### 5. TECHNIQUES IN LOCAL PROGRAMME EXECUTION

#### Annexes

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Dog Catching and Restraining Loop

- Aluminum pipe or bamboo pole 4-5 cm diameter
- Rope snare 1 cm x 120 cm long
- Metal band and ring

Place rope loop over dog's head and draw tight over neck by pulling through hollow handle (see Annex 5.1, page 2)
Dog Catching and Restraining Loop

Figure 1: Place rope over dog's head

Figure 2: Draw loop tight over neck by pulling through hollow handle
Animal Patrol Cages for Large Pickup Truck

All cages are constructed of chain link fence 4-5 cm with solid sheet steel floors. Space between double ceilings, walls and doors of rabies suspect cage is 5 cm. Doors should have locks.
Animal Patrol Cages for Small Pickup Truck

All cages are constructed of chain link fence 9.5 cm with solid sheet steel floors. Space between double ceilings, walls and doors of rabies suspect cage is 5 cm. Doors should have locks.
All cages are constructed of chain link fence 4.5 cm with solid sheet steel floors. Space between double ceilings, walls and doors at rabies suspect cage is 5 cm. Doors should have locks.
Design of Dog Pound with Communal Dog Cages, Rabies Suspect Cage, Carbon Monoxide Chamber and Office

Locations of cage building and office building may be modified to location site. Computor must be verified and access limited through a secure gate.

Water level in carbon monoxide scrubber must be below outlet pipe and show of inlet pipe. Water should be changed weekly.

Carbon monoxide scrubber and chamber must be gas tight for erriotic function and protection of personnel.

Annex 5-1
Details for Cages for Animal Pound

- **Front View**
  - Cage sizes are for different species of animals.
  - Small cages are for small dogs and cats.
  - Large cages are for larger animals such as horses.

- **End View**
  - Solid sheet metal walls and doors.
  - Doors can be locked for safety.

- **Top View**
  - Suggested spaces for different types of cages.

**Rabies suspect cages must be of sturdy construction with double doors and back walls. Special purpose cages are for identification or purebred dogs. For placement, assume for vicious dogs which cannot be placed in "compound cages."**
Annex 5-4

The use of strychnine in order to poison dogs and jackals

1. Baits prepared for the purpose of poisoning dogs and jackals contain strychnine which is one of the most dangerous poisons. A very small amount of this poison is enough to kill a human being or an animal, including birds.

You are therefore requested to handle this material with the utmost caution.

2. The cachets and tablets of strychnine must be kept in well closed metal boxes. Be careful not to wet them or crush them. Under no circumstances are you allowed to give them to other people.

3. On the box containing this poison a label must be fixed stating: "POISON". The box must always be kept in a drawer of a cupboard shut up with a lock.

4. Immediately after handling cachets or tablets of strychnine you should wash your hands with soap and water.

THE POISONING OF DOGS

5. To prepare a bait for poisoning dogs take a small piece of meat or cheese. With the help of a knife in a small make a small hole in the middle of the piece and put the bait within it. Press with your fingers on both sides so that the poison will be kept well within the bait. The size of the hole should not be more than 0.5 cm. Otherwise the dog will not be able to swallow it properly, and will know it and bite or the better part of it.

6. Before going out to poison dogs prepare off one or two baits for use within the next ten minutes.

7. When approaching a dog first throw him a piece of meat or cheese without the poison. After he has swallowed this, put the bait before him and go back a few steps. Watch the dog carefully to be sure that he has properly swallowed the bait.

8. In case the bait has not been taken up or swallowed or brought up again and thrown out from the dog's mouth, you must collect it and bury it deeply in the soil or take it with you in a second metal box for safe destruction.

9. Dogs which have been destroyed by poison must be buried soon afterwards, otherwise there is the danger that wild birds will find the carcass and get poisoned too.

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Editorial note

The editorial group of these guidelines does not advocate the use of strychnine, but feels obliged to include this example of a national code of practice, which points out the precautions to be observed by persons handling this dangerous poison.
DESTRUCTION OF OTHER WILDLIFE CARNIVORES

10. For the destruction of wild carnivores, e.g. jackals, you can use remnants of meat, spoiled sausages, day-old chicks or pieces of poultry bowels. Put the tablet or cachet within the mouth of the chicken or within the bowel and close it from both sides by a loop.

11. Put the baits in the afternoon near dung heaps or near a lane or a path on which footprints of wild carnivores have been seen. Put a small stick near each bait in order to find it in the morning when not eaten during the night, so that it can be taken back. Cover it with a few leaves so that carrion birds will not find it. Do not put more than ten baits in one location and keep a distance of two metres at least between the baits.

12. In the morning return to the baited place in order to get an estimate of the success of the action, to look for dead animals which should be buried, and to take back baits which have not been eaten.

PUBLIC WARNING

13. A warning "DANGER" notice announcing the intention to kill jackals and stray dogs in the district should be posted one to two days before the action.

* Editorial note

The indiscriminate use of poison baits may pose a risk to humans and endangered species.
Brochures for Rabies Control

RABIES

A DISEASE WHICH CAN BE CONTROLLED IN:

- can be prevented by vaccinating dogs
- is usually spread to people bitten by dogs
- will not be spread by vaccinated dogs

- have your dogs vaccinated when they are 3 months old
- revaccinate your dogs 1 yr. later and then every 2-3 yrs.
- keep your dogs in your compounds or yards

- over 25 000 dogs die of rabies each year
- over 100 000 people take antirabies vaccine yearly
- over 200 people die of rabies each year

Vaccinating teams from the City Health Office will be in
from ____________ to ____________. Have your dogs ready and help the vaccinators.
Rabies

Rabies is caused by a virus. In this country, dogs are the important animals which spread rabies. Infected dogs may be very ferocious in the early stage of the disease and may bite other dogs, spreading the virus to them. Later the infected dogs become paralyzed but may still bite and spread the virus until they die. Infected dogs may bite other animals as well as dogs, including cats, pigs, cattle, carabaos, horses, goats and sheep. Dogs which have rabies, and also cats, pigs, horses and occasionally other animals bite people and expose them to rabies. In some countries, wild animals also spread rabies to other wild animals, to livestock and pet animals and to people, but fortunately in this country, wild animals do not maintain the spread of rabies.

People who are bitten by any animals should first wash the wound well, then report to their nearest physician or health center. If the biting animal is a dog or cat, it should be confined so that it cannot bite any more people and should be observed for 10 days. If the dog or cat has rabies, it will surely die within this period. A veterinarian or animal inspector should be asked to see if the animal has rabies or to send the head of the animal to a laboratory for diagnosis. If a biting animal is suspected or proven to have rabies, then the victim must be given a series of vaccine injections by a physician or at the health center, and even then, some may not be protected and may become sick and die from rabies.

If dogs are vaccinated against rabies, they will be protected from the disease, and if at least 80% of the dogs in a community are vaccinated, the disease will disappear. It is important that every dog above 3 months of age be vaccinated during community vaccination campaigns. Many dogs are born each year and they must be vaccinated too. Keep dogs from wandering around by keeping them in your compounds or fenced yards. Keep your communities clean of garbage, wastes and any other food dogs could eat, as well as of unused buildings, cars or wood piles under which dogs could live. In this way, there will not be dogs wandering around your community, at risk of being exposed to rabies and of spreading the disease to ---- you.
Brochure for Rabies Control
الخطط العاملية مبادلة تقوم بها صاحب
الكلب البيطري في مختلف جهات الجماعية,
الكلب المقلوب لا يتعين للنقل من طرف انسان
العرفة أو الأسري الوطني.

ساحر على التحول مباشرة إلى
القرب مركز ملاك قد دام الكلب، زيادة
على ذلك يجب احترام توقيات المركز الخاصة
فمما يتعلق بحدود مرات الحق الشريحي.

هذا الداء منتشر حالياً بال全长 العربية
لكن يمكن أن يصع السماه إذا ما طبقنا
القانون التالي:

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كلب تجوب المدن والرياضات
مواطنون تفضّبهم الكلاب كل عام
مواطنون يموتون بدءاً أكلب كل سنة

اربطوا كلابكم
ولقحوها

1,000,000
10,000
40
Posters for Rabies Control
SUS BUENOS SENTIMIENTOS PUEDEN TRAICIONARLO.

los perros callejeros deben ser eliminados

COMBATA LA RABIA

PROVINCIA DE BUENOS AIRES
MINISTERIO DE SALUD PÚBLICA
Annex 5-7

RADIO ANNOUNCEMENT*

1. Protect yourself and your neighbours from rabies. Have your dog vaccinated when the vaccinators come to your barrio. A vaccinated dog will not spread rabies even if it should bite you or your neighbour.

2. Stop rabies in Dumaguete City. Rabies is spread by dogs that have not been vaccinated. A vaccinated dog will not spread rabies. Have your dog vaccinated against rabies when the vaccinators come to your barrio.

3. Rabies is a serious problem in Dumaguete City. Many people are bitten each year by dogs with rabies and are forced to take injections so that they will not die of rabies. Sometimes the injections do not save the person from rabies. Dogs which are vaccinated against rabies will not spread rabies even if they might bite someone. Have your dogs vaccinated.

4. Dogs which are vaccinated against rabies will not spread rabies even if they might bite someone. People bitten by vaccinated dogs do not have to take anti-rabies injections. Protect yourself and your neighbours. Stop rabies at the source. Vaccinate your dogs instead of being forced to take anti-rabies injections.

5. Stop rabies. Dogs can be vaccinated against rabies with only one injection. This injection will protect them for three years. The dog is the most important source of rabies. Have your dog vaccinated when the vaccinators come to your barrio.

6. Help your city stamp out rabies. The City Health Office will be sending teams of vaccinators to your barrio soon. Have your dog vaccinated. Rabies can be eradicated if you all cooperate.

7. Rabies is a dangerous disease. People bitten by dogs with rabies must be given many injections to protect them from the disease. Once a person is sick with rabies, he will surely die. Stop rabies in dogs by having them vaccinated.

8. Eradicate rabies in dogs in Dumaguete City. Have your dogs vaccinated when the vaccinators come to your barrio. Report the presence of stray dogs to the police department. Vaccinated dogs cannot spread rabies to the people.

9. Have your dogs vaccinated against rabies. Protect your barrio from rabies. If every dog in your barrio is vaccinated against rabies, this disease can be eradicated from your barrio. Have your dogs vaccinated when the vaccinators come to your barrio.

* Editorial Note

This radio announcement was specifically prepared to announce a local control campaign. It is quoted as an example.
10. The rabies problem in Dumaguete City is serious. Let us eradicate this disease here. The City Health Office is sending teams of vaccinators to each barrio. When the vaccinators come to your barrio, have your dogs vaccinated. If every dog in Dumaguete City is vaccinated, rabies will be eradicated.

11. Protect yourself. Don't get rabies by being bitten by your dog. Don't let your dogs give rabies to anyone else. Have your dogs vaccinated against rabies when the vaccinators come to your barrio.

12. Help stop rabies in Dumaguete City. Rabies is a fatal disease. It kills dogs, other animals and people. Dogs are the animals which usually spread rabies. Stop rabies in dogs. Have your dogs vaccinated. Report stray animals to the police.
adequate public awareness of the risk of exposure, and rapid assessment of the risk of infection is necessary in view of increased travel and the international transfer of animals.

In many countries the technical services for collection and shipment of specimens of suspected cases in animals and man to the laboratory are still inadequate (see Section 5.3.5) and particular attention must be paid to the requirements of effective surveillance systems, making use of existing services and laboratory facilities.

It is thought to be of advantage to have one national centre for diagnosis and surveillance. Should it prove to be necessary to establish more than one centre, those sub-centres should be able to report their data quickly to the main centre.

Due to modern international traffic of humans and animals, however, national surveillance alone will not suffice. For the protection of travellers (tourists and others) as well as for the indirect protection of humans by the control of animal border traffic, authorities should be informed quickly about the rabies situation in countries other than their own. This can be made possible by founding international centres for certain regions of the world, such as the WHO Centre for Rabies Surveillance and Research in Tübingen, Federal Republic of Germany. Most important also is the direct reporting of cases across borders between local veterinary and medical services of adjacent countries.

It is recognized that canine rabies can constitute a constant problem in countries with limited economic and professional resources for the implementation of large-scale surveillance programmes. Under such conditions, dispersion of efforts, resources and manpower should be avoided, and the total effort directed towards strategically important areas. To rid an area of endemic or epidemic canine rabies requires a well organized programme for the registration of owned dogs, their swift mass immunization, the elimination of stray dogs and health education.

Before these measures can be put into effect, the situation has to be properly appraised by disease surveillance in order to avoid overaction.

In national programmes of canine rabies eradication, priority should be given to areas that are known to be principal foci of the disease. Such foci are often in the major metropolitan areas (e.g., in South America), or in rural areas (e.g., in East Africa), where emphasis should be given to carrying out the mass vaccination of dogs in the shortest possible time. All dogs should receive their primary vaccination at the age of 3-4 months and a booster approximately one year later. Since rabid cats may present a serious problem, cat owners should be encouraged to have these pets immunized as well and cats should be included, if possible, in mass immunization programmes.

5.4.2 Reporting of animal cases

A reporting system should make use of existing administrative units and governmental institutions. In this section the terms "community", "district", "province", "central government" and "Ministry" are used, as being applicable to most national administrative structures.

Every person should be bound legally to report to the local authority any animal or carcass suspected of being affected with rabies (see legislation - Section 4.2). Possible reasons for suspecting an animal of having rabies should be made public through the mass media and by printed matter.
Local authorities in this context are:

- the district authorities, which should include a veterinarian and a medical officer. Both should be empowered to deputize legally any local practitioner of their profession.

- the communal authorities, e.g. the mayor's office. Those should be equipped with standardized containers for the shipment of samples or carcasses to the diagnostic centre. They should also be furnished with sufficient supply of standard forms to accompany the samples in the containers (see Annex 5.18).

- The local posts of constabulary or police; they should immediately relay reports received to "local authorities".

- Hunters, foresters, rangers, game-keepers and wardens, who should be employed in the same way as local police and constabulary.

Every report of a suspected case of rabies in animals should be registered at the district veterinarian's office. He has to keep records for the district and should map the location of cases on large-scale district maps. In cases of human contact, he should immediately notify the medical officer (this section does not deal with cases where direct medical assistance is required).

Killed animals or animals found dead and being suspected of having been affected with rabies should be shipped to the diagnostic centre. There, another record should be kept according to the data given by the accompanying report forms.

In cases of living suspects, the decision to kill or to detain and observe the animal rests with the district veterinarian. Any killed animals sent in for diagnosis must be accompanied by a report form.

In this way all suspected cases are recorded at the national centre. Of course, an unknown number of unreported cases will remain. This number must be kept as low as possible by means of public information, education and instruction.

The national centre should be part of the ministry responsible for the control of animal infectious diseases and also coordinate the surveillance activities of the medical services. The ministry should have direct control of all infectious diseases of animals, e.g., foot and mouth disease, rabies, anthrax, etc. The district veterinarian should report periodically to his provincial veterinary service headquarters. The national centre should report back diagnostic results to the province, the district, the community and in many cases to the owner or sender. A report should at the same time be sent to the veterinary and the medical department of the Ministry.

5.4.3 Reporting of human rabies cases

In the interest of the validity of a reporting system one should make sure that the following rules are followed when reporting:

- human bites by non-rabid animals should be recorded by the clinic but should not be reported to the rabies unit,

- human bites by rabid animals (highly suspected or proven by the veterinary laboratory) should be reported as exposures,
human rabies (with clinical symptoms and verification through identification of intracytoplasmic virus or fluorescent antibody staining of certain tissues) are reported as cases.

(a) Every human case of rabies, and bites by rabid or rabies-suspected animals, should be reported to the health authorities of the district where the incident occurred. Within 24 hours the name, age and address and all relevant details of the victim should be reported to the health and veterinary authorities (see Annex 5.19).

(b) The following persons should be legally bound to report:

- every physician
- nursing personnel
- heads of institutions, house holders
- headmasters of schools or kindergartens, teachers and other school employees
- tenants of flats
- publicans, hotel-managers, etc.
- veterinarians, police and constabulary, game-keepers and foresters, such cases coming to their knowledge while working in their professions or trades
- coroners
- landlords or their caretakers.

(c) When authorities have been advised of cases as in (a), they should send physicians to make the necessary examinations and inquiries.

(d) Rabies-infected, rabies-suspected persons and contacts should be bound to:

- give all information required
- permit themselves to be examined medically
- allow the taking of samples.

(e) For the diagnosis of rabies in humans, the patient should be hospitalized and specimens sent to a rabies diagnostic laboratory.

(f) On confirmation of a human case of rabies, communal authorities should put control measures into effect.

(g) In human lethal cases, which are suspected to have been caused by rabies, health authorities have to issue an order for a post-mortem. The samples taken at autopsy should be brought to the diagnostic centre, preferably by one of its experts, assisting at the autopsy.

(h) Humans bitten by rabies or rabies-suspected animals should be brought for treatment immediately.

(i) Physicians carrying out post-exposure vaccinations must complete a report form as in Annex 5.19 and distribute it as follows:

- keep one copy for filing,
- one to the provincial government, and
- one to the Ministry.
Completed report forms serve also to establish vaccination statistics, and to complete WHO Rabies Questionnaire no. 19.

(j) A vaccination certificate (as in Annex 5.20) should be issued to the vaccinated person.

(k) Communities, police and constabulary, vaccination posts and physicians must report all cases of dog bites to the district authorities; who advise their provincial government, who on their part send a monthly report together with the report on contagious diseases, sending copies to:

- the provincial government
- the district authorities.

5.4.4 Reporting by rabies diagnostic laboratories

Three priorities of diagnostic specimens exist:

Category A: cases of human exposure

These may be divided into major and minor bites or exposures.

Category B: cases in domestic animals and susceptible pets

In these cases it is advisable to assume human exposure even when reports do not say so.

Feral dogs and cats should be included here, too. Difficulties may arise in countries where the terms "wildlife" and "pet" overlap (e.g. skunks, racoons, monkeys).

Nevertheless, the principle idea is to be on the safe side where human health could be involved.

Category C: wildlife cases without human exposure

As these are purely of interest for surveillance and control they have the lowest priority.

The means of communication in reporting should be:

- telegram
- telephone or radio
- report forms.

If the possibility exists to use teletype networks of the police on 24 hour duty, the opportunity should be taken, especially after duty hours or during weekends: the local constables know where to find doctors or veterinarians.

Carcasses and samples arriving at the laboratory in the morning mail or rail delivery should be grouped and dealt with according to priorities.

Diagnostic work relating to category A and B specimens should start immediately, so that the results of FA-tests can be reported back to the district veterinarian on the same day. Reports on the results of the mouse-inoculation-test are necessarily delayed; it takes approximately 21 days for this confirmatory test to be completed.
It is essential to set a deadline for the daily results to be ready for transmission. Transmission by telegram or telex is to be preferred, with confirmatory written reports sent later.

In exceptionally urgent cases, district veterinarians should be contacted directly by telephone in order to enable them to act quickly. Again confirmatory written reports should follow.

Diagnostic work on specimens of category C may follow as soon as work on A and B specimens is finished. Generally, the results should be ready for transmission on the day following arrival, at the latest.

In wildlife epizootics large numbers of cases will have to be diagnosed and about 85 to 86 per cent may prove to be negative. It is understood that positive cases will be reported back to the district veterinarian within 72 hours after submission of the specimen, the negative reports could be issued in weekly, or bi-weekly lists.

Reports on diagnoses of categories A and B as well as C should go to the district veterinarian directly.

In category A cases he will report to the district medical officer. In category B cases the district veterinarian should receive the report.

Category C cases are also dealt with by the district veterinarian, who will decide on the course of action.

At the diagnostic centre each case must be registered in a ledger or case book in a prescribed form.

Report forms should be copied to the following:
- the district veterinarian
- the medical rabies institute
- the veterinary officer of the provincial government
- the veterinary department of the ministry
- the medical department of the ministry
- to the owner or sender of the sample or carcass, if the address is known.

The diagnostic centre prepares quarterly maps of positive cases and quarterly reports. These are sent to:
- the international centre of the region
- the veterinary department of the ministry
- the medical rabies institute.

Copies of the quarterly report of international diagnostic centres are sent to national diagnostic centres and to national governments.

5.4.5 Data to be reported

In order to give quick and precise information, case reports should meet minimal requirements and be submitted together with the specimen. The data required for national rabies surveillance is as follows:
- number of animal rabies cases
- number of human rabies cases
- number of post-exposure treatments in man
- number of pre-exposure treatments in man
- post-vaccinal complications
- control measures applied.

Animal rabies cases reports should include the name of owner and sender, address, date, animal species, location and other information as to whether the submitted animal was killed or found dead, and on possible exposures of humans and other animals.

The location will preferably be the smallest administrative unit within the country (generally the community), or part thereof, where the case occurred.

Any other additional relevant information available to the reporting officer should be added, especially if it is possible or intended to have the data stored, processed and evaluated by means of computerized techniques through a regional or international WHO reference centre. An animal rabies case reporting form as being used in the European Rabies Surveillance System is given in Annex 5.21.

Human rabies cases should be well documented. Reports on human rabies should be divided into actual rabies cases, post- and pre-exposure vaccinations and post-vaccinal complications, giving information for each as follows:

(i) Rabies cases
- person exposed (name, age, sex, address)
- details of exposure (date, place, type, site)
- source of exposure (animal species, disease status at time of exposure, clinical and laboratory diagnosis)
- circumstances of exposure (provoked attack, other people exposed)
- post-exposure treatment (local and systemic, type and batch number of vaccine and serum, course of treatment, vaccine reactions)
- clinical course (incubation period, period of illness, treatment, final outcome, diagnosis).

(ii) Post-exposure treatments
- the person exposed (age, sex, address)
- treatments (local wound, vaccine, serum)
- post-vaccinal complications
- source of exposure (animal species, disease status at time of exposure, clinical and laboratory diagnosis)

A suggested case recording form is given in Annex 5.19.

(iii) Pre-exposure immunizations
- the person (name, age, sex, occupation, address)
- the vaccination (type and batch number of vaccine, immunization schedule, seroconversion, booster doses)
- vaccine reactions and complications.

(iv) Post-vaccinal complications. General allergic reactions and CNS-disorders (e.g. meningitis serosa, myelitis, encephalomyelitis, neuritis)
related to the anti-rabies treatment should be reported on separate forms containing information on

- the person (name, age, sex, occupation, address)
- details of exposure
- post-exposure treatment
- clinical course and diagnosis
- final outcome.

5.4.6 Processing of surveillance data at receiving centres

In order to present the accumulating rabies data in a given format, the data have to be processed and presented in a certain manner. The procedures involved may be divided into 5 categories, irrespective of whether the data are processed by hand or through an electronic data processing unit. The steps are as follows:

(a) Data preparation

- preliminary data screening to check for obvious errors or non-intelligible characters on the reporting forms
- transformation of data into computer-compatible form (coding).

(b) Entry of data into the computer or into main-files.

(c) Data editing

- detection of errors
- resolution of errors (deletion of records, adding records, or changing characters of information).

(d) Data analysis

- simple analysis (averages, standard deviations, frequency counts)
- complex analyses (mapping, multivariate statistics, time-series resolutions)
- retrieval of data for various purposes from the master or subsidiary files.

(e) Display of output

Data may be presented in various forms (tables, maps) for use in routine reports, epidemiological analyses, and statistics. By use of varying computer programmes the data may be presented in many ways, e.g., (i) the capability of displaying information on geographical distribution; (ii) period that is needed for epidemiological analysis (from months up to years); (iii) infected areas; (iv) densities of cases; (v) number of cases per location, number of cases per given species, or a combination of species. The computer can produce all these types of information output quickly if it is correctly programmed.
5.4.7 Reporting of data from surveillance centres

The principle function of surveillance centres should be to exchange information on rabies and to provide veterinary and other public health authorities with the essential data for the most effective application of control measures in animals.

The information distributed from surveillance centres should include the following categories of data on animal and human rabies:

i. Data on epidemiological conditions including mapping of animal rabies cases for immediate action in rabies control, prevention, and post-exposure treatment of humans.

ii. Information on human exposure, vaccination, post-vaccinal accidents, and human cases of rabies.

iii. Retrospective analyses of the spread of the disease, control operations, and human treatment.

iv. Information on current outbreaks or enzootics.

The information distributed from surveillance centres should be designed to assist local officials in their decisions on animal rabies control operations and prevention of rabies in man, and to give information on long-term trends of the disease.

Countries with inadequate surveillance services should be encouraged to collect data systematically. Appropriate evaluation and reporting of such information would encourage better cooperation between countries and should be disseminated through the WHO Collaborating Centres. There should be close cooperation with the International Office of Epizootics (OIE) and the Food and Agriculture Organization of the United Nations (FAO). WHO rabies surveillance helps to develop and improve national systems by bringing all countries up to a common acceptable standard. National and international systems could be coordinated so that the same forms and reporting systems are used. There should be quick exchange of information from country to country through a common data pool.

5.5 Vaccine procurement and delivery

5.5.1 Selection of type of vaccine

The widespread use of modified live virus (MLV) and inactivated nervous tissue vaccines prepared from fixed virus has significantly reduced the incidence of rabies in many countries.

Annex 5-22 summarizes the main animal rabies vaccines as used in 1980. The same types of vaccines are still in use today without any major modifications.

A world-wide, up-to-date (April 1982) inventory of available animal rabies vaccines as compiled by the WHO Mediterranean Zoonoses Control Centre is given in Annex 5.23. The detailed list specifies necessary information such as
producing laboratory, type of vaccine, virus strain, irrelevant duration of immunity, required potency tests, etc. The list is not complete and is still lacking some important vaccines for different countries.

In planning and implementing a national rabies control program, the following factors need consideration in selecting the type of vaccine to be used: (1) modified live virus (MLV) vaccine; (2) natural species to be vaccinated in addition to dogs; (3) whether vaccination will be done predominantly by mass vaccination of veterinarians' surrogates; (4) provisions of local dog licensing regulations and control procedures; (5) adequacy of storage and distribution system.

(a) Established types of vaccines for dogs

For dogs two main types of vaccines are commonly used throughout the world: modified live virus and inactivated virus vaccines.

(i) Modified live virus (MLV) vaccine

Most of the vaccines currently used for dogs contain the Flury strain either as low or high egg passage virus (LEP/HEP), the Serum Aluminum Buffer (SAB) strain, or its derivative, ERA and Vakovac; or the Pasteur strain, derivatives such as the Challenge Virus Standard (CVS) or Human-Mouse (HM) strains. It must be borne in mind that if a virus is not entirely and definitely inactivated by physical or chemical methods, it must be considered as a live virus vaccine. Without doubt many of the so-called "phase-killed" or "Ferni-type" vaccines still used in many countries are of this type. They usually contain from 10 to 100 million to one million input virions.

- Flury MLV vaccines

The LEP strain is no longer recommended for MLV vaccine production, although it is still in occasional use.

The HEP strain can be produced from chick embryos incubated at the 7th day of egg incubation, harvested 14 days later and made into a 10% tissue suspension. The average yield is 2-4 dog doses per harvested embryo. It can also be produced from primary or two passage fibroblasts or cell lines of dog or hamster kidney origin. Monolayer cells are incubated after 24-28 hours of culture and harvested 7-9 days later, the average yield being 1-4 dog doses per 10ml of infected cell culture fluid.


Production of lamb or kid brain vaccine is detailed in Annex 5.24. Research requirements in this field are summarized in Annex 5.27.
- SAD derived MLV vaccines

Two SAD derived strains are commonly used. The ERA strain virus cultivated in porcine, canine, bovine or hamster cells and used in many countries. The Vnukovo virus cultivated in hamster kidney cells is mainly used in the USSR.

These vaccines are usually produced in monolayer cell-cultures

(ii) Inactivated virus vaccines

These vaccines are obtained by complete inactivation of rabies virus grown either "in vivo" (nerve tissue) or "in vitro" (cell culture).

- Nerve tissue vaccines

Fixed strains are recommended for use in the production of vaccines, but also strains isolated from local epidemics may be considered.

Animals inoculated can be of various species: laboratory mice, guinea-pigs and rabbits as well as farm animals such as sheep, goats, cattle or even horses and asses. Very young animals, preferably new-born, should be preferably used: the neuro-allergenic factor of brain tissue is much lower in the newborn and the titres obtained from such brains are 10 to 1000 times higher than from adult animals. Average yield, in these cases, is for instance about 3000 dog doses per lamb brain and 5-10 dog doses per suckling mouse brain.

Inactivation of vaccine virus can theoretically be achieved by physical inactivation (heat, ultra-violet radiation) or more safely by chemical agents. For practical purposes, only chemical treatment by either betapropiolactone or the immines is reliable and results in complete inactivation of the virus with negligible degradation of the virus antigen.

- Cell culture vaccines

The virus strains used are either the Pasteur derived strains, PV, CVS, Pitman-Moore or the Flury strain viruses. From a theoretical point of view any other rabies strain of well documented passage history and defined antigen properties (see below) can be used provided the virus yields from cell culture are sufficient.

Most of the cells used for rabies virus propagation originated from hamster cell lines, but sometimes primary explants of chick embryo fibroblasts, porcine, bovine or canine kidney cells are used. Cultivation of rabies virus on micro-carriers (sephadex beads) is under development and could increase the virus yield from cell culture.

Inactivation is generally achieved by chemical agents (see Annex 5.24).

Rabies vaccines are often combined with other bacterial or viral antigens (e.g. distemper, panleucopenia, hepatitis, leptospirosis, etc.). Such polyvalent vaccines are acceptable provided each of their components, and particularly the rabies antigen, has been properly tested for potency.

Adjuvants (e.g. aluminium hydroxide) added to rabies vaccines proved very useful and should be recommended in order to increase the duration of immunity in dogs.
(b) Advantages and disadvantages of different types of vaccines

Any vaccine that has been properly tested for safety, purity and potency and has been shipped, stored and administered according to instructions is capable of providing adequate immunity in the recommended species.

There are, however, factors that may alter the selection of one type of vaccine over another according to local conditions such as high temperature, lack of appropriate cold chain, storage, transportation facilities, etc.

(i) MLV vaccines

- Advantages

When properly produced and administered, MLV vaccines usually provide a long lasting immunity to dogs, without adjuvants and after a single injection.

MLV vaccines have proved superior in reducing the incidence of canine rabies to a low level in the United States (59 cases reported in 1978 versus 360 cases in 1957) and other countries. Two MLV products currently licensed have been shown to protect at least 95 percent of vaccinated dogs for at least 7 years after vaccination. A 100 percent survival rate, with 100 percent of control dogs.

Many countries are still accustomed to the use of such vaccines and can produce MLV in satisfactory condition and at low cost, with local equipment and already trained personnel.

- Disadvantages

MLV vaccines share the general disadvantages of any live virus vaccine. They must be stored properly. Stabilizers added by various manufacturers differ; some vaccines in past years were shown to have had significant loss of virus titer when stored in improperly functioning refrigerators. Recently, however, the stabilizer formulae have improved vaccine storage characteristics.

The MLV vaccines are lyophilized; reconstituting diluent must be added before use. Adventitious contaminants more readily survive with lyophilization than with inactivating procedures.

It is necessary that MLV vaccines be given only intramuscularly at one site in the thigh. Other intramuscular sites of administration are potentially unsafe, and may result in clinical rabies. Subcutaneous vaccination has been shown to be less efficacious. Reported cases of suspected vaccine-produced rabies are to be investigated and monoclonal antibodies have been beneficial in differentiating vaccine-induced rabies from vaccine failures. The MLV vaccines are prone to the deleterious effects of improperly cleansed syringes and needles, or disinfectants applied to the site of injection.

In a very limited number of dogs and cats there is circumstantial evidence that severely immuno-suppressed animals may react to modified live virus vaccination in an aberrant manner. Consider postponing vaccination of dogs when high levels of steroids have been administered or vaccinating with an inactivated vaccine when leukemia is present.
(ii) **Inactivated virus vaccines**

**Advantages**

Inactivated vaccines have advantages that are attractive. Vaccine-induced rabies is not a problem with properly inactivated products. Subcutaneous administration appears feasible when intramuscular injection is painful or difficult, such as in toy breeds. Intramuscular inoculation, however, remains the route of choice. Combining rabies antigens with other antigens is feasible after proof of non-interference has been established. Inactivated products for use in dogs do not present safety problems when administered to exotic species. Although accidental inoculation of MLV vaccines has not been shown to cause rabies in humans, less anxiety is produced if inadvertent injection with inactivated products occurs. Multi-dose vials of inactivated products can be stored after initial use and do not rapidly deteriorate if all doses are not administered within a short time.

**Disadvantages**

Inactivated products also have disadvantages. Some products, particularly vaccines prepared from adult animal brain tissue, are of marginal potency. Inactivated products are not all free of stability problems when improperly stored. The production of potent inactivated vaccines is somewhat different from that of MLV-vaccines, requiring more sophisticated equipment and training.

Neuro-allergenic sequellae are more common in dogs when given inactivated products with a high proportion of neural tissue. This defect has been substantially eliminated in recent years with the introduction of products of cell culture or neonatal neural tissue origin.

**(c) Facilities needed for use of different types of vaccines**

Production facilities vary depending on whether the vaccine is of tissue culture origin or neurogenic origin. The first requires increased incubator space, glassware preparation area, and tissue culture production facilities. The second requires increased animal space.

Facility requirements will also vary for modified live and inactivated vaccines. Production of a live virus product will require a lyophilizer and increased freezer space for storage prior to lyophilization. Inactivated vaccines can be stored at 4°C prior to bottling.

**Handling of vaccines**

To be efficient, MLV vaccines (including "Fermi-type") have to be inoculated at a minimum titre specific for each strain used, for instance $10^2$ but not exceeding $10^{2.7}$ MICLD50*/0.03 ml for Fermi-type, $10^{3.3}$ for Flury and $10^4$ for ERA vaccines.

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*MICLD50 = Mouse intra-cerebral lethal dose killing 50% of test animals.
Vaccines below the required titre are unlikely to be efficient. Therefore, great care must be taken to avoid decrease of this titre particularly by thermal inactivation. Cold chain conditions must be ensured all along from release and control of each batch by the manufacturer until the day of inoculation. Lyophilized vaccines must be used without delay (within 30 minutes) after dilution, and not kept at outside temperature or exposed to sunlight.

MV must be injected by the intramuscular route and never be mixed with other products.

Inactivated virus vaccines are much more resistant to thermal inactivation. Short accidental exposure to temperatures above 40°C does not appear to destroy but can reduce their protective activity. They must, therefore, be kept at low temperature (especially for liquid vaccines) but not frozen.

Inactivated vaccines can usually be mixed with other inactivated (or even live) vaccines and can be used either simultaneously or intramuscularly. The last route is recommended for vaccines without adjuvants.

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Cost of production at different volumes

The evaluation of the cost of vaccine production is difficult, in any case, due to the variation of a number of parameters depending on:

- The local conditions (price of basic supplies, laboratory materials, personnel expenditures, etc.). The production background (number of vaccine doses produced, number of existing laboratories, depreciation of furniture and equipment, etc.). Variations of these parameters can increase the cost of production from 2 to 10. The cost of production, including overheads, of a tissue culture origin inactivated vaccine at a cost of $5.00 per 100 doses has been estimated at $7 and $13.95 per 100 doses. The higher cost for vaccines with a single use container, although production costs for other types of vaccines are not available, the market price of the different vaccines are comparable, about $0.60 per dose. This would indicate that actual production costs are very similar.

In any case it appears advisable to ask national and international companies for price estimates prior to the purchase of vaccine. The corresponding offers will usually reflect the current market situation and the lower prices usually offered will be in the range of $0.1 to $0.95 dollars per dose for inactivated vaccines, and from $0.2 to $0.95 dollars for MV vaccines.

As batch numbers increase, the production costs decrease. This is due mainly to a decrease in the costs of quality control testing as it is spread over more doses. A point to notice, however, where because of size, the loss of a batch of vaccine due to an unsatisfactory test result becomes a severe economic impact. If a high unsatisfactory test rate is anticipated, then purchase of vaccine that has already been found satisfactory may be cost effective.
(e) Stability

Stability is an important consideration in the selection of a vaccine to be used in a rabies control programme. Vaccines should be monitored for loss of potency during the storage period. The time between release and expiry date of a vaccine, sometimes referred to as its shelf-life, is usually not more than 6 months for liquid inactivated vaccines, but much longer, at least 18 to 24 months, for lyophilized inactivated or MLV vaccines.

It should be noted that MLV vaccines in particular, may be adversely affected by improper treatment during storage and shipment. The cold chain must be ensured from the moment the vaccine is released until it is used. This calls for special attention from and briefing of officials in various services concerned (i.e. airline and customs officials, freight agents, field agents).

5.5.2 Quality control

Quality control of rabies vaccines is of utmost importance in any programme of rabies control: vaccinations using uncontrolled vaccines should be avoided under all circumstances, because of the severe consequences of any false confidence in an animal’s immunity.

No producer can assume that his product is safe and potent: every batch of rabies vaccine has to be controlled before release. This control comprises identification, safety, potency and stability of the vaccine.

(a) Control authorities

Quality control of rabies vaccines should be conducted at two levels: by the manufacturer and by a national control authority, e.g. the National Rabies Laboratory or National Veterinary Services Laboratory.

Tests required of the manufacturer (Annexes 5.25 and 5.26) comprise those on the master seed virus and serial lots of vaccine as well as those for viral and bacterial contaminants on all ingredients used in the vaccine or during production such as cells, nutrient serum, trypsin, etc.

It is considered beneficial for the quality control department of the manufacturer to be under different management from the production department.

Quality control conducted at the national laboratory should consist of tests on the master seed virus and selected tests on some batches of vaccine. The depth of testing conducted by the national laboratory is influenced by the degree of correlation present in results obtained from the manufacturer and the national laboratory. As the incidence of batches being determined satisfactory by the manufacturer (but unsatisfactory by the national laboratory) increases, the testing rate also increases.

Besides testing batches of vaccine at the time of manufacture, it is also the responsibility of the national laboratory to test batches of vaccine at the expiration date. This is accomplished by maintaining a repository of samples from each batch of vaccine produced. When a vaccine demonstrates a below-minimum result at the expiration date, the manufacturer is required to
shorten the period during which the vaccine can be marketed. This is accomplished by monitoring the potency at 6-month intervals and re-establishing the duration of acceptable potency. An alternative to shortening the dating is to increase the minimum required potency level at the time of manufacture.

Another function of the national laboratory is to supply the reagents necessary to conduct the various tests required of the manufacturers. Of primary importance is the reference vaccine used in the VN test. The reference for veterinary vaccines (as for example a VSU vaccine produced on chick embryo cells or suckling mouse brain) should be prepared, standardized and distributed by the national laboratory (see Section 5.5.7). The vaccine should be lyophilized, and potency from batch to batch is controlled by the manufacturer to reconstitute the vaccine and by additional dilution if necessary.

(h) General procedures for quality control

Although many new methods of testing rabies vaccines have been proposed during the last decade, the main types of test remained unaltered:

(i) Identification. This test is the same, whatever the type of vaccine. It verifies that a vaccine protects animals (usually mice or guinea pigs) against challenge infection with a known rabies virus strain. In addition, the virus strain should be identified by monoclonal antibodies.

(ii) Safety. Specific tests must be performed for different types of vaccine. For example, inactivated rabies vaccines must be tested for safety by direct inoculation of dogs at different dilutions.

(iii) Efficacy testing. Many different tests are proposed for evaluation of the antigenic value of inactivated vaccines (see below). Apart from this routine test, compulsory for each commercial batch, the immuno-genic value, i.e. the ability to induce neutralizing antibodies in the target animal, should be regularly assessed in dogs. This assessment will clear two important points: the level of antibodies between three weeks after the recommended vaccination and the antibody profile during the expected duration of immunity. No vaccine should be approved for use in the field unless an appropriate standardized experiment has demonstrated a duration of immunity of at least one year in the dog. The protective value, i.e. the ability to induce a resistance against a rabies virus should be assessed at least once for each new rabies vaccine. This challenge should be performed with a street rabies strain isolated from the country where the vaccine is to be used.

(iv) Vaccine virus strains and antigenic variants

The strains of virus presently used in veterinary vaccines are derivatives of Challenge Virus Standard (CVS), Albert Voite (PV), Suckling-Alabama-Dufferin (SAD) and Glory Low and High Egg Passage (LEP and HEP) and Yellow viruses. These strains have proved effective in protecting against street virus exposure in dogs when potent vaccines have been properly administered. There is no proof of the existence of street virus strains against which these vaccine strains will not protect in most parts of the world.
The question of antigenic variation among rabies viruses from different parts of the world has been studied by monoclonal antibodies (see also Sections 1.1 and 5.3.7(b)) and is of two categories:

- **Virus strain variation.** The use of monoclonal anti-nucleocapsid and/or anti-glycoprotein antibodies makes it possible to show minor though significant differences between any two or more given virus strains under test. No differences in protection by established vaccine strains are noted.

- **Serotype variation.** This group of viruses has been shown by both types of monoclonal antibodies and by cross-protection tests to represent major antigenic variants of rabies virus being distinct serotypes.

The viruses of the latter group have been found so far to originate exclusively from the African continent from bats, cats and a dog. For Africa it may therefore be necessary to include local viruses in their vaccines, in order to protect dogs, cats, and possibly humans from exposure to aberrant field strains.

There are indications that viruses exist also in other parts of the world showing a low degree of serotypic variation. When assayed by cross-protection tests in mice, these animals proved to be not fully protected. The importance of these findings is not clear as yet. Do we need in such cases to include local virus strains in certain types of vaccines? Are failures seen in the post-exposure treatment of man due to antigenic variants or are they a result of late or inadequate vaccination? Carefully constructed experiments employing dogs and other animals, as well as variant strains for immunization and challenge should partially answer these questions.

(c) **Quality control for MLV vaccines**

Modified live virus vaccines are usually tested in respect of their identification, safety, and potency (see Annex 5.25).

(i) **Safety tests:** Several types of safety tests have been prescribed by national authorities for the various vaccines. Specific tests are described in the third edition of 'Laboratory Techniques in Rabies'. Contaminating agents (e.g. parvovirus) can be a problem in this type of vaccine, probably due to sera or trypsin used in vaccine manufacture, and must therefore be carefully controlled.

(ii) **Potency tests:** Standard potency tests for MLV vaccines and the general principles underlying them are described in Annex 5.25. It is recommended that samples from the field that are approaching their expiry date be tested again.

(d) **Quality control for inactivated vaccines**

Recommended control procedures dealing with identification, safety and potency test are described in Annex 5.26.

(i) **Safety tests:** Claims made that a vaccine contains only inactivated virus should be substantiated by adequate tests for residual live virus: each vaccine batch must be shown to be free of living agents including residual rabies virus, regardless of whether other safety requirements are followed.
(ii) Potency tests: A variety of potency tests are now available (see Annex 5.26) among which the Habel and NIH test are still the most widely used tests.

Among other tests proposed, only the antibody-binding test (measuring the capacity of a vaccine to bind a constant amount of virus-neutralizing antibody) and the antibody assay in immunized mice (measuring the antibody levels in mice as induced by serial dilutions of the vaccine) have proved to be of practical value for the assay of the antigenic value of inactivated vaccines. When carried out in cell culture the antibody-binding test is a fast (overnight) and reproducible procedure and is highly recommended for use by the manufacturer as a during-process control method, and for quick re-evaluations of vaccines before expiration.

5.5.3 Distribution system

Rabies vaccines for animal use, either live or inactivated, should never be freely available for sale, but should be distributed by and to veterinarians with possible control at a national level. This system would allow general surveillance of quantities and quality of the vaccine distributed all over the country.

The vaccines, either manufactured in the country or imported, must be stored according to general recommendations, particularly by maintaining the cold chain during storage and further shipment. The stock must be regularly monitored in order to avoid delivery of batches after or near the date of expiry. This can be easily achieved if a national 'Drug Store' exists or if manufacturers or importers take this responsibility.

The circumstances of vaccination (date, type of vaccine, batch number, etc.) need to be filed in standard registers irrespective of whether the vaccine is applied to individual animals or used during mass campaigns. This may facilitate a retrospective evaluation of possible vaccine failures.

5.5.4 Responsibility for supply

Supply of vaccine is often one of the thorny points during mass vaccination campaigns. Roughly, two main sources of vaccine supply exist:

- vaccines manufactured in the country
- vaccines imported from foreign countries.

In both instances the national government should always have a direct control upon the quality and quantities of rabies vaccine, in order to organize rabies control at a national level.

As far as animal vaccine is concerned, the responsibility should be with the Ministry of Agriculture having

- legislation to control the production or importation of vaccines used throughout the country
- practical facilities for the implementation of quality control of these vaccines.
5.5.5 Training of personnel making vaccine

Senior staff personnel having the responsibility for producing rabies vaccine should be qualified by education and experience, preferably at university level, with major fields of study in veterinary medicine, microbiology and immunology. All personnel employed in the preparation of biological products should be competent in good laboratory techniques through education or training so as to consistently prepare high quality products.

One major difficulty in manufacturing rabies vaccines is the training of personnel. Actually most of the producers are located in developed countries, where this responsibility lies upon private companies. These are often reluctant to train people or to teach them technical procedures (except if a specific financial contract is foreseen), particularly for licensed vaccines. Therefore, in most of these cases, the training of personnel must be planned via bi- or multilateral cooperation contracts between governments where state laboratories produce rabies vaccines. This training can be achieved either by visits of people in charge of production, or by consultation with experts in their country, or by both.

The training and transfer of technical assistants to work on seed viruses, cell lines, formulas for stabilizers, etc. should also be accomplished in collaboration with rabies research laboratories or WHO Collaborating Centres. Researchers from these laboratories are prepared to work closely with institutions intended for vaccine production by giving guidelines, arranging for training courses and, most efficiently, by on-the-spot training. Once started, these researchers should be contracted to control vaccine production during the next years and to serve as consultants when problems arise.

5.5.6 Sources of supply for local vaccine production

The decision to produce a certain type of vaccine locally is much influenced by the necessary laboratory space, equipment and personnel as well as by the availability of supplies in the country. In general, inactivated brain tissue vaccines are easier to produce with local supplies than are high quality cell culture products.

(a) Brain tissue vaccines

The main source of virus is nerve tissue collected from young infected animals: mice, rats, rabbit, sheep, goats or even cattle or horses.

Any of these sources are convenient provided that a sufficiently high titre of virus is obtained, i.e., at least $10^7$ mouse LD50 per gram of neural tissues. Lower titres are acceptable for "Fermi type" vaccines since the minimum required residual virulence ($10^2$ but not exceeding $10^{2.7}$ MICLD50) is far below the expected titres from brain tissue whatever the age of the animals.

The choice of animal species to be used depends upon their availability in the country in sufficient number and quality, and at the required times for vaccine production. For instance suckling mice can be obtained throughout the year whereas lamb brains are available only in the spring. About 300 to 600 suckling mouse brains are necessary to equate to the yield of one lamb's brain.
In addition, maintaining a mouse colony of pregnant females that will provide suckling mice in large numbers on a regular basis for suckling mouse brain vaccine is a complex undertaking that requires highly trained and skilled personnel and strict adherence to rigorous isolation procedures.

Stock colonies should be free at least from LCM and ectromelia, however, other endogenous viruses such as Sendai, mouse hepatitis and others may impose occasional but serious problems.

(b) Vaccines from cell cultures

An easy method of obtaining tissues for cell culture use is to arrange with a local abattoir to supply the desired organs, pack them in ice, and deliver them to the laboratory by the most expeditious means. This approach, however, can lead to difficulties. Porcine kidney has probably been the source of porcine parvovirus and rotavirus contamination. Bovine kidney is known to be the source of BVD, PI-3, reovirus, and bovine herpesvirus (e.g. 18B) contamination.

If feasible, the use of cell lines that have been tested for the presence of extraneous agents (and found clean) is much preferable to using primary tissues. Research laboratories, WHO Collaborating Centres and the American Type Culture Collection are able to supply starter cultures of baby hamster kidney, porcine kidney, bovine kidney, and similar cell lines that have proved useful for vaccine production.

Cell culture media and other ingredients may either be imported (autoclavable stock media are available as dry powder) or prepared from biological or chemical products manufactured in the country.

For MLV vaccines care must be taken that contaminants or even pathogens are not introduced via biological sources.

Bovine viral diarrhea (BVD) virus and mycoplasma sp. are two contaminants that are frequently encountered in volume vaccine production using cell cultures. Strict attention must be paid to how these vaccine ingredients are harvested and pooled. It is suggested that trypsin of porcine origin be examined using the procedure of Craghan, et al., Applied Microbiology 26; 431-433, Sept. 1973) to detect any extraneous parvoviruses. A dependable continuing source of BVD-free foetal bovine serum is a very difficult thing to locate.

5.5.7 Assistance by WHO Collaborating Centres

The necessity of support through WHO, and WHO Collaborating Centres in the field of training, advice and technical assistance has repeatedly been emphasized in this chapter.

For the control of inactivated vaccines (locally produced or imported) WHO does provide every country with the "3rd International Reference Preparation to be used in the NIH test". This will allow the expression of test results in "International Units" which is useful for the biological standardization of rabies vaccines throughout the world. In order to comply with the amount of reference vaccine required for daily routine use, each country is advised to prepare its own reference stock and to standardize it against the international reference preparation.
Similarly, an "International Reference Serum" for the titration of sera of vaccinated animals is available on request.

Seed virus stock for vaccine production (fixed strains) can be obtained through WHO Collaborating Centres which can also assist in the identification either of already used strains or of candidate strains isolated from the country since this strain may be more immunogenic against infection by indigenous street viruses.

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WORLD HEALTH ORGANIZATION

5. TECHNIQUES IN LOCAL PROGRAMME EXECUTION

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Dog Catching and Restraining Loop

- Aluminum pipe or bamboo pole 4-5 cm diameter
- Rope snare 1 cm x 120 cm long
- Metal band and ring

Place rope loop over dog's head and draw tight over neck by pulling through hollow handle (see Annex 5.1, page 2)
Dog Catching and Restraining Loop

Figure 1: Place rope over dog's head

Figure 2: Draw loop tight over neck by pulling through hollow handle
Animal Patrol Cages for Large Pickup Truck

All cages are constructed of chain link fence 4.5 cm with solid sheet steel floors. Space between double ceilings, walls and doors of rabies suspect cage is 5 cm. Doors should have locks.
All cages are constructed of chain link fence 6 ft. high with solid sheet metal floors. Spacers between double rollings, walls and doors of cages should have locks.
Animal Patrol Cages for Jeep

All cages are constructed of chain link fence 4.5 cm with solid sheet steel floors. Space between double ceilings, walls and doors of rabies suspect cage is 5 cm. Doors should have locks.
Design of Dog Pound with Communal Dog Cages, Rabies Suspect Cages, Carbon Monoxide Chamber and Office

Locations of cage building and office building may be modified to local site. Components must be vaulted and access limited through a secure gate. Water lines in carbon monoxide scrubber must be below outlet pipe and away of inlet pipe. Water should be changed weekly.

Carbon monoxide scrubber and chamber must be gas tight for airtight function and protection of personnel.
Details for Cages for Animal Pound

<table>
<thead>
<tr>
<th>Cage Size</th>
<th>Material</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large</td>
<td>Steel</td>
<td>For large dogs</td>
</tr>
<tr>
<td>Medium</td>
<td>Steel</td>
<td>For medium dogs</td>
</tr>
<tr>
<td>Small</td>
<td>Steel</td>
<td>For small dogs</td>
</tr>
</tbody>
</table>

- **Front View**
- **End View**
- **Top View**

Rabies suspect cages must be of sturdy construction with double doors and back walls. Special purpose cages are for identified or purebred dogs for placement, or for vicious dogs which cannot be placed in `compound cages`. 

Page 2

Annex 5-3
The use of strychnine in order to poison dogs and jackals

1. Baits prepared for the purpose of poisoning dogs and jackals contain strychnine which is one of the most dangerous poisons. A very small amount of this poison is enough to kill a human being or an animal, including birds.

You are therefore requested to handle this material with the utmost caution.

2. The cachets and tablets of strychnine must be kept in well-closed metal boxes. Be careful not to wet them or crush them. Under no circumstances are you allowed to give them to other people.

3. On the box containing this poison a label must be fixed stating: "POISON". The box must always be kept in a drawer of a cupboard shut up with a lock.

4. Immediately after handling cachets or tablets of strychnine you should wash your hands with soap and water.

THE POISONING OF DOGS

5. To prepare a bait for poisoning dogs, take a small piece of meat or cheese. With the help of a knife or a ball make a small hole in the middle of the piece and put the strychnine in it. Press with your fingers on both sides so that the poison will be kept well inside the ball. The size of the ball should not exceed about 1 1/2 cm. Otherwise the dog will not be able to swallow it properly. Be still and wait and bite or the bitter poison, and then do not eat it.

6. Before going out to poison dogs prepare off-line baits for use within the next ten minutes.

7. When approaching a dog first throw him a piece of meat or cheese without the poison. After he has swallowed this, put the bait before him and go back a few steps. Watch the dog carefully to be sure that he has properly swallowed the bait.

8. In case the bait has not been taken up or swallowed or brought up again and thrown out from the dog's mouth, you must collect it and bury it deeply in the soil or take it with you in a second metal box for safe destruction.

9. Dogs which have been destroyed by poison must be buried soon afterwards, otherwise there is the danger that wild birds will find the carcass and get poisoned too.

---

Editorial note

The editorial group of these guidelines does not advocate the use of strychnine, but feels obliged to include this example of a national code of practice, which points out the precautions to be observed by persons handling this dangerous poison.
DESTRUCTION OF OTHER WILDLIFE CARNIVORES*

10. For the destruction of wild carnivores, e.g. jackals, you can use remnants of meat, spoiled sausages, day-old chicks or pieces of poultry bowels. Put the tablet or cachet within the mouth of the chicken or within the bowel and close it from both sides by a loop.

11. Put the baits in the afternoon near dung heaps or near a lane or a path on which footprints of wild carnivores have been seen. Put a small stick near each bait in order to find it in the morning when not eaten during the night, so that it can be taken back. Cover it with a few leaves so that carrion birds will not find it. Do not put more than ten baits in one location and keep a distance of two metres at least between the baits.

12. In the morning return to the baited place in order to get an estimate of the success of the action, to look for dead animals which should be buried, and to take back baits which have not been eaten.

PUBLIC WARNING

13. A warning "DANGER" notice announcing the intention to kill jackals and stray dogs in the district should be posted one to two days before the action.

---

* Editorial note

The indiscriminate use of poison baits may pose a risk to humans and endangered species.
Annex 5-5

Brochures for Rabies Control

RABIES

A DISEASE WHICH CAN BE CONTROLLED IN:

- can be prevented by vaccinating dogs
- is usually spread to people bitten by dogs
- will not be spread by vaccinated dogs
- have your dogs vaccinated when they are 3 months old
- revaccinate your dogs 1 yr. later and then every 2-3 yrs.
- keep your dogs in your compounds or yards
- over 25,000 dogs die of rabies each year
- over 100,000 people take antirabies vaccine yearly
- over 200 people die of rabies each year

Vaccinating teams from the City Health Office will be in from ________ to ________. Have your dogs ready and help the vaccinators.
Rabies

Rabies is caused by a virus. In this country, dogs are the important animals which spread rabies. Infected dogs may be very ferocious in the early stage of the disease and may bite other dogs, spreading the virus to them. Later the infected dogs become paralyzed but may still bite and spread the virus until they die. Infected dogs may bite other animals as well as dogs, including cats, pigs, cattle, carabaos, horses, goats and sheep. Dogs which have rabies, and also cats, pigs, horses and occasionally other animals bite people and expose them to rabies. In some countries, wild animals also spread rabies to other wild animals, to livestock and pet animals and to people, but fortunately in this country, wild animals do not maintain the spread of rabies.

People who are bitten by any animals should first wash the wound well, then report to their nearest physician or health center. If the biting animal is a dog or cat, it should be confined so that it cannot bite any more people and should be observed for 10 days. If the dog or cat has rabies, it will surely die within this period. A veterinarian or animal inspector should be asked to see if the animal has rabies or to send the head of the animal to a laboratory for diagnosis. If a biting animal is suspected or proven to have rabies, then the victim must be given a series of vaccine injections by a physician or at the health center, and even then, some may not be protected and may become sick and die from rabies.

If dogs are vaccinated against rabies, they will be protected from the disease, and if at least 80% of the dogs in a community are vaccinated, the disease will disappear. It is important that every dog above 3 months of age be vaccinated during community vaccination campaigns. Many dogs are born each year and they must be vaccinated too. Keep dogs from wandering around by keeping them in your compounds or fenced yards. Keep your communities clean of garbage, wastes and any other food dogs could eat, as well as of unused buildings, cars or wood piles under which dogs could live. In this way, there will not be dogs wandering around your community, at risk of being exposed to rabies and of spreading the disease to ---- you.
حملة
مقاومة
داء الكلب

الجمهورية التونسية
وزارة الفلاحية
ادارة الانتاج الزراعي
ادارة التعليم والبحوث والارشاد الفلاحي

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كلب تجوب المدن والارياض
مواطن تقضى هواة الكلاب كل عام
مواطن يموتون بداء الكلب كل سنة

اربطوا كلابكم
ولقحوها
Posters for Rabies Control

- Annex 5-6
- page 2

طريقة الحماية

- لا تشربوا الامطاراء والماء من الكلاب
- فمها قد تكون مسماً للتهام العرق
- كل عقدة كل أو طاعة كل أو مناعاً للالتهاب الجلبي من الكلاب
Sus buenos sentimientos pueden traicionarlo.

Los perros callejeros deben ser eliminados

Combata la rabia

Provincia de Buenos Aires
Ministerio de Salud Pública
Annex 5-7

RADIO ANNOUNCEMENT*

1. Protect yourself and your neighbours from rabies. Have your dog vaccinated when the vaccinators come to your barrio. A vaccinated dog will not spread rabies even if it should bite you or your neighbour.

2. Stop rabies in Dumaguete City. Rabies is spread by dogs that have not been vaccinated. A vaccinated dog will not spread rabies. Have your dog vaccinated against rabies when the vaccinators come to your barrio.

3. Rabies is a serious problem in Dumaguete City. Many people are bitten each year by dogs with rabies and are forced to take injections so that they will not die of rabies. Sometimes the injections do not save the person from rabies. Dogs which are vaccinated against rabies will not spread rabies even if they might bite someone. Have your dogs vaccinated.

4. Dogs which are vaccinated against rabies will not spread rabies even if they might bite someone. People bitten by vaccinated dogs do not have to take anti-rabies injections. Protect yourself and your neighbours. Stop rabies at the source. Vaccinate your dogs instead of being forced to take anti-rabies injections.

5. Stop rabies. Dogs can be vaccinated against rabies with only one injection. This injection will protect them for three years. The dog is the most important source of rabies. Have your dog vaccinated when the vaccinators come to your barrio.

6. Help your city stamp out rabies. The City Health Office will be sending teams of vaccinators to your barrio soon. Have your dog vaccinated. Rabies can be eradicated if you all cooperate.

7. Rabies is a dangerous disease. People bitten by dogs with rabies must be given many injections to protect them from the disease. Once a person is sick with rabies, he will surely die. Stop rabies in dogs by having them vaccinated.

8. Eradicate rabies in dogs in Dumaguete City. Have your dogs vaccinated when the vaccinators come to your barrio. Report the presence of stray dogs to the police department. Vaccinated dogs cannot spread rabies to the people.

9. Have your dogs vaccinated against rabies. Protect your barrio from rabies. If every dog in your barrio is vaccinated against rabies, this disease can be eradicated from your barrio. Have your dogs vaccinated when the vaccinators come to your barrio.

---

* Editorial Note

This radio announcement was specifically prepared to announce a local control campaign. It is quoted as an example.
10. The rabies problem in Dumaguete City is serious. Let us eradicate this disease here. The City Health Office is sending teams of vaccinators to each barrio. When the vaccinators come to your barrio, have your dogs vaccinated. If every dog in Dumaguete City is vaccinated, rabies will be eradicated.

11. Protect yourself. Don’t get rabies by being bitten by your dog. Don’t let your dogs give rabies to anyone else. Have your dogs vaccinated against rabies when the vaccinators come to your barrio.

12. Help stop rabies in Dumaguete City. Rabies is a fatal disease. It kills dogs, other animals and people. Dogs are the animals which usually spread rabies. Stop rabies in dogs. Have your dogs vaccinated. Report stray animals to the police.
Annex 5-8

RADIO INTERVIEW

Q. Is rabies usually a disease of dogs, Dr? 

A. Yes it is. At least, dogs are the most dangerous animals in its spread to people. You see, dogs live close to people's homes, so they have many chances to bite people. Dogs with rabies frequently, but not always, become furious when they have rabies and try to bite anything near them.

Q. Since you were fairly recently bitten by a rabid dog, would you tell a little about this experience.

A. Well, being bitten by a rabid dog is nothing any of us is proud of, though I am sure many of you in our radio audience have experienced this. Like most people, I suppose I thought that other people may be bitten but that it would probably never happen to me. I was bitten while examining a dog for rabies.

Q. Did the dog have rabies? Was it really furious?

A. Yes, the dog had rabies all right. But it wasn't furious, at least not at first. The dog seemed almost normal. It took a few bites of food, though it seemed to have trouble swallowing them. The owner patted the dog and it seemed normal. But when it suddenly turned on me, it bit twice before I could escape.

Q. Then what happened to the dog?

A. Well by the next day, the dog was weak and had no more interest in eating. The following day the dog quietly died. We removed the brain from the dog in our laboratory, placed a tiny portion on a glass slide, coloured it with dyes prepared for the purpose, and examined it under the microscope. There we saw the signs so characteristic of rabies.

Q. So the dog died of rabies. Obviously you didn't die of rabies. What did you do after being bitten, Dr?

A. I remember the first thing we did was to run to the house and scrub the bites with plenty of running water and soap. Not that I'm selling soap but it is very important that bites be washed at once with plenty of water and soap. Wash gently but thoroughly to remove the rabies virus left in the wound by the dog.

Q. Did you take the anti-rabies injections?

A. Yes, I did, as I suppose many of you in our radio audience have also done. This vaccine is given in many injections, one each day.

Q. Now I understand that the anti-rabies injections are to prevent rabies, not to treat the person who is already sick with rabies. Do the injections always protect the person from rabies?

A. You are right, that the injections are given to prevent rabies. We give them after the person has been bitten but before the person comes down with the disease. We must work fast and give large amounts of vaccine to stop the rabies virus before it reaches the brain. Once the
virus reaches the brain, there is no more hope and the person will surely die. Bites on the face and neck are especially dangerous as they are so close to the brain. People bitten on the face and neck often die of rabies even if injections are given. Unfortunately, too, some people who take all the injections also die of rabies.

Q. Why do some people who are properly vaccinated still die of rabies.

A. I don't really know. There are always a few people who are not protected by any vaccine. But it is more than this. Last year when three people were bitten by a rabid dog here in Valenzuela, Marikina, Oriental, they all took the proper injections, but all died of rabies. I'm afraid the vaccine we have available and our effort just isn't powerful enough to protect everybody.

Q. I have heard that the rabies injections are also quite dangerous. Is this really true?

A. Unfortunately, it is true of the types of vaccine we commonly have available. People occasionally develop an allergic reaction to the injections and this reaction may be very severe. We have had five such cases within the last few years at the University Medical Center. One was so severe that we were forced to stop the injections or the patient would have died from the reaction. The patient just had to take the chance that he would not come down with rabies and fortunately he did not. I do know of at least five cases in the Philippines where people have died from the injections. In one case in Iloilo the patient died and the dog did not even have rabies. This is very tragic.

Q. If there are dangers in taking the anti-rabies injections and if the injections are not always effective, what is the solution to the rabies problem?

A. I believe there are two solutions. For the person who is bitten by a rabid dog, there is no choice but to take the anti-rabies injections. The best control of rabies, I believe, is to stop rabies in dogs; then the disease will not be spread to people.

Q. How can rabies be stopped in dogs?

A. Dogs can be protected from rabies by vaccinating them.

Q. Is the vaccine for dogs used in the same way as the vaccine used in people who are exposed to rabies?

A. No, the vaccine available for dogs is given before the dog is bitten by another rabid animal, while the vaccine given to people is given after they are bitten. The best dog vaccine is given in only one injection and it protects a dog from rabies for three years.

Q. Where can we get our dogs vaccinated?

A. Until now, there have been two places, the Provincial Veterinarian's Office and the University Medical Centre. Now the City Health Office will be vaccinating dogs all over our city to implement a new municipal ordinance to eradicate rabies here.
Q. So we take our dogs to the City Health Office for vaccination?
A. No, it will not be necessary to take your dog to be vaccinated for the City Health Office will bring the vaccine to your dog. A series of vaccination campaigns are being planned in . . . . . . . . of the city. The time and place to get your dogs vaccinated is when the vaccinators come to your house.

Q. But won't our dogs bite while being vaccinated?
A. Please have your dog tied or confined at your home when the vaccinators come. If you do, I think that we can safely handle them. We have several devices to catch and hold dogs and to keep them from biting anyone during vaccination. The simplest of these is a muzzle that fits over the dogs' mouth. Our vaccinators will be trained to inject the dogs quickly and with very little pain.

Q. Of course, we hope that every dog will be confined for the vaccinators, but we see some dogs wandering around our city with no owners to have them vaccinated.
A. Yes, we really want to vaccinate every dog three months or older. If you feed a dog or if it sleeps under your house, even if you do not own it, confine it for the vaccinators. The vaccinators are going to have to catch and vaccinate dogs running around our city without owners.

Q. Now after all dogs will have been vaccinated, what should a person do who may be bitten by a dog?
A. First, at least make sure that the dog was wearing a license tag. If possible, get the number on the dog's tag. Then wash the wound where the dog bit you. This is always important in any bite. Wash the wound well with plenty of soap and water. The new city ordinance requires that all persons bitten by dogs should notify the City Health Office for record purposes. Physicians there will decide whether any treatment is needed.

Q. How will we know when the vaccination clinic will be held in our area?
A. The news will be spread by local officials. Also, just before the vaccination clinic, the Department will show a movie telling about rabies and its control. The news will also be given over the radio. The important thing is when the vaccination campaign is held in your area that you will have all your dogs vaccinated. We can eradicate rabies if we all work together. Let us do it!

Closing remarks.
A VACCINATED DOG

WILL NOT GET RABIES

ANG BINAKONAHANG IRO

DILI Gayud MATAKDA Sa RABIES

Posters for dog vaccination

WILL NOT GET RABIES
SAMPLE OF A POSTER USED IN AN ARAB COUNTRY

RABIES = DANGER!

DEFEND YOURSELVES!

PROTECT YOUR FAMILY

VACCINATE YOUR DOG AGAINST RABIES!

EVERY DOG WHICH HAS NOT BEEN VACCINATED WILL BE DESTROYED!
NO PETS

The British isles are still free from rabies and wish to remain so. Any animal landed in Britain must have an import licence and undergo quarantine, even if it has been vaccinated.

If you are visiting Britain, you are advised not to take your pets with you. If you travel in your own boat, any animals must be kept in confinement on board throughout your visit.

Smuggling of animals is punishable by heavy fines and up to a year's imprisonment. Any illegally imported animal is liable to be destroyed.
Children's Colouring Book
encouraging vaccination of pets

VACCINATE
Your Dog
Against RABIES

Protect
☆ Your dog
☆ Your family
☆ Your neighbors
Inday has a new dog.
The dog's name is Bantay.
Inday and Bantay like to play together.
Bantay is being vaccinated against rabies. The man is from the City Health Office. Both the man from the City Health Office and Inday are happy because now Bantay will not get rabies.
When Bantay gets vaccinated, he is given a dog tag to wear on his neck. Bantay is happy because he can play with Inday with no danger of rabies.
What you should know about rabies

Rabies is a disease which can affect all warm-blooded animals; this includes man. It is spread by a bite from an infected animal or by its saliva entering an open wound. Once symptoms develop it is always fatal.

The British Isles are free from rabies, but it is widespread in most of the rest of the world.

Life in our cities and in the countryside would change for the worse, and friendly contact with animals would cease.

All animals coming into this country must have an import licence and spend six months in quarantine.

Rabies could be brought from abroad by tourists and workers smuggling in animals, so there are heavy penalties for breaking these rules.

How you can help keep rabies out:
- If you go abroad, don't take your pets with you.
- Don't bring any animals with you when you return.
- If you suspect that an animal is being or has been smuggled into Britain, report it to the police or Customs.
- Make sure your friends know the rules, too.

Remember:
- When you are abroad, avoid contact with stray animals.
- If you are bitten or scratched, wash the wounds with soap and water immediately and see a doctor.
- Tell your own doctor what has happened when you get home.

Keep rabies out since 1965, rabies has been spreading across Britain.

Annex 5-12
EDUCATIONAL CHART
What you should know about rabies

**DOG-BITE?**
Then, here's what you do

- Stimulate bleeding, promptly wash with soapy water
- Seek medical advice: if ordered, take injections
- Tie up & Observe dog for 10 days
- If dog is alive, he is free of RABIES
- If dead, rush carcass to Diagnostic Laboratory

ANTI-RABIES DRIVE
PREPARATION OF ZENKER'S-ACETATE FIXATIVE

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>Potassium Dichromate</td>
<td>2.5 g.</td>
</tr>
<tr>
<td>Mercuric Chloride</td>
<td>5.0 g.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100 ml</td>
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</tbody>
</table>

PREPARATION OF 50% GLYCEROL-SALINE

Add equal parts of chemically pure glycerol to physiological saline. Place solution in small bottles or jars with screw-capped tops, autoclave and store at room temperature.
SELLER'S STAIN FOR NEGRI BODIES

Stock solutions

A. (i) Methylene blue 2 g.
   (ii) Absolute methyl alcohol (acetone free) 200 ml

B. (i) Basic fushsin 1 g.
   (ii) Absolute methyl alcohol (acetone free) 100 ml

Staining solution

<table>
<thead>
<tr>
<th>Methylene blue (A)</th>
<th>2 parts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic fuchsin (B)</td>
<td>1 part</td>
</tr>
</tbody>
</table>

Mix (A) and (B) thoroughly and do not filter. Store in screw-capped container. The mixed stain improves after standing for 24 hours and keeps indefinitely if protected from evaporation.

Staining procedure

Smears of fresh brain tissue preferably from the hippocampus major are made on glass slides. No fixation is required. While the smear preparation is still moist, immerse in Seller's stain for 1-5 seconds depending on the thickness of the preparation. Rinse quickly in running water or better in distilled water buffered to pH 7.0 and dry in air without blotting.

Results:

Negri bodies are stained magenta or heliotrope to bright red and show dark blue or black basophilic internal bodies. All parts of the nerve cell are stained blue. Interstitial tissue stains pink and the erythrocytes a coppery red colour.
FAST GREEN - ACID SAFRANIN (Smith, E.E.G., 1953)

Slices of tissue 1-2 mm thick are fixed in Zenker acetate for 12-24 hours. Alternatively, the whole brain may be fixed in bulk for easier handling; thin slices are then fixed for shorter periods.

Wash the fixed tissue slices in running water for 6-8 hours (Negri bodies in inadequately washed material stain black instead of green). Cut paraffin sections and bring down to water. Remove mercury salts with iodine and thiosulphate.

Stain for 20 minutes in:

<table>
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<tr>
<th>Solution</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>Fast Green FCF.</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Ammonium acetate</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Distilled water to 100 ml</td>
<td></td>
</tr>
</tbody>
</table>

Wash with tap water; sections are now a deep bluish-green. Differentiate in acid alcohol (1 per cent. HCl in 70 per cent. ethyl alcohol) until almost all the green colour is removed from the section. Decolourisation is fairly slow, but with practice the endpoint is easily determined with the naked eye.

Counterstain for 5 minutes in:

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<tr>
<th>Solution</th>
<th>Quantity</th>
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</thead>
<tbody>
<tr>
<td>Safranin O</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Distilled water to 100 ml</td>
<td></td>
</tr>
</tbody>
</table>

Wash thoroughly in tap water to remove excess safranin, dehydrate rapidly and mount in Canada balsam or Gurr's Neutral Mounting Medium.

Results

Negri bodies are stained an intense bluish green; high-power examination (1.8 mm) shows characteristically arranged vacuoles or inner bodies. Erythrocytes also stain green but have no internal structure. Microglial nuclei and nucleoli of nerve cells are bright red. Cytoplasm and general background are pale pink or grey.
BASIC EQUIPMENT AND SUPPLIES FOR RABIES DIAGNOSTIC LABORATORIES

General. Most of the materials listed below are required for all the specific techniques.

A. Equipment

1. Incubator, 37°C
2. Refrigerator, +4°C
3. Refrigerator, -20°C
4. Refrigerator, -70°C
5. Sterilizer
6. Incinerator
7. Weighing balance
8. Womson gas burner
9. Safety cabinet with UV lamp unit
10. Autolav
11. pH meters

B. Reagents

1. Alcohol (70%)
2. Distilled water
3. Phosphate buffered saline (PBS), pH 7.2-7.4
4. Disinfectants (lysol, iodine, etc.)

Specific. The following materials are required for each method described below in addition to those listed under "General".

Autopsy

A. Equipment

1. Post-mortem cable
2. Sharp autopsy knife or large scalpel
3. Sharp saw (preferably hack saw)
4. Bone-cutting forceps
5. Bone-holding forceps (lion-jaw type)
6. Carpenter's vice
7. Surgical instruments viz. rat-tooth thumb forceps, scalpels, sharp-pointed scissors
8. Glassware or plasticware (tubes, flasks, pipettes, petri dishes etc.)
9. Plastic bags for containing carcasses or animal tissue for incinerator
10. Large water-tight container
11. Smaller water-tight container
12. Cracked ice
13. Protective clothing (heavy rubber or plastic autopsy aprons, close-fitting autopsy gloves, rubber boots, masks and goggles).
14. Labels
15. Marking, waterproof pens.
B. Reagents

1. Zenker's fixative (Annex A)
2. 50% Glycerol-saline (Annex B)
3. Disinfectants for wound treatment (45-70% alcohol, 1% soap solution or 5-7% iodine solution can kill rabies virus within 1 minute).
4. Anti-rabies serum or human gamma globulin sufficient for two persons (store at 4°C)

Seller's Staining Technique

A. Equipment

1. One light, binocular microscope
2. Glass slides
3. Scissors, scalpel and spatula, forceps
4. Coplin jars
5. Petri dishes

B. Reagents

1. Seller's stain (Annex C)
2. 50% glycerol-saline (Annex B)
3. Immersion oil

Fluorescent Antibody Test

A. Equipment

1. One fluorescence microscope (preferably with incident lighting)
2. Glass slides
3. Slide boxes, for storing control slides
4. Coplin jars
5. Cover slips
6. Scissors, scalpels, forceps and spatula

B. Reagents

1. Conjugate (commercial)*
2. Normal mouse brain suspension stored at 4°C
3. Infected mouse brain suspension
4. Positive control smears
5. Negative control smears stored at -70°C
6. Test smears
7. Acetone
8. Immersion oil
9. Glycerol mounting medium

* The required amount depends on volume of work
* e.g. Baltimore Biological Laboratories (USA), Behringwerke, Marburg, (FRG), Cappel Laboratories, Cochranville, Pa. (USA), Pasteur Institute, Paris (France), Wellcome Research Laboratories, UK.
PROCEDURE

I. Laboratory animals. Swiss albino mice. Two litters of weaning mice (1-2 days old). 10 per litter and six adult mice are required for each specimen. An estimated amount should be preserved weekly from a well-kempt animal colony.

II. Equipment

1. Cage with clean, dry bedding and water for drinking
2. Mouse food and water
3. Anaesthetic jar with air-tight lid
4. Gauze for cocking ether in jar
5. Scissors, scalpels, forceps and syringes
6. Syringes (1/4 ml)
7. Needles (27 gauge)
8. Mortar and pestle (one each)
9. Sterile sand
10. Protective clothing (gown, mask, aseptic gowns)

III. Reagents

1. Clinical specimens dry or with preservative
2. Normal saline
3. Inactivated normal rabbit serum
4. Ether
5. Penicillin (crystalline)
6. Streptomycin

Available commercial sources for most of the equipment and supplies mentioned are given in Annex 5.
ENVOI D'UN PRÉLÈVEMENT
AU CENTRE NATIONAL D'ÉTUDES SUR LA RAGE
EN VUE D'UN DIAGNOSTIC ÉPIDÉMILOGIQUE

(Les rubriques signalées par un astérisque sont à remplir OBLIGATOIREMENT en CAPITALES)

SERVICES VÉTÉRINAIRES

Département de: ____________________________
Prélèvement expédié le: ____________________________
N° d'enregistrement: ____________________________
Espèce: ____________________________________________ Sexe: ____________________________________________
Trouvé mort - mort - abattu, le: ____________________________

Commune*: ____________________________________________
Canton*: ____________________________________________
Arrondissement*: ____________________________________________
Département*: ____________________________________________

Origine:

N° d'enregistrement: ____________________________________________
Espèce: ____________________________________________

Sexe: ____________________________________________
Trouvé mort - mort - abattu, le: ____________________________

Commune*: ____________________________________________
Canton*: ____________________________________________
Arrondissement*: ____________________________________________
Département*: ____________________________________________

Nom du propriétaire*: (le cas échéant) ____________________________________________
Adresse: ____________________________________________

Nom du vétérinaire*: (le cas échéant) ____________________________________________
Rue*: ____________________________________________
Code postal et localité*: ____________________________________________

Observations: (animal vacciné ou non, date de la vaccination, de la contamination, symptômes, etc.)
__________________________________________
__________________________________________
__________________________________________
__________________________________________
__________________________________________

CET ANIMAL N'A PAS ENTRAÎNÉ DE CONTAMINATIONS HUMAINES

Date et signature

CADRE RÉSERVÉ AU LABORATOIRE

1) IMMUNOFLUORESCENCE

POSitive NÉGative IMPossible ILLisible Le:

2) HISTOLOGIE

Non Faite

POSitive NÉGative IMPossible ILLisible Le:

L'inoculation aux souris est-elle décidée ou en cours: OUI / NON / IMPossible

1ère réponse: ____________________________

3) BIOLOGIE

IF

POSitive NÉGative INInterprétable Le:

* jour * jour

2ème réponse: ____________________________
## Annex 5 *

### SUGGESTED CASE RECORD FOR HUMAN RABIES EXPOSURE

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Referred by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person bitten</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Date of bite</td>
</tr>
<tr>
<td>Age</td>
<td>Geographical locality of biting episode</td>
</tr>
<tr>
<td>Sex</td>
<td>Scratch of bite on the body</td>
</tr>
<tr>
<td>Home address</td>
<td>Nature of bite</td>
</tr>
<tr>
<td>Other persons, if any, bitten by the same animal</td>
<td></td>
</tr>
<tr>
<td>(Names and addresses)</td>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
</tr>
</tbody>
</table>

### Treatment

- Local wound treatment

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of vaccine (either or both), if given</td>
<td>Source of serum Human Anti-Rabies Immune Globulin</td>
</tr>
</tbody>
</table>

### Previous rabies vaccine application? Previous serum treatment? |

| Date | Type | Date | Type |

Were there complications of treatment? If so, specify treatment and outcome |

### Status of exposed person after 6 months:

- Sex
- Date of death

### Status of other persons bitten by same animal, if known | 1. |

### Biting animal

- Kind of animal
- Description
- Breed | Age | Sex | Weight |

Animal vaccinated against rabies? |

If so, type of vaccine |

### Outcome

- Date |

- Result of laboratory examination:
- Healthy
- Dead

- Results of laboratory examination:
- Enzyme-linked immunosorbent assay (ELISA) positive
- Neutralization test (NNT) positive
- Other tests

SUGGESTED VACCINATION CERTIFICATE FOR MAN *

The certificate described here should be kept carefully by the vaccinee with his personal health documents. Blank forms should be supplied by the manufacturer of the vaccine.

RABIES VACCINATION CERTIFICATE

Name ............................................................... ............................................................... ............................................................... ............................................................... ............................................................... ............................................................... ............................................................... ............................................................... ............................................................... ............................................................... ............................................................... ............................................................... ............................................................... ............................................................... ............................................................... 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### PRE-EXPOSURE VACCINATION

#### Primary vaccination

<table>
<thead>
<tr>
<th>Date</th>
<th>Dose</th>
<th>Type of vaccine Origin/Batch no.</th>
<th>Vaccination centre</th>
<th>Signature of physician</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

The label of the vaccine box may be stuck here.

Serum titre, if determined: ....................................................

#### Booster

<table>
<thead>
<tr>
<th>Date</th>
<th>Dose</th>
<th>Type of vaccine Origin/Batch no.</th>
<th>Vaccination centre</th>
<th>Signature of physician</th>
</tr>
</thead>
<tbody>
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</tr>
</tbody>
</table>
POSTEXPOSURE TREATMENT

1. Serum or rabies immune globulin (human origin)

<table>
<thead>
<tr>
<th>Date</th>
<th>Dose (IU)</th>
<th>Origin</th>
</tr>
</thead>
</table>

2. Vaccine

<table>
<thead>
<tr>
<th>Date</th>
<th>Dose</th>
<th>Type of vaccine</th>
<th>Vaccination centre</th>
<th>Signature of physician</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

Recommendation for postexposure treatment of persons vaccinated previously:

In the case of new exposure: give 1 booster dose of vaccine.

In the case of severe exposure or when there is doubt about the potency or immunization schedule previously used, additional booster doses of vaccine must be given, e.g., on days 0, 3 and 7.

Passive immunization (serum or RIG) should not be given.
<table>
<thead>
<tr>
<th>DATE</th>
<th>LOCATION CODE</th>
<th>LOCATION NAME</th>
<th>SPEC CODE</th>
<th>SPECIES NAME</th>
<th>HE</th>
<th>AK</th>
<th>AGE</th>
<th>SEX</th>
<th>MISCELLANEOUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>05/01/82</td>
<td>06.02.12.8</td>
<td>URBENHAUS</td>
<td>01</td>
<td>REDFOX</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>4</td>
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</tr>
<tr>
<td>05/01/82</td>
<td>06.02.13.3</td>
<td>BAD STETTEN</td>
<td>01</td>
<td>REDFOX</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>06/01/82</td>
<td>07.02.11.0</td>
<td>VIENNA, VILLARS</td>
<td>01</td>
<td>REDFOX</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td></td>
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<tr>
<td>07/01/82</td>
<td>08.02.14.6</td>
<td>VIENNA, VIENNA</td>
<td>01</td>
<td>REDFOX</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>4</td>
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</tr>
<tr>
<td>08/01/82</td>
<td>08.02.14.3</td>
<td>CHÂTEAU, DABÉLLON</td>
<td>01</td>
<td>REDFOX</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>09/01/82</td>
<td>09.02.13.0</td>
<td>SAVOY, CARLO</td>
<td>01</td>
<td>REDFOX</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>09/01/82</td>
<td>09.02.13.0</td>
<td>WINTERTHUR</td>
<td>01</td>
<td>REDFOX</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

**HE (human exposure) and AK (animal killed):** 0 = no, 1 = yes, 9 = not known.

**AGE:** 1 = young, 2 = adult, 9 = not known. **SEX:** 1 = female, 2 = male, 9 = not known.
Explanation of Case Reporting Form

1. Reporting country, e.g. Switzerland

2. Reporting unit, e.g. University of Berne

3. Reporting period, e.g. monthly, quarterly with the appropriate dates

4. Date - arrival of animal for diagnosis

5. Location code and location name, e.g. the town, district or regional name of where the animal was found and its corresponding location code
e.g. in Switzerland
   25 - Canton Zürich
   0228 - community of Turbenthal

6. Species code and species name - see "Animal Code Numbers" on adjoining list

7. Human exposure, animal killed, age and sex - use the appropriate numbers as listed at the bottom of the Case Reporting Form.
<table>
<thead>
<tr>
<th>Code</th>
<th>Genus</th>
<th>Species</th>
<th>Mammal</th>
<th>Other Wildlife</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>Mus</td>
<td>mouse</td>
<td>man</td>
<td>not rabid</td>
<td>no 1/4</td>
</tr>
</tbody>
</table>

**WILD ANIMALS**

<table>
<thead>
<tr>
<th>Code</th>
<th>Genus</th>
<th>Species</th>
<th>Mammal</th>
<th>Other Wildlife</th>
<th>Notes</th>
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<tbody>
<tr>
<td>01</td>
<td>Canis</td>
<td>red fox</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>Canis</td>
<td>Arctic fox</td>
<td>Ermine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>Vulpes</td>
<td>jackal</td>
<td>Jackal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>Canidae</td>
<td>wolf</td>
<td>Loup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>Canidae</td>
<td>raccoon</td>
<td>Loup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>Felidae</td>
<td>wild cat</td>
<td>Loup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>07</td>
<td>Mustelidae</td>
<td>badger</td>
<td>Loup</td>
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</tr>
<tr>
<td>08</td>
<td>Mustelidae</td>
<td>stone marten</td>
<td>Loup</td>
<td></td>
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</tr>
<tr>
<td>09</td>
<td>Mustelidae</td>
<td>pine marten</td>
<td>Loup</td>
<td></td>
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<tr>
<td>10</td>
<td>Mustelidae</td>
<td>polecat</td>
<td>Loup</td>
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<td>11</td>
<td>Mustelidae</td>
<td>ferret</td>
<td>Loup</td>
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</tr>
<tr>
<td>12</td>
<td>Mustelidae</td>
<td>fish otter</td>
<td>Loup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Mustelidae</td>
<td>large weasel</td>
<td>Loup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Mustelidae</td>
<td>house otter</td>
<td>Loup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Ursidae</td>
<td>brown bear</td>
<td>Loup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Ursidae</td>
<td>raccoon</td>
<td>Loup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Ursidae</td>
<td>other wild carnivores</td>
<td>Loup</td>
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**UNGULATE**

<table>
<thead>
<tr>
<th>Code</th>
<th>Genus</th>
<th>Species</th>
<th>Mammal</th>
<th>Other Wildlife</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Cervidae</td>
<td>roe deer</td>
<td>Renard roux</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Cervidae</td>
<td>red deer</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Cervidae</td>
<td>fallow deer</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Cervidae</td>
<td>moose</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Cervidae</td>
<td>other cervidae</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Bovidae</td>
<td>wild boar</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Bovidae</td>
<td>European bison</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Bovidae</td>
<td>moufflon</td>
<td>Renard rouge</td>
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<td></td>
</tr>
<tr>
<td>29</td>
<td>Bovidae</td>
<td>ibex</td>
<td>Renard rouge</td>
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<td></td>
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<tr>
<td>30</td>
<td>Bovidae</td>
<td>chamois</td>
<td>Renard rouge</td>
<td></td>
<td></td>
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<tr>
<td>31</td>
<td>Equidae</td>
<td>wild horse</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Equidae</td>
<td>wild donkey</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Equidae</td>
<td>other ungulates</td>
<td>Renard rouge</td>
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**INSECTIVORA**

<table>
<thead>
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<th>Code</th>
<th>Genus</th>
<th>Species</th>
<th>Mammal</th>
<th>Other Wildlife</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>34</td>
<td>Erinaceus</td>
<td>hedgehog</td>
<td>Renard rouge</td>
<td></td>
<td></td>
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<tr>
<td>35</td>
<td>Erinaceus</td>
<td>mole</td>
<td>Renard rouge</td>
<td></td>
<td></td>
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<tr>
<td>36</td>
<td>Erinaceus</td>
<td>shrews</td>
<td>Renard rouge</td>
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**CHIROPTERA**

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<th>Code</th>
<th>Genus</th>
<th>Species</th>
<th>Mammal</th>
<th>Other Wildlife</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>Hipposideroidea</td>
<td>insectivorous bats</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Hipposideroidea</td>
<td>insectivorous bats</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>Hipposideroidea</td>
<td>other bats</td>
<td>Renard rouge</td>
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<td></td>
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**RODENTIA**

<table>
<thead>
<tr>
<th>Code</th>
<th>Genus</th>
<th>Species</th>
<th>Mammal</th>
<th>Other Wildlife</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>Sciuridae</td>
<td>squirrel</td>
<td>Renard rouge</td>
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<td></td>
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<tr>
<td>41</td>
<td>Sciuridae</td>
<td>marmot</td>
<td>Renard rouge</td>
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<tr>
<td>42</td>
<td>Castoridae</td>
<td>beaver</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Castoridae</td>
<td>dormouse</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>Castoridae</td>
<td>hamster</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>Castoridae</td>
<td>muskrat</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>Castoridae</td>
<td>voles</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>Castoridae</td>
<td>other small rodents</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>Leporidae</td>
<td>wild rabbit</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>Leporidae</td>
<td>hare</td>
<td>Renard rouge</td>
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</table>

**HUNTING BIRDS**

<table>
<thead>
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<th>Code</th>
<th>Genus</th>
<th>Species</th>
<th>Mammal</th>
<th>Other Wildlife</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Falco</td>
<td>hunting birds</td>
<td>Renard rouge</td>
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<td></td>
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</table>

**OTHER WILDLIFE**

<table>
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<th>Genus</th>
<th>Species</th>
<th>Mammal</th>
<th>Other Wildlife</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>Vulpes</td>
<td>other wildlife</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ANIMAL CODE NUMBERS**

<table>
<thead>
<tr>
<th>Code</th>
<th>Genus</th>
<th>Species</th>
<th>Mammal</th>
<th>Other Wildlife</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>Vulpes</td>
<td>any</td>
<td>Renard rouge</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**EXTRA INFORMATION**

- Red fox (Vulpes vulpes)
- Arctic fox (Vulpes lagopus)
- Other fox species (Vulpes vulpes)
- European bison (Bison bonasus)
- Musk ox (Ovibos moschatus)
- European roe deer (Capreolus capreolus)
- European muntjac (Muntiacus reevesi)
- European addax (Addax nasomaculatus)
- European bison (Bison bonasus)
- Musk ox (Ovibos moschatus)
- European roe deer (Capreolus capreolus)
- European muntjac (Muntiacus reevesi)
- European addax (Addax nasomaculatus)
### Annex 5-21

**DOMESTIC ANIMALS**

| 71 | dog | Haushund | chien |
| 72 | cat | Katze | chat |
| 73 | other domesticated carnivores | andere domestizierte Fleischfresser | autres carnivores domestiques |
| 74 | horse | Pferd | cheval |
| 75 | donkey | Esel | âne |
| 76 | mule | Maultier | âne |
| 77 | hinny | Maulenel | bardot |
| 78 | pig | Schwein | porc |
| 79 | cattle | Rind | bovin |
| 80 | sheep | Schaf | mouton |
| 81 | goat | Ziege | chèvre |
| 82 | other domesticated herbivores | andere domestizierte Pflanzenfresser | autres herbivores domestiques |
| 83 | domesticated rabbit | Hauskaninchen | lapin domestique |
| 84 | dog, stray | streunender Hund | chien errant |
| 85 | dog, living wild | verwilderte Katze | chien sauvage |
| 86 | cat, living wild | verwilderte Katze | chat harné |
| 87 | unspecified | | |
| 88 | | | |
| 89 | | | |
| 90 | | | |
| 91 | | | |
| 92 | | | |
| 93 | | | |
| 94 | | | |
| 95 | | | |
| 96 | | | |
| 97 | | | |
| 98 | | | |
| 99 | | | |

- Canis familiaris
- Felis domestica
- Equus caballus
- Equus asinus
- Muus domesticus
- Ovis aries
- Capra hircus
- Orzytopus cuniculus
### TABLE 1: CURRENT RABIES VACCINES FOR USE IN ANIMALS

<table>
<thead>
<tr>
<th>Vaccine: Generic Name</th>
<th>For Use in</th>
<th>Dosage</th>
<th>Age at Primary Vaccination</th>
<th>Booster Recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A) MODIFIED LIVE VIRUS VACCINES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken Embryo Origin</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>every 3 years</td>
</tr>
<tr>
<td>Low Egg Passage, Flury Strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canine Cell Line Origin</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>every 3 years</td>
</tr>
<tr>
<td>High Egg Passage, Flury Strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine Tissue Culture Origin</td>
<td>Cats</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>every 3 years</td>
</tr>
<tr>
<td>High Cell Passage, SAD Strain</td>
<td>Cattle</td>
<td>1 ml</td>
<td>4 months &amp; 1 year later</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Horses</td>
<td>1 ml</td>
<td>4 months &amp; 1 year later</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>1 ml</td>
<td>4 months &amp; 1 year later</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>1 ml</td>
<td>4 months &amp; 1 year later</td>
<td>annually</td>
</tr>
<tr>
<td>Canine Tissue Culture Origin</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>every 3 years</td>
</tr>
<tr>
<td>High Cell Passage, SAD Strain</td>
<td>Cats</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>annually</td>
</tr>
<tr>
<td>Bovine Kidney Tissue Culture Origin</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>annually</td>
</tr>
<tr>
<td>High Cell Passage, SAD Strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamster Cell Line Origin</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>annually</td>
</tr>
<tr>
<td>High Cell Passage, Kissling Strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamster Kidney cell origin</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>annually</td>
</tr>
<tr>
<td>(Vnukovo-32 strain)</td>
<td>Cats</td>
<td>1 ml</td>
<td>as required</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>1 ml</td>
<td>as required</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Horses</td>
<td>1 ml</td>
<td>as required</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>1 ml</td>
<td>as required</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>1 ml</td>
<td>as required</td>
<td>annually</td>
</tr>
</tbody>
</table>
## INACTIVATED VIRUS VACCINES

### Nervous tissue

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>For Use</th>
<th>Dosage</th>
<th>Age at Vaccination</th>
<th>Booster Recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Animal Brain Origin</td>
<td>Dogs</td>
<td>2 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Cats</td>
<td>2 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td>Suckling Animal Brain Origin</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>every 3 years</td>
</tr>
<tr>
<td></td>
<td>Cats</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
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</table>

### Cell Culture

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>For Use</th>
<th>Dosage</th>
<th>Age at Vaccination</th>
<th>Booster Recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster Cell Line Origin</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td>High Cell Passage, Kissling Strain</td>
<td>Cats</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td>Hamster Cell Line Origin PM strain</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>every 3 years</td>
</tr>
<tr>
<td></td>
<td>Cats</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>1 ml</td>
<td>4 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Horses</td>
<td>1 ml</td>
<td>4 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>1 ml</td>
<td>4 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>1 ml</td>
<td>4 months</td>
<td>annually</td>
</tr>
<tr>
<td>Hamster Cell Line Origin PM strain</td>
<td>Cats</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td>with Panleucopenia</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td>with Leptospirosis</td>
<td>Cats</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>1 ml</td>
<td>4 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>2 ml</td>
<td>4 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>2 ml</td>
<td>4 months</td>
<td>annually</td>
</tr>
<tr>
<td>Hamster Cell Line Origin LEP Flury</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Cats</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td>Hamster Cell Line Origin HEP Flury</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Cats</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td>Chick Cell Origin, LEP Flury strain</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Cats</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td>Vaccine: Generic Name</td>
<td>For Use in</td>
<td>Dosage</td>
<td>Age at Primary Vaccination</td>
<td>Booster Recommended</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------------</td>
<td>--------</td>
<td>-----------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Porcine Kidney, LEP Flury strain</td>
<td>Dogs</td>
<td>2 ml</td>
<td>3 &amp; 4 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Cats</td>
<td>2 ml</td>
<td>3 &amp; 4 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>2 ml</td>
<td>as required</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Horses</td>
<td>2 ml</td>
<td>as required</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>2 ml</td>
<td>as required</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>2 ml</td>
<td>as required</td>
<td>annually</td>
</tr>
<tr>
<td>Porcine cell line origin, SAD</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>every 3 years</td>
</tr>
<tr>
<td></td>
<td>Cats</td>
<td>1 ml</td>
<td>3 months &amp; 1 year later</td>
<td>every 3 years</td>
</tr>
<tr>
<td>Porcine cell line origin, SAD</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td></td>
<td>Cats</td>
<td>1 ml</td>
<td>3 months</td>
<td>annually</td>
</tr>
<tr>
<td>Monkey cell line origin, SAD</td>
<td>Dogs</td>
<td>1 ml</td>
<td>3 months</td>
<td>every 3 years</td>
</tr>
<tr>
<td></td>
<td>Cats</td>
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<td>3 months</td>
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</tr>
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<td>TRADE NAME OF VACCINE</td>
<td>TYPE OF VACCINE</td>
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<tr>
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<td>(la Plata)</td>
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<td>PV fixed</td>
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<td>Public Health Institute</td>
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<tr>
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<td>Smith Kline</td>
<td>Flourimex</td>
<td>Live att.</td>
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<td>Merial S.A.</td>
<td>(La Paz)</td>
<td>Inactiv. SMB</td>
<td>C55-91</td>
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<td>Herbal</td>
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<tr>
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<td>T.B.I.P.V.</td>
<td>(Sofia)</td>
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<td>Connaught</td>
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<tr>
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<td>Zhenanou</td>
<td>Verc Med Lab.</td>
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<td>RVF</td>
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* see page 7 for abbreviations
<table>
<thead>
<tr>
<th>COUNTRY</th>
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<th>TRADE NAME OF VACCINE</th>
<th>TYPE OF VACCINE</th>
<th>ANIMAL &amp; TISSUE USED FOR VIRUS PRODUCTION</th>
<th>VIRUS STRAIN</th>
<th>ORIGIN OF VIRUS STRAIN</th>
<th>INACTIVANT</th>
<th>FURTHER TREATMENT</th>
<th>ADJUVANT</th>
<th>DOSE VOLUME</th>
<th>NO. OF DOSES</th>
<th>DURATION OF INCUBATION</th>
<th>POTENCY TEST</th>
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<td>Instituto Nacional de Salud</td>
<td>Inactiv.</td>
<td>Suckling mouse brain</td>
<td>51-91-COS Inst. Bacter. de Chile</td>
<td>Ultra-violet rays</td>
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<td>2 ml</td>
<td>1</td>
<td>2 years</td>
<td>NIH-M</td>
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<tr>
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<td>Carlos Finlay</td>
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<td>1 ml</td>
<td>no data</td>
<td></td>
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<td>1 ml</td>
<td>2 ml</td>
<td>1</td>
<td>2 years</td>
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<td></td>
<td></td>
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<td>1-2 years</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
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<td>C E F'</td>
<td>LEP-C23 U S A</td>
<td>B P L</td>
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<td>1 ml</td>
<td>1 year</td>
<td></td>
<td></td>
<td>NIH</td>
</tr>
<tr>
<td></td>
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<td>Inactiv.</td>
<td>BHK cells</td>
<td>Flury LEP</td>
<td>A E I</td>
<td>liquid</td>
<td>Alum. Hydros.</td>
<td>2 ml</td>
<td>1 ml</td>
<td>1 year</td>
<td></td>
<td></td>
<td>NMD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inactiv.</td>
<td>BHK</td>
<td>Flury LEP</td>
<td>A E I</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Inactiv.</td>
<td>NIL 2 - RE</td>
<td>GS Hlstar</td>
<td>B P L</td>
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<td>1 ml</td>
<td>3 years</td>
<td></td>
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<tr>
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<td>BHK-C 13 cells</td>
<td>RV-3 Pasteur</td>
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<td>1 ml</td>
<td>2 years</td>
<td></td>
<td></td>
<td>NMD</td>
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<tr>
<td></td>
<td></td>
<td>Inactiv.</td>
<td>BHK 21 - C13</td>
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<td>1 ml</td>
<td>1 year</td>
<td>NIH-M</td>
<td>1 year</td>
<td></td>
<td></td>
<td>Mice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inactiv.</td>
<td>Chick embryo</td>
<td>LEP, RE</td>
<td>Pasteur</td>
<td>-</td>
<td>Alum. Hydros.</td>
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<td>1 ml</td>
<td>1 year</td>
<td></td>
<td></td>
<td>Mice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inactiv.</td>
<td>live</td>
<td>BHK</td>
<td>Pasteur</td>
<td>-</td>
<td>Alum. Hydros.</td>
<td>2 ml</td>
<td>1 ml</td>
<td>18 months</td>
<td></td>
<td></td>
<td>Neutral</td>
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<tr>
<td></td>
<td></td>
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<td>live</td>
<td>Vnukovo</td>
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<td>-</td>
<td>Alum. Hydros.</td>
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<td>1 ml</td>
<td>2 years</td>
<td></td>
<td></td>
<td>Neutral</td>
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<td>live</td>
<td>Sheep brain</td>
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<td>-</td>
<td>Alum. Hydros.</td>
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<td>1 ml</td>
<td>1 year</td>
<td></td>
<td></td>
<td>Mice</td>
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TABLE OF RABIES VACCINES PRODUCED ALL OVER THE WORLD FOR ANIMAL USE

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<thead>
<tr>
<th>COUNTRY</th>
<th>LABORATORY</th>
<th>TRADE NAME OF VACCINE</th>
<th>TYPE OF VACCINE USED FOR VIRUS PRODUCTION</th>
<th>VIRUS STRAIN</th>
<th>ORIGIN OF VIRUS STRAIN</th>
<th>INACTIVANT</th>
<th>FURTHER TREATMENT</th>
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<th>DOSE VOLUME</th>
<th>NO. OF DOSES</th>
<th>DURATION OF IMUNIZATION</th>
<th>POTENCY TEST</th>
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<td>India</td>
<td>Institute Biol. Prod.</td>
<td>Rasalpura</td>
<td>Simple Sheep brain</td>
<td>P3AV</td>
<td>CRI, Kasauni</td>
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<td>14</td>
<td>1 year</td>
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<td>liquid</td>
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<td>14</td>
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<td>none</td>
<td>2-5 ml</td>
<td>1</td>
<td>1 year</td>
<td>Nabil</td>
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<td>phenol</td>
<td>none</td>
<td>3 ml</td>
<td>1</td>
<td>1 year</td>
<td>Guineapig</td>
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<td>RBF</td>
<td>Pasteur-Paris</td>
<td>B P L</td>
<td>phenol</td>
<td>none</td>
<td>2 ml</td>
<td>1</td>
<td>2 years</td>
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<td>RBF</td>
<td>Pasteur-Paris</td>
<td>B P L</td>
<td>phenol</td>
<td>none</td>
<td>3 ml</td>
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<td>1 year</td>
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<td>1 year</td>
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<td>none</td>
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<td>1</td>
<td>6 months</td>
<td>-&quot;-</td>
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<td>Inactivant</td>
<td>Further Treatment</td>
<td>Adjuvant</td>
<td>Dose Volume</td>
<td>No. of Doses</td>
<td>Duration of Immunization</td>
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<td>Sekeley</td>
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<td>Embryonated eggs</td>
<td>PVA, HEP</td>
<td>WHO Stock</td>
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<td>Lyophilized</td>
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<td>Chicken embryo</td>
<td>PVA, WHO</td>
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<td>BPL</td>
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<td>-</td>
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<td>1 year</td>
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<td>2</td>
<td>1 year</td>
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<td>RVF</td>
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<td>2</td>
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<td>COUNTRY</td>
<td>LABORATORY</td>
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<td>TYPE OF VACCINE</td>
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<td>ORIGIN OF VIRUS STRAIN</td>
<td>INACTIVANT</td>
<td>FURTHER TREATMENT</td>
<td>ADJUVANT</td>
<td>DOSE VOLUME</td>
<td>NO. OF DOSES</td>
<td>DURATION OF IMMUNIZATION</td>
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<tr>
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<td>----------------------------</td>
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<tr>
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<td>Vet. Res. Institute</td>
<td>(Date unspecified)</td>
<td>Live</td>
<td>Hamster cell line</td>
<td>Flury HEP</td>
<td>Unknown</td>
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<td>1</td>
<td>3 years (dogs)</td>
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<tr>
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<td>(Date unspecified)</td>
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<td>Canavox</td>
<td>LEP</td>
<td>Pasteur, Paris</td>
<td>Lyophilized</td>
<td>none</td>
<td>2 ml</td>
<td>1</td>
<td>2-3 years</td>
<td>mice</td>
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<tr>
<td>Spain</td>
<td>Inst. Bayer (Barcelona)</td>
<td></td>
<td>Modified</td>
<td>CEF</td>
<td>LEP</td>
<td>Pasteur, Paris</td>
<td>Lyophilized</td>
<td>none</td>
<td>2 ml</td>
<td>1</td>
<td>3 years</td>
<td>mice</td>
</tr>
<tr>
<td>Tunisia</td>
<td>Institute Pasteur, Tunis</td>
<td></td>
<td>Inactivated</td>
<td>Lamo brain</td>
<td>PV 11</td>
<td>Inst. Pasteur</td>
<td>B.P.L.</td>
<td>Lyophilized</td>
<td>5 ml</td>
<td>2</td>
<td>1 year</td>
<td>Rabies</td>
</tr>
<tr>
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<td>Elik V.C.R. Institute</td>
<td>(Date unspecified)</td>
<td>Live</td>
<td>CEF</td>
<td>Israel</td>
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<td>1 ml</td>
<td>1</td>
<td>1 year</td>
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<tr>
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<td>Beecham</td>
<td>(Date unspecified)</td>
<td>Killed v.</td>
<td>Hamster cell line</td>
<td>Kissing</td>
<td>CDC, Atlanta</td>
<td>B.P.L.</td>
<td>Concentr.</td>
<td>1 ml</td>
<td>1</td>
<td>1 year</td>
<td>NIH</td>
</tr>
<tr>
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<td>Norden Lab.</td>
<td>(Date unspecified)</td>
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<td>Vistar Inst.</td>
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<td>Mouse</td>
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<tr>
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<td>(Date unspecified)</td>
<td>Inactivated</td>
<td>B.H.K. cell</td>
<td>Kissling</td>
<td>Pasteur, CVS</td>
<td>B.P.L.</td>
<td>None</td>
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<td>1</td>
<td>1 year</td>
<td>NIH</td>
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<td>NIH, Bethesda</td>
<td>B.P.L.</td>
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<td>1 ml</td>
<td>1 ml</td>
<td>1</td>
<td>3 years</td>
<td>NIH</td>
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<tr>
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<td>Suckling mouse brain</td>
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<td>B.P.L.</td>
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<td>1 ml</td>
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<td>3 years</td>
<td>NIH</td>
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<tr>
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<td></td>
<td>Inactivated</td>
<td>B.H.K. cell</td>
<td>NIH, Bethesda</td>
<td>B.P.L.</td>
<td>None</td>
<td>1 ml</td>
<td>1 ml</td>
<td>1</td>
<td>3 years</td>
<td>NIH</td>
</tr>
<tr>
<td>US.A.</td>
<td>Wellcome Animal Health and Disease</td>
<td>(Date unspecified)</td>
<td>Killed v.</td>
<td>Monkey cell line</td>
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<td>Concentr.</td>
<td>A1,9 gel</td>
<td>1 ml</td>
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<td>1 year</td>
<td>NIH</td>
</tr>
<tr>
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<td>Porcine cell line</td>
<td>HPC SAD</td>
<td>Concentr.</td>
<td>Algel</td>
<td>1 ml</td>
<td>1 ml</td>
<td>1</td>
<td>1 year</td>
<td>NIH</td>
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<td>(Date unspecified)</td>
<td>Killed v.</td>
<td>Suckling mouse brain</td>
<td>NIH, Bethesda</td>
<td>B.P.L.</td>
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<td>1 ml</td>
<td>1 ml</td>
<td>1</td>
<td>1 year</td>
<td>NIH</td>
</tr>
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</table>
### Mediterranean Zoonoses Control Centre

**Table of Rabies Vaccines Produced All Over the World for Animal Use**

<table>
<thead>
<tr>
<th>Country</th>
<th>Laboratory</th>
<th>Trade Name of Vaccine</th>
<th>Type of Vaccine</th>
<th>Animal &amp; Tissue Used for Virus Production</th>
<th>Virus Strain</th>
<th>Origin of Virus Strain</th>
<th>Inactivant</th>
<th>Further Treatment</th>
<th>Adjuvant</th>
<th>Dose Volume</th>
<th>No. of Doses</th>
<th>Duration of Immunization</th>
<th>Potency Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S.A.</td>
<td>Schering Corp.</td>
<td>Biomb-3</td>
<td>killed v.</td>
<td>Suckling mouse brain</td>
<td>CVS</td>
<td>NJU, Bethesda</td>
<td>BPL 10% stabilizer</td>
<td>-</td>
<td>1 ml</td>
<td>1</td>
<td>1 year</td>
<td>NIH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fort Dodge</td>
<td>Trismuno</td>
<td>inactiv.</td>
<td>-</td>
<td>CVS 27</td>
<td>Pasteur Inst.</td>
<td>BPL</td>
<td>yes</td>
<td>1 ml</td>
<td>1</td>
<td>2 years</td>
<td>NIH</td>
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<tr>
<td>Venezuela</td>
<td>Institute &quot;Pablo Angel&quot; Caracas</td>
<td>Rabical</td>
<td>modified live</td>
<td>C E P</td>
<td>Flury 182</td>
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<td>-</td>
<td>lyophilized</td>
<td>none</td>
<td>1 ml</td>
<td>1</td>
<td>1 year</td>
<td>NIH</td>
</tr>
<tr>
<td>Egypt</td>
<td>VLVAX</td>
<td>-</td>
<td>modified live</td>
<td>C E P</td>
<td>Flury LEF</td>
<td>Pasteur, Paris</td>
<td>-</td>
<td>lyophilized</td>
<td>none</td>
<td>2 ml</td>
<td>1</td>
<td>1-3 years</td>
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<tr>
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<td>Vacuna antirrábica</td>
<td>inactiv.</td>
<td>Suckling mouse brain</td>
<td>CVS 51, 51</td>
<td>Institute Agrobiologia de Chile</td>
<td>UV rays</td>
<td>none</td>
<td>none</td>
<td>2 ml</td>
<td>1</td>
<td>3 years</td>
<td>NIH</td>
</tr>
<tr>
<td></td>
<td>Santos S.A.</td>
<td>Vacuna antirrábica</td>
<td>inactiv.</td>
<td>Suckling mouse brain</td>
<td>CVS and Nachbars</td>
<td>UV rays</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>2 ml</td>
<td>1</td>
<td>1 year</td>
<td>Hotel</td>
</tr>
</tbody>
</table>
### Table of Rabies Vaccines Produced All Over the World for Animal Use

**Countries Which Do Not Produce Any Rabies Vaccines for Animal Use (1982):**

1. Australia
2. Cyprus
3. Denmark
4. Finland
5. German Democratic Republic
6. Holland
7. Ireland
8. Israel
9. Lebanon
10. Malta
11. Switzerland
12. Syria

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**Abbreviations:**

- **H C P:** High Cell Passage
- **H E F:** Hamster Embryo Fibroblast
- **C E F:** Chicken Embryo Fibroblast tissue
- **B H K:** Baby Hamster Kidney cells
- **S M B:** Suckling Mouse Brain
- **A E I:** Acetylenimine
- **B E I:** Binary Ethylenimine
- **R V F or PV (F):** Rabies Virus Fixed Pasteur
- **C V S:** Challenger Virus Strain
- **S A D:** Street Alabama Dufferin strain of rabies virus
- **H E P:** High Egg Passage
- **L E P:** Low Egg Passage
- **R B A V:** Rabbit Brain Adapted Virus (Pasteur)
- **B P L:** Beta-Propiolactone
- **CEPANZO:** Panamerican Zoonoses Centre (Buenos Aires)
- **C D C:** Centre for Disease Control (Atlanta, Georgia, USA)
- **N I H:** National Institute of Health (Bethesda, Maryland, USA)
- **ISIPV:** Scientific Institute of Immunology and Veterinary Production, Sofia, Bulgaria
- **KILPAN:** Veterinary Institute of Infectious and Parasitic Diseases, Athens, Greece
METHOD FOR THE PREPARATION OF AN INACTIVATED-VIRUS VACCINE FROM LAMB OR KID BRAIN*

Possible methods of vaccine production for dogs are reported by Kaplan and Koprowski in the third edition of "Laboratory Techniques in Rabies" (8). Simple-type, formal-type, phenolized and freeze-dried sheep brain vaccine, guinea pig, mouse or rabbit brain vaccines, chick embryo vaccine and tissue culture vaccines.

In countries where tissue culture equipment and supplies between five or large scale animal breeder are not available among these vaccines, very much laboratories have selected the lamb (5-7-5) to produce the rabbit virus used for vaccine production. There is generally very extensive sheep (or goat) farming in the countries affected by canine rabies. In addition, the use of heteroprotective for inactive has eliminated most of the disadvantages arising from the use or formalized or phenolized vaccines (1-4-10) and the addition of adjuvant alone allows a longer duration of post-vaccinal immunity. The following procedure, adapted and modified after several experiments in tropical conditions, can be recommended to prepare an inactivated virus vaccine (per lamb or kid) strain. The seven consecutive stages:

1. Selection of Lamb (or kid);
2. Vaccination of the animal by intracranial injection of the fixed strain equivalent virus + serum (1 CVS) on the 1st day (10);
3. Sacrifice of the animal;
4. Harvesting the brains of the sacrificed animal;
5. Grinding and inactivation of the ground material (10);
6. Addition of albumin, hyperimmune serum of the vaccine in flask (10);
7. Testing for safety and potency, in the flask (3).

All these operations are carried out by personnel vaccinated against rabies, and under the safety equipment recommended for handling of rabies virus.

a) Selection of the lamb or kid.

Selection of the animal for propagation of the vaccine is of prime importance. Young lambs or kids are recommended, as viral harvest from adult sheep or goat is very poor and its inactivation would yield a vaccine of inadequate immunogenic value when tested for potency.


** The freeze-dried CVS strain may be requested from a WHO collaborating centre. Before use it should be passaged twice (intracerebral passage) in mice to yield a raised titre. The strain should be stored preferably at -70°C or at -20°C and repassaged on mice every six months to maintain its titre and virulence.
Consequently, the lambs or kids selected should be in good health and less than 6 weeks old. To reduce feeding costs it is preferable that these animals are already separated from their mothers and able to drink from a bucket.

b) Inoculation of the lambs or kids

The viral inoculum is 0.5 ml of a suspension of "working CVS" virus (20% ground mouse brain in distilled water containing 2% 100 serum stored at -30°C in a final dilution of 10^{-1}). The CVS suspension employed should have a titre of at least 10^{6} Mouse Intracerebral Lethal Dosis (MICLD50)/0.03 ml; consequently the animal will receive 10^{6.2} MICLD50.

Figure 1

The preferred site for this performance is on the lateral aspect of the frontal bone, the skin of the area should first be washed, shaved (or finally clipped) and disinfected with tincture of iodine.

The point of performance lies in the middle of a line extending from the base of the ear to the centre of an imaginary line connecting the crest of the two orbital arches (Fig. 1).

At this point, the skin and the underlying tissues are first cut through to the bone. The drilling, which is easily accomplished, is halted when bone resistance ceases; the inoculum is then introduced using a 16 mm 5/10 needle (Fig. 2).

There is no need to protect the skin incision (except by clip or adhesive dressing if it is big or suspected to be contaminated.)
c) **Sacrifice of the animals**

The inoculated animals reach the preagonal phase between the fourth and the seventh day after inoculation. This moment is determined in the following way: the animal is held in a standing position on all four feet by the handler, and then released; if it falls without any locomotor movement, even an uncoordinated one, the time has come to sacrifice it by slitting the throat.

As the simultaneous sacrificing of all the animals is not always possible, the heads of the first animal sacrificed may be stored, if necessary at +4°C (for no longer than 24 hours) or at −30°C (for no longer than 6 weeks).

The expected date of sacrifice of the animals should, of course, fall on a working day.

![Figure 3](image1.jpg) **Figure 3**

![Figure 4](image2.jpg) **Figure 4**

d) **Harvesting brains**

At this stage, all handling operations are carried out wearing gloves, mask, hat and protective goggles. The head is detached from the body by section at the base of the neck. The decapitated corpse and the emptied cranium will subsequently be incinerated.

The whole of the skull is skinned and the entire bared zone is disinfected by painting with tincture of iodine solution.

The whole brain is then extracted in as aseptic a way as possible following opening of the skull with a paring knife and hammer by the technique used for the diagnosis of rabies (6). For this purpose two V-shaped slits are cut in the frontal bone pointing towards the ears and meeting in the middle of the line extending from one orbital arch to the other, after which two other parallel slits are cut at the base of the skull, extending from the ends of the two previous slits and meeting on the level of the occipital foramen (Figure 4 and Figure 5).
The rhombic flap thus marked out is tilted forwards to disclose the whole of the cerebrum, the cerebellum and the medulla oblongata, which are aseptically extracted without delay from the meninges and placed, after removal of bone fragments, in sterile containers with lids. If it is not possible to deal with the brain* and its appendages immediately after their removal, they are to be stored at +4°C for 24 hours at the most, or at -30°C for longer storage (15 days).

Figure 5

e) Grinding and inactivation of the ground tissue

All these operations are carried out in a sterile zone, with the use of gloves, mask, hat and protective goggles, by personnel already vaccinated against rabies and having an antibody titre of at least 0.5 IU/ml.

The prepared brains (time should be allowed for thawing if they have been stored at -30°C) are collected together and weighed (weight = W). In practice, no more than 10 brains should be processed at the same time. The brains are then coarsely chopped with a surgical knife and placed in a sterile container with 2 x W ml of distilled water at 4°C.**

This mixture is then ground for five minutes using an "Ultra-Turrax" shaft-type grinder*** (Figure 6 and Figure 7).

* The brain is here defined as all the nerve centres contained in the cranium (in particular the cerebrum, the cerebellum and the medulla oblongata).

** The choice of distilled water rather than a "buffer" solution is conducive to the lysis of infected cells and the liberation of intra-cellular virions.

*** Ultra-Turrax model T45 (or, for small batches of vaccine, model, TP 18/10 56) from Janke and Kunkel GmbH and CO KG - IKA Werk D 7813 STAUFEN (Germany).
The depth of the container selected should be such that the shaft of the Ultra-Turrax can reach the bottom. Where the rod enters the container it should be connected to it by a makeshift joint and additionally surrounded by sterile cotton wool to avoid the diffusion of virulent aerosols.

As an additional precaution the whole of the container should be enclosed in a double plastic bag carefully gathered together around the shaft of the Ultra-Turrax (elastic band and adhesive tape: Figure 6). This is made possible by the fact that this shaft is motionless: the brain suspension is ground by rotation of the internal spindle of the shaft.

The operator should stand some distance away from the apparatus throughout the grinding operation so as to avoid inhaling any aerosol, which is highly dangerous. On completion of grinding, the 1/2 suspension obtained is made up with one volume of “sterile” buffer pre-heated to 37°C, yielding a final suspension containing roughly five parts of brain in 100 parts of diluent, or 16 x W, ml. This suspension can be homogenized and ground again with the Ultra-Turrax for three minutes.

Following addition of the adjuvant (aluminium hydroxide) the vaccine will thus contain 3.75 parts of brain substance in 100 parts of diluent.

Important note: This figure of five percent suspension is applicable only if the brain suspension before inactivation has a titre of at least $10^7$ MICLD50 in 0.03 g. of undiluted brain material (which is $10^{5.7}$ MICLD50 when diluted to five parts in a hundred).

When filtering 100 ml of this virulent five percent suspension through an 0.4 mm mesh nylon filter no pieces of unground debris should adhere to the mesh. If this occurs the whole suspension must be ground again. The whole 5 per cent suspension is then poured directly into the container (pre-heated to 37°C) in which it is to be inactivated. The receiving container has a magnetic bar (or screw-type rotor stirrer) that is sufficiently powerful to homogenize the whole volume of prepared vaccine.

* Buffer formula:

- NaCl : 8.77 grams
- K$_2$HPO$_4$ : 16.11 grams
- KH$_2$PO$_4$ : 0.68 grams
- Sucrose : 50 grams is added only if the vaccine is to be freeze-dried

Distilled or double distilled water to make up to 1 litre. The pH of the solution lies between 7.5 and 7.6 (if necessary, adjust with 1M NaOH).

* Betapropiolactone purum (O-CH$_2$-CH$_2$-Co) from Fluka AG BUCHS, Switzerland. May be purchased in Europe, e.g. through O.S.I. 146 rue de Javel, 75015 Paris, France.
A sample (1 ml) of the suspension is taken, drying to measure the
virulence titre of the batch, which (as above) should be at least 10³.7
MICLD50/0.03 ml.

The suspension should in sterile fluid on nutrient agar) or contain
no more than 10 bacteria.

The suspension is then incorporated by the addition of 1 part in 4000
(0.023 of a part) in 3000 ml benzalkonium chloride (30B).

AII is added in the form of a sterile powder 1% solution in
water. The procedure must be carried out very rapidly but this concentration
analysis of AII occurs in less than 30 minutes.

Immediately after the AII has been added, the container is
hermetically sealed and vigorously agitated to ensure perfect mixing of the
product and neutralization of all the viral particles.

The container is then maintained at 37°C (in an incubator or warm
water bath) with agitation of its contents for 60 minutes (water bath), either
by gentle stirring (or magnetic stirring) for 1 hour at 30°C.

At this stage the suspension might be contaminated during subsequent
handling or storage, since the 3% concentration of benzalkonium chloride
parabanic acid) should therefore be added to neutralize any possible
contamination (not to neutralize the rubber valve, which is already completely
neutralized).

This operation is always carried out where provision of sankling, to
yield an end content of 1 part in 4000 (0.023 of a part) in the latter addition of
adjuvant, the final concentration will be approximately 100%; 100%.

The suspension is then transferred to 0°C, at which temperature it is maintained
for between 12 and 24 hours (overnight), with stirring of possible.
Annex 5-24

All equipment that has been in contact with virulent products should be chemically or physically decontaminated (preferably by autoclaving).

f) Addition of aluminium hydroxide

Aluminium hydroxide should be in gel form, rather than powder or crystal form. The gel may be purchased ready for use from: R. Bellon, 37190 Azay-le-Rideau, France, or BAIF, P.O. Box No. 1, Wagholi, Poona 412 207, India. It may be replaced by aluminium gel manufactured from aluminium sulphate and ammonia as indicated below:

The suspension of five parts in 100 previously obtained must be sterile (bacteriological control) at this stage. One part of sterile aluminium hydroxide to three parts of vaccine is added at the same time, except if the vaccine is to be lyophilised (see buffer formula) in which case adjuvant must be added separately to the vaccine solvent.

PREPARATION OF ALUMINIUM GEL, VACCINE ADJUVENT

1. Preparation of the gel:

Dissolve 1 kg of aluminium sulphate in 20 litres of demineralized or distilled water.

Precipitate the resultant solution with 2 litres of 20% ammonia.

Add this ammonia solution gradually to the 20 litres of aluminated solution stirring from time to time; a white precipitate forms.

Filter through Chardin or Dumas filter paper, leaving to drain overnight.

On the following day, collect up the aluminium hydroxide gel deposited on the filter, dissolve it in 10 litres of demineralized water to wash it, and refilter.

Leave to drain for a week.

Collect the creamy white deposit on the filter in a container; it is aluminium hydroxide gel.

2. Preparation of a gel solution containing two parts of 100 of dry extract

The content of gel (previously obtained) in the dry extract should first be determined.

For this purpose place a 10 g sample of this gel in a pre-weighed disc and dry it in an electric oven for half a day.

Weigh frequently until the weight becomes constant; the end point gives the quantity of dry extract present in the sample (and enables the dry extract to be calculated in percent or per mille); a gel solution containing 2% of dry extract may then be prepared*, flasked and sterilized at 120°C before addition to the vaccine.

* Example: for one litre of 2% or 20% gel solution, 20 grams of dry gel or 20 000 mg are needed.

For 10 grams of wet gel there are, for example, 1050 mg of dry gel; for 1 gram of wet gel, there are 100 mg of dry gel.
The amount of dry gel needed for one litre of solution will therefore be:

\[ \frac{18 \times 20,000}{100} = 2000 \text{ grams of wet gel} \]

(Note: Technique used at the Laboratoire Central de l'Elevage - B.P. 862, Antananarivo, Madagascar).

The pH of the vaccine should not be below 7.5 after all these operations; it should if necessary be be readjusted by the addition of soda.

The vaccine may be flanked immediately, continuing to stir during these operations or stored at 4°C during testing for safety and potency.

g) Testing for safety and potency

The safety of the vaccine is tested by the intracerebral injection of mice with 0.03 ml of vaccine. When kept under observation for 30 days all the mice should remain healthy, except some accidental death during the 5 days after inoculation.

The potency of the vaccine may be verified by a simplified test (2) and by the NIH test (6) summarised in annex.

In the simplified test two batches of 10 mice (R and T) are vaccinated, one batch with a reference preparation having a titre of 1 IU/ml (batch R), the other with the vaccine diluted to 1/100 (batch C).

The vaccination is carried out by intraperitoneal injection (0.5 ml) on D0 with a booster of D7.

On D14 the two batches are challenged by intracerebral injection.

After observation for 14 days there should be no more than seven mice dead (or ill) in batch T, and one in batch R.

Should this be the case, the batch may be regarded as having a titre (P. 0.95) of more than 1 IU/ml, and it is passed.

If not, the batch must be subjected to a full NIH test which will give its precise titre in IU. This titre should be at least 1 IU/ml in a month after manufacture*.

The subcutaneous or intramuscular injection of a prescribed dose, or intradermal injection of a reduced dose with a "dermo-jet" (11), should lead to the reduction of neutralizing antibodies in a titre equal to or greater than 0.1 IU/ml of serum for a group of at least five vaccinated subjects.

* Because antigenic value of liquid vaccine decreases rapidly these should be used as soon as possible (within 3 months after manufacture. Lyophilised vaccine can be kept longer (18 months).
REFERENCES


LIST OF MATERIALS AND EQUIPMENT REQUIRED FOR THE MANUFACTURE OF INACTIVATED RABIES VACCINE FROM LAMB BRAIN

List given as a guide for the manufacture of a minimum 100,000 and a maximum of 1,000,000 doses of vaccine per annum.

1. GENERAL FACILITIES : BUILDINGS

Laboratories Three rooms, each 12-15 m
- one for the handling of the virulent material
- one for the inactivation and hatching of the vaccine
- one for vaccine control or mice
  (with supply of electricity/gas, hot and cold water, etc.)

Animal House
- Shelter for young animals, about 1 m² per animal * + food store
- The third laboratory room may be used to house the mice if they are brought in. If they are reared (bred), provision must be made for one or two additional rooms (rearing room and food store).

Comment: Allow for 1 box + lid for 10 mice.

2. HEAVY EQUIPMENT : LABORATORY EQUIPMENT

For refrigeration: - Either a large refrigerator (250 litres + a large freezer compartment, 1/3 the capacity of the refrigerator).
  - Or a small cold room + freezer (-30°C)

For sterilization: - Either a small vertical autoclave (gas, electricity or petrol) + a small Pasteur oven.
  - Or a Pasteur oven of at least 1 m³ + 10 litre pressure cooker.

For vaccine preparation:
- A shaft-type Ultra-Turrax grinder + 3 long shafts (30 cm)
  + 2 100 ml containers
- A large magnetic stirrer + 5 magnetic stirring bars
- A balance to weigh quantities between 1 and 500 grams + 0.1 gram
- A manual distributor (with sterilizable circuit) adjustable to + 1 cm³, for quantities up to 50 m³. (This apparatus is essential only for production in excess of 100,000 doses per year).
- A flask sealer (if more than 100,000 doses are produced annually) or 2 hand-operated cappers.
- Distillation apparatus (3 litres/hour) or, in its absence, a bacteriological filter with the same output.
- A bacteriological oven adjustable to 37°C + 1°C.

* The vaccine "yield" is between 1000 and 3000 doses of "canine" vaccine per lamb or kid and every animal is kept for 5-8 days (inoculation until harvesting of the brain).
3. GLASSWARE AND SUNDRIES

**Glassware**

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5000 ml pyrex flasks</td>
<td>5</td>
</tr>
<tr>
<td>1000 ml pyrex flasks</td>
<td>10</td>
</tr>
<tr>
<td>100 ml pyrex flasks</td>
<td>30</td>
</tr>
<tr>
<td>30 ml pyrex flasks</td>
<td>50</td>
</tr>
<tr>
<td>Test tubes</td>
<td>100</td>
</tr>
<tr>
<td>30 ml plain glass flasks (+ stopper + capsule)</td>
<td>1 for every 20 doses of vaccine</td>
</tr>
</tbody>
</table>

Large test tubes, 5000 ml(3), 2000 ml(3), 500 ml(3) and 100 ml (10)

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ml pipettes</td>
<td>50</td>
</tr>
<tr>
<td>5 ml pipettes</td>
<td>50</td>
</tr>
<tr>
<td>1 ml pipettes</td>
<td>100</td>
</tr>
<tr>
<td>2 ml syringes</td>
<td>50</td>
</tr>
<tr>
<td>1 ml syringes (with 0.1 ml graduations)</td>
<td>50</td>
</tr>
<tr>
<td>Large and small funnels</td>
<td>5 x 2</td>
</tr>
</tbody>
</table>

**Sundries:** General laboratory equipment

- 0.42 mm diameter mesh nylon filter or guaze filter (vaccine filtration)
- pH indicator paper
- "Camping-gas" + spare cylinder
- throwaway gloves
- protective goggles
- protective mask (gauze)
- 90° alcohol
- iodine
- strong plastic boxes, test tube racks, test tubes
- plastic bags
- absorbent cotton-wool - adhesive plaster - disinfectant (bleach and ammonia water)
- sterile distilled (or filtered) water
- Chardin filter paper

4. MEDICO-SURGICAL EQUIPMENT

- one drill + four 2 mm diameter bits (for trepanation of sheep)
- butchers knives (3)
- paring knives (10) - hammers - sharpening stone - 16 cm curved scissors (5 pairs)
- surgical knives (10) - needles, 25 mm 30/11 (type BD 19 G 11/4) and 20 mm 25-6 (type BD 23 G 1).

5. SPECIAL CHEMICAL OR BACTERIOLOGICAL PRODUCTS

Merthiolate - Thiomersal (allow 15 g for 100 000 doses of vaccine)

Betapropioactone (allow 100 ml for 100 000 doses of vaccine)

Ready-prepared aluminium hydroxide (0.25 ml per vaccine dose) or aluminium sulphate + ammonia (8 kg and 16 litres respectively for 100 000 doses of vaccine)

Dehydrated bacteriological control medium (aero-anaerobic agar-agar).
6. BIOLOGICAL MATERIAL

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb or kids*</td>
<td>Depending on the age and size of the animal, the virus titre of its brain, and the antigenic value of the end product, the &quot;yield&quot; per animal ranges from 1000 to 3000 ml at 1 IU/ml (minimum) = 1 &quot;canine dose&quot;.</td>
</tr>
<tr>
<td>Mice:</td>
<td>The calculation should be based on at least 2 mice for the control of 1000 vaccine doses (safety and potency).</td>
</tr>
<tr>
<td>Virus:</td>
<td>Stored, freeze-dried CVS strain used after passage through mice and stored in a frozen state.</td>
</tr>
</tbody>
</table>

* The most potent vaccines may be obtained from unweaned animals. Consequently, the ewe or goat may be slaughtered following the death of the young, and the meat possibly consumed.
### EQUIPMENT RECOMMENDED IN FRANCE (1981)

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Approximate cost (US dollars)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General installations</strong> (laboratories, animal houses)</td>
<td></td>
</tr>
<tr>
<td>No estimate given (depends on local conditions, i.e. whether laboratory facilities already exist or have to be set up)</td>
<td></td>
</tr>
<tr>
<td><strong>Heavy equipment</strong></td>
<td></td>
</tr>
<tr>
<td>Refrigerator, large model</td>
<td>500</td>
</tr>
<tr>
<td>Freezer</td>
<td>500</td>
</tr>
<tr>
<td>Autoclave</td>
<td>500</td>
</tr>
<tr>
<td>Pasteurizer</td>
<td>500</td>
</tr>
<tr>
<td>Ultra-Turrax grinding mill, with fittings</td>
<td>800</td>
</tr>
<tr>
<td>Magnetic stirrer</td>
<td>150</td>
</tr>
<tr>
<td>Balance</td>
<td>100</td>
</tr>
<tr>
<td>Manual dispensing apparatus</td>
<td>100</td>
</tr>
<tr>
<td>Bottle-capping apparatus (manual)</td>
<td>50</td>
</tr>
<tr>
<td>Distilling apparatus</td>
<td>400</td>
</tr>
<tr>
<td>Bacteriological incubator</td>
<td>300</td>
</tr>
<tr>
<td><strong>Glassware</strong></td>
<td></td>
</tr>
<tr>
<td>Complete set</td>
<td>3 200</td>
</tr>
<tr>
<td><strong>Miscellaneous equipment</strong></td>
<td></td>
</tr>
<tr>
<td>Complete set</td>
<td>600</td>
</tr>
<tr>
<td><strong>Medico-surgical equipment</strong></td>
<td></td>
</tr>
<tr>
<td>Complete set</td>
<td>300</td>
</tr>
<tr>
<td><strong>Special chemical or bacteriological products</strong></td>
<td></td>
</tr>
<tr>
<td>Depends on vaccine quantity prepared (approximately US $1.00 per 1 000 doses)</td>
<td></td>
</tr>
<tr>
<td><strong>Biological material</strong></td>
<td></td>
</tr>
<tr>
<td>No estimate given (depends on local conditions - approximately US $50.00 per sheep and US $1.00 per mouse)</td>
<td></td>
</tr>
<tr>
<td><strong>Approximate total (not including installations and their operation) of equipment investment</strong></td>
<td>8 000</td>
</tr>
<tr>
<td><strong>Total basic equipment for the vaccines manufactured (not including staff salaries and depreciation)</strong></td>
<td>Approximately US $1.00 per 20 doses.</td>
</tr>
</tbody>
</table>
QUALITY CONTROL IN MODIFIED LIVE VIRUS VACCINE

(Condensed from British Pharmacopoeia, 1977).

Rabies Veterinary Vaccines. Living are preparations of chick-embryo tissue or cell culture infected with a suitable attenuated strain of rabies virus. The vaccines are prepared immediately before use by reconstitutions from the dried vaccines with a suitable sterile liquid.

Caution - Care must be taken when handling living strains during preparation of the vaccines. Infected animals should be handled with care and the advisability of immunising staff should be considered. Aerosol infection with the fixed strain has been recorded during production and testing.

The vaccines reconstituted as stated on the label comply with the following requirements:

Identification: Protect guinea-pigs against a subsequent inoculation of rabies street virus or fixed virus. The vaccines are distinguished from inactivated rabies vaccine by their ability to produce rabies encephalitis when injected intracerebrally into mice.

Safety: (i) Inject each of twenty guinea-pigs, intramuscularly with 0.25 ml of a suspension, prepared by dispersing the contents of one container in 20 ml of Water for Injections, and observe for twenty-one days; no significant local or systematic reaction develops.

(ii) Inject each of not fewer than two healthy susceptible animals of each of the species in which the vaccine is intended to be used, by the route stated on the label with twice the appropriate vaccinating dose and observe for not less than twenty-eight days; no abnormal reaction develops.

Potency: Reconstitute each of two containers (2 dog doses) of dried vaccine in 3 ml of Water for Injections containing 2 per cent normal horse serum and pool the contents. Add 3 ml of the suspension to a further 17 ml of diluent. Inoculate each of not fewer than ten guinea pigs; intramuscularly on the inside of a hind leg with 0.25 ml of the diluted vaccine. Three weeks later challenge the animals as well as each of not fewer than five control animals by intramuscular injection (in the other hind leg) with either canine street virus or fixed (CVS) strain of rabies virus using half the amount of virus found necessary to kill 100 per cent of guinea-pigs in a preliminary titration: 80 per cent of the control animals die of rabies within twenty-one days after challenge with street virus and within fourteen days after challenge with CVS virus; 70 per cent of the vaccinated animals survive challenge inoculation without showing any signs of rabies.
Rabies Veterinary Vaccine, Inactivated is a suspension of a fixed strain of rabies virus inactivated in such a manner that antigenic activity is retained. It may be propagated in animal neural tissue or in cell culture. The vaccine may be issued as a dried product which is reconstituted, immediately before use, with an appropriate quantity of a suitable sterile liquid. The vaccine complies with the following requirements:

Identification: Appropriate doses protect mice against subsequent inoculation of a suitable challenge strain of fixed rabies virus.

Safety:

(a) Brain tissue vaccine

(i) Inject each of five mice, intracerebrally, with not less than 0.03 ml of the vaccine and observe for not less than twenty-one days; not less than three of the mice survive and all the mice remain free from symptoms of encephalitis or meningitis.

(ii) Inject each of two healthy animals of the species used for virus production in preparing the vaccine, intracerebrally, with an appropriate dose and observe for at least twenty-one days; all the animals remain free from symptoms of encephalitis or meningitis.

(iii) Inject each of not fewer than two healthy susceptible animals of each of the species in which the vaccine is intended to be used by the route stated on the label with twice the appropriate vaccinating dose and observe for not less than twenty-eight days; no abnormal reaction develops.

(b) Cell culture vaccines

(i) Inject each of five mice, intracerebrally, with not less than 0.03 ml of the vaccine and observe for not less than twenty-one days; not less than three of the mice survive and all the mice remain free from symptoms of encephalitis or meningitis.

(ii) Inject each of not fewer than two healthy susceptible animals of each of the species in which the vaccine is intended to be used by the route stated on the label with twice the appropriate vaccinating dose and observe for not less than twenty-eight days; no abnormal reaction develops.
Potency: Prepare a series of fivefold dilutions of the vaccine. Inoculate 0.5 ml of each dilution, intraperitoneally, into each of a group of ten susceptible mice, four to six weeks old, using a different group for each dilution, repeat the injection after seven days. After a further seven days challenge all the mice as well as not fewer than ten control animals by the intracerebral inoculation of a dilution of a standard challenge strain of fixed virus estimated to contain between 5 and 50 LD50 of virus. Observe the mice for fourteen days. Mice showing signs of rabies (paralysis, convulsions) and dying after five days and mice showing paralysis but surviving at fourteen days are counted as failing to resist the challenge. Estimate the potency by comparison with the Standard Preparation. The vaccine being examined has an antigenic value not less than that of the Second International Reference Preparation.

Standard Preparation: The Standard Preparation is the Third International Reference Preparation, established in 1978, consisting of a freeze-dried suspension of cell culture infected with fixed rabies virus and inactivated, or another suitable preparation, the potency of which has been determined in relation to the International Reference Preparation.

* Evaluated by the National Institute of Health test.

** Recommended dilutions for usual vaccines are:

10^-0.7; 10^-1.05; 10^-1.4; 10^-1.75; 10^-2.1; 10^-2.1; and 10^-2.8.

*** Use preferably neo-probit graph for Comparison: see model on next page.
Research on dog rabies vaccination should be undertaken or intensified in the following areas:

1. Research on the safety, efficiency and stability of inactivated cell-culture vaccines for animals.

2. Efforts should be directed towards:
   - decreasing the cost of animal cell culture vaccines, while maintaining the potency and safety of present products; and
   - the use of adjuvants and alternate routes of administration, such as the intradermal, should be further investigated.

3. Improvement of assay procedures for the potency of vaccines - particular emphasis should be placed on the comparison of the NIH test with other tests, including the significance of test results in homologous and heterologous systems (production strain different from challenge strain).

4. Research in possibly better adapted strains (using monoclonal antibody techniques) for vaccination of dogs in determined geographic areas.

5. Further development of a "package" of simple techniques for the determination of dog population parameters (census, turnover, dog accessibility) for the planning and management of canine rabies control programmes.

6. Further studies on the feasibility of oral immunization of dogs with:
   a) live virus strains EHM/BHK21
   b) recombinant vaccines.

7. Collaborative studies on the specificity, sensibility of simple laboratory techniques for rabies diagnosis (i.e. rapid enzyme-immuno-diagnosis).

8. Research on the efficiency and safety of a simple technique for brain tissue sampling and shipment for rapid diagnosis.