**Validated Methods**

- **Scientifically Validated Methods**
- **Recommendations for Method Deletions**
- **Regulatory Acceptance**

**Tracking System for Alternative test methods (TSAR)**

### Scientifically Validated Methods

1. **Two in vitro skin irritation tests: EpiDerm SIT and SkinEthic RHE assay**
   - Date of the ESAC statement: 05 November 2008
   - Remarks: stand-alone tests for the replacement of the Draize Skin Irritation Test
   - Status: ongoing development of Draft OECD Test Guideline and EU Test Method B.46
   - **Links:** ESAC Statement

2. **Acute Toxic Class (ATC) Method for acute oral toxicity testing**
   - Date of the ESAC statement: 31 October 2007
   - Remarks: replacement of the acute oral toxicity test (the LD₅₀ method)
   - Status: regulatory accepted (see section below)
   - **Links:** ESAC Statement

3. **Fixed Dose Procedure (FDP) for acute oral toxicity testing**
   - Date of the ESAC statement: 31 October 2007
   - Remarks: replacement of the acute oral toxicity test (the LD₅₀ method)
   - Status: regulatory accepted (see section below)
   - **Links:** ESAC Statement

4. **Up-and-Down Procedure for acute oral toxicity testing**
   - Date of the ESAC statement: 31 October 2007
   - Remarks: replacement of the acute oral toxicity test (the LD₅₀ method)
   - Status: regulatory accepted (see section below)
   - **Links:** ESAC Statement

5. **Artificial skin models (EPISKIN®, EpiDerm®) for skin irritation testing**
   - Date of the ESAC statement: 27 April 2007
   - Remarks: stand-alone test (EPISKIN®) for the replacement of the Draize Skin Irritation Test
   - Status: ongoing development of Draft OECD Test Guideline and EU Test Method B.46
   - **Links:** ESAC Statement

6. **Reduced Local Lymph Node Assay (rLLNA) for skin sensitisation**
   - Date of the ESAC statement: 27 April 2007
   - **Links:** ESAC Statement

7. **The Bovine Corneal Opacity and Permeability (BCOP) and the Isolated Chicken Eye (ICE) test methods for eye irritation**
   - Date of the ESAC statement: 27 April 2007
   - Remarks: the ESAC statement is based on the U.S. ICCVAM retrospective study
   - Status: ongoing development of Draft OECD Test Guideline and EU Test Method
   - **Links:** ESAC Statement

8. **Micronucleus Test as an alternative to the In Vitro Chromosome Aberration Assay for genotoxicity testing**
   - Date of the ESAC statement: 17 November 2006
   - Status: ongoing development of Draft OECD Test Guideline 487 and EU Test Method
   - **Links:** ESAC Statement

9. **SkinEthic™ Human Skin Model for skin corrosivity testing**
    - Date of the ESAC statement: 17 November 2006
    - Remarks: accepted for its use within OECD Test Guideline 431 and EU Test Method B.40 bis
    - **Links:** ESAC Statement

10. **Five In Vitro Pyrogen tests**
    - Date of the ESAC statement: 21 March 2006
    - Remarks: all five tests are indicated in the ESAC Statement
    - **Links:** ESAC Statement

11. **Testing Strategy to reduce the use of fish in acute aquatic toxicity testing**
    - Date of the ESAC statement: 21 March 2006
    - Status: ongoing development of OECD Guidance Document
    - **Links:** ESAC Statement
The Colony Forming Unit-Granulocyte/Macrophage (CFU-GM) Assay for predicting acute neutropenia in humans
Date of the ESAC statement: 21 March 2006
Links: INVITTOX Protocol, ESAC Statement

ELISA test for batch potency testing of erysipelas vaccines
Date of the ESAC statement: 28 June 2002
Status: regulatory accepted (see section below)
Links: ESAC Statement

Embryonic Stem Cell Test (EST) for embryotoxicity
Date of the ESAC statement: 01 May 2002
Links: INVITTOX Protocol, ESAC Statement

Micromass (MM) embryotoxicity assay
Date of the ESAC statement: 01 May 2002
Links: INVITTOX Protocol, ESAC Statement

Whole Rat Embryo embryotoxicity assay
Date of the ESAC statement: 01 May 2002
Links: INVITTOX Protocol, ESAC Statement

CORROSITEX assay for skin corrosivity
Date of the ESAC statement: 06 December 2000
Remarks: the ESAC statement is based on the outcome of the U.S. ICCVAM study
Status: regulatory accepted (see section below)
Links: INVITTOX Protocol, ESAC Statement

ELISA test for batch potency testing of tetanus vaccines for human use
Date of the ESAC statement: 06 December 2000
Status: regulatory accepted (see section below)
Links: ESAC Statement

Toxin Binding Inhibition (ToBI) test for batch potency testing of tetanus vaccines for human use
Date of the ESAC statement: 06 December 2000
Status: regulatory accepted (see section below)
Links: ESAC Statement

Local Lymph Node Assay for skin sensitisation (LLNA)
Date of the ESAC statement: 21 March 1999
Remarks: the ESAC statement is based on a retrospective data analysis
Status: regulatory accepted (see section below)
Links: ESAC Statement

3T3 Neutral Red Uptake (NRU) phototoxicity test
Date of the ESAC statement: 20 May 1998, 03 November 1997
Status: regulatory accepted (see section below)
Links: INVITTOX Protocol, ESAC Statement

In vitro production of monoclonal antibodies
Date of the ESAC statement: 14 May 1998
Remarks: the ESAC statement is based on a retrospective data analysis
Links: ESAC Statement

EPISKIN™ skin corrosivity test
Date of the ESAC statement: 03 April 1998
Status: regulatory accepted (see section below)
Links: INVITTOX Protocol, ESAC Statement

Rat Transcutaneous Electrical Resistance (TER) skin corrosivity test
Date of the ESAC statement: 03 April 1998
Status: regulatory accepted (see section below)
Links: INVITTOX Protocol, ESAC Statement

EpiDerm™ skin corrosivity test
Date of the ESAC statement: 21 March 1998
Status: regulatory accepted (see section below)
Links: INVITTOX Protocol, ESAC Statement
Recommendations for Method Deletions

- The batch potency testing of erythropoietin concentrated solution
  Date of the ESAC statement: 28 June 2002

- The relevance of the target-animal safety test for batch safety testing of vaccines for veterinary use
  Date of the ESAC statement: 28 June 2002
  Remarks: The ESAC statement is based on a retrospective data analysis.

Regulatory Acceptance

- In vitro tests for percutaneous absorption
  Links: 440/2008/EC, (Corrigendum), OECD

- CORROSITEX assay for skin corrosivity
  Regulation: OECD Test Guideline 435, adopted in July 2006
  Remarks: the ESAC statement is based on the outcome of the U.S. ICCVAM study
  Links: OECD

- The relevance of the target-animal safety test for batch safety testing of vaccines for veterinary use
  Regulation: The target animal safety test can now be waived when a sufficient number (e.g. 10) of batches has been found to comply with the test. European Pharmacopoeia monograph: Vaccines for veterinary use (0062;2005)
  Links: EDQM/European Pharmacopoeia

- ELISA test for batch potency testing of erysipelas vaccines
  Regulation: The method has been included in the monograph on swine erysipelas vaccine (European Pharmacopoeia, 4.6; No 012/2004:0064)
  Links: EDQM/European Pharmacopoeia

- Local Lymph Node Assay for skin sensitisation (LLNA)
  Regulation: Method B.42 of Annex to 440/2008/EC (EU Test Methods Regulation) and its Corrigendum, the method has originally been adopted in April 2004; updated OECD Test Guideline 429, adopted in April 2002
  Remarks: the ESAC statement is based on a retrospective data analysis
  Links: 440/2008/EC, (Corrigendum), OECD

- ELISA test for batch potency testing of tetanus vaccines for human use
  Regulation: The method has been included in the general text 2.7.8 Assay of tetanus vaccine (adsorbed) and adopted by the European Pharmacopoeia Commission in March 2003.
  Links: EDQM/European Pharmacopoeia

- Toxin Binding Inhibition (ToBI) test for batch potency testing of tetanus vaccines for human use
  Regulation: The method has been included in the general text 2.7.8 Assay of tetanus vaccine (adsorbed) and adopted by the European Pharmacopoeia Commission in March 2003.
  Links: EDQM/European Pharmacopoeia

- Deletion of the Acute Oral Toxicity test, Lethal Dose (LD₅₀)

- Up-and-Down Procedure for acute oral toxicity testing
  Regulation: OECD Test Guideline 425 adopted December 2001
  Remarks: replacement of the acute oral toxicity test (the LD₅₀ method)
  Links: OECD

- 3T3 NRU phototoxicity test
  Regulation: Method B.41 of Annex to 440/2008/EC (EU Test Methods Regulation) and its Corrigendum, the method has originally been adopted in April 2000; OECD Test Guideline 432, adopted in April 2004.
  Links: 440/2008/EC, (Corrigendum), OECD

- EpiDerm™ skin corrosivity test
• **EPISKIN™ skin corrosivity test**
  Regulation: Method B.40 bis of Annex to 440/2008/EC (EU Test Methods Regulation) and its Corrigendum, the method has originally been adopted in April 2000; OECD Test Guideline 431, adopted in April 2004.
  
  Links: 440/2008/EC, (Corrigendum), OECD

• **Rat TER skin corrosivity test**
  Regulation: Method B.40 of Annex to 440/2008/EC (EU Test Methods Regulation) and its Corrigendum, the method has originally been adopted in April 2000; OECD Test Guideline 430, adopted in April 2004.
  
  Links: 440/2008/EC, (Corrigendum), OECD

• **Acute Toxic Class Method for acute oral toxicity testing**
  Remarks: replacement of the acute oral toxicity test (the LD_{50} method)
  
  Links: 440/2008/EC, (Corrigendum), OECD

• **Fixed Dose Procedure for acute oral toxicity testing**
  Regulation: Method B.1 bis of Annex to 440/2008/EC (EU Test Methods Regulation) and its Corrigendum, the method has originally been adopted in July 1992, updated in April 2004; updated OECD Test Guideline 420, adopted in December 2001.
  Remarks: replacement of the acute oral toxicity test (the LD_{50} method)
  
  Links: 440/2008/EC, (Corrigendum), OECD