Rabies vaccine
OIE terrestrial manual chapter 2.1.13

National Veterinary Assay Laboratory, MAFF
Koichiro GAMOH, DVM, PhD
1. Ensuring the quality of veterinary medicinal products
   - National assay
   - Preparation of standards
National Veterinary Assay Laboratory

2. Examination and consultation for applications
   - Examinations for approval
   - Inspection of laboratories (GLP, GCP, GMP)
   - Consultation services for approval
National Veterinary Assay Laboratory

3. Emergency management and ensuring food safety
   - Monitor and control drug-resistant bacteria derived from animals
   - Environmental impact assessment of veterinary drugs
4. International cooperation
   - VICH
   - OIE Collaborating Center (2010~)
   - Technical cooperation
Rabies vaccine is the most useful tool to control and to eradicate rabies

- Rabies vaccine is effective against any rabies virus variant of phylogroup 1
Rabies outbreak in Bali, Indonesia in 2008
Rabies outbreak in Bali, Indonesia in 2008

- Dog population: 425,000
- Bali government banned rabies vaccination before the outbreak.

Rabies spread rapidly all over the island.

- Some mass vaccinations
- Culling of rabies suspected dogs

Rabies is almost eradicated.
Rabies, countries or areas at risk

Japan has been free of rabies since 1958.
Rabies cases of dogs in Japan

Enforcement of Rabies prevention law
Rabies Prevention Law in Japan

Rabies Prevention Law was enforced in 1950
- Compulsory vaccination
- Registration of dogs
- Animal quarantine

Rabies was eradicated in 1958.
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      b. Final products
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3. Major test items
# 1. Rabies vaccine

(1) International veterinary rabies vaccines

<table>
<thead>
<tr>
<th>Product name</th>
<th>Produced by</th>
<th>Adjuvant</th>
<th>Booster recommended</th>
<th>For use in</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nobivac</td>
<td>MSD</td>
<td>Aluminium phosphate</td>
<td>3 years later</td>
<td>Dogs, Cats, Ferrets</td>
</tr>
<tr>
<td>Canigen</td>
<td>Virbac</td>
<td>Aluminium phosphate</td>
<td>3 years later</td>
<td>Dogs and Cats</td>
</tr>
<tr>
<td>IMRAB 3</td>
<td>Merial</td>
<td>Aluminium phosphate</td>
<td>1 year later &amp; triennially</td>
<td>Dogs, Cats, Sheep, Cattle, Horses, Ferrets</td>
</tr>
<tr>
<td>IMRAB 1</td>
<td>Merial</td>
<td>Aluminium phosphate</td>
<td>Annually</td>
<td>Dogs and Cats</td>
</tr>
<tr>
<td>DEFENSOR 1</td>
<td>Pfizer</td>
<td>Aluminium phosphate</td>
<td>Annually</td>
<td>Dogs and Cats</td>
</tr>
<tr>
<td>DEFENSOR 3</td>
<td>Pfizer</td>
<td>Aluminium phosphate</td>
<td>1 year later &amp; triennially</td>
<td>Dogs, Cats, Sheep, Cattle, Horses, Ferrets</td>
</tr>
</tbody>
</table>
1. Rabies vaccines

(2) Japanese veterinary rabies vaccines

— Tissue Culture Inactivated Vaccine —

- One product registered in 1985
- Four manufacturers
- Compulsory vaccination every year by law
- About 20 lots, 5.0 million doses are used in a year
- The most used vaccine for mammals in Japan
- Vaccine strain: RC-HL strain
  (low pathogenicity, originated from Pasteur strain)
1. Rabies vaccines
   (2) Japanese veterinary rabies vaccines

1. Efficacy
   - Potency: $\geq 1.0^a$ IU/dose
     1.0$^a$ IU/dose: recommended by OIE for veterinary vaccine
   - Induce 0.5IU/mL$^b$ one year after vaccination
     0.5IU/mL$^b$: adequate antibody titer to prevent rabies(OIE/WHO)
1. Rabies vaccines
   (2) Japanese veterinary rabies vaccines

2. Safety
   - Adjuvant is not included
   - Protein nitrogen content test
     Criteria for veterinary rabies vaccine: ≤100 μg/ml
     Set for rabies vaccine and horse vaccines

Incidence rate of Serious Adverse Event:
Rabies vaccine: 0.02 cases/10,000 heads
The other canine vaccines: 0.05 cases/10,000 heads
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3. Major test items
Quality assurance system
Principle of Veterinary vaccine production, OIE terrestrial manual chapter 1.1.6

Production control
- Standards for facilities
- GMP

Quality Control
- In process products
- Final Products

National Assay

Manufactures
Government
(1) Production control
a. Standards for facilities

Facilities used for the production of vaccines should be designed to protect the purity of the product throughout the production process and to safeguard the health of the personnel. They must be constructed:

• They can be readily and thoroughly cleaned.
• They provide adequate separation of preparation rooms
• They have adequate ventilation
• They have ample clean hot and cold water and efficient drainage and plumbing
• They have dressing rooms and other facilities for personnel that are accessible without passing through biological product preparation areas.
(1) Production control

a. Standards for facilities

Facilities must be adequate to provide for all applicable production functions, such as:
- Storage of master seeds, ingredients, and other production materials
- Preparation of growth media and cell cultures
- Preparation of glassware and production equipment
- Inoculation, incubation, and harvest of cultures
- Storage of in-process materials
- Quality control of testing of in-process materials and final products
- Research and development
(1) Production control
b. Good Manufacturing Practice (GMP)

**GMP**: A system for ensuring that products are consistently produced and controlled according to quality standards.
Standards Documents that describe manufacturing and quality control procedures, necessary matters, etc. Such as:
- Product specification document
- Manufacturing control standards document
- Sanitation control standards document
- Quality control standards document

All the operations should be conducted based on the standards documents
Summary of GMP

GMP regulates 2 independent departments and 3 personnel

- Manufacture supervisor
  - supervise manufacturing control manager and quality control manager
  - Responsible for dealing with complains, product recall procedures and self-inspection
  - responsible for manufacturing
  - check the results

- Manufacturing control manager

- Quality control manager
  - responsible for quality control
Summary of GMP③

Dealing with complains
• Manufacturing supervisor shall investigate the cause if there is a complaint.

Product recall procedures
• In the recall of a product, manufacturing supervisor shall investigate the cause.

Self inspection
• Manufacturing supervisor shall periodically conduct self inspection.
Quality assurance system
Principle of Veterinary vaccine production, OIE terrestrial manual chapter 1.1.6
(2) Quality control
a. In process product

- Cell culture
- Virus culture
- Purification/concentration
- Inactivation
- Stock solution
- Final bulk
- Final product

Tests:
- Test of cell culture
- Virus content test
- Inactivation test
- Potency test
- Sterility test
- Protein nitrogen content test
(2) Quality control

b. Final Products

- Sterility test
- Description test
- Macrogol quantification test
- Thimerosal quantification test
- pH measurement test
- Protein nitrogen content test
- Safety test
- Potency test
Quality assurance system
Principle of Veterinary vaccine production, OIE terrestrial manual chapter 1.1.6

Production control
- Standards for facility
- GMP

Quality Control
- In process products
- Final Products

National Assay

Manufactures

Government
(3) National assay

Batch/Serial release for Distribution, OIE Terrestrial manual, chapter 1.1.6

“In countries that have national regulatory programmes that include official control authority retesting of final products, samples of each batch/serial should also be submitted for testing in government laboratories by competent authorities.”
(3) National assay

Manufacturers

In house test assay

Application for National assay

Local Governments

Notice of the results

National Veterinary Assay Laboratory

National assay
(3) National assay

Veterinary vaccines cannot distribute or sell unless pass the national assay by NVAL.

**Rabies vaccine**

- Sterility test
- Inactivation test
- Abnormal toxicity test
- Potency test: ELISA
(3) National assay

Some important assays are conducted both by manufacturers and NVAL

Rabies vaccine

<table>
<thead>
<tr>
<th>Assay</th>
<th>In process product</th>
<th>Final product</th>
<th>National assay by NVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterility test</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Inactivation test</td>
<td>●</td>
<td>—</td>
<td>●</td>
</tr>
<tr>
<td>Potency test</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Protein nitrogen content test</td>
<td>●</td>
<td>●</td>
<td>—</td>
</tr>
</tbody>
</table>
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      b. National assay

3. Major test items
3. Major test items for rabies vaccine

Abnormal Toxicity test

“Test to make sure that a vaccine does not cause abnormal actions when injected in small laboratory animals.”

Materials:

- Testing substances (vaccines)
- Ten mouse and 2 guinea pigs
3. Major test items for rabies vaccine
   Abnormal Toxicity test

Methods:
- Ten mouse and 2 guinea pigs were injected with 0.5ml and 5ml of testing substances by Intra-peritoneal route, respectively.
- Test animals and control animals are observed for 7 days.
3. Major test items for rabies vaccine

Abnormal Toxicity test

Judge:

Any symptoms caused by vaccines must not develop in the test animals.
3. Major test items for rabies vaccine

Sterility test

“Test to make sure that a vaccine does not include either bacteria or fungus.”

Materials:

- Testing substances (vaccines)
- Thioglycolate medium (TGC medium)
- Soybean-casein digest liquid medium (SCD medium)
3. Major test items for rabies vaccine
Sterility test

Methods:
1. Bacteria test
   - Inoculate testing substances into TGC medium
   - Incubate at 30-32°C for 14 days
   - Judge: turbidity must not be observed.
3. Major test items for rabies vaccine

Sterility test

Methods:

2. Fungal test

- Inoculate testing substances into SCD medium/TGC medium
- Incubate at 20-25°C for 14 days
- Judge: turbidity must not be observed.
3. Major test items for rabies vaccine

Inactivation test

“Test to make sure that a vaccine is inactivated.”

Materials:

- Testing substances (vaccines)
- Suckling mouse younger than 3 days old.
3. Major test items for rabies vaccine
   Inactivation test

Methods:

- Vaccine 0.02ml is injected intracerebrally into each of 10 test mouse.
- The 10 test mouse are observed for 14 days with 2 control mouse.

Judge:

Any symptoms caused by rabies virus must not develop in the test mouse.
3. Major test items for rabies vaccine

Potency test

NIH potency test: A prescribed potency test by OIE and WHO

Materials:

- Reference vaccine provided by OIE and WHO
- Test vaccines
- Challenge virus, CVS strain
- Mouse
3. Major test items for rabies vaccine

Potency test

Methods:
Reference and test vac. are diluted (1/5, 1/25, 1/125, 1/625)
Each dilution: 16 mouse

- Vac., ip route, twice.
- One week after vac., challenged with CVS strain.
3. Major test items for rabies vaccine

Potency test

Judge:

- After 2 weeks observation, ED50 was calculated.
- IU of test vaccine was estimated by comparing ED50 of test vaccine with that of Reference vac.
  - Human vac. ≥ 2.5 IU
  - Veterinary vac. ≥ 1.0IU
3. Major test items for rabies vaccine

Potency test

Concerns:

- It requires long time (4 weeks)
- It requires many animals (150 mouse)
- It gives variable results, with up to 400%
- It is against animal welfare.
  - the intracerebral injection accompanies pain
  - clinical symptoms of rabies develop in unprotected animals
Serological Potency Assay

Serum Neutralization Test:
for Potency test for rabies vaccine

- More cost and time effective (3 weeks)
- Reduce the number of animals
- Avoid the pain and distress of unprotected animals
Serological Potency Assay

The European Directorate for the Quality of Medicines and HealthCare (EDQM) conducted collaborative study

They confirmed accuracy and inter-laboratory transferability.

Serological potency assay can substitute for NIH potency test
Potency assay by ELISA in Japan

Detect protective antigen content in test vaccines and determine the potency of test vaccines by comparing the ELISA value of a test vaccine to that of a reference vaccine.

Defined as two parallel line assay with three doses by use of a reference vac.
Potency assay by ELISA

Principle of ELISA

- POD labeled MAb
- RV
- Mab to catch RV
- Blocking solution
Potency assay by ELISA

Materials: Reference vaccine

- Freeze dried, kept at -80°C
- Effective for 10 years
- 2.81 IU/ml by NIH test
- Confirm antibody induction in dogs
- NVAL provides the vaccine to manufacturers (3550 yen ≈ $35).
Flowchart of ELISA

1. Pre-treatment
2. Gel filtration
3. ELISA
4. Calculation
Potency test by ELISA

1. Pretreatment
   Sonication and Centrifugation
Potency test by ELISA

2. Gel filtration

Sample: 4ml
Flow rate: 1ml/min.
Collection of samples: 2ml/tube
Take 8ml of this fraction

Potency test by ELISA

2. Gel filtration

ELISA

Gs-protein
Potency test by ELISA

3. ELISA

Measure O.D.

Test Vaccine

Reference Vaccine

1 : 2 : 4
Potency test by ELISA

4. Calculation

1. Validity test
   To confirm that the two lines are parallel
2. Determine the potency, \( P \geq 0.683 \)
Introduction of ELISA as the national assay in 1996

- No veterinary rabies vaccine has been rejected by the national assay.
- Protein nitrogen content was reduced.
  - Before the introduction of ELISA: 50 μg/ml (1994)(mean, 37 lot)
  - After the introduction of ELISA: 24 μg/ml (2010)(mean, 20 lot)
Rabies Antibody Detection Kit

- VNA : 0.5 IU/mL ≤
  
  The appearance of only the control line indicates the sample is positive. (0.5 IU/mL ≤)

- VNA : < 0.5 IU/mL
  
  The appearance of both test line and control line indicates the sample is negative. (< 0.5 IU/mL)
# Result

<table>
<thead>
<tr>
<th>Dog • Cat</th>
<th>VNA by RFFIT (IU/mL)</th>
<th>&lt;0.5</th>
<th>0.5 ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAPINA(+)</td>
<td>137</td>
<td>2</td>
<td>139</td>
</tr>
<tr>
<td>RAPINA(−)</td>
<td>0</td>
<td>135</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>137</td>
<td>137</td>
<td>274</td>
</tr>
</tbody>
</table>

(+) The appearance test line indicates the sample VNA is negative (<0.5 IU/mL)
(−) The appearance only the control line indicates the sample is positive. (0.5 IU/mL ≤)

- Sensitivity: 100% (137/137)
- Specificity: 98.5% (135/137)
- Accuracy: 99.2% (272/274)
THANK YOU!