COLLECTION AND SUBMISSION OF DIAGNOSTIC SPECIMENS TO THE FAO WORLD REFERENCE LABORATORY FOR RINDERPEST

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Introduction

In October 1994, the FAO World Reference Laboratory for Rinderpest (WRLR) was established at the Institute for Animal Health, Pirbright, UK. The main aims of the WRLR are to provide a diagnostic service for all countries involved in the Rinderpest Eradication Campaigns; establish a library of rinderpest virus strains; undertake molecular epidemiological studies to identify origins of outbreaks and further understanding of the epidemiology of the disease and to introduce standardisation of the diagnostic techniques used in rinderpest diagnosis. This rinderpest diagnostic service is provided free to all member countries of the FAO. In order to fulfill this role, it is imperative that the WRLR receives as many samples, in the best possible condition, as possible to create a library of rinderpest virus strains and enable molecular epidemiological studies.
Collection of Specimens

The laboratory confirmation of rinderpest can be achieved by several methods which detect live virus, virus antigens, virus genetic material or antibodies directed against the virus. Characterisation of rinderpest viruses relies primarily on isolating live virus and/or examining viral RNA. Maximising the chances of a successful result and information gained requires different samples. Paired samples should be submitted, one chilled sample, in transport medium if indicated, for virus isolation or further pathogenesis studies, and another fixed in 10 per cent buffered formol saline. The latter will survive extremes of temperature and still allow molecular biological studies and strain differentiation. Every effort must be made to obtain both fresh and formalin fixed material.

The key to diagnostic success is the examination of as many samples as possible from several sick animals. The crucial factor is the selection of suitable donor animals.

Donor Selection

Virus is first shed in the excretions and secretions of the infected Animals towards the end of the incubation period, before the onset of illness. It continues to be shed throughout the prodromal fever, the mucosal erosion phase and the diarrhoeic phase. Shedding of virus stops in early convalescence, a few days after the fever has regressed. The infectious period, therefore, lasts at most from 10 to 16 days. The optimal period for collecting suitable diagnostic specimens, however, is much shorter because viral titres peak before or at the onset of fever and antigens peak early in the mucosal erosion phase. Dead animals are poor donors of diagnostic samples and are best avoided unless no other source of samples is available. Similarly, animals late in the course of disease, distressed by mucopurulent nasal and ocular discharges, and soiled animals voiding fetid, fluid faeces are less likely to yield positive diagnostic samples. The most suitable live donors of samples for virus isolation or antigen detection are febrile, have mucosal erosions and clear lachrymal secretions.

The first rinderpest-specific humoral antibodies belong to the IgM class of immunoglobulins and emerge toward the end of the mucosal erosion phase or in the early diarrhoeic phase of the disease. A few days later, rinderpest-specific antibodies are also found in the IgA and IgG classes of animals which have been affected for the last 10 days.

Samples for live animal
The specimens required for diagnosis from the selected live animals are biopsy samples of peripheral lymph nodes, gum debris, tears, and clotted blood. All tissue samples or gum debris should be kept chilled and transport medium (phosphate buffered salin pH 7,6 (PBS) containing antibiotics and fingizone - BUT NO GLYCEROL) may be added to help preserve the specimens. For virus isolation samples should be transported as quickly as possible chilled, not frozen. If samples are to be stored for long periods, they should be frozen at -70° C not -20° C.

Sample as many animals and as many tissues as possible, to increase the chance of virus isolation and detection.

**Lymph nodes**

A peripheral lymph node should be located and grasped firmly through the skin. A wide-bore needle (18 gauge) with its stilette in place should be thrust into the parenchyma of the node. Remove the stilette and attach a 10 or 20 ml syringe, pre-wetted with a drop of Heparin Injection BP. Aspirate a plug of tissue into the syringe and eject into a suitable container containing 0,5 ml of transport medium.

**Gum debris**

Collect the necrotic debris coating eroded gums on a spatula or finger rubbed across the gums and inside the lower and upper lips. Scrape off into a convenient container.

**Tears**

Collect tears onto cotton buds swabs inserted into and twirled around the conjuctival sac behind the eyelids. Break off the bud or swab into a suitable container (glass or plastic universal bottle) and add 150 µl of PBS (Appendix 2).

**Clotted blood**

Collect blood for serum into plain, sterile containers, preferably silicone-coated, and allowed to clot undisturbed for a period of at least 24 hours. Separate serum by centrifugation, transfer to suitable clean, sealed containers and store at 4° C or below.

**Post-mortem specimens**

**Slaughtered animals**

Select the least two animals for slaughter and proceed as follows:

1. Select the slaughter site carefully to avoid contaminating the carcass, organs and tissues.
2. Stun, pith, exanguinate and necropsy the selected animals.

3. Remove the spleen and cut it into strips sufficient to fill three labelled and chilled 30-ml screw-capped bottles. Identify, seal and immerse the bottles immediately in wet (i.e. melting) ice in an insulated box of flask. Cut thin slices of the spleen (no more than 1 cm thick) and immerse them in formol saline (Appendix 1).

4. Using similar sterile precautions, Collect enough carcass lymph nodes, particularly mesenteric lymph nodes, to fill three labelled and chilled screw-capped bottles. Identify the bottles and place them in wet ice. As before, cut thin slices for fixation in formol saline.

5. Dissect out as much tonsilla tissue as possible and distribute aliquots among three labelled and chilled screw-capped bottles, which should then be identified and inserted into the wet-ice container. Fix thin slices of tonsillar tissue in formol saline. Portions of affected mucosae in the alimentary, respiratory and urogenital tracts should also be collected and fixed in formol saline.

**Dead animals**

Where possible, all dead animals should be necropsied. Fresh, clean carcasses of animals that have died early in the course of the disease are worth sampling by collecting aliquots of spleen, lymph nodes and tonsils for antigen-detection tests. Slices of these tissues together with portions of effected mucosae should also be fixed in formol saline for histopathological examination. Animals that have died late in the course of the disease are soiled and fetid and are usually emaciated and dehydrated. They should be examined to supplement the clinical findings but their tissues should only be collected for histopathology. Decomposed animals are not worth examining.

**Labelling and storage of samples**

Sample bottles must be labelled clearly in waterproof ink on good quality adhesive tape. These should be done at the time of collection, together with the writing of comprehensive field notes (see Appendix 4). Tissues placed in fixative require no further care other than ensuring that screw-caps are tightly in place and that gross overheating does not occur.

Serum samples can be stored indefinitely in clean crew-capped bottles at -20 °C or, if a freezer is not available, for several weeks at 4 °C.

All the samples should remain chilled on ice until they are returned to the laboratory. Transport medium is not strictly necessary for trasporting solid tissues, as these contain sufficient protein to protect the virus and are not amenable to buffering. However, a small amount of transport medium (PBS with high levels of antibiotics and fungistat may be added to combat surface contamination and dehydration. Glycerol should not be included because it kills rinderpest virus.
Packaging and Despatch of Biological Materials to the WRLR

The UK Ministry of Agriculture Fisheries and Food licence allow importation of biological specimens to IAH-Pirbright by AIR FREIGHT, normally to London, Heathrow Airport. This must be followed by customs clearance and collection by authorised staff from IAH-Pirbright Laboratory.

In order to achieve the safe and speedy passage of biological materials from overseas countries to the Pirbright Laboratory it is necessary to observe the following:

1. Contents

Before sending materials, the sender must contact the WRLR by facsimile or telephone notifying the intention to send samples and to check the samples required and the conditions for despatch. The parcel must only contain material which is to be processed at the Pirbright Laboratory, as none of the contents are allowed to be sent to other laboratories in the United Kingdom from the Pirbright Laboratory.

2. Packaging

The material must be packed in a leak-proof container in such a way that the contents arrive in a satisfactory condition and that, should a container break in transit, none of its contents will leak and contaminate the outside layer of the package. To achieve this the primary container must be placed in a strong secondary container and the space between the two containers hold sufficient absorbent material to completely absorb the fluid which is present in the primary container. The lids on both containers must make effective, leak-proof seals. Sealed freezer packs should be used for refrigeration. The ideal primary container is a glass "universal" bottle (25 to 30 ml capacity) with a metal screw cap fitted with a strong rubber washer; plastic disposable "universal" bottles are also suitable. Tape should be wound tightly around the cap to prevent leakage. The bottle should be wrapped in absorbent cotton wool or corrugated paper to protect both the sides and the ends of the bottle. The wrapped bottle should then be inserted into a secondary container, with further padding. The secondary container should be leak-proof, preferably with a screw-cap and rubber washer. The secondary container should then be packed in a solid outer covering to prevent distortion. Labels should be clear and comply with International Transport Regulations.
3. Labelling

The external label must be clearly addressed and indicate that the packet contains Animal Pathological Material of no commercial value and that it is to be collected from the airport by the Addresse. **Use appendix 6 for the external label.**

4. Despatch

All such materials **MUST** be sent by **AIR FREIGHT. BEFORE** despatch the sender **MUST** notify the WRLR Secretary at Pirbright (Fax. No. +44-1483-232621; Tel. No. +44-1483-232441/232446) of details of the Airway Bill Number, the flight number and the time and date of its arrival in the UK. The Secretary then arranges for **OUR** freight handlers at the airport to "clear" the parcel through customs and subsequently hand the parcel over to our driver - hence no commercial freight companies are involved other than the airline. Normally such parcels should be sent to London Heathrow (or London Gatwick if the airline concerned does not fly to Heathrow).
Appendix 1

Preparation of 10% neutral buffered formalin

Mix one part 1 formalin (40% formaldehyde solution) with 9 parts of phosphate buffered saline pH 7.6.

Appendix 2

Preparation of phosphate buffered saline (calcium, magnesium free)

Sodium chloride 8.00 g
Potassium chloride 0.20 g
Sodium hydrogen orthophosphate 1.15 g
Potassium dihydrogen orthophosphate 0.20 g

Dissolve in pure water and make up to 800 ml.

Appendix 3

Transport medium

Phosphate buffered saline pH 7.6 (PBS) containing antibiotics and fungizone -
Appendix 4

Field record sheet

TO BE COMPLETED BY FIELD OFFICER

<table>
<thead>
<tr>
<th>Your reference:</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAME AND ADDRESS</td>
</tr>
<tr>
<td>OF OWNER:</td>
</tr>
<tr>
<td>LAT. AND LONG.:</td>
</tr>
<tr>
<td>NUMBER, SPECIES AND</td>
</tr>
<tr>
<td>BREED OF STOCK HELD:</td>
</tr>
<tr>
<td>TYPE OF HUSBANDRY:</td>
</tr>
<tr>
<td>Material sent</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>DETAILS INCLUDING AGE</td>
</tr>
<tr>
<td>OF STOCK AFFECTED:</td>
</tr>
<tr>
<td>EXTENT, SEVERITY AND</td>
</tr>
<tr>
<td>DURATION OF OUTBREAK:</td>
</tr>
<tr>
<td>POSSIBLE ORIGIN:</td>
</tr>
<tr>
<td>PREVIOUS HISTORY OF INFECTION, VACCINATION, INCLUDING TYPES (if known):</td>
</tr>
<tr>
<td>REMARKS (wildlife contact etc.):</td>
</tr>
<tr>
<td>NAME AND SIGNATURE:</td>
</tr>
<tr>
<td>DESIGNATION:</td>
</tr>
</tbody>
</table>
Appendix 5

Packaging

Packaging biological material to be sent to Pirbright Laboratory, UK.

- Sample in watertight primary container
- Wrap each container individually
- Labelled watertight secondary packaging (crush and leak-proof)
- Absorbent material
- Outer packaging (strong box or container)

To: Institute for Animal Health
Pirbright Laboratory
UK
Appendix 6

Label for submitting samples