INSTRUCTIONS FOR COLLECTION OF SPECIMENS FOR FMD DIAGNOSIS AT THE WORLD REFERENCE LABORATORY

Suitable samples for virus isolation are epithelium and vesicular fluid from unruptured and ruptured vesicles (a suggested minimum of 2 cm² of fresh tissue from recently affected areas), heart muscle from myocarditis cases, 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 should only be submitted if others are not available. All samples should be kept cool during primary submission from the field to the laboratory where they are to be repacked for onward transmittal to the World Reference Laboratory for FMD (WRLFMD).

EPITHELIUM/VESICULAR FLUID/HEART MUSCLE

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 I), preferably with added antibiotics (Appendix 2). There will be considerable loss of infectivity if samples are sent in buffer outside of this pH range. If to be stored for periods of more than a week prior to submission to WRLFMD, the samples should be kept at -20°C (the glycerol will prevent freezing). Specimens can be dispatched to WRLFMD at room temperature or with cool packs if the ambient temperature is high.

WHOLE BLOOD

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 WRLFMD and sent with cool packs if the ambient temperature is high.

PROBANG SAMPLES

Prior to sampling, a 2 ml amount of 0.08 M phosphate buffer¹ 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 probang should be poured into a previously labeled bijou bottle for transport.

Between collections from each animal, probangs should be disinfected in a bucket containing 4% Na₂CO₃ 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.

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. Preferably, samples should be frozen immediately after collection using solid CO₂ and solid CO₂ must be used to keep the samples frozen whilst in transit to WRLFMD.

SEMEN SAMPLES

Semen should be collected under sterile conditions, frozen at -70°C or lower as soon as possible and packaged as described for probang samples.

SERUM SAMPLES

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, but refrigeration or freezing is an added safeguard against spoilage.

CELL CULTURE ISOLATES

¹ 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
Supernatant fluids from cell cultures showing FMDV induced cytopathic effect should be submitted frozen and solid CO₂ must be used to keep the samples frozen whilst in transit to WRLFMD.

**INFORMATION ABOUT THE SAMPLES**

It is essential that each sample is accompanied by a submission form detailing relevant information about the species of origin, date of collection, history of the outbreak, clinical and epidemiological data, use of vaccination, etc and an electronic submission form is available from our website at [http://www.wrlfmd.org/find_diagnosis/sample_submission.htm](http://www.wrlfmd.org/find_diagnosis/sample_submission.htm)

**REFERENCE**


**Appendix 1**

**COLLECTING MEDIUM FOR SPECIMENS OF EPITHELIUM**

<table>
<thead>
<tr>
<th>0.04 M phosphate buffer</th>
<th>Add 3.05 gm Na₂HPO₄.2H₂O</th>
<th>Add 0.39 gm KH₂PO₄</th>
<th>to 500 ml sterile distilled water.</th>
<th>Add 1 ml 1% phenol red.</th>
<th>Add antibiotics –see Appendix 2.</th>
<th>Adjust pH to 7.2-7.6 with HCl.</th>
</tr>
</thead>
</table>

**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

**Probang and epithelium**

To each 500 ml of 0.08 M or 0.04 M phosphate buffer add the following amounts of reconstituted antibiotics:

- Penicillin 2.5 ml (final concentration 1000 units/ml)
- Mycostatin 1.0 ml (final concentration 100 units/ml)
- Neomycin 1.0 ml (final concentration 80 units/ml)
- Polymyxin 0.5 ml (final concentration 50 units/ml)

**Appendix 3**

**COLLECTING MEDIUM FOR PROBANG SAMPLES**

<table>
<thead>
<tr>
<th>0.08 M phosphate buffer</th>
<th>Add 6.11 gm Na₂HPO₄.2H₂O</th>
<th>Add 0.78 gm KH₂PO₄</th>
<th>to 500 ml sterile distilled water.</th>
<th>Add 1 ml 1% phenol red</th>
<th>Add antibiotics –see Appendix 2.</th>
<th>Adjust pH to 7.2-7.4 with HCl.</th>
</tr>
</thead>
</table>