



European Commission
Joint Research Centre
Institute for Health & Consumer Protection
ECVAM Unit
21020 Ispra (VA)
Italy

Tel: +39 0332 785996
Fax: +39 0332 785336

ECVAM European Centre for the Validation of Alternative Methods

STATEMENT ON THE VALIDITY OF A SEROLOGICAL METHOD (ELISA) FOR THE BATCH POTENCY TESTING OF INACTIVATED SWINE ERYSIPELAS VACCINES

At its 18th meeting, held on 3 June 2002 at the European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy, the ECVAM Scientific Advisory Committee (ESAC)¹ unanimously endorsed the following statement:

The results obtained with the ELISA² procedure in the international validation study on alternative methods for the batch potency testing of inactivated swine erysipelas vaccines were reproducible, both within and among the participating laboratories that performed the tests. The ELISA procedure proved applicable to testing a diverse group of inactivated swine erysipelas vaccines, of different potencies, compositions and combinations. The concordances between the potencies derived from the *in vitro* serological (ELISA) data and from the *in vivo* data were very good. The test was able to distinguish between highly potent and less potent vaccines. The Committee therefore agrees with the conclusion from this formal validation study that the ELISA test is scientifically validated for use as a replacement for the challenge procedure in the batch potency testing of inactivated swine erysipelas vaccines.

The Committee welcomes the fact that the ELISA method has already been incorporated into the draft European Pharmacopoeia monograph on inactivated swine erysipelas vaccine³ and recommends its adoption.

The ESAC has been regularly kept informed of the progress of the study (initiated and managed by the Paul Ehrlich Institute, Langen, Germany), and this endorsement was based on an assessment of various documents,^{4,5} including, in particular, the report on the results and evaluation of the validation study by the Management Team,⁶ which was published in *Vaccine*.⁵

This validation study was conducted in accordance with the general principles laid down in the report of the CAAT²/ERGATT² workshop held in 1990,⁷ guidelines contained in the report of an ECVAM/ERGATT workshop held in 1995,⁸ criteria laid down by ECVAM and the ECB,^{2,9} the ICH^{2,10,11} guidelines and WHO^{2,12} guidance on the validation of analytical test methods, and the recommendations of ECVAM/AGAATT² workshop held in 1997.¹³

Michael Balls
Head of Unit
ECVAM
Institute for Health & Consumer Protection
Joint Research Centre

Eva Hellsten
Head of Unit C.3
DG Environment
European Commission
Brussels, Belgium

Ispra, Italy

28 June 2002

1. The ESAC was established by the European Commission, and is composed of representatives of the EU Member States, industry, academia and animal welfare, together with representatives of the relevant Commission services. The following members of the ESAC were present at the meeting on 3 June 2002:

Dr Bas Blaauboer (ERGATT)	Mr Michael Balls (ECVAM - Chairman)
Dr Philip Botham (ECETOC)	Mr Jürgen Vogelgesang (DG ENV)
Dr Argelia Castaño (Spain)	Mr Juan Riego Sintes (ECB)
Dr Bernward Garthoff (EFPIA)	Mr Enrico Sabbioni (ECVAM)
Professor André Guillouzo (France)	Mr Andrew Worth (ECVAM - Secretary)
Dr Maggy Jennings (EUROGROUP for Animal Welfare)	
Professor Elisabeth Knudsen (DK)	
Dr Roman Kolar (EUROGROUP for Animal Welfare)	
Dr Odile de Silva (COLIPA)	
Professor Horst Spielmann (Germany)	
Dr Annalaura Stammati (Italy)	
Professor Eric Tschirhart (Luxembourg)	
Dr Matti Viluksela (Finland)	
Professor Erik Walum (Sweden)	

2. AGAATI: Advisory Group on Alternatives to Animal Testing in Immunobiologicals; CAAT: Center for Alternatives to Animal Testing, Baltimore, USA; ECB: European Chemicals Bureau, Ispra, Italy; ELISA: Enzyme Linked Immunosorbent Assay; ERGATT: European Research Group for Alternatives in Toxicity Testing, Utrecht, The Netherlands; ICH: International Committee of Harmonisation; WHO: World Health Organization, Geneva, Switzerland.
3. Council of Europe (2002). Swine erysipelas vaccine (inactivated). *Pharmeuropa* **14**, 69-70.
4. Roskopf-Streicher U, Johannes S, Wilhelm M, Gyra H & Cussler K. (1999). Potency testing of swine erysipelas vaccines by serology - results of a prevalidation study. *ALTEX* **16**, 123-128.
5. Roskopf-Streicher U, Johannes S, Wilhelm M & Cussler K. (2000). Quality control of inactivated erysipelas vaccines: Results of an international collaborative study to establish a new regulatory test. *Vaccine* **19**, 1477-1483.
6. PEI (1999). Validation of a serological method for the potency testing of erysipelas vaccines. Final Report BMBF Research Project No: 0311200. 57 pp. Paul Ehrlich Institute.
7. Balls M, Blaauboer B, Brusick D, Frazier J, Lamb D, Pemberton M, Reinhardt C, Roberfroid M, Rosenkranz H, Schmid B, Spielmann H, Stammati AL & Walum E. (1990). Report and recommendations of the CAAT/ERGATT workshop on the validation of toxicity test procedures. *ATLA* **18**, 303-337.
8. Balls M, Blaauboer BJ, Fentem JH, Bruner L, Combes RD, Ekwall B, Fielder RJ, Guillouzo A, Lewis RW, Lovell DP, Reinhardt CA, Repetto G, Sladowski D, Spielmann H & Zucco F.

- (1995). Practical aspects of the validation of toxicity test procedures. The report and recommendations of ECVAM workshop 5. *ATLA* **23**, 129-147.
9. Balls M & Karcher W. (1995). The validation of alternative test methods. *ATLA* **23**, 884-886.
 10. EMEA (1995). CPMP/ICH/281/95: *Note for Guidance on Validation of Analytical Procedures: Methodology*. 9 pp. London: EMEA.
 11. EMEA (1995). CPMP/ICH/381/95: *Note for Guidance on Validation of Analytical Methods: Definitions and Terminology*. 5 pp. London: EMEA
 12. WHO (1997). WHO/VSQ/97.02 A *WHO guide to good manufacturing practice requirements*. Hoofdstuk 15. Validation of analytical assays. pp. 65-69.
 13. Hendriksen CFM, Spieser J-M, Akkermans A, Balls M, Bruckner L, Cussler K, Daas A, Descamps J, Dobbelaer R, Fentem JH, Halder, M, van der Kamp M, Lucken R, Milstien J, Sesardic D, Straughan D, Valadares A. (1998). Validation of alternative methods for the potency testing of vaccines. The report and recommendations of ECVAM Workshop 31. *ATLA*, **26**, 747-761.

General information about the validation of a serological method (ELISA) for the batch potency testing of erysipelas vaccines:

- A. The study was initiated by the Paul Ehrlich Institute (PEI; Langen, Germany) and funded by the German Federal Ministry of Education and Research (BMBF project number 0311200). It was co-ordinated by Ute Roskopf-Streicher and Dr Sigrid Johannes (PEI), with Dr Manfred Hansper (Forschungszentrum Jülich GmbH, Jülich, Germany) and Dr Jean-Marc Spieser (European Directorate for the Quality of Medicines, Strasbourg France) as observers. Twelve laboratories (including ECVAM, represented by Dr Marlies Halder) of seven European and North American countries, representing industrial and national control laboratories, participated in this validation study.
- B. Swine erysipelas is a bacterial disease of great economic importance and world-wide distribution. Vaccination is the most efficient tool for preventing disease in animals. The quality control tests for the batch release of vaccines are prescribed in Monograph 064 of the European Pharmacopoeia (*Ph. Eur.*). At the time when the validation study was initiated, a multi-dilution vaccination-challenge test was stipulated for the batch potency testing of inactivated erysipelas vaccines, which was carried out with the reference vaccine and the test vaccine. It required at least 106 mice per batch (1) and caused severe suffering. In Europe, at least 10,000 mice *per annum* were used for this purpose. The current *Ph. Eur.* monograph still stipulates a single-dilution vaccination-challenge test to be carried out with the test vaccine and reference vaccine in two groups of ten mice, whereas an additional group of 10 mice is needed as control for the challenge preparation (2).
- C. In the early 1990s, several working groups described the protective antigens of the infectious agent, *Erysipelothrix rhusiopathiae*, and the PEI developed an indirect ELISA, by using an alkaline extract of *E. rhusiopathiae* as the coating antigen (3, 4), for detecting protective serum antibodies of immunised mice. The serum antibody levels are estimated by comparing the titration curves of the test serum and reference serum. The values obtained are expressed as relative potencies, and the reference serum is given the arbitrary value of 1. Vaccines which induce antibody levels ≥ 1 pass the batch potency test, whereas vaccines inducing antibody levels < 1 fail the test.
- D. In 1998, the PEI initiated a prevalidation study involving eight laboratories (including ECVAM). Nine coded serum samples were tested with the ELISA and compared to the reference serum. The ELISA results showed very good agreement between the laboratories. The intralaboratory variation was 15%, and the interlaboratory variation was 22%. *In vitro/in vivo* comparisons revealed that it was possible to distinguish and rank the vaccines with the ELISA procedure according to the *in vivo* derived potencies provided by the PEI (5). Based on these promising results, it was decided to validate the ELISA procedure in a formal validation study.
- E. The design of the validation study was agreed with the 12 participating laboratories, including ECVAM. Nine vaccines from various manufacturers and of different compositions were distributed to the participants. For each vaccine, a group of 10 mice were immunised with 1/10th of the pig dose. After three weeks, the animals were bled and the antibodies against erysipelas were estimated with the ELISA procedure. ECVAM conducted no *in vivo* studies, but received coded serum samples from the PEI, which had been collected in an independent immunisation trial. The ELISA results showed very good agreement between the laboratories in the ranking of the vaccine

potencies. The intralaboratory and interlaboratory variations were in the same range of as in the prevalidation study (5, 6).

F. The participants, organisers and observers of the study concluded that the ELISA procedure had been scientifically validated, and recommended its consideration for regulatory acceptance.

G. Promotion to regulatory acceptance:

- In order to support and promote the regulatory acceptance, a reference coating antigen for the ELISA procedure was prepared within the Biological Standardisation Programme of the EDQM. The *Ph. Eur.* Commission adopted the antigen as a new European Biological Reference Preparation at the 110th session (June 19-21, 2001) (7).
- At the beginning of 2002, the newly revised monograph, now including the ELISA procedure for batch potency testing, was published in *Pharmeuropa* (8)

References:

1. Council of Europe (1997). Swine erysipelas vaccine (inactivated), Monograph No 64. European Pharmacopoeia, 3rd Edition.
2. Council of Europe (2001). Swine erysipelas vaccine (inactivated), Monograph No 64. Supplement 2001 to European Pharmacopoeia, 3rd Edition.
3. Beckmann R & Cussler K (1994). Wirksamkeitsprüfung von Rotlaufimpfstoffen an der Labormaus. ELISA kontra Infektionsversuch. *ALTEX Supplement I*, 39-45.
4. Rosskopf-Streicher U, Johannes S, Hausleithner D, Gyra H & Cussler K. (1998). Suitability of an ELISA for the batch potency testing of erysipelas vaccines. *Pharmeuropa Special Issue, BIO 98-1*, 65-70.
5. Rosskopf-Streicher U, Johannes S, Wilhelm M, Gyra H & Cussler K. (1999). Potency testing of swine erysipelas vaccines by serology - results of a prevalidation study. *ALTEX* **16**, 123-128.
6. Rosskopf-Streicher U, Johannes S, Wilhelm M & Cussler K. (2000). Quality control of inactivated erysipelas vaccines: Results of an international collaborative study to establish a new regulatory test. *Vaccine* **19**, 1477-1483.
7. Cussler K, Rosskopf-Streicher U, Volkens P & Milne C. (2001). Collaborative study for the establishment of Erysipelas ELISA Coating Antigen European Biological Reference Preparation Batch No 1. *Pharmeuropa Special Issue BIO 2001-1*, 75-88.
8. Council of Europe (2002). Swine erysipelas vaccine (inactivated). *Pharmeuropa* **14**, 69-70.