

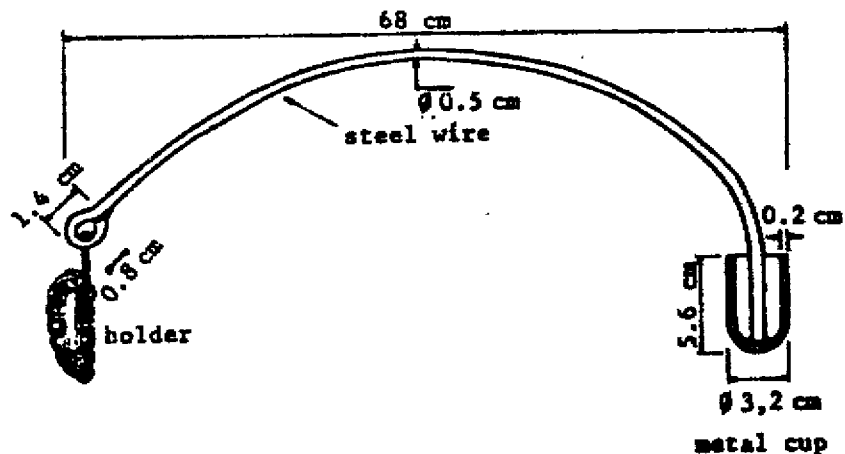
Clinical Disease Suspected

Specimen

- |                             |                                                                                                                                 |
|-----------------------------|---------------------------------------------------------------------------------------------------------------------------------|
| 30. Sheep Pox               | Blood (clotted and unclotted);<br>smears from fresh lesions; skin<br>lesions (fixed and fresh/<br>chilled for viral isolation). |
| 31. Swine Vesicular Disease | See Foot-and-Mouth Disease.                                                                                                     |
| 32. Teschen Disease         | Blood (clotted and unclotted);<br>brain, half thru mid-line<br>(fresh, frozen for viral isolation)                              |
| 33. Vesicular Exanthema     | See Foot-and-Mouth Disease.                                                                                                     |
| 34. Vesicular Stomatitis    | See Foot-and-Mouth Disease.                                                                                                     |

- 4.2 Cleaning before taking sample material - If the areas from which samples are taken are dirty e.g. feet, it is advisable to wash the area with water. Do not use soap, alcohol or other disinfectants as any existing virus may be destroyed.
  - 4.3 Opening a vesicle - A vesicle should be opened with a sharp-pointed pair of scissors; be cut along the edge of the lesion and the piece of epithelium removed with a pair of forceps.
  - 4.4 Weight of sample material - The sample material from an animal should weigh about 5 grams (equivalent to 2 square inches —13 sq cm) of tongue epithelium. The sample may be made up of several pieces of epithelium from the same animal in order to provide the required weight.
  - 4.5 Mixing of sample material - Each bottle must contain samples from only one animal. It is recommended that material from the mouth (tongue, gums, lips, palate) should not be mixed with material taken from the udder and feet.
  - 4.6 Conservation medium - After the material is collected it is immediately placed into a bottle with conservation medium (see Point 11. Preparation of phosphate buffered glycerine). The bottle should be opened only momentarily. When a bottle with conservation medium is not on hand, use a bottle that has been cleaned and heat sterilized. When cooled, place the sample in the bottle.
5. Blood or sera for serology:
- A blood sample should be taken from individually identified infected animals during the initial investigation to attempt virus isolation in case epithelial samples of good quality are not available. If 2 to 3 weeks have already elapsed examine for secondary cases in order to detect new lesions. If no secondary cases exist, quite likely it is not FMD. You may confirm negative diagnosis by a widespread serologic survey.
- DO NOT RELY ON SERA FOR DIAGNOSIS.**

6. Procedure for collecting specimens from bovine and small ruminants using the probang (see the design)



- 6.1 The virus of FMD can become established in the surface epithelium of the pharyngeal region and multiply, creating a carrier status. This can occur in vaccinated animals exposed to virus, animals recovered from the infection and non-immune animals exposed to small quantities of virus. Carrier animals may or may not be serologically positive.
- 6.2 The following simple technique should be used to collect specimens from bovines using the probang:
- 6.2.1 The animal should be well restrained with the head held in a straight forward position and alightly elevated.
- 6.2.2 The operator should face the animal, insert the probang cup into the mouth, then push the cup over the dorsal prominence of the tongue. With gentle pressure, the animal will swallow the cup.
- 6.2.3 Allow the cup to pass into the oesophagus and by moving it back and forth it can be seen or palpated along the side of the neck, assuring that the cup is in the oesophagus and not in the trachea.
- 6.2.4 Move the cup back and forth in the oesophageal-pharyngeal (OP) region with some vigour for five or six times.

- 6.2.5 Withdraw the cup with a firm steady pressure, resulting in little or injury to the animal. As the cup is withdrawn, care should be taken to keep the cup in an upright position to prevent spillage.
- 6.2.6 After passage(s) of the probang, the specimen in the cup should be poured into the specimen container until 10 ml or more of specimen has been obtained. It is important that immediately after obtaining the desired amount of specimen an equal volume of the conservation medium should be added to the specimen and the contents of the specimen container should then be vigorously shaken for about one minute. About 1 ml of OP fluid will be obtained from small ruminants. Add to this 5 ml conservation medium. (See point II of this Annex.)
- 6.2.7 It should be noted that the probang should not be used in such a way that haemorrhages are produced, since blood in the specimen is undesirable. The saliva per se is not what is required in the specimen, since it contains very little virus.

If the animal vomits during the collection procedure the material obtained should be discarded and a new material collected.

Preparation for shipment from Field to Laboratory:

- 7.1 The bottles should be sealed with masking tape or sealing wax. They should be labelled with masking tape and the following information on the label should be written in pencil (ink tends to be illegible when wet):
- (1) Property or place where the sample was collected;
  - (2) Special
  - (3) Classification of Animal and Herd sampled:
    - (a) Sex
    - (b) Age
    - (c) Identification if any
    - (4) Date of collection
- 7.2 The outer surfaces of all bottles containing sample material should be quickly cleaned with a 4 percent sodium carbonate solution, then rinsed with pure water.
- 7.3 It is advisable to keep the bottles of sample material frozen or shipped

under refrigeration. Samples in bottles without conservation medium must be shipped frozen or on ice. Freezing may be readily accomplished by using a mixture of dry ice and alcohol and then maintained during shipment with ice mixed with sodium chloride (common salt). The bottles of sample material should be well protected to avoid damage during transport.

8. Dispatch of samples from Field to Laboratory:

The samples must be sent by safe and reliable means for personal delivery to the Veterinary Diagnostic Laboratory. In the event of a delay, the samples should be kept under refrigeration. Samples in bottles without conservation medium must be kept frozen or on ice at all times.

9. Epidemiological Report:

The batch of samples should be accompanied by an epidemiological report.

10. Cleaning and Disinfection after taking samples:

All personnel involved in the taking of samples must take care to thoroughly wash with a generous supply of water and soap. Hands, shoes and all equipment must be disinfected before leaving the property.

11. Instructions for the preparation of conservation medium:

Phosphate buffered glycerin is recommended for the conservation of epithelial tissues only.

11.1	<u>Formula</u>	-	Monobasic potassium phosphate ( $\text{KH}_2\text{PO}_4$ )	1.80 g
			Diabasic potassium phosphate ( $\text{K}_2\text{HPO}_4$ )	2.30 g
			Distilled water.	1000 ml

11.2 To this solution should be added an equal quantity of neutral glycerine.

11.3 pH. This glycerine buffer solution normally has a pH of 7.4 - 7.8. If the pH proves too high, more monobasic potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) should be added. In the case of a too low pH, this could be corrected by adding more diabasic phosphate ( $\text{K}_2\text{HPO}_4$ ).

This formula could be replaced by any other glycerine-phosphate mixture with a pH of 7.4 - 7.8.

PROCEDURE FOR SUBMISSION OF SPECIMENS TO THE  
PLUM ISLAND ANIMAL DISEASE CENTER (PIADC)

1. No shipments are to be made without prior arrangement including USDA Permit.
2. Commercial Attache at the US Embassy, PAHO/WHO Country Representative or the Director of the PIADC (Tel. 516-323-2500) will be contacted to arrange for a permit if not available. Such permits are now available at the PIADC. The permit number can be obtained by calling the above number.
3. An appropriate shipping container, with instructions for packaging is kept in the Emergency Storage room of the Veterinary Division.
4. Specimens should be refrigerated and shipped at 4°C, with freeze packs if they can reach the PIADC within 48 hours after collection from freshly killed animals. If not, specimens should be shipped frozen with freeze packs.
5. See list of diseases and corresponding tissues to be collected (Appendix
6. Written history (Sample Submission Form) must accompany all shipments.
7. If possible, Polaroid pictures of actual field cases should accompany specimens.
8. In all cases there should be no changes in airline routing where the sample is hand-carried or not.
9. Prior to despatch by any means, the Director PIADC must be informed by telephone giving airline estimated time of arrival, flight number of aircraft and Airway Bill number carrying consignment to enable PIADC staff to receive specimens at New York Airport. If hand-carried the name of the individual and a description (Airway bill number is not required). The person must also give his office and home telephone numbers as well as those of the Director of Veterinary Services so that contact can be made from PIADC.
10. If shipped the address label should read as follows:

Director PIADC  
c/o Mr. Bifano  
Cargo Building 80  
JFK, New York.

PROCEDURE FOR SUBMISSION OF SPECIMENS TO THE  
PAN AMERICAN FOOT-AND-MOUTH DISEASE CENTER (PANAFTOSA)

1. Packing bottles of sample material: For shipment of samples to PANAFTOSA, the bottles with sample material should be packed in sufficient cotton or other packing material to prevent damage, yet ensure complete absorption of the liquid medium should there be leakage from any source. (See Appendix Iata Regulations).
2. A firm and secure wooden, metallic or other suitable container with a tightly fitting cover should be used to hold the wrapped sample bottles, with absorbent material used to fill any excess spaces thus immobilizing the bottles from each other and within the container.
3. Addressee:

Director  
Centro Pan-Americano de Febre Aftosa  
Caixa Postal 589  
20000 Rio de Janeiro, RJ  
Brasil
4. The following words should be written clearly and in a visible place on the outside of the package:

MUESTRA BIOLÓGICA - ENTREGA PREFERENCIAL - URGENTE  
OF NO COMMERCIAL VALUE - FRAGILE

Labels may be obtained on request from PANAFTOSA
5. One of the most efficient means of forwarding samples suspected of FMD is by a courier travelling the most direct route to the Pan American Foot-and Mouth Disease Center in Rio de Janeiro, Brazil. It is strongly recommended that this means of transportation be used where there are no other effective established means of shipment of such samples.
6. Otherwise, an aeroplane making a direct flight to Rio de Janeiro should be used for dispatching samples and the package handed to a member of the crew who should be requested to place it in the refrigerator. If this arrangement is not possible, the most rapid air service should be used.
7. The shipping documents prepared by the Airline Company should be presented to the Brazilian Consulate to obtain the authorization for the dispatch to

Brazil. In case of any difficulty, reference should be made to the following decrees authorizing the unrestricted entry of samples, etc., destined for the Center:

Atas Internacionais 341, Article No.19, 3rd paragraph, letter b of Decret 32180 Federal Government of Brazil, January 3rd 1953 (D.O. 5/2/53, Fls. 1850-51).

- f. At the time that the sample is mailed, a cable should be sent to the Center, indicating the date of the dispatch, name and flight number of airline used and the amount of samples sent. Cable should be addressed to: PANAFITOSA, RIO DE JANEIRO. Telex No. is: (021) 30253 CPFA BR. If the dispatch is delayed the samples should be kept refrigerated.

LABEL - FOR SHIPMENT TO PANAFITOSA

**CAUTION**

**MATERIAL BIOLÓGICO  
SIN VALOR COMERCIAL**



**URGENTE**

(c) If either ice or dry ice need to be used as a refrigerant, it must be placed outside the secondary packaging(s). Interior supports must be provided to hold the secondary packaging(s) in its original position after the ice or dry ice has been dissipated. If normal ice is used, it must be packed in a leakproof outer packaging. If dry ice is used, the outer packaging must permit the release of Carbon dioxide gas.

**1.3 On Cargo only Aircraft (IATA Paking No.696)**

Maximum quantity in one pakago is 4 litres and must be packed as follows:

Each package containing an etiologic agent in volumes exceeding 50 millilitres must be designed and constructed so that there would be no release of the contents to the environment, and the effectiveness of the packaging would not be significantly reduced. In addition, etiologic agents (infectious substances) shall be packed as follows:

(a) They shall be placed in securely closed, water-tight primary receptacles (test tubes, phials, etc.) which shall be enclosed in a durable, water-tight secondary packaging. Several primary receptacles may be enclosed in a single secondary packaging provided that the total volume of all the primary receptacles so enclosed does not exceed 500 millilitres. The space at the top, bottom, and sides between the primary receptacles and secondary packagings shall contain sufficient non-particulate absorbent material such as, cotton wool, to absorb completely the contents of the primary receptacle(s) in case of breakage or leakage. Each set of primary receptacles and secondary packagings shall then be enclosed in an outer packaging constructed of corrugated fiberboard, wood, or other material of equivalent strength. The space at the top, bottom, and sides between the secondary packaging(s) and the outer packaging shall include a shock absorbent material, in volume at least equal to that of the absorbent material between the primary receptacles and secondary packagings. Not more than eight secondary packagings may be packed in a single outer packaging. The maximum gross volume

of etiologic agents (infectious substances) that may be carried in any one package is 4 litres.

- (b) If either ice or dry ice need to be used as a refrigerant, it must be placed outside the secondary packaging(s). The shock absorbent material shall be so placed that the secondary packaging(s) does not become loose inside the outer packaging after the ice or dry ice has been dissipated. If normal ice is used, it must be packed in a leakproof outer packaging. If dry ice is used, the outer packaging must permit the release of Carbon dioxide gas.

Shipper's Certificate - is required for each shipment. All laboratories should have a supply of blank certificates. Please contact Caribbean Epidemiology Centre (CAREC) if you need to replenish your stocks.

Always use the following numbers:-

<u>Article Number</u>	<u>Class</u>	<u>IATA Packing No.</u>
736	Etiol. Ag. 6	695 (Passenger/Cargo)
		696 ( Cargo only )

Note that the proper shipping name of the Article is -  
ETIOLOGIC AGENT to be followed in brackets by the actual name or composition of the article e.g. ETIOLOGIC AGENT (Virus in liquid medium).

### 1.5 Label

- (a) Note new labelling system (Figure 1) Biohazard labels should no longer be used. CAREC will supply new Infectious Substance labels.
- (b) Address.
- (c) Mark package to include refrigerants, wet ice, dry ice.
- (d) For packages containing more than 50 ml. which have to be transported by cargo only planes. A cargo only label should be attached.

FIGURE 1.



**1.6 Transport**

Etiologic Agents must be located in an aircraft in a place which is inaccessible to persons other than crew members. Etiologic agents shall neither be carried in the same compartment occupied by passengers, nor shall they be carried in passengers or crew checked or carry-on baggage.

2. DIAGNOSTIC SPECIMENS

2.1 Definition is any human or animal material including but not limited to excreta, secreta, blood and its components, tissue and tissue fluids being shipped for purpose of diagnosis. If such materials do not contain, or are reasonably believed not to contain an etiologic agent, they are not considered as restricted articles under these Regulations.

2.2 Packing Notes

Primary and secondary packaging as Etiologic Agents.

2.3 Labels

(a) Diagnostic Specimen.

(b) Address.

2.4 Transport

No restriction except that dry ice in quantities, not exceeding 2½ kilograms (6 pounds) per passenger may be used to pack perishables as carry-on baggage only.

CONTENTS OF EMERGENCY ANIMAL DISEASE KIT
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ITEM	QUANTITY
1. Tubes, Screw cap, Sterile	4
2. Tubes, Blood collection, sterile	4
3. Tubes, Blood collection, with anticoagulant	4
4. Cotton swabs, sterile in screw cap tubes	4
5. Specimen jars, screw cap, sterile	2
6. Specimen jars, screw cap, sterile with 10% formaline	2
7. Physiological saline, sterile	1 quart
8. Glycerol, Phosphate buffered in 30 ml. screw cap vials	6
9. Formalin, full strength	1 pint
10. Ethyl alcohol, 95%	1 pint
11. Microscope slides, pre-cleaned	1 gross
12. Hand soap	2 cakes
13. Caustic soda (lye)	1 can
14. Plastic bags and ties to seal tubes and jars	20
15. Cotton to pack materials	1 roll
16. Blue ice (freezer pads)	2, 1 quart size
17. Insulated box	2
18. Round friction top, tin cans, one gallon	2
19. <u>Post Mortem Kit</u> : Knife, hack saw, Face Shield, Steel axe, dissection pan, scissors, director grooved, tissue, forceps, apron, sharpening stone, disposable gloves, boots.	