00.

2.0.2 *M.bovis* is a serious zoonotic infection. Post mortem examination must only be carried out on a down-draught table by operatives wearing a powered respirator to EN146 Class THP3 specification.

2.0.3 Post mortem examinations can only be carried out at VLA sites meeting the above safety requirements and possessing adequate numbers of trained staff.

3.0 MATERIALS

Reagents

3.0.1 The following reagents are required:

- Methanol (GPR)
- 1% aqueous cetyl pyridinium chloride (CPC)
- 10% formal saline

Equipment

3.0.2 The following equipment is required:

- High efficiency powered respirator to EN146 Class THP2/3
- Down-draught table
- Disposable gloves (arm length and wrist length)
- Cut-proof gloves or cut-resistant glove liners
- Post-mortem knife
- Pointed scissors and scalpel
- Forceps - rat tooth
- Microscope slides
- Universal containers
- Packaging materials
- Instrument stand
- Approved disinfectant
- Pot containing 80% ethanol (for DNA sample)
- Labels derived from database

Forms

3.0.3 The following forms are required:

- RCT 9 - Badger Post Mortem and TB lesion Report form. (See Appendix 1)
- RCT 10 - Badger culture and histopathology Report form. (See Appendix 2)

4.0 CARCASE RECEIPT, IDENTIFICATION AND HANDLING

Receipt of badgers

4.0.1 Badgers should only be delivered during normal working hours allowing sufficient time for unloading/checking at the time of delivery. The WLU must telephone VLA regional laboratories (RLs) to let them know the time of the expected delivery. Out of hours delivery may only take place to those RLs where prior arrangement has been agreed to allow for provision of keys and security clearance.

4.0.2 On delivery, badgers should be counted off the van and agreed by a WLU and VLA staff member. Badgers must be labelled using either of the labels shown in Appendix 3 (form RCT 7) or Appendix 4 (Form RCT 8). Unlabelled badgers should not be accepted; these should be reported to the responsible WLU Unit manager who should issue a fresh label or decide what must be done and put that decision in writing and fax it to the laboratory concerned.

In order to assist RL staff will store unlabelled badgers but not process these until the RL has received a suitable identification in writing from the WLU.

4.0.3 If any badgers are delivered outside VLA hours to those RLs with appropriate facilities it will be the WLU staff member who will be responsible for checking the numbers and filling in a form

confirming the delivery, signing the form (VLA – Badger DIN 006.00) and leaving a copy at the delivery site.

4.0.4 If a badger is found to be unlabelled from an out-of-hours delivery this must be recorded immediately on the delivery form by the VLA officer and reported to the responsible WLU Unit manager who must decide on the action to be taken, putting that decision in writing. This decision must be faxed to VLA regional laboratories, as soon as possible. The badger will be labelled with a photocopy of the delivery form, to which should be added the date, time and by whom it was put into chilled storage. It will then be stored, but not processed, until the RL has received a suitable identification in writing from the WLU.

4.0.5 If badgers are received at a RL directly from the field, without having been chilled, then the date and time that the carcass was placed in the RL chiller must be entered on the RCT 9 form. This section should not be completed if the badger carcass was chilled before submission.

4.1 OFFICE PROCEDURE

Badger number

4.1.0 On receipt of the carcass, the VLA regional laboratory office should transfer the unique badger reference number given on the badger carcass label to the badger post mortem (RCT 9) and TB bacteriological results report (RCT 10) forms. The badger number will consist of a two letter code, either [] or [], followed by a five digit number. In addition, the badger will be identified according to the VLA regional laboratory where it underwent post mortem examination. This is done by the addition to the badger number of a two digit suffix, as follows:

- [] 16 • [] 24
- [] 17 • [] 26
- [] 21 • [] 27
- [] 22 • [] 28
- [] 12 • [] 29

For example, a badger trapped by [] WLU and examined at VLA [] would be numbered "[] [XXXXX]/22"

5.0 STORAGE

Storage prior to post mortem examination

5.0.1 Following completion of labelling procedures, carcasses must be placed in refrigerated storage at 0 to 4 °C preferably in a single layer on the floor or on suitable shelving. Post mortem examination should take place within 72 hours of receipt, wherever possible.

5.0.2 The VLA Project Leader, in discussion with the National Trial Manager, will decide whether carcasses must be stored frozen prior to post mortem examination. Where freezing is necessary it must be at –20 °C. The relevant box on the RCT9 should be completed for frozen badgers.

6.0 EXTERNAL EXAMINATION

Recording data

6.0.1 Information, including the laboratory reference number, must be recorded on report forms RCT 9 and 10.

Physical characteristics.

6.0.2 The following information must be recorded:

- carcass weight (in kg to one decimal point)
- nose tip to tail base length (cm)
- age group, i.e. cub or adult
- sex
- teat condition
- tooth wear

Toothwear assessment.

6.0.3 A visual assessment of toothwear is made on a five point scale described in Appendix 5.

Teat condition

6.0.4 The condition of teats must be assessed to indicate as to whether the teats are extended and whether milk can be expressed.

Age groups

6.0.5 Assigning badgers to the adult/cub age groups requires a subjective judgement which may be difficult, especially when cubs reach adult weight towards the end of their first year. The assessment is best made using criteria of body weight, tooth wear and general appearance. Categorisation is limited to the following two broad groups:

- cub
- adult

Ignore the “aged” box on the RCT 9 form.

Mud contamination

6.0.6 The heavy contamination of a carcass with mud over the limbs, head and flank areas must be recorded as a Y or N in the appropriate box of the post mortem examination report form.

Confirmation of post despatch checks

6.0.7 Following the despatch of a badger, field staff must undertake checks to ensure that the badger is dead. Confirmation that such checks have been completed are signified by marker. A check must be made as to whether or not spray markings are present and the findings reported. A Y or N should be recorded in the appropriate box of the post mortem examination report form.

Number of bullet entry wounds

6.0.8 To assess despatch procedures the head must be examined to assess the number of bullet entry wounds. The appropriate box of the post mortem examination report form should be completed to record either single or multiple entry wounds.

Illegal interference

6.0.9 The presence of suspected illegal damage e.g. injuries due to snares or shooting must be recorded as a Y or N in the appropriate box of the post mortem examination report form. Such damage must be brought to the attention of the National Trial Manager in writing, who will, where appropriate, advise the authorities.

Recording of bite wounds

6.0.10 Open bite wounds must be recorded on both forms RCT 9 and RCT 10.

Trap related injuries

6.0.11 For welfare reasons, particular care must be taken to record injuries resulting from trapping. Typically such injuries will be to the paws, limbs, claws, muzzle, teeth, or head and be fresh in appearance.

Assessment of injuries

6.0.12 Injuries should be assessed and recorded as follows:

area of the body: claws, lower limbs, upper limbs, teeth alone, teeth plus broken jaws, snout and other parts of a head, a tick must be completed in the appropriate boxes of the post mortem examination report form.

- in the case of the lower and upper limbs, lesions must be recorded as:

minor: some hair loss, some redness, but all lesions 2cm or less

major: lesions are more than 2 cm with red or broken skin and hair loss

Where no injuries are identified a tick should be placed in the appropriate box of the post mortem examination report form.

7.0 TISSUE SAMPLES FOR DNA ANALYSIS

Sample

7.0.1 The sample is taken before the carcass is opened and, for each badger, will comprise the distal 1cm cut from either ear and the appropriate box completed on the post mortem examination report form.

Submission of samples

7.0.2 The ear tip should be placed in the pot provided, using a separate pot for each sample. The pot will already contain 80% ethanol.

The pot must be sealed and labelled with the:

- badger unique ID

7.0.3 The pots should be retained at room temperature and forwarded to CSL at [REDACTED] monthly or when 100 samples have been collected, whichever is sooner. Details of label production and protocol for despatch of samples will be provided to VLA by the CSL.

8.0 TISSUE EXAMINATION

Avoiding cross contamination

8.0.1 A clean set of instruments and fresh set of disposable gloves should be used for each badger.

Opening the carcass

8.0.2 The badger should be laid on its back and:

- the mammary glands should be examined and two longitudinal incisions should be made to the right and left mammary glands. If milk exudes from the cut surfaces report form RCT 9 should be noted accordingly.
- the skin to then be deflected and the thoracic and abdominal cavities opened so that the peripheral lymph nodes, the thoracic and abdominal viscera may be examined.

Internal examination sequence

8.0.3 Examination should be in the following order:

- a) peripheral lymph nodes
- b) thoracic and abdominal contents

Examination of lymph nodes

8.0.4 The following lymph nodes* (both right and left where applicable) must be incised at least once and the cut surfaces examined:

- Submaxillary
- Retropharyngeal
- Prescapular
- Axillary
- Bronchial
- Mediastinal
- Hepatic
- Gastric
- Renal (when located)
- Mesenteric
- Internal iliac
- External iliac
- Superficial inguinal
- Popliteal

* where not located, report form RCT 9 should be annotated with Code 5 indicating that the lymph node was not examined. An appropriate code must be entered in the appropriate boxes of the post mortem examination report form.

9.0 THORACIC AND ABDOMINAL CONTENTS*Examination of thoracic contents.*

9.0.1 Make multiple longitudinal incisions to the lungs at intervals of approximately 1 cm. The surface of the pericardial sac must be examined.

Examination of abdominal contents.

9.0.2 The following abdominal viscera should be incised in several places and the cut surfaces examined:

- liver (3 to 4 slices)
- kidneys (2 to 3 longitudinal sections)

The surface of the kidneys should be examined after peeling off the outer membrane.

An appropriate code must be entered in the appropriate boxes of the post mortem examination report form.

If the badger is female, examine the uterus and determine if the animal is pregnant. Tick the relevant box in section 6 of the post mortem report form.

The macroscopic appearance of lesions

9.0.3 The macroscopic appearance of lesions found in tissues (other than skin) must be recorded. To avoid contamination of paperwork or the need to remove gloves, an assistant may be required to complete the form. The following numerical codes must be used:

CODE	LESION
0	No lesion
1	Single lesion
2	2 to 3 lesions
3	More than 3 lesions
4	Lesions profusely throughout tissues
5	Not examined / not found

10.0 COLLECTION OF SPECIMENS FOR TB CULTURE AND HISTOPATHOLOGY

Specimens for culture

10.0.1 Each carcass must be categorised as a result of the gross post mortem findings and a tissue sample collected and placed in 1% CPC as follows:

CARCASE CATEGORY	SAMPLE REQUIRED
No visible lesion	One half of each retropharyngeal and the entire bronchomediastinal chain. (NB This is defined as the standard sample.)
Bite wounds present	A section of the bite wound into a separate universal container.
Lymph node/visceral lesion present	Lesion and the standard sample in the <u>same</u> universal container.

10.0.2 All tissue specimens for culture must be submitted as follows:

- unfrozen, in 1% CPC transport media.
- in a universal container with 1% CPC in a ratio not greater than one part tissue to five parts CPC.

- material stored in CPC, if not despatched on the day of collection, should be left at room temperature overnight, then at approximately 4 °C thereafter. TISSUES IN CPC MUST NOT BE FROZEN.
- Despatch to culture laboratory on day of collection whenever possible.

11.0 SPECIMENS FOR HISTOPATHOLOGY

At the regional laboratory carrying out the post mortem examination.

11.0.1 A portion of each visible lesion should be placed in 10% formal saline unless the lesion is so small that it would compromise a tissue sample being collected for culture. If in doubt collection for culture is the priority.

11.0.2 The container should be identified with the laboratory reference number and the fixed samples should be dispatched to VLA [REDACTED] together with the CPC samples for culture or, when the latter are sent to VLA [REDACTED], separately.

At the culture laboratory

11.0.3 All fixed samples from visible lesions will be processed to block stage. All blocks will be archived according to the VLA Standard Operating Procedure. Sections will subsequently be prepared from those samples that do not yield *Mycobacterium bovis* on culture.

11.0.4 VLA-[REDACTED] will prepare and read H+E and ZN stained histological sections and complete the RCT 10 report.

12. ARCHIVING OF TISSUES

12.0.1 The VLA is responsible for archiving and maintaining a record of the following archived material:

- tissue homogenates arising from culture
- positive mycobacteria cultures
- histological blocks and stained sections derived therefrom

13.0 DESPATCH OF SAMPLES

13.0.1 All specimens must be accompanied by the completed RCT 10 form and should be sent to either:

- VLA - [REDACTED]
- VLA - [REDACTED]

Submitting laboratories will be given direction as to where their samples should be sent.

13.02 All samples sent through the post must comply with the current Post Office packaging requirements for pathological specimens and in accordance with the UN 602 regulations. A “red spot” placed on the outer cover of the package under the address label will alert the receiving laboratory to the nature of the contents and the appropriate safety procedures can be initiated. Non-trial samples should not be included in the same package.

14.0 DISPOSAL OF CARCASSES AND WASTE

14.0.1 Disposal of carcasses and associated clinical waste must be by incineration and in accordance with local waste disposal policies.

TOOTHWEAR ASSESSMENT

1. A visual assessment of toothwear is undertaken by the inspection of incisors. Incisors are more readily inspected and easier to view in profile. Molars may wear at a similar rate but visual assessment of their degree of wear is more difficult to assess. Only in aged animals does the effect of wear on molars become more readily apparent as they become worn level with the gums.
2. Classification must be in accordance with the scale set out in the following table:

CATEGORY	DESCRIPTION
0	This applies in cubs only. Incisors are tricuspid with sharp edges.
$\frac{1}{4}$	Cubs will usually only show this amount of toothwear in their second year (cubs at ██████████ in their first year are always recorded with zero toothwear). Incisors are no longer tricuspid. They will be worn flat across the tips but tooth height will not have been significantly reduced.
$\frac{1}{2}$	The flattened area of the incisors will be much more conspicuous than in the previous category as it extends into the broadening section of the tooth. Tooth height will have been reduced by 25-50%. Canines may show significant signs of wear but this can vary greatly from individual to individual within this category. Damage to canines may be common. Discoloration may be fairly common but shows considerable variation and is not a reliable indicator.
$\frac{3}{4}$	Wear to incisors will have reduced tooth height by 50-80%. Discoloration may be more extensive as will broken and worn canines.
1	Incisors will have been worn down close to or level with the gums and tooth height, consequently reduced by more than 80%. Discoloration will be very widespread as will be damaged and blackened teeth. By this stage molars may also be worn down close to gum level.

3. It is recognised that spatial variation in diet will influence the interpretation of toothwear as an indicator.

Badger tooth wear assessment

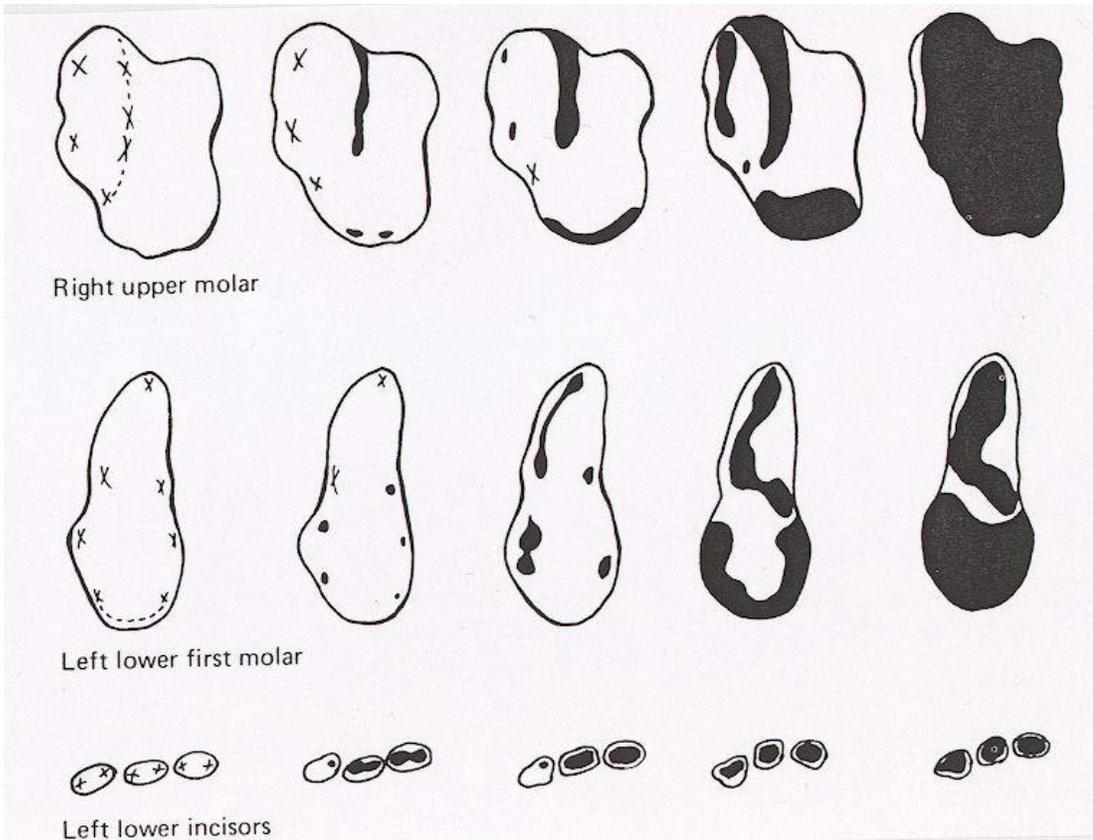
0

1/4

1/2

3/4

1



Safe working procedures for receipt, handling, post mortem and disposal of badgers

1. HAZARDS AND RISKS

1.10. Infectious agents

When handling badger carcasses there is the obvious potential risk from *Mycobacterium bovis* but the presence of other infectious agents is also a possibility. Susceptibility to these agents depends on the resistance of the individual, the route of entry into the body and the virulence and total dose of the organism. Main routes of infection are:-

- i) Contact - some infectious agents can penetrate unbroken skin or gain entry through mucus membranes, as well as through cuts and abrasions which may be so small as to escape notice.
- ii) Ingestion - ingestion of organisms can easily occur by the accidental transfer of infected material from fingers, equipment and pencils, pens, etc. to the mouth.
- iii) Inhalation - this is a common route of laboratory infection, some organisms can remain airborne, in aerosols, and infective for long periods. The type of work done in the post mortem room has considerable potential for aerosol production.

In the PM room, infection by *M. bovis* is most likely to be via inhalation, infection through ingestion is also a possibility but unlikely.

More detailed information on zoonotic infections is in **Inset 46 Section H**.

1.20. Physical agents

Apart from the risk of infection from biological agents, there is risk of physical injury from cutting instruments, sharp ends of bones, slippery floors, lifting and handling bags of carcasses and waste, disinfectants and electricity. Cut injuries are also susceptible to infection, stabs and those caused by bones particularly so.

2. CONTROLS AND PRECAUTIONS

Rigorous application of the following controls and precautions will ensure, as far as is reasonably practicable, that risk to staff from the hazards above are minimal.

2.10. BCG vaccinations

All staff who regularly handle animals, or material from animals, where the presence of tuberculosis is suspected must have received a BCG vaccination or have had a positive skin test. Tetanus inoculations should also be up-to-date.

2.20. Restricted entry - lone working

Entry to the PM room is restricted to authorised Veterinary, Scientific and Technical staff only. All others must be made fully aware of the restrictions and appropriate signs posted.

The number of staff in the PM room should be limited to those necessary to carry out the work but it is recommended that a minimum of two staff are present. Regular checks should be made on staff working alone. New or inexperienced staff should never work in the PM room alone.

2.30. Personal hygiene

A high degree of personal hygiene is necessary. Particular attention must be paid to the cleanliness of hands and of protective clothing. Hands must be washed thoroughly with soap and water following work in the PM room and before leaving the post mortem room area.

Staff must not smoke or consume food or drink of any kind while working in the PM room and care should be taken to avoid hand to mouth contact, e.g. by sucking or chewing ends of pens and pencils etc.

It is very important that all cuts, abrasions or other skin lesions are adequately protected with waterproof dressings.

2.40. Personal protection

Staff must wear the appropriate protective clothing in the post mortem room. This should be issued on a personal basis and be dedicated to PM room use.

Appropriate clothing will be waterproof and disinfectable, and may consist of jackets, trousers, gowns or aprons. Boots with slip resistant soles and gloves should also be worn. Gloves are either to be disposable PVC/vinyl or reusable rubber/nitrile. Airstream helmets are available and should be used.

Where knives and other sharp instruments are to be used it is strongly recommended that cut resistant liners be worn under the glove on the non-cutting hand.

Clothing should be stored clean in an ante-room or other designated clean area and always put on before entering the post mortem room. Protective clothing must be cleaned and disinfected after use.

No one should enter the PM room when work is in progress unless they are wearing protective clothing or leave the PM room while wearing contaminated clothing.

All Personal Protective Equipment (PPE) must be well maintained, regularly checked and be in a "ready to use" state at all times. PPE must be replaced as soon as it becomes damaged, worn or otherwise ceases to be effective.

2.50. Good housekeeping

Staff must be tidy and methodical in their work and limit the accumulation of equipment and materials particularly that which may hinder the cleaning of the room.

Paperwork or reports relating to badger carcasses which may subsequently have to be handled by others should not be allowed in the PM room.

Only sufficient equipment and materials for immediate use should be kept in the PM room, it should not be used as a storeroom. Any equipment (including pens and pencils) used in the PM room may become contaminated. For this reason it should be dedicated to PM use only.

3. RECEIPT

Badger carcasses should be submitted double bagged and appropriately labeled. The second bag must be sealed and the outside kept free of contamination. On arrival they should be taken directly to the post mortem room or, if not to be examined immediately, to the cold store.

Care should be taken in lifting and handling bags and safe lifting techniques employed. Staff should seek assistance where they are in any doubt about their ability to lift safely. Bags must not be stacked on top of each other.

4. EXAMINATION

The post mortem examination and collection of samples should be carried out according to the Randomised Badger Culling Trial (RBCT) SOP 11.

Bags containing badger carcasses must be opened in the PM room and transferred to a down draft table before commencing any examination. Instructions on the use and maintenance of down draft tables are provided in a separate document.

A quantity of fresh disinfectant* appropriately diluted ready for use should be available at all times for swabbing contaminated surfaces and in a separate container for discarding used instruments. Gloves and eye protection must be worn when handling concentrated disinfectants.

All work should be done carefully and methodically to avoid the production of aerosols.

Samples selected for sending to the TB culture laboratory should be taken according to the RBCT SOP 11. Great care must be taken to avoid contaminating the outside of sample containers and they should be placed in a clean polythene bag which should be sealed before being passed to the packing room.

4.10. Use of sharps

Precautions to be observed when using knives and other sharp instruments include:

- a) Staff working on specimens must be aware of the risk to themselves and others from sharp instruments and ends of cracked bone.
- b) Instruments and equipment must only be used for their intended purpose e.g. knives must not be used as chisels.
- c) Knives should be kept very sharp.
- d) Blunt ended scissors, in preference to pointed, and scissors, in preference to scalpels, should be used wherever practical.
- e) Ensure specimen or sample is properly secured before work on it commences.

- f) Instruments should not be passed hand to hand during an examination and no attempt should be made to arrest the fall of dropped instruments.
- g) Cutting movements upwards and/or toward the body should be avoided.
- h) A cut-resistant or chain mail glove should be worn on the non-cutting hand; this will considerably reduce the risk of cuts.
- i) Disposable blades and other disposable sharps must be placed immediately into an approved sharps container.

5. DISPOSAL

Badger carcasses should be incinerated on site or otherwise disposed of according to local carcass waste disposal procedures.

No more than three carcasses should be placed in one bag (**Note** - three large carcasses could exceed the 25kg lifting limit).

Clinical waste, including sharps, should be disposed of as soon as possible and not stored. If, in exceptional cases, clinical waste has to be stored this must be in a dedicated area in a lockable leak-proof container.

6. CLEANING AND DISINFECTION

At the end of work all work surfaces and the floor should be thoroughly disinfected and cleaned.

Instruments and other re-usable equipment should be placed in disinfectant* for a period of 30 minutes. They should then be sterilised by autoclaving, prior to final cleaning.

The down draft tables should be decontaminated and cleaned following SOP GE455.A.

Cleaning and disinfection of the PM room must be done thoroughly but carefully. Hosing down especially when water is directed at contaminated surfaces may raise aerosols and the water flow should never be violent. The risk of eye contamination by disinfecting chemicals must be borne in mind. Great care must be taken to avoid wetting or splashing electrical apparatus and connections and outlets should never be hosed down.

** The chosen disinfectant must be approved for use in respect of Tuberculosis Orders. "Virkon" is not. FAM 30 (and most other iodophors) is approved but will eventually stain cabinet and equipment yellow. Phenolics are effective and most are approved - "Hycolin" is a clear phenolic that is reasonably easy to use.*

7. GOOD PRACTICE

Staff must abide by rules which govern the operation of the PM room. They should not put themselves or colleagues at risk by practicing faulty techniques, being careless with tools and equipment, or deviating from the accepted system of entry and exit. Any lowering of standards could result in the spread of contamination to clean areas of the laboratory, office or even beyond.

NB: Staff should be fully familiar with information on laboratory/PM room safety in

“Safety in Veterinary Laboratories and COSHH Risk Assessments before any work is undertaken.

Thawing of frozen badgers.

1. Remove from the freezer the number of badgers required for a day's post mortem examinations and place in the cold store at 4°C; preferably on the shelves. If it is not possible to put them onto the shelves spread them on the floor with space between each.
2. Leave for four days to thaw.
3. At the end of the fourth working day remove the badgers from the cold store and spread them on the floor of the post mortem room with space between them. Leave them overnight for processing the next day.
4. Proceed with the post mortem examination according to the protocol described in SOP 11 and this VISI.