Collection and transportation of specimens for vesicular virus investigation

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Summary: A description is given of the recommended methods for submitting specimens to the World Reference Laboratory, Pirbright, for the investigation of vesicular virus infection. Specimens of epithelium, vesicular fluid, whole blood with anticoagulant, semen or probang samples are suitable for virus isolation. Serum samples may be submitted for estimation of vesicular virus antibody levels. Emphasis has been placed on the security of the packaging of specimens and the conditions under which they must be maintained in order to ensure a satisfactory result.

KEYWORDS: Aphthovirus - Diagnostic techniques - Research institutes - Specimen handling - Standardization - Swine vesicular disease virus - Vesicular stomatitis virus - Viral diseases.

INTRODUCTION

In 1958 the World Reference Laboratory for Foot-and-Mouth Disease (FMD) was established at the Research Institute (Animal Virus Diseases) at Pirbright — later renamed the Animal Virus Research Institute (1963) — following negotiations between the Government of Great Britain and the Food and Agriculture Organization of the United Nations (FAO). Subsequently, in 1960, it was also recognized by the Office International des Epizooties as the World Reference Laboratory for FMD.

The World Reference Laboratory undertakes as its primary function the investigation of specimens from outbreaks of vesicular disease to establish whether FMD virus or one of the other vesicular viruses (swine vesicular disease virus, vesicular stomatitis virus) is involved. In consequence, the Laboratory now holds and maintains the most comprehensive collection of vesicular viruses, particularly FMD virus strains, from throughout the World. Reference antisera to many of these strains of virus are available and can be supplied to other laboratories to enable them to undertake the identification of their own local isolates of virus. Since its establishment in 1958, the World Reference Laboratory has received in excess of 14,200 samples from more than 98 countries for diagnostic purposes.

This diagnostic service for vesicular disease of animals is provided free to all member countries of the FAO. Results of tests are reported rapidly, usually by telex. The FAO is also informed as a matter of routine, as is the OIE, which transmits this information to its Member Countries.

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The accumulation of FMD virus isolates at the World Reference Laboratory provides a valuable library of different virus strains past and present, and allows for the rapid identification of suitable vaccine strains for the control of outbreaks. It also provides epidemiological data on the risk of spread of disease through regions and can warn those countries which vaccinate against FMD of the approach of emergent strains not adequately covered by their routine vaccination regime. It can also advise those countries that do not normally vaccinate of any approaching threat to their livestock.

In addition, the World Reference Laboratory can undertake the examination of sera for FMD, swine vesicular disease and vesicular stomatitis disease virus antibodies to determine freedom from infection, or otherwise, of animals involved in international trade movement. Probang (oesophageal-pharyngeal) samples and also semen samples can be tested for evidence of FMD virus. A charge is, however, made for the testing of sera, semen and probangs.

This paper is intended to update previous protocols described by Brooksby, Davie and Hedger (1) for the submission of suspected vesicular virus specimens and serum samples to the World Reference Laboratory. The paper is also in response to the numerous requests from visitors to the Laboratory for detailed descriptions of how best to submit samples both to ensure a satisfactory result and to minimize the risks associated with the transportation of infectious material.

**ESSENTIAL REQUIREMENTS FOR ALL SPECIMENS**

The sending of pathological material and biological products to the World Reference Laboratory, whether from within the UK or overseas, is subject to the special rules concerning packaging stipulated for perishable biological material by the Universal Postal Convention established by the Universal Postal Union (Article 1.3.6.3. Measures Concerning International Transfer of Pathological Material and Biological Products). Airline companies carrying pathological material and biological products may also have special requirements and these should be consulted prior to shipment.

These regulations, although designed to prevent leakage and consequent contamination by materials sent in this way, are also important in helping to ensure that the specimens arrive in a satisfactory state for laboratory examination. An improperly packed or identified specimen is not only illegal but may also cause considerable inconvenience and wastage of time both for those involved in its collection and dispatch as well as for the staff of the World Reference Laboratory.

The basic principles are that any specimen is fresh, within a suitable container (and transport medium if appropriate), and securely contained in sturdy packing of which at least two layers should be watertight containers. The inner container should be labelled with a description of the specimen and its origin (Fig. 1). The outside of the final layer of packing material must have the address of the World Reference Laboratory, indicate that the package contains pathological material, that it is fragile, and have instructions as to the temperature at which it should be kept.
INSTRUCTIONS FOR COLLECTION AND PACKAGING OF SPECIMENS

Suitables samples for virus isolation are epithelium and vesicular fluid from unruptured and ruptured vesicles (a suggested minimum of 2 cm$^2$ of fresh tissue from recently affected areas), whole blood with anticoagulant, semen and probang samples. Sera only should be sent for estimation of vesicular virus antibody levels. Tissues such as muscle and lymph nodes from infected animals may contain virus but the amount of infectivity may be insufficient to isolate virus or identify antigen by *in vitro* procedures and, as negative results may be misleading, such specimens are not accepted for investigation.

EPITHELIUM/VESICULAR FLUID

**Collection**

The specimens should be suspended in a mixture of equal amounts of glycerine and 0.04 M phosphate buffer pH 7.2-7.6 (Appendix 1), preferably with added antibiotics (Appendix 2). There will be considerable loss of infectivity if samples are sent in buffer outside of this pH range.

**Packaging** (Fig. 1)

![Fig. 1](image)

*Fig. 1*  
Items recommended for use in packaging epithelium, vesicular fluid, whole blood or serum
Starting with the innermost container, the recommended procedure for packing samples is as described below. Alternative methods of packing are acceptable if they provide a similar level of security against breakage and/or leakage.

1. A strong glass container should be used with a metal screw cap fitted with a strong rubber washer or wad. The best container of this type is a 20 ml Universal bottle. Tape should be used around the cap in order to prevent leakage of fluid.

2. Sufficient information for the identification of the material should be written on a piece of waterproof adhesive tape attached to the bottle. The outside of the bottle should then be disinfected before proceeding further.

3. The bottle should be wrapped in absorbent cotton wool or lint or in corrugated paper, arranged to protect the ends as well as the sides of the bottle. The wrapping of the bottle in the various containers should be completed in clean surroundings.

4. The wrapped bottle should be inserted in a metal container in which it fits snugly. If collection takes place at a point which is some distance from the point of dispatch by air, the sample should be kept refrigerated. This container should also be labelled.

5. The metal container should be fluid-tight, preferably with a screw cap and a rubber washer. If such a container is not available, a tin with a tight-fitting lid which can be soldered on should be used.

6. The metal container should be placed in a solid outer covering to prevent distortion. A solid cardboard tube closed with a metal cap at each end is to be preferred but, as an alternative, a wooden box with a metal cap is equally satisfactory.

7. Sturdy wrapping paper secured by adhesive tape or string should be used and labels should be clear and comply with the International Transport Regulations.

8. Information that should be included on the label:

PATHOLOGICAL MATERIAL OF NO COMMERCIAL VALUE
World Reference Laboratory for Foot-and-Mouth Disease, Institute for Animal Disease Research, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey GU24 0NF UNITED KINGDOM.
PERISHABLE FRAGILE TO BE COLLECTED AT AIRPORT BY ADDRESSEE KEEP AT 4°C CENTIGRADE

WHOLE BLOOD

Collection

Blood samples should be collected under sterile conditions and mixed as soon as possible with the anticoagulant heparin (0.1-0.2 mg per ml of whole blood). Sequestrene EDTA can be used as an alternative anticoagulant (30 mg of Sequestrene EDTA in one ml of 0.7% aqueous solution sodium chloride per 20 ml of whole blood). The sample should be kept at 4°C until dispatched to the World Reference Laboratory.

Packaging

As described for specimens of epithelium/vesicular fluid.
PROBANG SAMPLES

Collection

Prior to sampling, a 2 ml amount of 0.08 M phosphate buffer* containing 0.01% bovine serum albumin, phenol red (0.002%) and antibiotics adjusted to pH 7.2 (Appendix 3) should be added to one bijou bottle or similar container for each of the animals to be sampled. Each bijou bottle should be identified with a waterproof label.

After collection, the sample should be poured from the "probang" cup into a wide-necked bottle such as a 20 ml Universal bottle and examined visually for quality. Two ml, which should contain some visible cellular material, should then be added to the previously prepared bijou bottle containing an equal volume of buffer and thoroughly mixed by gentle shaking. The final pH of a normal sample thus treated should be ±pH 7.6.

Samples taken from some animals may be heavily contaminated with ruminal contents — these should be discarded and the animal's mouth should be flushed with water or physiological saline solution before repeat sampling.

Samples from sheep tend to be small, mucoid and difficult to detach from the probang. The easiest procedure is to insert the probang cup directly into a disposable Universal 20 ml bottle or similar container into which has been dispensed 3 ml of buffer solution. The probang is then shaken in the buffer solution to free the sample from the cup, and the sample together with the buffer should be poured into a previously labelled bijou bottle for transport.

Between collections from each animal, probangs should be disinfected in a bucket containing 4% Na$_2$CO$_3$ or 0.2% citric acid. After disinfection, the probang should be thoroughly rinsed in running tap water or at least three separate buckets of clean water placed in series.

Packaging

It is important to avoid thawing and refreezing at any stage, so packing should be done quickly and complete packages should be kept at −70°C or lower.

1. The outside of the bijou bottle containing the sample should be disinfected by immersion in 4% Na$_2$CO$_3$ or 0.2% citric acid, dried off, labelled and then frozen immediately in solid CO$_2$ within an insulated box or in a −70°C refrigerator.

2. For shipment the bijou bottles should be placed in a metal container within an insulated box of approximately 1 ft$^3$ (0.3 m$^3$), which is filled with dry ice. The container should be placed in the middle of the dry ice. The dry ice must be sufficient to last the journey to IADR. The insulated box should be wrapped and sealed and properly labelled. It should permit some escape of gas, otherwise there is a risk of explosion (see packaging of specimens of epithelium, paragraph 8.)

* Instead of phosphate buffer, tissue culture medium (e.g. Eagle's MEM or Earle's LYH) containing 0.04 M HEPES buffer has also been found satisfactory.
3. If insufficient dry ice has been used, and it has been exhausted by the time the samples are unpacked at IADR, further samples will be requested.

**SEmen Samples**

**Collection and Packaging**

Semen should be collected under sterile conditions, frozen at \(-70^\circ\text{C}\) or lower as soon as possible and packaged as described for probang samples.

**Serum Samples**

**Collection**

Serum, rather than whole blood, must be submitted. A minimum of 4 ml is essential. Should lesser amounts be submitted there is a risk that re-sampling may be required with subsequent delay.

It is essential that sterile containers be used. If sera have been collected under sterile conditions they can normally travel satisfactorily without refrigeration.

It is imperative that those submitting sera appreciate that a period of three weeks is required from the time sera are received at IADR before results will be available — this time period has been found necessary to allow for possible retesting or re-submission of sera. At least ten working days advance notice of submission of serum for testing is required.

**Packaging**

If sterile, samples can be submitted without refrigeration, but refrigeration or freezing is an added safeguard against spoilage. Samples of serum should be packaged as described for specimens of epithelium.

**PROCEDURE FOR DISPATCH OF SPECIMENS TO THE WORLD REFERENCE LABORATORY**

1. Dispatch from outside the United Kingdom should only be by air freight. The World Reference Laboratory makes arrangements to collect material from the London airports at Heathrow and Gatwick and has the necessary import permits to facilitate customs clearance.

Wherever possible, containers should be sent by British Airways so that airport clearance charges incurred by the World Reference Laboratory can be kept to a minimum.

2. The flight number, date and time of arrival and airway bill number should be sent by telex to the World Reference Laboratory in advance of arrival (Telex No. 859137 AVRIG).

3. A letter should accompany the specimens giving as much history and epidemiological information as possible. In addition, forms similar to those shown in Appendix 4 and Appendix 5 should be sent with specimens for virus isolation or antibody assay, respectively.
4. Accompanying information must include the name, address and telex number of the sender and instructions for notification of results.

5. It is emphasized that the continued success of this service very much depends upon the active participation of all countries to ensure that the data bank of the Laboratory is up-to-date, thus making possible the provision of the most recent and relevant information on particular foot-and-mouth disease field situations.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the valuable advice provided by staff of the World Reference Laboratory, in particular Mr I.T.R. Barnett and Mr N.P. Ferris.

REFERENCES


Appendix 1

COLLECTING MEDIUM FOR SPECIMENS OF EPITHELIUM

0.04 M phosphate buffer
Add 3.05 gm Na$_2$HPO$_4$.2 H$_2$O
0.39 gm KH$_2$PO$_4$
to 500 ml sterile distilled water.
Add 1 ml 1% phenol red.
Add antibiotics — see Appendix 2.
Adjust pH to 7.2-7.6 with HCl.

Appendix 2

ANTIBIOTICS

Reconstitution

Penicillin phial of 500,000 units add 2.5 ml sterile distilled water
Mycostatin phial of 500,000 units add 10 ml sterile distilled water
Neomycin phial of 500,000 units add 10 ml sterile distilled water
Polymyxin phial of 350,000 units add 7 ml sterile distilled water
Appendix 3

COLLECTING MEDIUM FOR PROBANG SAMPLES

0.08 M phosphate buffer
Add 6.11 gm Na₂HPO₄·2H₂O
0.78 gm KH₂PO₄
to 500 ml sterile distilled water.
Add 1 ml 1% phenol red.
Add antibiotics — see Appendix 2.
Adjust pH to 7.2-7.4 with HCl.

Appendix 4

Specimen of Submission Form to accompany samples to the World Reference Laboratory for vesicular disease diagnosis

<table>
<thead>
<tr>
<th>FOOT-AND-MOUTH DISEASE SAMPLE FOR TYING</th>
</tr>
</thead>
<tbody>
<tr>
<td>From: (Territory)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FOR LABORATORY USE ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description of Sample</td>
</tr>
<tr>
<td>ELISA</td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
<tr>
<td>3.</td>
</tr>
<tr>
<td>4.</td>
</tr>
<tr>
<td>5.</td>
</tr>
<tr>
<td>Remarks:</td>
</tr>
</tbody>
</table>
TO BE COMPLETED BY FIELD OFFICER.

<table>
<thead>
<tr>
<th>Your Ref.:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NAME AND ADDRESS</td>
<td>OF OWNER: —</td>
</tr>
<tr>
<td>LAT. AND LONG.: —</td>
<td></td>
</tr>
<tr>
<td>NUMBER, SPECIES, AND BREED OF STOCK HELD: —</td>
<td></td>
</tr>
<tr>
<td>TYPE OF HUSBANDRY: —</td>
<td></td>
</tr>
<tr>
<td>MATERIAL SENT</td>
<td>DATE COLLECTED</td>
</tr>
<tr>
<td>DETAIL(S), INCLUDING AGE, OF STOCK AFFECTED: —</td>
<td></td>
</tr>
<tr>
<td>EXTENT, SEVERITY, AND DURATION OF OUTBREAK: —</td>
<td></td>
</tr>
<tr>
<td>POSSIBLE ORIGIN: —</td>
<td></td>
</tr>
<tr>
<td>PREVIOUS HISTORY OF INFECTION, OR VACCINATION, INCLUDING TYPES, IF KNOWN: —</td>
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</tr>
<tr>
<td>REMARKS: GAME CONTACTS, ETC: —</td>
<td></td>
</tr>
<tr>
<td>SIGNATURE: —</td>
<td></td>
</tr>
<tr>
<td>DESIGNATION: —</td>
<td></td>
</tr>
</tbody>
</table>
Submission Form to accompany samples from animals for international trade purposes to World Reference Laboratory (Exportation of Animals)

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>DATE OF LETTER</th>
<th>LABORATORY REF:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SENDER'S REF:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DATE RECEIVED:</td>
</tr>
</tbody>
</table>

**EXPORTATION OF ANIMALS**

**OWNER OF ANIMALS:**

**ADDRESS:**

**VETERINARIAN'S**

**ADDRESS:**

**TRADING COMPANY:**

**ADDRESS:**

**TELEPHONE No.**

**TELEX No.**

**CABLE ADDRESS**

**ANIMAL SPECIES**

**NUMBER**

**COUNTRY OF ORIGIN**

**DESTINATION**

**DATE OF QUARANTINE**

**EXPECTED DATE OF EXPORTATION**

**TYPE OF SAMPLES:**

<table>
<thead>
<tr>
<th>SERUM</th>
<th>SEMEN</th>
<th>PROBANG</th>
<th>OTHER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(SPECIFY)</td>
</tr>
</tbody>
</table>

**DATE OF SAMPLE COLLECTION**

**TEST REQUIRED:**

- **SERUM ASSAY:**
  - FMD (SPECIFY TYPE)
  - SVD
  - VSV

- **VIRUS ISOLATION:**
  - FMD
  - SVD
  - OTHERS (SPECIFY)

**TYPE OF TEST REQUIRED:**

- SERUM NEUTRALIZATION TEST
- COUNTER IMMUNO ELECTROPHORESIS
- DOUBLE IMMUNO DIFFUSION
- ELISA

**NAME OF VETERINARY SURGEON SUBMITTING SAMPLES:**

<table>
<thead>
<tr>
<th>ANIMAL No.</th>
<th>TUBE/SAMPLE No.</th>
<th>BREED</th>
<th>SEX</th>
<th>AGE</th>
<th>LABORATORY No.</th>
</tr>
</thead>
</table>