



Towards the end of in vivo toxicity testing

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Reduced toxicity testing – in Ph. Eur.

Based on review and rationalisation of strategies, accumulated info and collaborative studies

	Development	Bulk	Final lot	Notes
Diphtheria vaccines	Test for specific activity in GP Removed	Absence of toxin Irreversibility of toxoid (<i>in vitro</i>)	-	Applicable as of 01/07/22 <i>No more in vivo test for toxicity</i>
Tetanus vaccines (human and vet)	Test for specific activity in GP Removed	Absence of toxin in GP Irreversibility of toxoid Removed	-	Applicable as of 01/01/21 Test for human and vet aligned. <i>In vivo still present but reduced</i>
Acellular Pertussis Vaccines	-	Residual toxin <i>in vivo</i> – replaced with <i>in vitro</i> Irreversibility of toxoid Removed	Residual toxin Removed	Applicable as of 01/01/20 <i>No more in vivo test for toxicity</i> BSP114 study <small>Isbruker et al. <i>Pharmeur Bio Sci Notes</i> 2016:97-114</small>
Abnormal Toxicity Test (ATT)	Deletion for regular release tests in > 80 monographs in 1998 Complete deletion based on lack of scientific relevance			Applicable as of 01/01/19 <i>No more ATT in Ph. Eur.</i>

Considerations prior to deleting a toxicity test (or any test):

- Is the test suitable for its intended use?
- Is there an alternative test that is equally or more sensitive/reliable?
- Is the test relevant for today's manufacturing requirement (GMP)?
- Is the test needed considering the overall testing strategy?
- Does historic testing results from routine testing or vaccine development support deletion?

Abnormal toxicity test (ATT) - Ph. Eur.

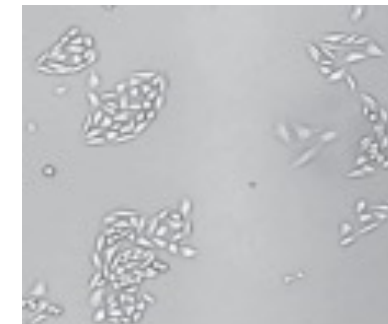
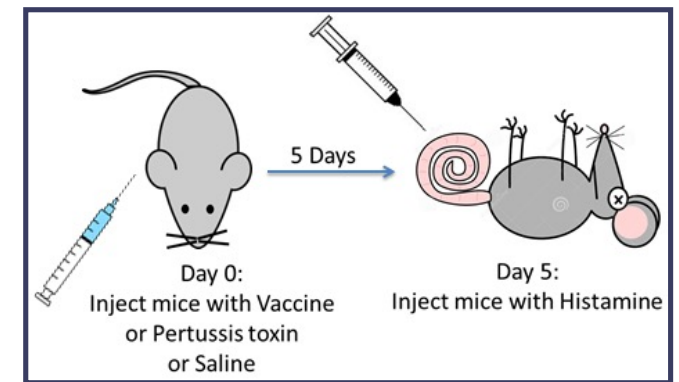
General safety test (FDA), innocuity test (WHO)

- Developed early 1900s:
 - Detection of toxic levels of phenol (preservative) in serum products (mice)
 - Detection of extraneous contaminants such as tetanus toxin and spores in sera (guinea pigs).
- 1940-ies: the two tests were combined to become a general safety test.
 - Unchanged since then, despite evolution of analytical techniques and manufacturing processes (GMP)
- Test principle:
 - inject batches of product into guinea pigs/mice. A batch passes the test if no animal shows any sign of illness or dies within a defined timeframe.
 - Routine testing → Considerable usage of animals: e.g. for vaccines, 5 mice and 2 guinea pigs per batch
- Retrospective analysis of data:
 - The test was neither specific, reproduceable, reliable nor suitable for the intended use (Duchow et al. 1994).
- Deleted as a routine test in 1998 in >80 Ph. Eur. monographs
- However, moved to the “Production section” = to be performed during vaccine development - in vaccine monographs.
- Workshop in 2016 with global stakeholders:
 - Conclusion: the ATT lacks scientific relevance, and its omission does not compromise the safety of biologics.
 - Consensus to strive for deletion of the ATT from regulatory requirements - globally
- **Decision to suppress the ATT in the all monographs – 2017 + deleted in “FDA regulation” and WHO guidance**

Acellular pertussis vaccine

Purified pertussis toxoid: Residual Pertussis toxin (PTx)

- Histamine sensitisation test (HIST):
 - Release test: residual pertussis toxin & irreversibility of toxoid
 - Large number of animals, high variability, several versions of HIST are required in different regions
- Replacement: CHO cell clustering assay
 - Clustering of non-confluent CHO cell cultures by active PTx
 - Sensitivity: ~ 0.006 IU PTx
 - Standardised method validated in a collaborative study
 - Isbrucker R, Daas A, Wagner L et al. Transferability study of CHO cell clustering assays for monitoring of pertussis toxin activity in acellular pertussis vaccines, Pharmeur Bio Sci Notes 2016:97-114
- **Ph. Eur. 2.6.33. RESIDUAL PERTUSSIS TOXIN**
 - Describes the CHO cell assay



No toxin



Clustering 0.05 IU toxin

Acellular pertussis vaccine

Other changes to PTx testing

- Deleted routine testing of irreversibility
 - Historic data verifies stability of PT toxoid
- During development:
 - Detoxification process should be demonstrated to avoid reversion.
- Deletion of residual PTx testing on final product.
 - A more sensitive and reliable method is performed on the purified PT toxoid

Diphtheria vaccines:

During vaccine development - deleted:

Specific toxicity. The production method is validated to demonstrate that the product, if tested, would comply with the following test: **inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea pigs**, each weighing 250-300 g that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals show signs of or dies from diphtheria toxaemia, the vaccine does not comply with the test. If more than one animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

Routine testing: Absence of toxin and irreversibility of toxoid – Cell based

Principle of the test:

Even very low levels of diphtheria toxin have a toxic effect on VERO-cells. pH indicator can be applied as read out.

The toxoid is incubated at $5 \pm 3^\circ\text{C}$ and 37°C for 6 weeks. The latter to monitor if the toxoid revert to toxin at elevated physiological temperature.

The bulk of purified toxoid passes the test if no toxicity that can be neutralised with anti-diphtheria toxin is found in either sample.



The cell-based routine test is more sensitive and reliable than the animal test

Tetanus vaccines:

During vaccine development: Specific activity in Guinea pigs - deleted

- **Specific toxicity.** The production method is validated to demonstrate that the product, if tested, would comply with the following test: **inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea pigs**, each weighing 250-300 g that have not previously been treated with any material that will interfere with the test. If within 21 days of the injection any of the animals shows signs of or dies from tetanus, the vaccine does not comply with the test. If more than one animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

Routine testing of purified toxoid: Irreversibility of toxoid – deleted

- **Absence of tetanus toxin and irreversibility of toxoid.** Using the same buffer solution as for the final vaccine, without adsorbent, prepare a solution of bulk purified toxoid at the same concentration as in the final vaccine. **Divide the dilution in to 2 equal parts. Keep one of them at $5 \pm 3^{\circ}\text{C}$ and the other at 37°C for 6 weeks.** Test both dilutions as described below. **Use 15 guinea pigs**, each weighing 250-350 g and that have not previously been treated with any material that will interfere with the test. Inject subcutaneously into each of the 5 guinea pigs 5 ml of the dilution incubated $5 \pm 3^{\circ}\text{C}$. Inject subcutaneously into each of the 5 guinea pigs 5 ml of the dilution incubated 37°C .
- Data showed that tetanus toxin lost its neurotoxic activity when incubated at 37°C !
- NO point in maintaining the incubation at 37°C = the irreversibility-element of the test.

Tetanus vaccines – cont.:

Bulk purified toxoid: Absence of tetanus toxin

- Absence of tetanus toxin – slightly rephrased.
- **Absence of tetanus toxin.** Inject subcutaneously 1 mL containing at least 500 L.f of bulk purified toxoid into each of **5 healthy guinea pigs** each weighing 250-350 g, that has not previously been treated with any material that will interfere with the test. If within 21 days of the injection any of the animals shows signs of or dies from tetanus, the toxoid does not comply with the test.
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- The test is still performed in animals.
- Alternative in vitro test (BINACLE) – currently evaluated in BSP collaborative studies.
- Whether the animal test can be replacement by the BINACLE test or any other in vitro test remains to be discussed.

Conclusion

Ph. Eur. Vaccine monographs – toxicity testing

- Applying scientific approaches adapted to the specific vaccine Ph. Eur. has improved testing requirements for toxicity in vaccine monographs without jeopardizing the safety.
 - ➔ Improved methods, more reliable testing strategies
 - ➔ Reduction, replacement, refinement = less suffering for animals

Thank you for your attention!

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