PathoQuest

RNA Next-Generation Sequencing transcriptomic analysis: an alternative validated method to replace animal *in vivo* tests for assessing the viral safety of cell based Biologics

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A Validated Assay within a Validation Strategy

"Method Validation": validating a component with pre-defined specifications

Applied to both laboratory and bioinformatics methods

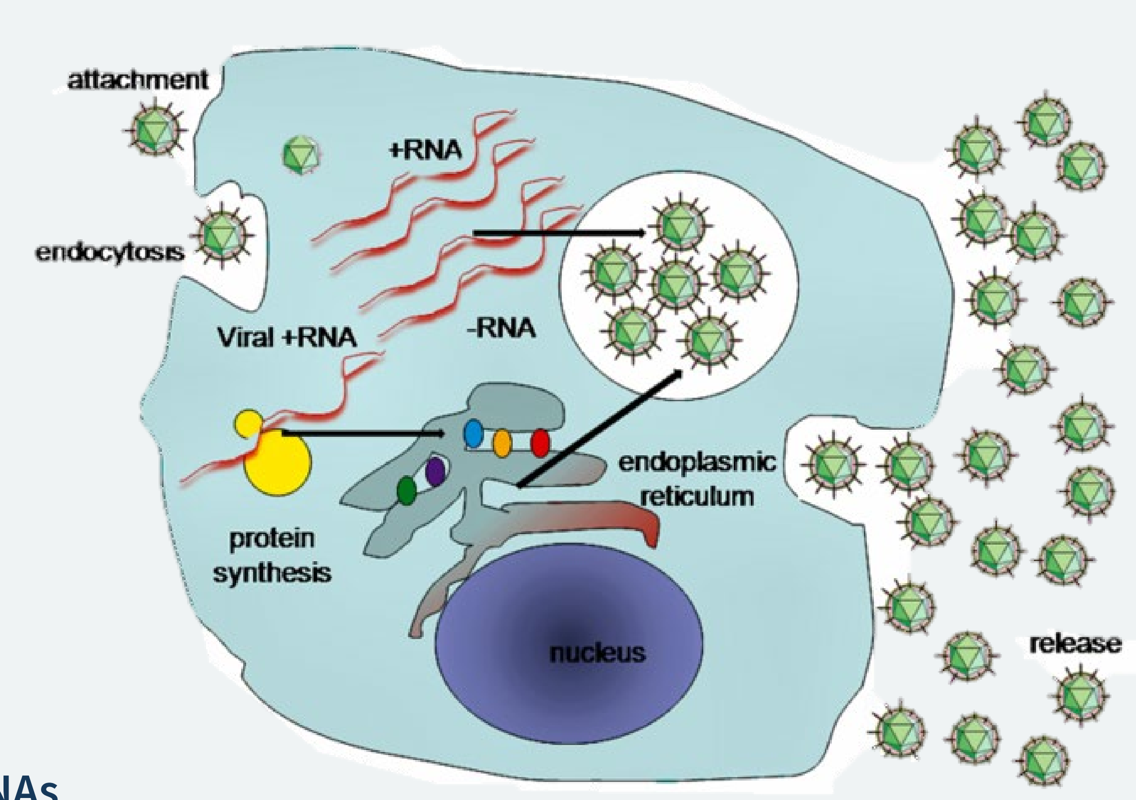
Extraction → Quantity & Quality Assessments → Library Construction → Quantity & Quality Assessments → Sequencing "Test" → Pipeline Analysis against DataBase (Yearly Update)

"System Validation" broadly encompasses a range of method validation (*e.g.* extraction through reporting)

Requirement: "must detect (replicative) adventitious viruses"

Why performing transcriptome analysis by NGS?

- Transcriptome = analysis of expressed RNAs
- Detects all types of viruses
  - RNA/DNA
  - Circular/linear genomes
  - Single & double-stranded
- Takes advantage of RNA phase of viral replication
  - Including DNA and most latent viruses
  - High levels of expression of viral RNAs : easy to detect
- Can differentiate replicating viruses from carryover:
  - Using strand info, RNA profiles and/or metabolic labelling of nascent RNAs
- Analysis can be agnostic or targeted
- Validated Methods



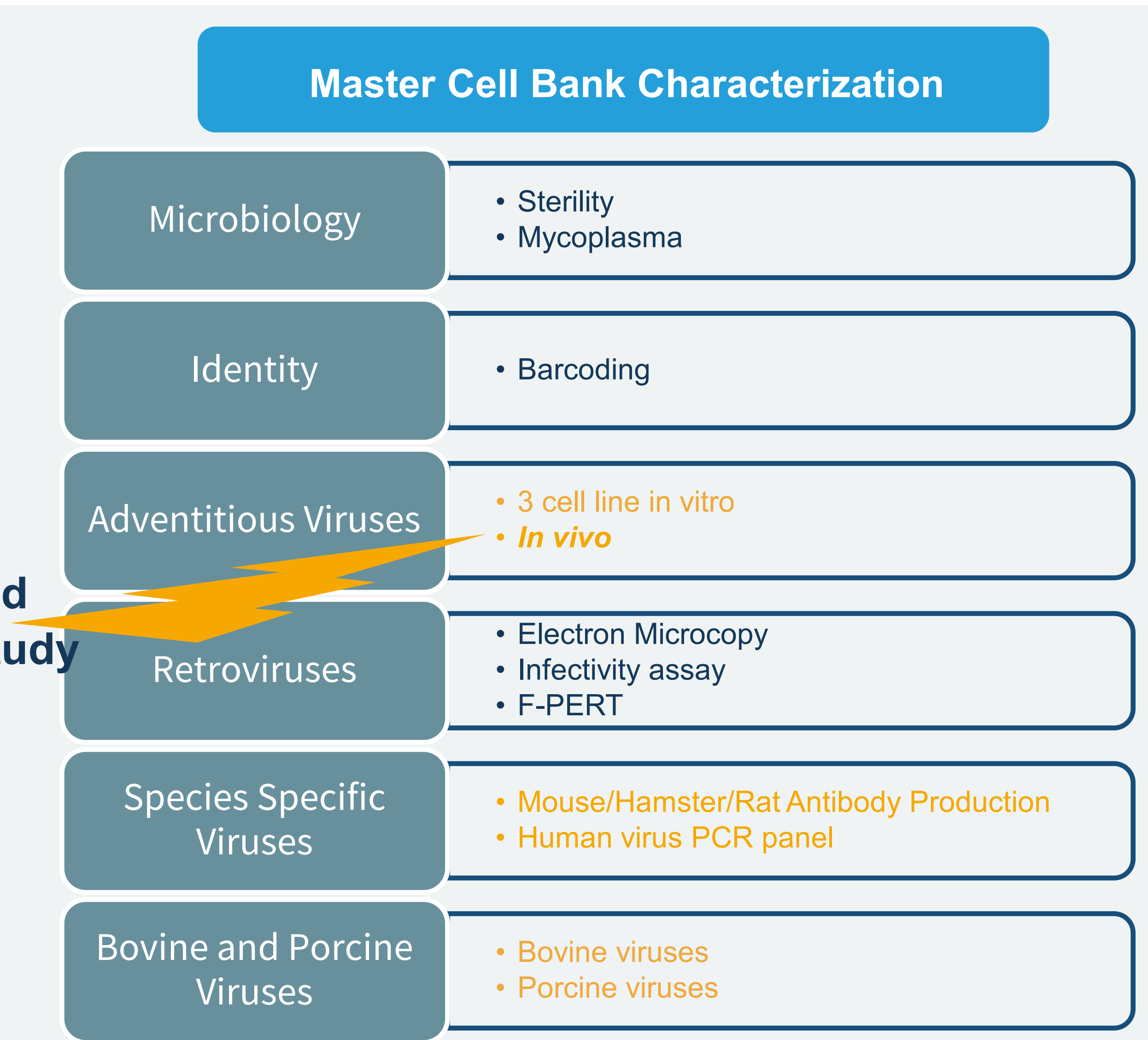
ICHQ5A(R2) Guideline

Redefining the role of NGS in viral safety testing

3.2.3: "NGS is encouraged as a replacement for *in vivo* assays"

3.2.5.2: "NGS can replace the *in vivo* tests with broad virus detection for unknown or unexpected virus species. NGS can also supplement or replace the *in vitro* cell culture assays for detection of known and unknown or unexpected virus species.”

3.2.5.2: “Furthermore, the assay may also be used for the detection of known viruses, and it can replace the HAP, MAP, and RAP tests and other virus-specific PCR assays."



Transcriptome

Transcriptomic Assay

	Phase I	Phase II	Phase III	Commercial
Cell banks for recombinant: • MCB • WCB • EoPCB				
ATMP Harvest, API: • Cell therapy • Ex-vivo gene therapy				
ATMP Drug Product: • Cell therapy • Ex-vivo gene therapy				
Starting Material				

GMP run +
Ref Standard included within GMP run

- 96 exogenous reference sequences (synthetic RNA molecules) of non-viral origin.
- Based on generic validation package

Model virus selection

(Gombold et al., 2014)

PathoQuest

- **Category A viruses:** Higher sensitivity with *in vivo compared to in vitro*
- **Category B viruses**: Lower sensitivity with *in vivo compared to in vitro*
- **Category C viruses:** Detected *in vitro only*

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Systematic evaluation of *in vitro* and *in vivo* adventitious virus assays for the detection of viral contamination of cell banks and biological products☆

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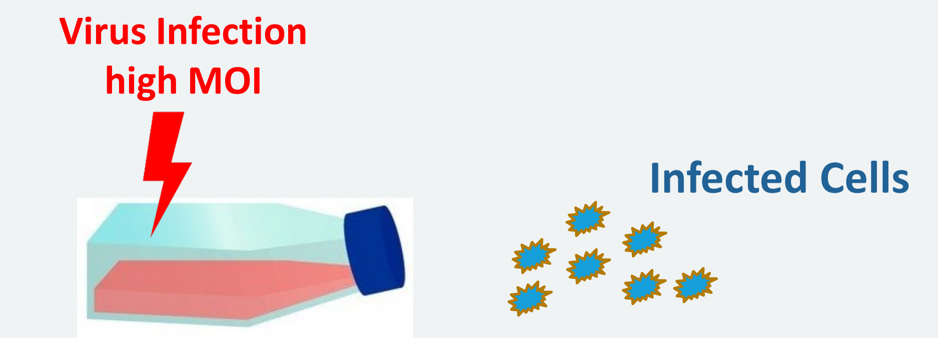
In Vivo | In Vitro

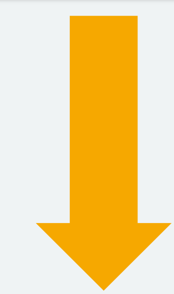
NT Ad5 * A549
NT Ad41
BVDV * BT
BPIV-3
Cox A
Cox B
Echo 11
Flu
HSV-1
Measles
Mumps
NT Rhino 2
Rubella * RK13
NT sCMV
NT SV40
VSV

10^{-3} 10^{-2} 10^{1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5} UN ND | ND UN 10^{5} 10^{4} 10^{3} 10^{2} 10^{1} 10^{0} 10^{-1} 10^{-2} 10^{-3}

Comparison of LOD for *In Vivo* and *In Vitro* Assays for Model Adventitious Viral Agents

Method comparison





- Virus specific cell substrates (e.g., VSV->Vero; Influenza->MDCK), infected at high MOI
- Harvested shortly after infection

Cell lysates were prepared for *in vivo* testing :

- following SOP/monographs/guidelines
- dilutions infected cell lysates in non-infected cell lysates
- adult mice, suckling mice, embryonated eggs

Intact cell pellets were prepared for NGS analysis:

- following PTQ SOP
- Dilutions of infected cells RNAs in non–infected cells RNA
- NGS RNAseq transcriptomic analysis

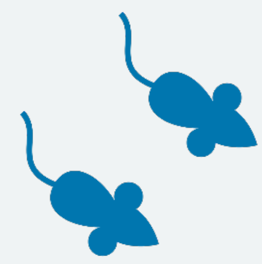
Note: Standardization: ratio of infected/ non-infected cells

Result Expression

RNA Seq : LOD expressed as the ratio of infected/non infected cells which provides NGS signal (reads) and equivalent $TCID_{50}$/mL

In vivo Study

- A dilution is considered positive if at least 20% of animals/eggs die
- LOD is the highest dilution that gives a positive result



- Category A
- Category B
- Category C

IN VIVO

NGS

CATEGORY A
NGS < *In Vivo*

VSV
Influenza A H1N1

CATEGORY B
NGS > or similar to *In Vivo*

HSV-1
Cox A16
Cox B3
Mumps

CATEGORY C
Undetectable in *In Vivo* assay

NT
NT
NT

Echo 11
Measles
BVDV1

10^{-13} 10^{-12} 10^{-11} 10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} UN ND

ND UN 10 10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7}

UN = Undiluted, ND = not detected, NT = not tested

Egg yolk
Egg allontoic
Suckling mice
Adult mice
NGS

Comparison of LOD for *In Vivo* and *NGS* Assays for Model Adventitious Viral Agents

Conclusion

- This is the **first comprehensive H:H comparison** of an RNAseq transcriptomic assay to *in vivo* tests applied to cells

- NGS-transcriptomic assay **detects 1 infected cell in a background of 10^3 to 10^7 virus-free cells**

  - For viruses detected at low sensitivity or not detected by *in vivo*, NGS shows a better analytical sensitivity and range of detection than *in vivo* and therefore ensures a better diagnostic sensitivity (=probability of detection)

  - For viruses detected at high sensitivity by *in vivo*, detection by NGS is highly efficient and ensures a high sensitivity of detection of cells infected by this type of highly productive viruses

- These results are obtained in challenging conditions using highly diluted infected cells, which underestimate the sensitivity of detection of tests applied to infected cells

- Replacement of *in vivo* tests by NGS would **increase the overall safety of the product**, while being **more rapid, less expensive** and **more ethical**.

Thank you

for your attention