European Regulatory Framework and Practices for Veterinary Leptospira Vaccine Potency Testing
Synopsis

• Role of
  → National Authorities
  → the EU
  → the Ph.Eur.

• Organization & Products of the Ph.Eur.

• The Pharmacopoeia
  • Elaboration
  • Content
  • Leptospira vaccine monographs

• Recent developments in Leptospira vaccine testing
The European Pharmacopoeia

• 1964: Convention on the elaboration of a European Pharmacopoeia

• Today:
  – 36 member states + the European Union
  – 24 observer countries and international organisations including World Health Organization (WHO)
Member States and Observers
European Union (EU)
Its legal status

- Lays down common, compulsory quality standards for all medicinal products in Europe, i.e. raw materials, preparations, dosage forms, containers
- Mandatory at the same date in 36 states (CoE) and the EU
- National pharmacopoeias to cover subjects of solely national interest
European Pharmacopoeia (Ph. Eur.)

Organization of the Ph. Eur.

• Commission
  • „Ph. Eur. Parliament“
Ph. Eur. Commission

- One delegation per member state or observer
- 36 Member States plus a delegation from the EU (a representative from DG Health & Consumer and the EMA);
- 24 Observer countries and World Health Organization (WHO).
- Persons come from health ministries, health authorities, pharmacopoeias, universities, or industry and are appointed by the national authorities on the basis of their expertise.
- Three sessions a year; texts are adopted by unanimous vote.
European Pharmacopoeia (Ph. Eur.)

Organization of the Ph. Eur.

• Commission
  • „Ph. Eur. Parliament“

• Expert groups
  • Biologicals methods and statistical analysis
  • Organic chemistry - Synthetic products
  • Veterinary sera and vaccines
  • …..

• Technical Secretariat (EPD)
European Pharmacopoeia (Ph. Eur.)

“Products”

- European Pharmacopoeia
  - Book
  - On-line (http://online.edqm.eu)
  - USB-stick
European Pharmacopoeia (Ph. Eur.)

“Products”

- European Pharmacopoeia
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  - USB-stick

- PHARMEUROPA
  - On-line (http://pharmeuropa.edqm.eu)

- Knowledge database
  - On-line

- Reference Standards
  - Biological Standardisation Committee
Elaboration of a Ph.Eur monograph

The European Pharmacopoeia Commission decides to elaborate/revise a monograph

Group of experts
a rapporteur prepares a draft monograph, which is evaluated by the experts

PHARMEUROPA
the draft monograph is published for public enquiry, which lasts 3 months

The national pharmacopoeia authorities process the comments received on the draft
The technical secretariat (EPD) compiles the comments

The group of experts examines the comments and revises the draft monograph accordingly

The draft is proposed to the European Pharmacopoeia Commission

adopts
does not adopt

EUROPEAN PHARMACOPOEIA:
the monograph is published about 6 months after adoption
European Pharmacopoeia (Ph. Eur.) → legal requirements

• General Notices
• Monographs
  • Vaccines for Veterinary Use (0062)
  • Vaccine specific monographs
• Supplementary texts
  • 2.6.1. Sterility
  • 5.2.4. Cell cultures for the production of veterinary vaccines
  • 5.2.6. Evaluation of safety of veterinary vaccines and immunosera
  • 5.2.7. Evaluation of efficacy of veterinary vaccines and immunosera
  • …. 
CANINE LEPTOSPIOSIS VACCINE (INACTIVATED)

Vaccinum leptospiritos canis inactivatum

1. DEFINITION
Canine leptospirosis vaccine (inactivated) is a preparation containing a killed or inactivated antigen of one or more strains of Leptospira interrogans serovar canicola. The vaccine is used to protect against leptospirosis. It is administered by injection in dogs.

2. PRODUCTION

2.1. PREPARATION OF THE VACCINE
The vaccine material is obtained from cultures of Leptospira interrogans serovar canicola. The cultures are grown in a suitable medium and then killed or inactivated to render the vaccine safe for use in dogs.

2.2. VACCINE COMPOSITION
The vaccine contains killed or inactivated Leptospira interrogans serovar canicola. The vaccine is administered by injection in dogs.

3. RATCH TESTS

3.1. Identification. The vaccine contains Leptospira interrogans serovar canicola. The vaccine is administered by injection in dogs.

3.2. Pathogenicity. The vaccine is tested for pathogenicity by inoculation of a specific medium. The vaccine is tested for pathogenicity by inoculation of a specific medium. The vaccine is administered by injection in dogs.

3.3. Safety. The vaccine is administered by injection in dogs.

3.4. Residual live bacteria. The vaccine is tested for residual live bacteria by inoculation of a specific medium. The vaccine is administered by injection in dogs.

3.5. Potency. The vaccine is tested for potency by inoculation of a specific medium. The vaccine is administered by injection in dogs.

See the information section on general monographs (cover page).
Canine leptospirosis vaccine (inactivated)

Batch potency test *(Hamster Test)*

It is not necessary to carry out the Potency test (test in dogs) for each batch of the vaccine if it has been carried out using a batch of vaccine with a minimum potency. Where the test is not carried out, an alternative validated method is used, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency. The following tests may be used.

2-3-1-1. *For vaccines with or without adjuvants.* If leptospiroa from more than one serovar (for example L. interrogans serovar canicola and serovar icterohaemorrhagiae) has been used to prepare the vaccine, carry out a batch potency test for each serovar against which protective immunity is claimed on the label. Use for the test 10 healthy hamsters not more than 3 months old, that do not have antibodies against the principal serovars of L. interrogans (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis) and which have been obtained from a regularly tested and certified leptospira-free source. Administer 1/40 of the dose for dogs by the subcutaneous route to 5 hamsters. Maintain 5 hamsters as controls. Challenge each hamster after 15-20 days by the intraperitoneal route with a sufficient quantity of a virulent culture of leptospiroa of the serovar against which protective immunity is claimed on the label. The vaccine complies with the test if not fewer than 4 of the 5 control hamsters die showing typical signs of leptospira infection within 14 days of receiving the challenge suspension and if not fewer than 4 of the 5 vaccinated hamsters remain in good health for 14 days after the death of 4 control hamsters.
Canine leptospirosis vaccine (inactivated)

Batch potency test (ctd.)

2-3-1-2. *For vaccines with or without adjuvants.* A suitable validated sero-response test may be carried out. Vaccinate each animal in a group of experimental animals with a suitable dose. Collect blood samples after a suitable, fixed time after vaccination. For each of the serovars present in the vaccine, an in vitro test is carried out on individual blood samples to determine the antibody response to one or more antigenic components which are indicators of protection and which are specific for that serovar. The criteria for acceptance are set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency.

**Sero logical Test**

2-3-1-3. *For vaccines without adjuvants.* For each of the serovars present in the vaccine, a suitable validated in vitro test may be carried out to determine the content of one or more antigenic components which are indicators of protection and which are specific for that serovar. The criteria for acceptance are set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency.

**Antigen Quantification Test**

European Regulatory Framework and Practices for Veterinary Leptospira Vaccine Potency Testing
Bovine leptospirosis vaccine (inactivated)

Batch potency test *(Serological Test)*

It is not necessary to carry out the Potency test (test in cattle) for each batch of vaccine if it has been carried out using a batch of vaccine with a minimum potency. Where the test is not carried out, an alternative validated method is used, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency. The following test may be used.

For each of the serovars for which protection is claimed, the antibody response from vaccinated animals is measured. Use not fewer than 12 guinea-pigs weighing 250-350 g that do not have antibodies against *L. borgpetersenii* serovar hardjo and the principal serovars of *L. interrogans* (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis) and that have been obtained from a regularly tested and certified leptospira-free source. The dose to be administered to the guinea-pigs is that fraction of a cattle dose which has been shown in the validation studies to provide a suitably sensitive test. Vaccinate each of 10 guinea-pigs with the suitable dose. Maintain not fewer than 2 guinea-pigs as controls. At a given interval within the range of 19-23 days after the injection, collect blood from each guinea-pig and prepare serum samples. Use a suitable validated method such as a micro-agglutination test to measure the antibodies in each sample.

The vaccine complies with the test if antibody levels are equal to or greater than those obtained with a batch that has given satisfactory results in the test described under Potency and there is no significant increase in antibody titre in the controls.
EDQM Workshops

on Alternatives to the Leptospira Vaccine potency test with participants from

• Licensing authorities
• Official Medicines Control Laboratories
• Industry
• Academia
• OIE reference laboratory

1999: Participants from Europe and U.S.A.
2012: Participants from Europe
Conclusions workshop 1999

- Ph.Eur. monograph is outdated
- Hamster Potency test has deficiencies
- Alternative methods should be based on efficacy tests in the target species
  - Monographs revised, Conclusions integrated as “door openers”

- Working group should be created
  - sharing knowledge and
  - to coordinate efforts to replace the hamster test
Conclusions workshop 2012

• **LPS immunodominant**
  - candidate antigen for antigen quantification
  - due to antigen variation no common reference material
  - mAbs may be obtained from Royal Tropical Institute Amsterdam (NL)
  - 2 manufacturers have CA approved vaccine on the market, tested for potency with an *in vitro* test

• **LipL32 no evidence for protection**
Conclusions workshop 2012

• *In vitro* assay may be used to assess
  • Antigen quantification
  • Potency
  • (Stability)

• No single, universal alternative method
  • due to the complexity of the vaccines
    → number of serotypes, number of serovars
    → specific antigens as protective agents
    → combinations
    → presence/absence of adjuvants
Conclusions workshop 2012

• Consistency approach may reduce final product testing

• EDQM might provide standard mAbs for ELISA test

• Press release
  http://www.edqm.eu/medias/fichiers/edqm_workshopleptospirosis_vaccine_batch_potency_t.pdf
Further steps

✓ Information of Ph. Eur. Commission
- Revision of the monograph for vaccines for dogs
  - On the agenda of expert group 15V
- Develop guidance on implementation of consistency approach
✓ Update of EPAA
✓ Update of EMA/CVMP/IWP
European Regulatory Framework and Practices for Veterinary Leptospira Vaccine Potency Testing

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