Viral Safety of Vaccines: How to Streamline the Adventitious Agent Testing Package?

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Analytical R&D Europe and North America
Outline

● Viral safety: three complementary approaches
● Routine testing strategy for viral adventitious agents
● One approach for streamlining the adventitious agent testing package
● What are the barriers against the waiving of \textit{in vivo} tests?
● What are the limitations of the \textit{in vivo} tests?
● Why do we need new technologies for detection of adventitious agents?
● New molecular methods for detection of adventitious agents
● Comparison example: performance evaluation between qPCR, NGS and \textit{in vitro} cell culture
● Discussion
● Conclusions and perspectives
Viral Safety: three complementary approaches*

- Selecting and testing cell lines and other raw materials, including media components, for the absence of undesirable viruses which may be infectious and/or pathogenic for humans.

- Assessing the capacity of the production processes to clear infectious viruses.

- Testing the product at appropriate steps of production for absence of contaminating infectious viruses.

* From ICH Harmonised Tripartite Guideline Q5A (Sep 1999) Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin
Routine testing strategy for viral adventitious agents

- **Testing of raw materials from animal origin (Serum, Trypsin)**
  - 9 CFR tests on indicator cells with CPE, HAd, and IFA read-outs

- **Broad, overlapping viral testing package on cell banks**
  - Non-Specific (known/unknown agents):
    - *In vitro* tests using indicator cells (CPE & HAd read-outs)
    - *In vivo* tests including adult & suckling mice, (guinea pigs, rabbits) embryonated eggs
  - Retrovirus detection/quantitation by TEM
  - Reverse transcriptase detection (PERT)/ Retrovirus Infectivity
  - Specific (known agents):
    - PCRs, *In vivo* antibody detection tests...

- **Testing of seed lots (vaccines), crude harvests/unprocessed bulks**
  - *In vitro* and *in vivo* tests for seed lots
  - *In vitro* tests using indicator cells, PCRs...
  - Control cells, control eggs (for vaccines)
One approach for streamlining the adventitious agent testing package

- **Risk assessment taking into account:**
  - The raw materials used in the manufacturing process of the cell banks and seed lots
  - History of the cell line and viral strains
  - The manufacturing process of the cell banks and seed lots
  - The ability of the manufacturing steps to eliminate/inactivate the potential adventitious agents

- **Definition of a testing strategy for all the steps (MCB, WCB, EOPC/ECB and MSL, WSL, Unprocessed bulk):**
  - Introduction of HTS technology in the testing strategy of cell banks and seed lots:
    - Waiving of *in vivo* tests and of NAT tests
    - Assessment of the *in vitro* cell culture tests (number of indicator cell lines...), possibility to replace the *in vitro* tests?
What are the barriers against the waiving of in vivo tests?

- Lack of harmonization of testing requirements
  - Subtle differences exist between the different regulations

- Full ICH Validation not available for the compendial tests
  - Compendial tests considered as validated (as described in the Ph. Eur. General Notices)
  - However full ICH validation data not available for in vivo tests
  - Only one study published (Gombold et al. 2014)

- Comparative study between existing in vivo tests and new methods
  - Difficult to launch additional validation studies for comparing in vivo compendial tests with new methods: ethical concerns
  - New technologies such as HTS do not detect the same characteristic of the virus
    - Genome or fragment of genomes versus pre-clinical observations of the effects viruses have on experimental animals with the in vivo tests
  - Qualitative (breadth of detection/specificity) and Quantitative (limit of detection) elements to be compared: which criteria will be used to demonstrate the comparability?
What are the limitations of the *in vivo* tests?

- For viral seeds, prior neutralization of the vaccine virus is needed
  - Challenges with some viruses (*e.g.* Poxvirus vectors)
  - Specificity of the neutralizing reagents
  - Interference or toxicity due to the matrix (*e.g.* vaccines based on poxvirus vectors or herpesvirus vectors in Suckling Mice)

- Poor detection of some viruses (even the ones, the *in vivo* tests are supposed to detect)
  - See Gombold *et al.*, 2014

- Detection redundancy within the Adventitious Agent Testing Package exists, however the breadth of detection of existing tests still has some limitations as demonstrated by the Porcine Circorvirus contamination event
Why do we need new technologies for detection of adventitious agents?

- Gaps in the current testing packages:
  - Viral families and their potential to be detected by the indicated test methods (from Rebecca L. Sheets and Paul A. Duncan, in “Vaccine Analysis: Strategies, Principles, and Control”, Springer-Verlag Berlin Heidelberg 2015)

<table>
<thead>
<tr>
<th>Virus Family</th>
<th>Embryonated eggs</th>
<th>Adult and suckling mice</th>
<th>Guinea pigs and rabbits</th>
<th>Routine in vitro cell cultures</th>
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</thead>
<tbody>
<tr>
<td>Adeno-</td>
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<td>Parvo-</td>
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<tr>
<td>Toga-</td>
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</table>

- Green indicating a generally suitable combination
- Yellow suggesting either limited applicability or need for unique conditions
- Red generally not considered suitable for detection

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SANOFI PASTEUR
Why do we need new technologies for detection of adventitious agents? “Circovirus crisis”

<table>
<thead>
<tr>
<th>Month</th>
<th>Event</th>
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</thead>
<tbody>
<tr>
<td>March 2010</td>
<td>Victoria <em>et al.</em> (Journal of virology): results demonstrating the presence of PCV1 viral sequences in Rotarix vaccine using a new high throughput molecular biology method (MPS)</td>
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<tr>
<td>May 2010</td>
<td>VRBPAC meeting was organized</td>
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<tr>
<td>Aug 2010</td>
<td>FDA letter to licensed vaccine manufacturers</td>
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</tbody>
</table>

Please describe any plans you may have to implement additional adventitious agent testing methods as part of your manufacturing process as these methods become available including, but not limited to, screening for PCV and PCV DNA as well as any additional in-process testing for adventitious agents that you may have recently added, but not reported to the agency. In this regard, please consider any animal derived materials (e.g., culture medium, albumin, enzymes, lipids, etc) and the point at which they are used in your product manufacture, any adventitious agent related quality control testing performed by the material vendor or done in-house, and any applicable viral clearance or inactivation steps provided by your manufacturing process.

- **Immediate impact for Sanofi Pasteur**
  - PCV screening by PCR implemented on all our products potentially impacted with PCV (focus on viral vaccines using porcine trypsin in the process)
  - Commitment from Sanofi Pasteur to accelerate the exploration of new molecular technologies for broad detection of adventitious agents
New molecular methods for detection of adventitious agents

- Family / Degenerate PCRs
- Broad range PCR combined with MS
- Microarrays
- PCR combined with Microarrays
- Next Generation Sequencing (NGS) / Massively Parallel Sequencing (MPS) / High Throughput Sequencing (HTS)
Comparison example: Performance evaluation between qPCR, NGS and *in vitro* cell culture*

Evaluation of Next-Generation Sequencing performance relative to qPCR and \textit{in vitro} cell culture tests

- Designed a study to compare the performance of an \textit{in vitro} cell culture assay with qPCR and NGS using hCMV as a model virus
  - Can the extended cell culture period be shortened by using qPCR or NGS as end-point analysis?
  - Can qPCR or NGS also improve upon the sensitivity of an \textit{in vitro} cell culture assay?

Results:

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Culture</td>
<td></td>
<td>10 CCID50/mL</td>
</tr>
<tr>
<td>qPCR</td>
<td></td>
<td>TBD CCID50/mL</td>
</tr>
<tr>
<td>NGS</td>
<td></td>
<td>TBD CCID50/mL</td>
</tr>
</tbody>
</table>
Evaluation of Next-Generation Sequencing performance relative to qPCR and *in vitro* cell culture tests

**Validation of the *in vitro* Cell Culture Assay**

- Examined 1, 10, 100 and 1000 CCID$_{50}$/mL hCMV spiked independently into 6 replicates of the same viral crude harvest preparation stock
- Monitored all samples for 28 days (or until CPE was observed)

<table>
<thead>
<tr>
<th>Test article</th>
<th>1 CCID$_{50}$/mL</th>
<th>10 CCID$_{50}$/mL</th>
<th>100 CCID$_{50}$/mL</th>
<th>1000 CCID$_{50}$/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate #1</td>
<td>Neg. @ Day 28</td>
<td>Day 28</td>
<td>Day 14</td>
<td>Day 14</td>
</tr>
<tr>
<td>Replicate #2</td>
<td>Day 28</td>
<td>Day 20-22</td>
<td>Day 20-22</td>
<td>Day 14</td>
</tr>
<tr>
<td>Replicate #3</td>
<td>Day 14</td>
<td>Day 14</td>
<td>Day 14</td>
<td>Day 14</td>
</tr>
<tr>
<td>Replicate #4</td>
<td>Neg. @ Day 28</td>
<td>Day 14</td>
<td>Day 14</td>
<td>Day 6-8</td>
</tr>
<tr>
<td>Replicate #5</td>
<td>Day 20-22</td>
<td>Day 14</td>
<td>Day 14</td>
<td>Day 6-8</td>
</tr>
<tr>
<td>Replicate #6</td>
<td>Day 20-22</td>
<td>Day 20-22</td>
<td>Day 6-8</td>
<td>Day 6-8</td>
</tr>
</tbody>
</table>

**LOD is 10 CCID$_{50}$/mL of test article incubated for 28 days**
Comparison between NGS, qPCR and *in vitro* assay

- **qPCR**
  - For most samples, qPCR was able to detect hCMV early than *in-vitro* cell culture assay
  - Unable to detect any hCMV in Replicate #4 even by Day 28

- **NGS**
  - Very similar results as qPCR
  - Able to detect hCMV in all samples with 1 CCID50/mL including Rep#4

- In addition, NGS was able to detect hCMV at 10 CCID50/mL without cell culture amplification.

<table>
<thead>
<tr>
<th>1 CCID50 / mL</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate #1</td>
<td>Cell Culture</td>
<td>qPCR</td>
<td>NGS</td>
</tr>
<tr>
<td>Replicate #2</td>
<td>Cell Culture</td>
<td>qPCR</td>
<td>NGS</td>
</tr>
<tr>
<td>Replicate #3</td>
<td>Cell Culture</td>
<td>qPCR</td>
<td>NGS</td>
</tr>
</tbody>
</table>
| Replicate #4  | Cell Culture | qPCR | NGS | | Positive

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Conclusion

- **Next-generation sequencing allows for better sensitivity for the detection of hCMV**
  - hCMV in test article without incubation (10 CCID<sub>50</sub>/mL), or
  - hCMV after cell culture amplification (1 CCID<sub>50</sub>/mL)
    - This is an improvement in sensitivity but not necessarily a reduction in time (considering the time needed for sample preparation and analysis).
  - Sensitivity is comparable to a qPCR designed specifically to detect hCMV
Discussion

● These new technologies are scientifically relevant
  ● NGS/HTS is a non-specific method with a large breadth of detection
  ● Illustrated by the « PCV crisis »
  ● Preliminary successful comparison data (and other examples in the literature)

● Need to admit that the in vivo tests for adventitious agent testing are not the “Gold Standard” nowadays

● Head-to-head comparison with in vitro tests on cell culture feasible with a defined panel of representative viruses but difficult to envisage Head-to-Head comparison between in vivo tests and new in vitro method (e.g. NGS/HTS)
  ● Regulations not harmonized for in vivo tests
  ● Ethical considerations for compendial methods (considered as validated)
  ● Scientific relevance of this comparison?

● Need to define a regulatory pathway for waiving the in vivo tests worldwide:
  ● No real guideline exists to define a « transition package » for the introduction of these new technologies
Conclusion and Perspectives

- **Ongoing activities**
  - Collaborative studies and spiking studies for performance evaluation of High Throughput Sequencing using appropriate well-characterized viruses and cells
  - Validation of HTS system and implementation in a GMP environment

- **Expected benefits**
  - Introduction of these new methods: an opportunity for convergence of regulations
  - Streamlining of our adventitious agent testing package with the potential removal/supplementation/replacement of *in vivo* & *in vitro* adventitious agent tests, NAT tests

- **Potential applications at various stages of the manufacturing process**
  - Cell seeds, pre-master cell banks, pre-master seed lots,
  - Cell banks,
  - Viral seed lots, viral harvests,
  - Raw materials, media,
  - Investigation/confirmation/identification of a putative viral contaminant during production

Need for a worldwide regulatory pathway
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