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SIMPLIFIED TECHNIQUE FOR THE COLLECTION, STORAGE AND SHIPMENT OF BRAIN SPECIMENS FOR RABIES DIAGNOSIS[1]

This document contains details of a kit designed for carrying out the simple and practical collection, preservation and shipment of brain specimens taken for the diagnosis of rabies. Specimens collected with this kit will enable laboratories to test for rabies using current laboratory techniques.

So far, the kit has been produced in small quantities and on a trial basis by the WHO Collaborating Centre for Research and Management in Zoonoses Control, Centre National d'Etudes sur la Rage et la Pathologie des animaux sauvages, Malzéville, France.

The WHO Collaborating Centre (see address below) would welcome receiving confirmation of your interest in the kit, including an estimate of your requirements. This will help to determine the need for large-scale production of the kit.

This document is intended for personnel in rabies diagnosis laboratories and in the health and veterinary services who are in charge of specimen collection in the field.

Additional copies of the document may be obtained on written request to: The Chief, Veterinary Public Health, Division of Communicable Diseases, World Health Organization, 1211 Geneva 27, Switzerland.

By: J. Barrat and J. Blancou, WHO Collaborating Centre for Research and Management in Zoonoses Control, Centre National d'Etudes sur la Rage et la Pathologie des animaux sauvages, P.O. Box No. 9, F-54220 Malzéville, France.

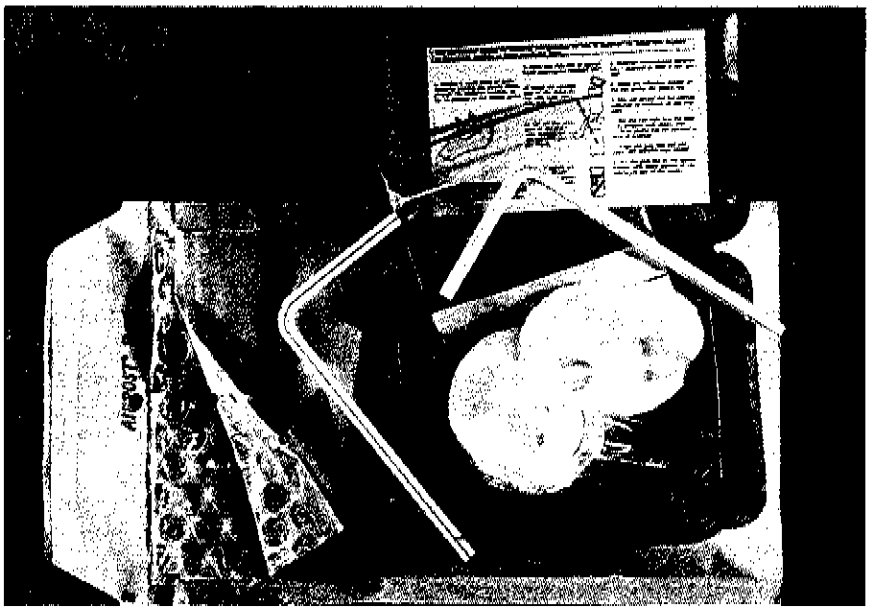

Figure 1

1. THE KIT (see figure 1) contains:

- 2 drinking straws for specimen collection;

- 2 tubes (with plastic stoppers) containing the preservation solution;

- cellulose wadding for absorbing the preservation solution in case the tubes break accidentally;

- a self-sealing plastic bag for packing the tubes and cellulose wadding;

- a plastic box (with a press-stud closing system and adhesive tape to prevent accidental reopening);

- a padded envelope (with an inner plastic layer containing shock-absorbing air bubbles);

- a despatch form to be adapted to local conditions (see Annex IV).

The estimated manufacturing cost is given in Annex III.

To obtain a reliable diagnosis the following steps must be closely observed when using the kit:

2. SPECIMEN COLLECTION

It has been shown[1] that diagnosis performed on a cylindrical specimen of brain tissue removed via the occipital foramen is as reliable for diagnosis as a specimen from the Ammon's horn obtained by opening the skull and dissecting the brain (see Annex II).

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[1] Barrat, J. and Halek, H.: Simplified and adequate sampling and preservation techniques for rabies diagnosis in Mediterranean countries. In: Comp. Immun. Microb. Inf. Dis. (1986), 9, (1).

A cylindrical specimen can easily be taken in the following way:

(a) Access to the skull:[1]

- if the carcass of the suspect animal is intact bend the head downwards and cut the cervical muscles transversally as far as the vertebrae behind the external occipital protuberance (see figure 2).

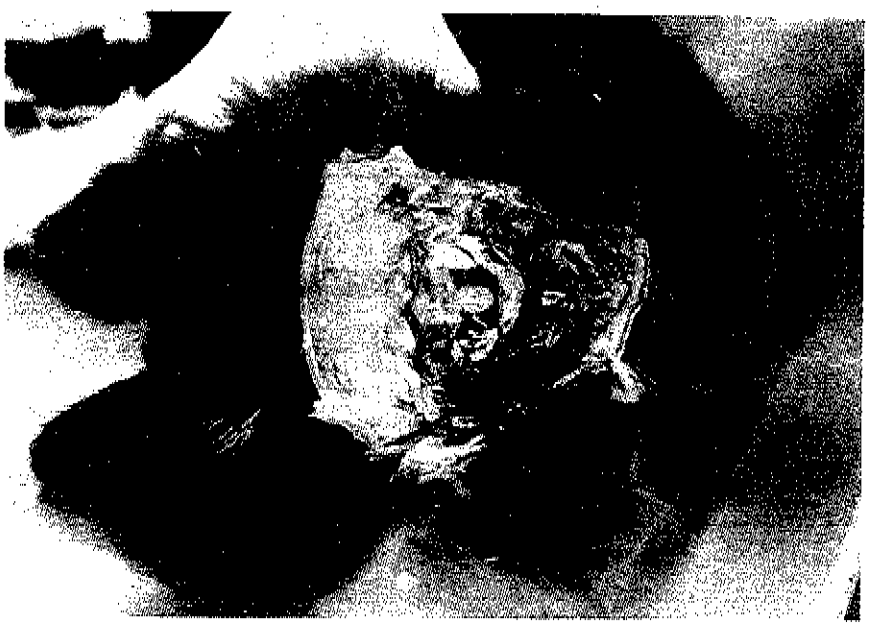

Figure 2

Leaving the dog's head in this position, open up the atlanto-occipital joint by incision of the dorsal atlanto-occipital membrane and then of the meninges.

- if the head has already been removed from the body, take the specimen in the same way via the occipital foramen exposed by decapitation.

(b) Taking specimens:

To take the specimens, insert the straw with a slight twisting movement through the occipital foramen, towards one of the eyes (see figure 3).

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[1] Caution: Persons handling the carcass of an animal with suspected rabies should wear disposable gloves and, if possible, goggles and a mask. Staff should preferably be vaccinated against rabies. In the event of accidental contamination (brain tissue or saliva squirted into the eye, or contact with any potentially virulent material through grazed skin), consult a rabies treatment centre.

Figure 3

The cylindrical brain specimen taken at this angle includes the medulla oblongata, the base of the cerebellum, the Ammon's horn region and finally, the cerebral cortex (see figure 4).

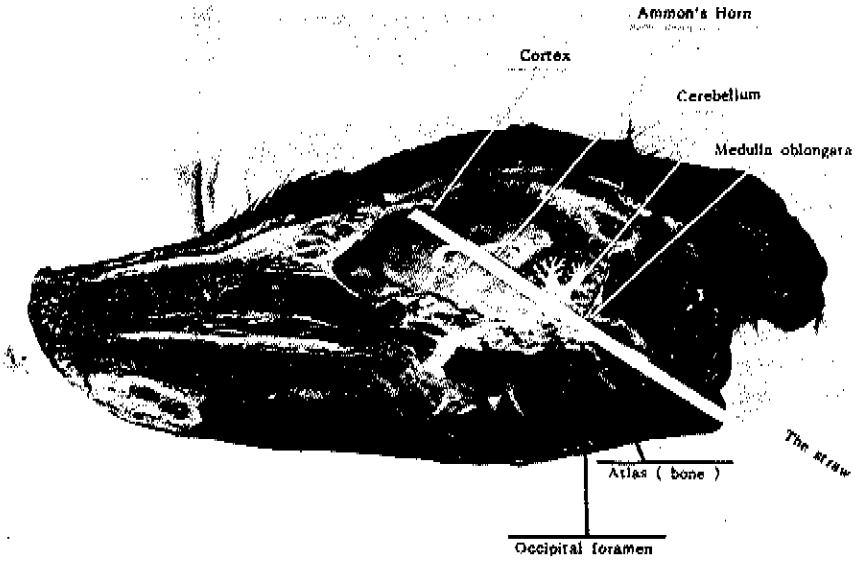

Figure 4

When inserting the straw into the brain a quantity of the brain tissue will enter the straw. There is no need to suck on the straw by mouth nor to use a suction apparatus (which could become contaminated).

3. PACKAGING

(a) Once the specimen has been taken, withdraw the straw containing the cylinder of brain tissue.

Place the straw in the tube containing storage solution[1]; cut the straw so that the stopper of the tube can be pushed in firmly (see figure 5). The composition of the preserving fluid is given below.

Figure 5

[1] "Nancy 871" solution based on glycerol and other chemicals whose efficacy against bacterial contamination and safety in respect of the rabies virus have been tested beforehand in the laboratory. 1000 ml of the fluid is made up as follows: pure glycerol (Prolabo Normapur) 500 ml, Merthiolate (thiomersal or thimerosal or sodium ethylmercurithiobenzoate) 100 mg. double distilled water pH7 ad 1000 ml. This composition may be changed, since further studies are in progress. If it is intended to produce the solution locally, the manufacturing laboratory should assure the quality of the products used, especially of the pure glycerol.

To ensure the sensitivity of subsequent diagnosis techniques (see Annex I), it is very important to leave the cylinder of brain tissue in the straw.

(b) Hermetically seal the tube containing the length of straw filled with brain tissue (see figure 6). For greater safety, surround the stopper with adhesive tape.

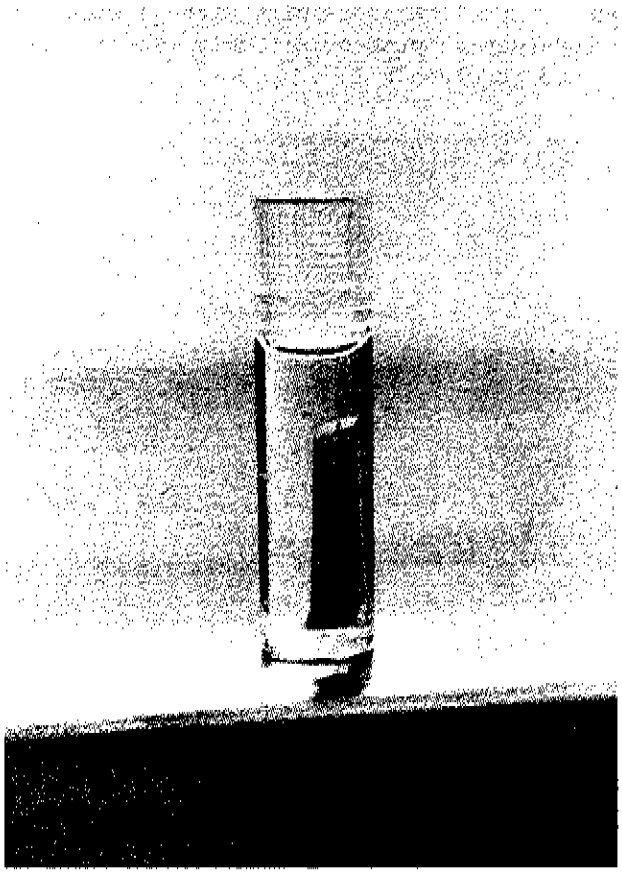

Figure 6

Label the tube with the reference number of the specimen.

The second tube can be used, either for a second specimen from the same animal, or for a specimen from another animal.

4. SHIPMENT

(a) The tube(s) should be wrapped in sufficient cellulose wadding to ensure absorption of the storage solution if the tube(s) should accidentally break.

(b) Place the labelled tube(s) containing the fragment(s) of straw wrapped in the cellulose wadding in the plastic bag, and mark the bag with the reference number(s).

(c) Then place the plastic bag inside the grey plastic box. Close the box and seal it with adhesive tape to prevent accidental re-opening.

(d) Then put the grey box inside the special padded envelope (provided with an inner plastic envelope containing shock-absorbing air bubbles) (see figure 7).

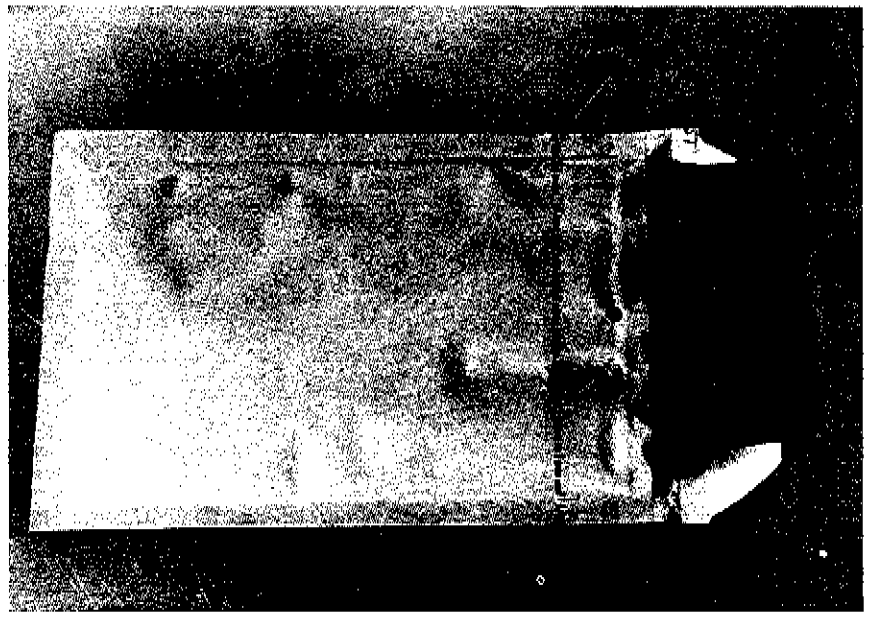

Figure 7

This envelope may be sent through the normal postal service, unless there are national regulations to the contrary.

(e) Enter full case details corresponding to the specimen(s) on the despatch form (see Annex IV) and include this in the envelope. A duplicate copy of this information should be mailed separately to the laboratory. Send the envelope to the diagnostic laboratory by the fastest route (e.g. airmail). In the event of any delay, store the kit in the sealed envelope at 4°C.

(f) Any equipment used for taking the specimen which may have come into contact with virulent material must be disinfected (autoclave or 5% ammonium IV), or destroyed by incineration.

ANNEX I

METHODS OF DIAGNOSIS TO BE APPLIED TO SPECIMEN(S) CONTAINED IN THE KIT

First, remove the cylinder of brain tissue from the length of "straw" with the aid of forceps, exerting pressure from the other end with a glass rod (see figure 8).

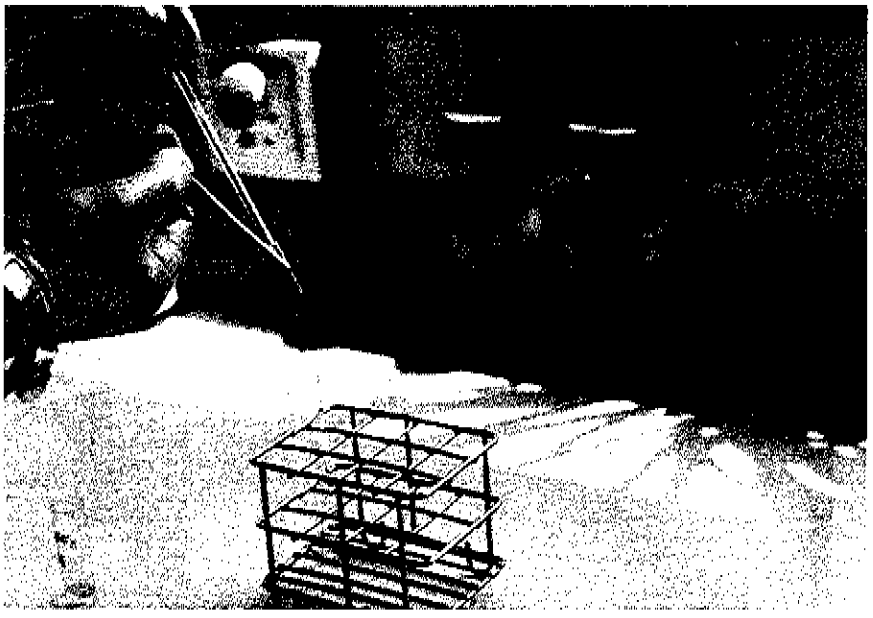

Figure 8

Then rinse the specimen for 10 minutes in a buffer solution (e.g. PBS) to remove the glycerol.

Three techniques can then be applied to the fragment of brain tissue thus collected and rinsed:

(a) Making impressions for the direct immunofluorescence test

Place the specimen on a tongue-depressor and "wipe" it with a second wooden tongue-depressor.

Then make the impressions. Staining is done by the conventional techniques.[1]

(b) Suspension prepared by grinding for inoculation into cells (murine neuroblastoma N2A) or inoculating mice

After making the impressions grind the sample:

- in a 50% (v/v) concentration in newborn calf serum, for inoculation into N2A neuroblastoma cells[2];

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[1] Kaplan, M.M. & Koprowski, H., ed. Laboratory techniques in rabies, 3rd ed., (Monograph Series No. 23), World Health Organization, Geneva (1973).

[2] Portnoi, D., Favre, S. & Sureau, P.: Use of neuroblastoma cells (MNB) for the isolation of street rabies virus from field specimens in: Rabies Information Exchange Memorandum, Centres for Disease Control, Atlanta, USA (1982), 35-36.

Annex I

OR

- in a 10% (w/v) concentration in Hanks' solution supplemented with antibiotics, for inoculation into mice by the conventional method.[1]

(c) Removal of a small fragment of brain tissue for performing the "rapid rabies enzyme immunodiagnostic" test (RREID)[2]

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[1] Kaplan, M.M. & Koprowski, H., ed. Laboratory techniques in rabies, 3rd ed., (Monograph Series No. 23), World Health Organization, Geneva (1973).

[2] Perrin, P., Rollin, P.E. & Sureau, P.: A rapid rabies enzyme immuno-diagnosis (RREID): a useful and simple technique for the routine diagnosis of rabies. In: J. biol. stand. (1986), 14, 217-222.

ANNEX II

RESISTANCE LIMITS OF THE SPECIMEN IN THE KIT

Storage of the brain tissue in the straw is better than by the conventional technique of shipping the Ammon's horn in 50% glycerol.

In 130 tests (immunofluorescence or neuroblastoma cell culture) peformed after storage of the cylinder of brain tissue in its straw fragment for eight days at either 20°C or 37°C, the diagnosis results agreed in all cases with those obtained with a fresh sample of the Ammon's horn removed after complete opening-up of the same animal's skull. When the Ammon's horn fragment was placed unprotected in glycerol, however, 18% of the positive tests on fresh samples proved negative after eight days of storage at 37°C.

ANNEX III

ESTIMATED MANUFACTURING COST OF 100 COMPLETE SHIPMENT KITS

	US$
100 boxes	57.6
100 envelopes	16.2
200 plastic bags	16.2
200 plastic tubes	16.4
200 straws	6.0
"Nancy 871" fluid for 100 kits	16.6
Total for 100 kits:	129.0

Approximately: US$ 1.30 per kit

ANNEX IV

DEPATCH FORM

BRAIN TISSUE SPECIMENS FOR RABIES DIAGNOSIS

Name and title of originator: . . . . . .

Institute: . . . . . .

Locality: . . . . . District: . . . . . Province: . . . . . .

Form number: . . . . . Date: . . . . . .

Information	Tube No. 1 Reference	Tube No. 2 Reference
Animal species		
Date animal died or Date animal was killed		
Main symptoms observed before death		
Place of origin of animal		
Name of owner (if any)		
Persons exposed (Yes or no)		
Other animal exposed (Yes or no)		
Date sample taken		
Place of sampling		
Date of despatch		
Storage conditions (if despatch delayed after sampling)		
Other relevant information		

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