Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Federal Department of Economic Affairs FDEA
Institute of Virology and Immunoprophylaxis IVI

Vaccine control

European Regulatory Framework and Practices for Veterinary Leptospira Vaccine Potency Testing

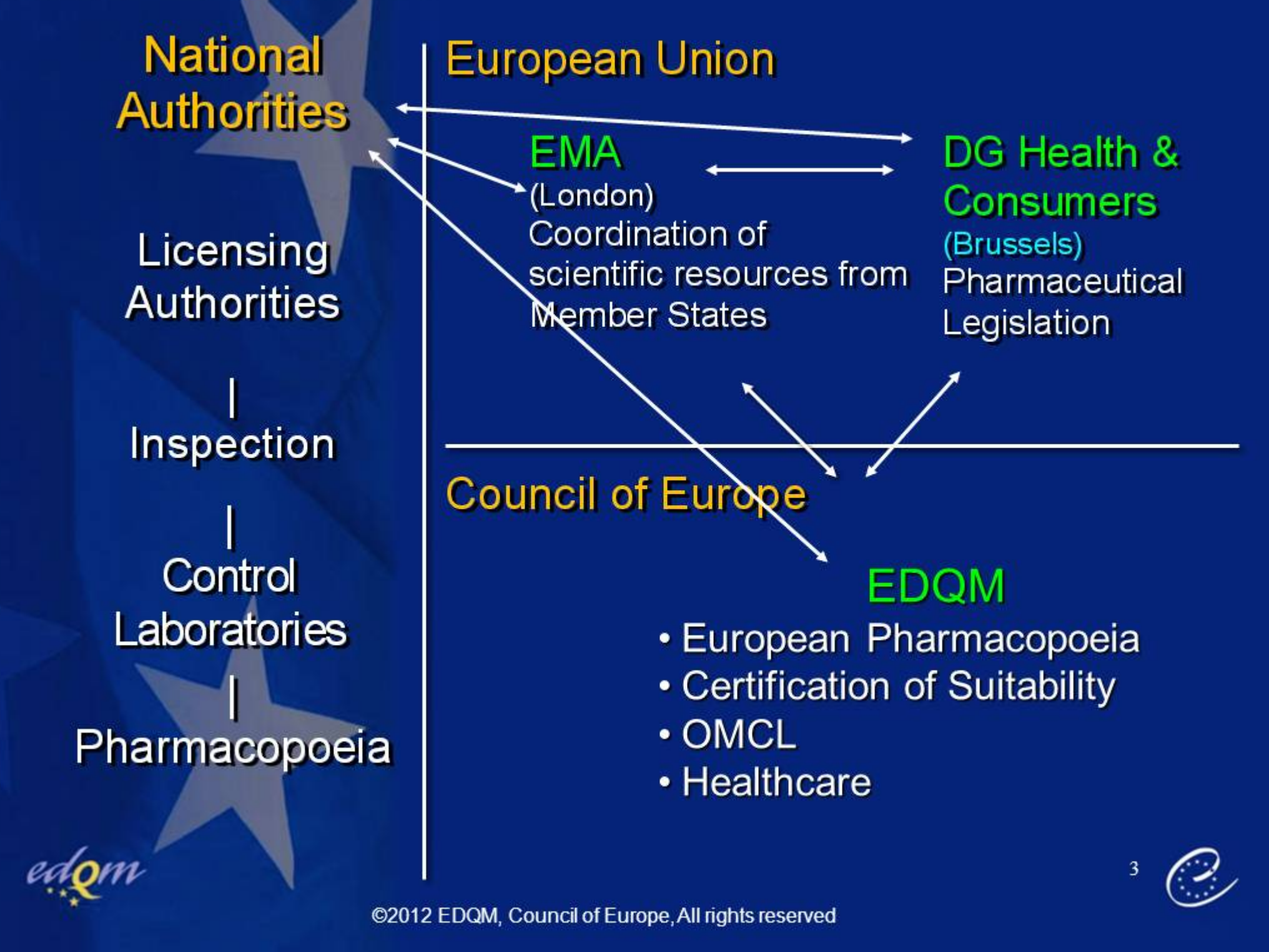
Lukas Bruckner

International Workshop on Alternative Methods for Leptospira Vaccine Potency Testing, Ames, IA, U.S.A., September 19- 21, 2012

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**

Regulatory Framework

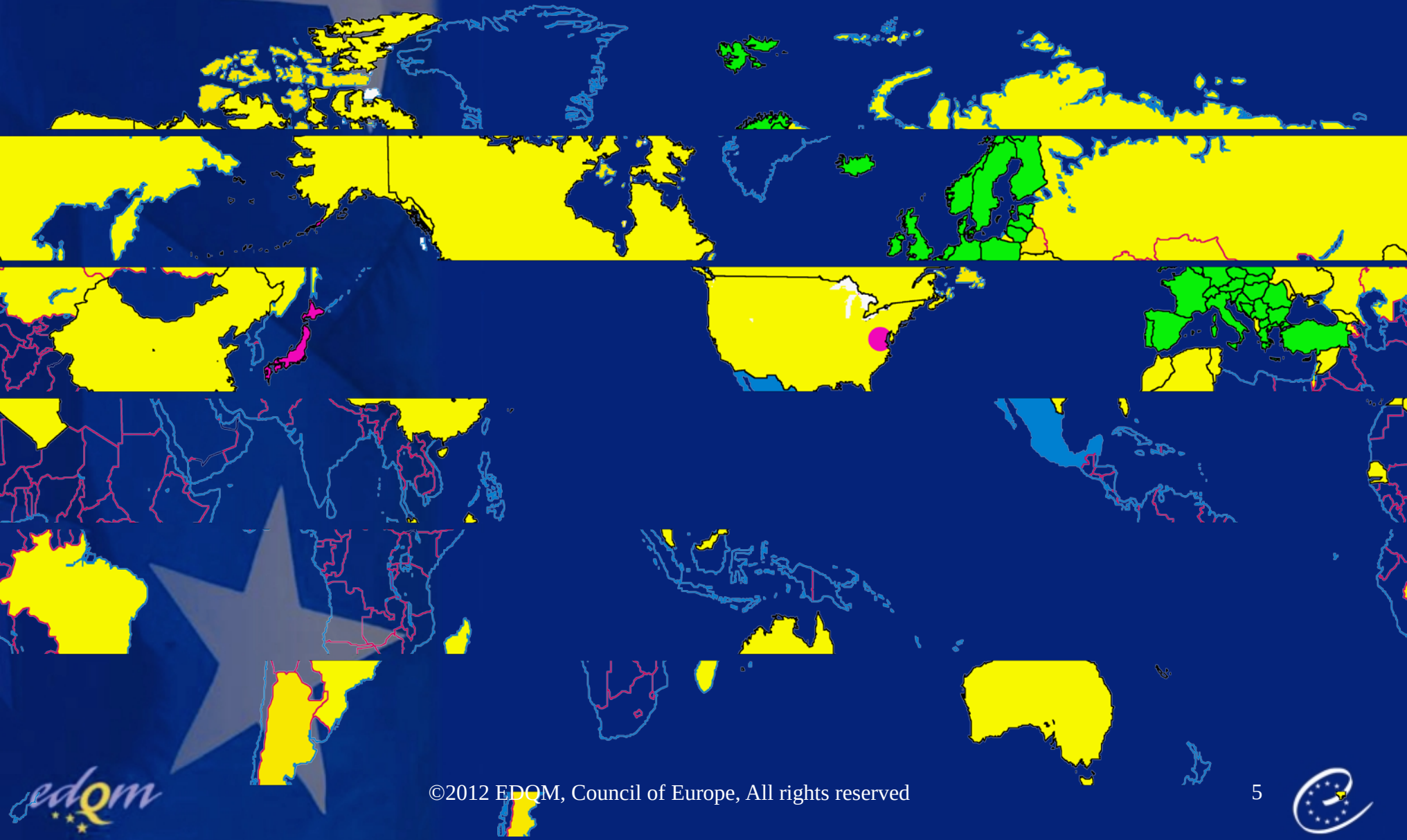


The European Pharmacopoeia

- 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)

Regulatory Framework

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
- 1975: Mandatory status reinforced in the EU pharmaceutical legislation for the EU/EEA member states (Directives 2003/63/EC, 2001/83/EC, 2001/82/EC)

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.)

Regulatory Framework

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.)

Regulatory Framework

“Products”

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

European Pharmacopoeia (Ph. Eur.)

Regulatory Framework

“Products”

- **European Pharmacopoeia**
  - → Book
  - → On-line (http://online.edqm.eu)
  - → USB-stick
- ***PHARMEUROPA***
  - → On-line (http://pharmeuropa.edqm.eu)
- **Knowledge database**
  - → On-line
- **Reference Standards**
  - → Biological Standardisation Committee

Council of Europe

edqm
European Directorate for the Quality of Medicines & HealthCare

Find information on...

- EDQM Vision, Mission & Values
- Ph. Eur. Members & Observers
- Control of Medicines
- Blood Transfusion
- Organ Tranplantation
- Pharmaceutical Care
- eTACT
- The Medicrime Convention
- Consumer Health Protection

Most viewed pages

- FAQ - Helpdesk List
- Databases
- EDQM Reference Standards
- European Pharmacopoeia 7th Edition
- Certification News

Visit the EDQM Store

Access for existing clients

- European Pharmacopoeia
- Blood and Organ Guides
- Standard Terms

Free access

- Pharmeuropa & Pharmeuropa Bio
- Email Alert
- RSS feed

The Vision of the EDQM

- a Directorate of the Council of Europe created in 1964.
- a leading organisation that protects public health by enabling the development, supporting the implementation and monitoring the application of quality standards for safe medicines and their safe use.
- Develops also guidance and standards in the areas of blood transfusion, organ transplantation and consumer health issues.

> What's new at the EDQM?

Latest News

12 September 2012
11th Symposium on Pharmaceutical Reference Standards
The Symposium brought together 146 experts from 27 countries representing authorities, pharmacopoeias, international organisations and industry.Proceedings available here.

10 August 2012
CEP July Monthly Report now available
The DCEP publishes on a monthly basis a report containing some relevant figures relating to its main activities.

09 August 2012
EDQM Publishes Annual Report for the year 2011
The report outlines its different activities and provides overview of achievements and results from 2011.

Read all the latest news >

Upcoming Events

27-29 September
IPA/ EDQM/ WHO Technical Conference "Current Challenges in Global Regulatory Compliance"
Mumbai, India

3-8 October 2012
FIP Centennial Congress
Amsterdam, The Netherlands

13 October 2012
European Day for Organ Donation &

Focus

Biologicals

The European Pharmacopoeia has its eye on the future

In the spirit of continuous improvement, the Ph. Eur. Commission is committed to embracing the current technological and regulatory trends, but also appropriately paves the way for the future. In response to this, the EDQM has undertaken an extensive consultation among regulatory authorities and Expert Groups.

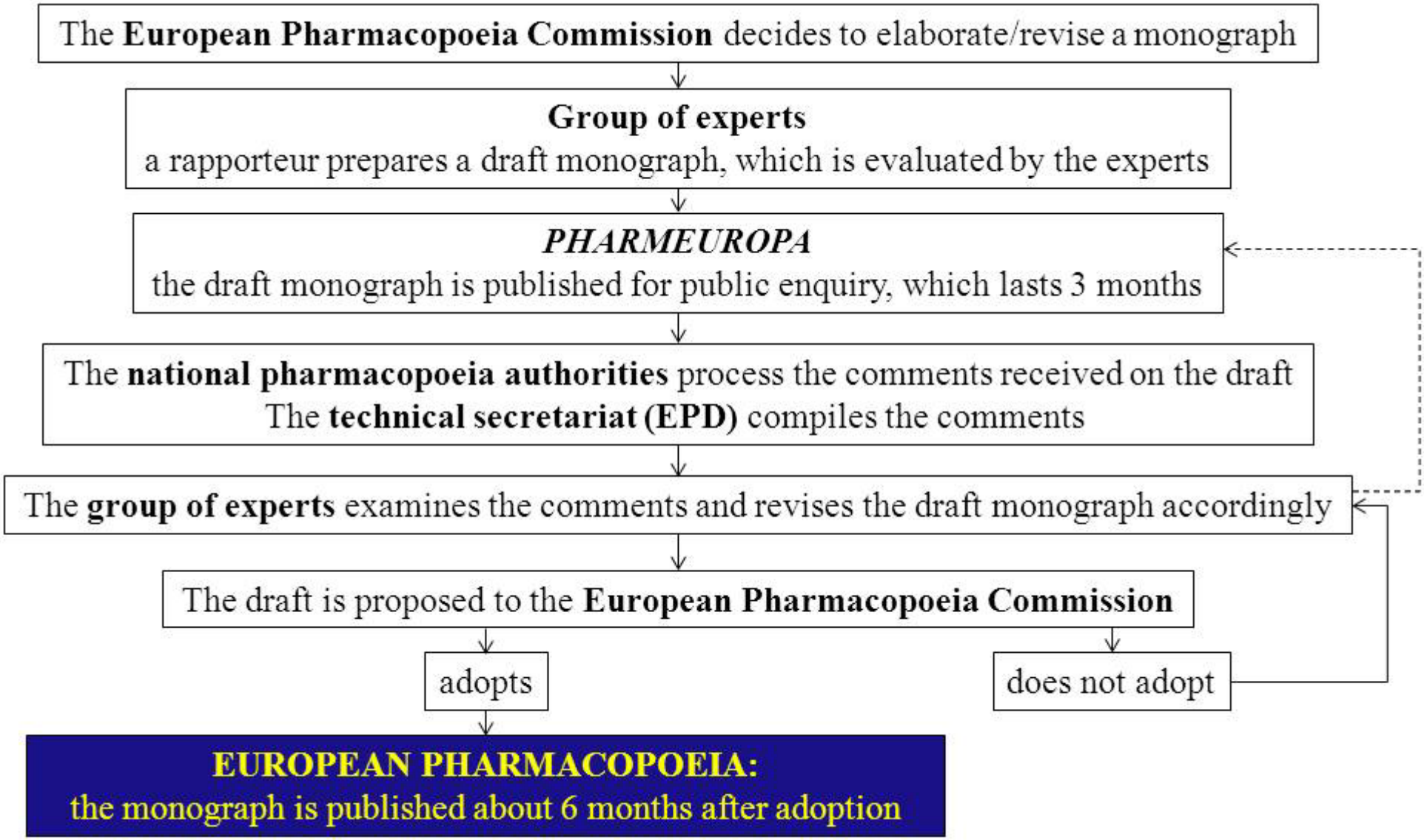
Read more on the European Pharmacopoeia

Products & Services

7th Edition Book
USB Stick & Online

The electronic versions contain all the European Pharmacopoeia texts and provide additional and unique features: fully searchable, hyperlinks in the text of a monograph give access to information on general methods...
The electronic versions are bilingual

Elaboration of a Ph.Eur monograph



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
  - ….

01/2008:0447

CANINE LEPTOSPIROSIS VACCINE (INACTIVATED)

Vaccinum leptospirosis caninae inactivatum

1. DEFINITION

Canine leptospirosis vaccine (inactivated) is a preparation of inactivated whole organisms and/or antigenic extract(s) of one or more suitable strains of one or more of *Leptospira interrogans* serovar canicola, serovar icterohaemorrhagiae or any other epidemiologically appropriate serovar, inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of dogs against leptospirosis.

2. PRODUCTION

2-1. *PREPARATION OF THE VACCINE*

The seed material is cultured in a suitable medium; each strain is cultivated separately. During production, various parameters such as growth rate are monitored by suitable methods; the values are within the limits approved for the particular product. Purity and identity are verified on the harvest using suitable methods. After cultivation, the bacterial harvests are collected separately and inactivated by a suitable method. The antigen may be concentrated. The vaccine may be adjuvanted.

2-2. *CHOICE OF VACCINE COMPOSITION*

The vaccine is shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) for the dogs for which it is intended. The following tests for safety (section 2-2-1) and immunogenicity (section 2-2-2) may be used during the demonstration of safety and efficacy.

2-2-1. **Safety.** Carry out the test for each route and method of administration to be recommended for vaccination and in dogs of each category for which the vaccine is to be intended. Use a batch of vaccine containing not less than the maximum antigen content and/or potency that may be expected in a batch of vaccine.

2-2-1-1. *General safety.* For each test, use not fewer than 10 dogs that do not have antibodies against the principal *L. interrogans* serovars (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis). Administer to each dog a double dose of the vaccine. If the recommended schedule requires a second dose, administer one dose after the recommended interval. Observe the dogs at least daily until 14 days after the last administration. Record body temperatures the day before each vaccination, at vaccination, 4 h later and daily for 4 days.

The vaccine complies with the test if no dog shows abnormal local or systemic reactions, signs of disease or dies from causes attributable to the vaccine.

2-2-1-2. *Safety in pregnant bitches.* If the vaccine is intended for use or may be used in pregnant bitches, use not fewer than 10 bitches at the stage of pregnancy in accordance with the schedule to be recommended or at different stages of pregnancy. Administer to each bitch a double dose of the vaccine. If the recommended schedule requires a second dose, administer one dose after the recommended interval. Observe the bitches at least daily until one day after whelping.

The vaccine complies with the test if no bitch shows abnormal local or systemic reactions, signs of disease or dies from causes attributable to the vaccine and if no adverse effects on the pregnancy or the offspring are noted.

2-2-2. **Immunogenicity.** For each type of the serovars against which protective immunity is claimed on the label, carry out a separate test with a challenge strain representative of that serovar.

Each test is carried out for each route and method of administration to be recommended for vaccination, using in each case dogs of the minimum age to be recommended. The vaccine administered to each dog is of minimum antigen content and/or potency.

Use for the test not fewer than 12 dogs that do not have antibodies against the principal serovars of *L. interrogans* (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis). Vaccinate not fewer than 6 dogs, according to the schedule to be recommended. Maintain not fewer than 6 dogs as controls. Challenge each dog after 25-28 days by the conjunctival and/or intraperitoneal route with a sufficient quantity of a suspension of the relevant pathogenic *L. interrogans* serovar. Observe the dogs at least daily for 28 days after challenge.

Examine the dogs daily and record and score clinical signs observed post-challenge and any deaths that occur. If a dog shows marked signs of disease, it is euthanized. Monitor body temperatures each day for the first week after challenge. Collect blood samples from each dog on days 0, 2, 3, 4, 5, 8 and 11 post challenge. Collect urine samples from each dog on days 0, 3, 5, 8, 11, 14, 21 and 28 post challenge. Euthanise surviving dogs at the end of the observation period. Carry out post-mortem examination on any dog that dies during the observation period and on the remainder when euthanized at the end of the observation period. In particular, examine the liver and kidneys for macroscopic and microscopic signs of leptospira infection. Take a sample of each kidney and test each blood, urine and kidney sample for the presence of challenge organisms by re-isolation or by another suitable method. Analyse blood samples to detect biochemical and haematological changes indicative of infection and score these.

The test is invalid if: samples give positive results on day 0; *L. interrogans* serovar challenge strain is re-isolated from or demonstrated by another suitable method to be present in fewer than 2 samples on fewer than 2 different days, to show infection has been established in fewer than 80 per cent of the control dogs.

The vaccine complies with the test if: at least 80 per cent of the vaccinates show no more than mild signs of disease (for example, transient hyperthermia) and, depending on the *L. interrogans* serovar used for the challenge, one or more of the following is also shown:

- where the vaccine is intended to have a beneficial effect against signs of disease, the clinical scores and haematological and biochemical scores are statistically lower for the vaccinates than for the controls,
- where the vaccine is intended to have a beneficial effect against infection, the number of days that the organisms are detected in the blood is statistically lower for the vaccinates than for the controls,
- where the vaccine is intended to have a beneficial effect against urinary tract infection and excretion, the number of days that the organisms are detected in the urine and the number of kidney samples in which the organisms are detected is statistically lower for the vaccinates than for the controls.

2-3. *MANUFACTURER'S TESTS*

2-3-1. **Batch potency test.** It is not necessary to carry out the Potency test (section 3-5.) 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 leptospira 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 leptospirae 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.

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.

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.

3. RATCH TESTS

3-1. **Identification.** When injected into healthy animals that do not have specific antibodies against leptospira serovar(s) present in the vaccine, the vaccine stimulates the production of such antibodies. If test 2-3-1-3 is used for batch potency test, it also serves to identify the vaccine.

3-2. **Bacteria and fungi.** The vaccine and, where applicable, the liquid supplied with it comply with the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062)*.

3-3. **Safety.** Use 2 dogs of the minimum age recommended for vaccination and that do not have antibodies against the leptospira serovar(s) present in the vaccine. Administer to each dog by a recommended route a double dose of the vaccine. Observe the dogs at least daily for 14 days.

The vaccine complies with the test if no dog shows notable signs of disease or dies from causes attributable to the vaccine.

3-4. **Residual live bacteria.** Carry out a test for live leptospirae by inoculation of a specific medium. Inoculate 1 mL of the vaccine into 100 mL of the medium. Incubate at 30 °C for 14 days, subculture into a further quantity of the medium and incubate both media at 30 °C for 14 days: the vaccine complies with the test if no growth occurs in either medium. At the same time, carry out a control test by inoculating a further quantity of the medium with the vaccine together with a quantity of a culture containing approximately 100 leptospirae and incubating at 30 °C; the test is invalid if growth of leptospirae does not occur within 14 days.

3-5. **Potency.** The vaccine complies with the requirements of the test mentioned under Immunogenicity (section 2-3-2.) when administered by a recommended route and method.

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 leptospira 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 leptospirae 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.

Ph. Eur.

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.

➢*Serological 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*

Ph. Eur.

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

EDQM

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

EDQM

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**

EDQM

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

EDQM

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

EDQM

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**

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Federal Department of Economic Affairs FDEA
Institute of Virology and Immunoprophylaxis IVI

Vaccine control

European Regulatory Framework and Practices for Veterinary Leptospira Vaccine Potency Testing

Lukas Bruckner

International Workshop on Alternative Methods for Leptospira Vaccine Potency Testing, Ames, IA, U.S.A., September 19- 21, 2012