PathoQuest

NEXT GENERATION SEQUENCING

THE NEW ALTERNATIVE TO IN VIVO TESTING

Method Comparision: Quality Control Testing of Biologics

In anticipation of upcoming ICHQ5A revision

In collaboration with Charles River Laboratories (CRL), we are now the first contract research organization (CRO) to conclusively show the superiority of next-generation sequencing (NGS) over conventional *in vivo* biosafety testing methods on cells. The ability of our RNA-sequencing based strategy to detect more broadly and deeply biological contaminants in biologics than *in vivo* testing is clear.

It is not surprising that both direct and indirect animal testing models that use suckling mice, adult mice, embryonated eggs, guinea pigs, *etc*. have rapidly fallen out of favor given the 3Rs model (reduction, refinement, and replacement) to ensure the ethical treatment of animals[2-4]. Combined with a legacy of no known *in vivo* testing positives[5] despite a history of known contamination events in biologics[6], an alternative testing strategy is warranted and long overdue. NGS fits that niche and should be your test of choice particularly in discussion with your agency partners.

KEY CRITERIA FOR VIRAL SAFETY TESTING TO EVALUATE NGS ADVANTAGES OVER ANIMAL TESTING

	Animal Testing	NGS
Detection range	✗	✓
Specificity for virus identification	✗	✓
Sensitivity	✗	✓
Turnaround time	✗	✓
3Rs compliance	✗	✓
Validation	✗	✓
Regulatory acceptance	✓	✓

BENEFITS OF SWITCHING TO NGS:

NGS combines the advantages of classical *in vivo* testing methods while overcoming many of their drawbacks and challenges, including specificity, sensitivity, and speed.

- NGS is **intrinsically agnostic**: no *a priori* information about the targets is required, it will directly detect both known and unknown biological agents of concern, helping you minimize the tests needed for critical decision making
- Unlike *in vivo* and *in vitro* testing, NGS is **not dependent on the susceptibility** of the test system to the agent of concern, streamlining the testing process
- NGS provides **clear nucleotide sequence level identification** of any biological contaminant(s) that may be present, giving you greater confidence and peace of mind
- NGS helps **discriminate between inert and active viruses** utilizing the nature of the sequence signatures and strand bias to provide greater context
- NGS has exceptionally **low sample requirements** preserving more of your sample for your own critical needs
- NGS is **fast**, providing critical data when you need it
- **Regulatory guidance already supports use of NGS** as an alternative to *in vivo* testing[2-4]

The many advantages of NGS over animal testing are clear and for the first time in industry history we have shown in a head-to-head comparison that:

- **The limit of detection** of an NGS assay for viruses infecting cell lysates is **similar or better than *in vivo* assays**
- NGS can detect minute amounts of infected cells among hundreds to millions of non-infected cells
- NGS can detect viruses in infected cells that are not detected by *in vivo* assays due to limitations in the susceptibility of the system to the target virus

STUDY DESIGN

The starting point for our comparative analysis was two-fold:

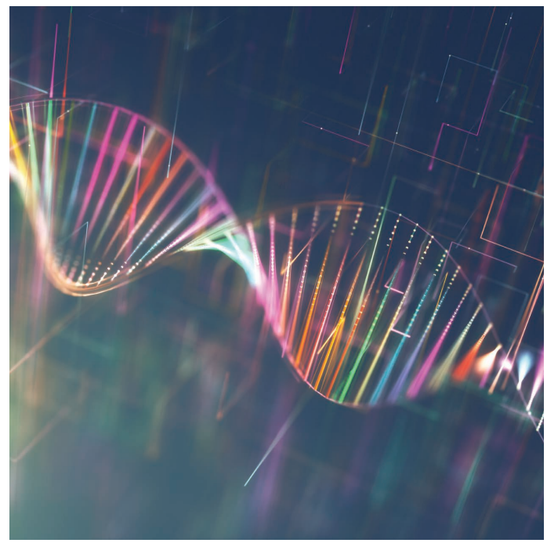
- **European Pharmacopoeia Chapter 5.2.14:** Substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines
- **Gombold *et al* (2014):** Systematic evaluation of *in vitro* and *in vivo* adventitious virus assays for the detection of viral contamination of cell banks and biological products

The viruses we selected for this comparative study align with those detailed in Gombold *et al*. and included the following three categories:
Category A: Vesicular Stomatitis Virus and Influenza A, both seen as the most challenging models for detection by NGS [↑ *in vivo* sensitivity, ↓ *in vitro* sensitivity]
Category B: Herpes Simplex Virus type 1, Coxsackie viruses A and B, and Mumps [↕(moderate to low) *in vivo* sensitivity, ↑ *in vitro* sensitivity]
Category C: Echovirus 11, Measles, and Bovine Viral Diarrhea Virus [undetectable by current *in vivo* assays illustrating a clear advantage for molecular methods]

We used an infected cell model (host cells infected with the respective virus) to better reflect the typical test matrix for the industry. This contrasts with the more common spiked-virus strategy in which viruses are added directly to a test matrix (*e.g.*, cell lysate). While the latter is quicker and simpler to generate in the laboratory, the impact and kinetics of an active infection can be lost leading to potential bias in detection.

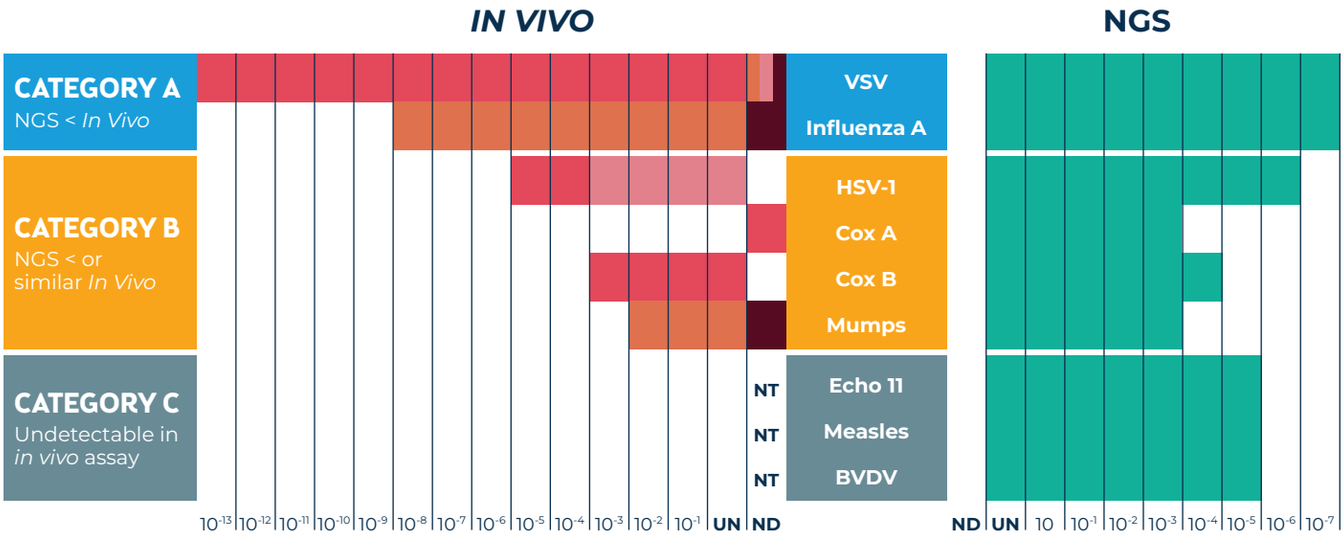
For the *in vivo* portion of our assessment, industry standard methodologies were used to inoculate the test systems (adult mice, suckling mice and eggs) with the infected cell lysates. The systems were subsequently monitored for signs of infection and/or death.

For the NGS portion of our test, RNA was extracted from the frozen [infected] cell pellets and used to prepare standard RNA-Seq (transcriptome) libraries for sequencing using an Illumina system.



COMPARISON OF LIMITS OF DETECTION FOR IN VIVO AND NGS ASSAYS FOR MODEL ADVENTITIOUS VIRAL AGENTS.

In vivo assays were performed using suckling mice, adult mice, and embryonated hens' eggs. For ethical purposes, we chose not to perform all *in vivo* tests. For each Gombold *et al.* virus category, only the most sensitive reported ones were used.[4]

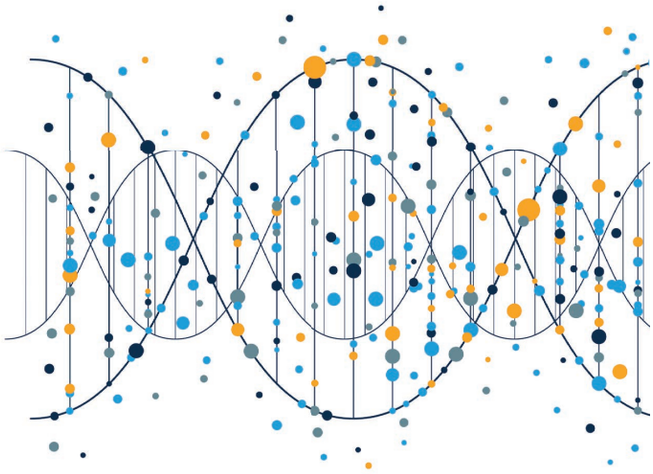


UN = Undiluted, ND = not detected, NT = not tested

In nearly all cases, NGS detected the target virus at the limit of detection, whereas detection via the *in vivo* assay was sporadic.

CONCLUSION

As demonstrated by our comparative analysis, our NGS-transcriptomic assay is well suited to replace traditional *in vivo* testing strategies and provide enhanced safety assurance via improved detection of virus contaminants. More importantly, this technology opens new possibilities for innovative biologics and breakthrough therapies to reach the patient faster.



1. UMI market test: Use of NGS to replace animal tests for the viral safety of biologics Dec.20/Feb.21
2. Legislation for the protection of animals used for scientific purposes. Directive 2010/63/ EU as amended by Regulation (EU) 2019/1010. European Commission Web site. Accessed May 12, 2021.
3. European Pharmacopoeia - general chapter 5.2.14 Substitution of *in vivo* methods by *in vitro* methods for the quality control of vaccines. European Pharmacopoeia. ninth ed. 2017. 9.3.
4. Gombold *et al.*, Vaccine. DOI :10.1016/j.vaccine.2014. 02.021. (Mar 2014).
5. PQ *In Vivo* Paper Reference
6. Victoria *et al.*, J Virol. DOI: 10.1128/JVI.02690-09 (Jun. 2010).

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