Viral Safety Testing of Vaccines and other Biological Products: a Change of Paradigm

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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 in vivo tests?
- What are the limitations of the 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 in vitro cell culture
- Discussion
- Evolution of regulations: a change of paradigm
- Conclusions and perspectives
Viral Safety: three complementary approaches*

* From ICH Harmonised Tripartite Guideline Q5A (Sep 1999) Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

- The safety tripod

  Patient safety
  
  Select  Test  Remove

* 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 Specificity of the neutralizing reagents
  - Interference or toxicity due to the matrix

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

- Detection redundancy within the Adventitious Agent Testing Package, however the breadth of detection of existing tests still has some limitations as demonstrated by the PCV 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: Detection Methods for Viral Families

<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>
<td>Green</td>
<td>Green</td>
<td>Red</td>
<td>Red</td>
</tr>
<tr>
<td>Arena-</td>
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<td>Yellow</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>Arteri-</td>
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<td>Green</td>
<td>Red</td>
<td>Red</td>
</tr>
<tr>
<td>Astro-</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Green</td>
<td>Green</td>
</tr>
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<td>Bunya-</td>
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<td>Calici-</td>
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<td>Red</td>
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</tr>
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<td>Circo-</td>
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<td>Green</td>
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<td>Flavi-</td>
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<td>Red</td>
<td>Red</td>
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<td>Hepadna-</td>
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<td>Red</td>
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<td>Red</td>
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<td>Papilloma-</td>
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<td>Green</td>
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<td>Paramyxo-</td>
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<td>Parvo-</td>
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<td>Red</td>
<td>Red</td>
</tr>
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<td>Picorna-</td>
<td>Green</td>
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<td>Red</td>
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<td>Polyoma-</td>
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<td>Green</td>
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<td>Retro-</td>
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<td>Red</td>
<td>Red</td>
</tr>
<tr>
<td>Rhabdo-</td>
<td>Green</td>
<td>Green</td>
<td>Red</td>
<td>Red</td>
</tr>
<tr>
<td>Toga-</td>
<td>Green</td>
<td>Green</td>
<td>Red</td>
<td>Red</td>
</tr>
</tbody>
</table>

- **Green** indicating a generally suitable combination
- **Yellow** suggesting either limited applicability or need for unique conditions
- **Red** generally not considered suitable for detection
Why do we need new technologies for detection of adventitious agents?

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
</tr>
</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>
</tr>
<tr>
<td>May 2010</td>
<td>VRBPAC meeting was organized</td>
</tr>
<tr>
<td>Aug 2010</td>
<td>FDA letter to licensed vaccine manufacturers</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
</tbody>
</table>

- Immediate impact for Sanofi Pasteur
  - 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 \textit{in vitro} cell culture\textsuperscript{*}

Evaluation of Next-Generation Sequencing performance relative to qPCR and *in vitro* cell culture tests

- Designed a study to compare the performance of an *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 *in vitro* cell culture assay?

**Results:**

<table>
<thead>
<tr>
<th>Cell Culture</th>
<th>Day 0</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 CCID50/mL</td>
<td>TBD CCID50/mL</td>
</tr>
<tr>
<td>qPCR</td>
<td>TBD CCID50/mL</td>
<td>TBD CCID50/mL</td>
</tr>
<tr>
<td>NGS</td>
<td>TBD CCID50/mL</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>Replicate</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.
Conclusion

- Next-generation sequencing allows for better sensitivity for the detection of hCMV
  - hCMV in test article without incubation (10 CCID$_{50}$/mL), or
  - hCMV after cell culture amplification (1 CCID$_{50}$/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

<table>
<thead>
<tr>
<th>Email Mark List</th>
<th>Cell Culture</th>
<th>Day 0</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>qPCR</td>
<td></td>
<td>10 CCID$_{50}$/mL</td>
<td>=&gt; 1 CCID$_{50}$/mL</td>
</tr>
<tr>
<td>NGS</td>
<td>10 CCID$_{50}$/mL</td>
<td>1 CCID$_{50}$/mL</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

- These new technologies are scientifically relevant
  - NGS/HTS is a non-specific method with a large breadth of detection
  - Illustrated by the PCV contamination event
  - 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
WHO TRS 978 Annex 3 (Recommendations for Cell Substrates):

New and sensitive molecular methods with broad detection capabilities are being developed. These are not yet in routine use but, as they become widely available and validated, they will play an increasing role in the evaluation of cell substrates. The sensitivity of these methods, as well as their breadth of detection, should be considered when evaluating their applicability. One of the advantages of some of these new methods is that they have the potential to discover new viruses. These new approaches involve either degenerate PCR for whole virus families or random-priming methods, which do not depend on a known sequence. Analysis of the resulting amplicons has employed sequencing, hybridization to oligonucleotide arrays, and mass spectrometry (103–105). The new generation of massively parallel sequencing (MPS) methods may have particular utility. They can be applied to detect virions following nuclease treatment to remove cellular DNA and unencapsidated genomes. In this mode, MPS has been used to discover new viruses in serum and other tissues and has revealed the contamination of human vaccines by porcine circovirus (103, 106–110). MPS can also be employed to screen cell substrates for both latent and lytic viruses by sequencing the transcriptome. In this mode, enormous quantities of data are generated and robust bioinformatic methods are required to detect viral sequences by either positive selection against viral databases or negative selection to remove cellular sequences (103, 110, 111). Care is required to exclude false “hits” to viruses due

Similar wording in WHO TRS for Yellow Fever Vaccine, Dengue, IPV, OPV, Rotavirus…
Evolution of regulations: a change of paradigm (2/2)

- Ph. Eur. Chapter 5.2.3: Cell Substrates for the production of vaccines for human use
  - Published in Pharmeuropa 26.4 in September 2014

  *Infectious extraneous agents. Cell lines for vaccine production shall be free from infectious extraneous agents and a testing strategy must be developed based on a risk assessment. One such strategy is given in Table 5.2.3-1, but alternate strategies could focus on more extensive testing at the MCB or WCB level. In any case, any strategy must be justified and lead to the same level of safety as outlined in Table 5.2.3-1. Novel, sensitive molecular techniques with broad detection capabilities are available, including Massively Parallel Sequencing (MPS) methods, degenerate PCR for whole virus families or random-priming methods (associated or not with sequencing), hybridisation to oligonucleotide arrays and mass spectrometry. These methods may be used either as an alternative to in vivo or specific NAT tests or as a supplement/alternative to in vitro culture tests, in agreement with the competent authority. If positive results are obtained with either broad molecular methods or NAT tests, a follow-up investigation must be conducted, in agreement with the competent authority, to determine whether detected nucleic acids are due to the presence of infectious extraneous agents and/or are known to constitute a risk to human health. For cell lines of insect origin, tests for specific viruses relevant to the species of origin of*

- Ph. Eur. Chapter 2.6.16: Tests for extraneous agents in viral vaccines for human use
  - Published in Pharmeuropa 28.2 in April 2016, similar wording

- Ph. Eur. Chapter 5.2.14: Substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines
  - Published in Pharmeuropa 28.2 in April 2016
  - New concept with several concrete examples of substitution of *in vivo* methods
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-substitution 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
Acknowledgments

- Eric Abachin
- Ali Azizi
- Virginie Courtois
- Hugues Graf
- Carine Logvinoff
- Rebecca Malott
- Bryan McNeil
- Sarmitha Sathiamoorthy
- Martha Schreiber
- Patrice Riou
- Reza Yacoob
- Joan Bevilacqua
- Clotilde Thiriart
- Tony D’Amore
- Marie-José Quentin-Millet
Thank you