A case study of false-positive results & the ATT in the context of modern quality control

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Time has moved on...
Case Study – Benzyl Alcohol

• Used in a wide variety of cosmetic or medicine formulations as fragrance component, preservative, antioxidant, solvent, and viscosity-decreasing agent

• In medicinal products from several therapeutic areas
  ⇒ Extensive human & animal safety data available at marketing approval and from post-marketing experience

• European excipient guideline for human medicinal products
  – 90 mg/kg daily

• WHO acceptable daily intake
  – 5 mg/kg
Study Design

• 10 mg/ml BA similar conc. as in formulation in some marketed drugs

• Species and strains
  – CD1 & Kunming mice, Hartley guinea pig

• Route of administration
  – ip and iv as per ATT & sc for comparison
  – 0.5 mL injection to mice and 0.5mL guinea pigs ip and sc
  – 0.1 mL for iv in mice (recommended highest best practice volume)
    \[ \Rightarrow 250 \text{ mg/kg in mice (50 mg/kg iv)} \]
    200 mg/kg in guinea pigs

• Measurements as per ATT over 7 days
  – mortality
  – body weight
  – clinical observation
**Study Results**

- **sc & iv administration**
  - No findings in either mouse strain or in guinea pigs (sc)

- **ip administration**
  - Transient and minimal behavioural changes (abnormal gait of hind legs) observed within 1-2 minutes post-dosing
  - Seen in both mouse strains and in guinea pigs

Effects not strain or species dependent and only after ip administration
Are The Results Relevant?

*Same findings to be expected in any drug containing similar levels of benzyl alcohol*

- Positive outcome, i.e. failed in the ATT?
  - Not according to the ‘old’ US criteria
  - but possibly in other countries when applying the letter of the pharmacopeia

- **The real question:** Indication of contamination?
  - No, because caused by a formulation component

Presence of benzyl alcohol may confound ATT interpretation and lead to false-positive results

Questions validity of ATT as QC test
ATT in the Context of Modern Quality Control
Drug development and control of contaminants today

- **Extended product characterization** during process development and process validation
  - Investigation of degradation profiles, product compatibility with various materials/surfaces

- **Advanced process understanding**, in-process controls, validation of the manufacturing process and release testing complying with international standards

- (Determination of safety/toxicity profile in *in vitro* assays and animal models as well as in clinical trials before marketing authorization in accordance with international and national guidelines)
How do we control contaminants today?

**Examples for Parenteral Preparations**

<table>
<thead>
<tr>
<th>Type of contaminant</th>
<th>Measure to verify the absence of contaminants in a product batch (Examples)</th>
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</thead>
<tbody>
<tr>
<td>Microbiological</td>
<td>• Bioburden test (in process control)</td>
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<tr>
<td></td>
<td>• Sterility</td>
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<tr>
<td>Pyrogen</td>
<td>• Validation of depyrogenization (as part of the process validation)</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>• Bacterial endotoxins (Limulus Amebocyte Lysate, LAL) test</td>
</tr>
<tr>
<td>DNA, Host Cell Protein (HCP)</td>
<td>• Monitored on Drug Substance Level</td>
</tr>
<tr>
<td>Residual contaminants</td>
<td>• Extended product characterization</td>
</tr>
<tr>
<td></td>
<td>• Manufacturing process validation</td>
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<td></td>
<td>• Batchwise QC testing to confirm batch to batch consistency</td>
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</table>

Today, reliable and validated tests for different potential contaminants are available
Validation of Analytical Procedures

General Consideration

ICH Q2(R1) “Validation of Analytical Procedures”:

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

Applicability to ATT

• No adequate positive control (e.g., reference standard) is available as the test seeks for unknown contamination and non-specific toxicity.

• The ATT lacks explicit acceptance criteria & specific endpoints in relation to its objective of detecting contaminants (“signs of ill health”\textsuperscript{a}, “significant signs of toxicity”\textsuperscript{b,c}, and “abnormal reaction”\textsuperscript{d} are not specific for contaminants).

\textsuperscript{a} Ph. Eur. (Immunosera/Vaccines)  
\textsuperscript{b} WHO (Vaccines)  
\textsuperscript{c} Russian Pharmacopoeia (Vaccines/Sera)  
\textsuperscript{d} Chinese Pharmacopoeia (Chemicals, Traditional Medicines)
<table>
<thead>
<tr>
<th>Validation Characteristics</th>
<th>Fulfilled by ATT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specificity(^a)</strong></td>
<td>No</td>
</tr>
<tr>
<td>ICH Q2(R1): Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.</td>
<td></td>
</tr>
<tr>
<td><strong>Reproducibility</strong></td>
<td>No</td>
</tr>
<tr>
<td>ICH Q2(R1): Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).</td>
<td></td>
</tr>
<tr>
<td><strong>Detection Limit(^a)</strong></td>
<td>No</td>
</tr>
<tr>
<td>ICH Q2(R1): The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.</td>
<td></td>
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</tbody>
</table>

\(^a\) ICH Q2(R1): Characteristic normally evaluated for impurity test (limit)
Reproducibility & Scientific Value Questionable

Kraemer et al.\textsuperscript{a}

- Identical batches tested in different laboratories $\Rightarrow$ significantly different test results
- Positive results

$\Rightarrow$ never correlation with product quality or contamination, and

$\Rightarrow$ same batches passed the ATT in subsequent test repetitions

**What is the value of the ATT?**

- Lack of evidence that ATT can fulfill its objective and detect contaminants for batches produced with adequate quality control measures in place
- Historical analyses by e.g. PEI, FDA, EFPIA $\Rightarrow$ lack of evidence that ATT resulted in meaningful data over decades

\textsuperscript{a} Kraemer B, Nagel M, Duchow K, Schwanig M, Cussler K. 1996. [Is the abnormal toxicity test still relevant for the safety of vaccines, sera and immunoglobulins?]. ALTEX 13(1):7–16.
ATT in context of Animal Welfare

- Substantial number of animals used cannot be justified in view of its unproven and questionable suitability to detect contaminants and increase the product safety*

- The *European Convention on the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes* has reviewed the test for replacement, reduction and refinement (3Rs)
  
  ➔ ATT test has been consequently deleted from numerous EP monographs

- EU adopted a new directive on the protection of animals used for scientific purposes (2010/63/EU38)

- EDQM is pushing forward the implementation of 3R (replacement, reduction and refinement) alternatives

- FDA removed ATT in CFR in 2015

• Developed as analytical “assay” in the early 1900’s - nowadays adequate and reliable analytical tools available

• False positive test results may lead to delayed supply of important medicines to patients

• Modern pharmaceutical manufacturers
  – extensive product characterization during development,
  – thorough control of manufacturing process & appropriate quality control (QC) in place.

• General lack of value & scientific rationale for ATT as QC test

• Most regulators no longer require ATT for most product classes based on historical reviews and adequate quality measures in place

• Substantial number of animals used for a test with unproven and questionable suitability to detect contaminants and increase product safety
Conclusion & Recommendation

- To ensure patient safety, attention should be on proper product characterization, process understanding.

- Absence of contaminants can be ensured via:
  - quality control measures using state-of-the-art analytical techniques and
  - control of manufacturing process confirming batch-to-batch consistency

ATT should be eliminated from all Pharmacopoeias and regulatory requirements worldwide.

No alternative assays or replacement for ATT are needed as adequate quality control measures are already in place.
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Doing now what patients need next
Abnormal Toxicity Test

Additional Readings

EFPIA Position Paper

“Rationale for Removing Abnormal Toxicity Testing”
Abnormal Toxicity Test

Additional Readings

Scientific Review


A Russian translation of this review is published in
Divergence in national regulations may delay patient supply with drugs

- Global manufacturers deliver products and generally the same batch to different countries

ATT for a biotechnological product?

- Batch (e.g., biotechnological product) released by a company and already available to patients (e.g., in EU, Canada, USA) would have to be tested for abnormal toxicity in other countries to be released for the local market

- False positive results may delay batch release and consequently patient supply with live-saving drugs
Doing now what patients need next