Application Note

Replacing the Mouse, Hamster and Rat Antibody Production Tests (MAP, HAP, RAP) with Next Generation Sequencing iDTECT® Transcriptome assay

Summary

ICH Q5A revision 2 (adopted November 2023) is the key regulatory guidance document for the viral safety evaluation of cell lines used in the manufacture of biologics. This guideline now specifically references next generation sequencing (NGS) as an advanced virus testing tool and an ethical replacement for virus testing in animal models.

In partnership with Charles River Laboratories, PathoQuest has generated data which underscores the superiority of their GMP validated iDTECT® Transcriptome assay over MAP/HAP/RAP in the characterization of rodent cell lines. This assay harnesses the power of NGS to screen cell lines for any replicating viral contaminants and allows the replacement of two mandatory animal-based assays: *in vivo* adventitious virus and MAP/HAP/RAP testing.

Our approach enables the faster, safer, animal free release of cell banks.

Applications: Rodent Cell Bank Characterization

- Monoclonal antibodies
- Recombinant proteins
- Subunit vaccines

iDTECT® Transcriptome Benefits

Faster	Accelerate manufacturing timelines by releasing cell banks at least 4 weeks earlier, without waiting on results of the animal based assays
Safer	Agnostic assay - broad range of virus detection of known viruses, unknown viruses and variants.
Animal free	Ethical, animal free alternative aligned with reduction, refinement, replacement (3Rs) goals
More robust	Low repeat rate, and can be repeated in as little as 3 weeks when required.
Efficient	One assay to replace *in vivo* adventitious virus testing and MAP/HAP/RAP.
Fit for purpose	• Accepted by regulators (see ANSM review here: https://pathoquest.com/ngs-ansm) • GMP validated • Head-to-head comparison with *in vivo* adventitious virus assay published [6] • Specific detection of HAP/MAP/RAP viruses demonstrated • Biologics Master File filed with the FDA/CBER • Includes controls for matrix-specific interference

Introduction

Species-specific testing remains an important safety consideration in the characterization of cells banks. Whilst viral contamination during the manufacture of biologics is rare [1], the most commonly reported contaminant of rodent cells is the rodent-specific pathogen MVM.

The classical mouse, hamster, and rat antibody production tests (MAP/HAP/RAP) are still used extensively in characterization packages for Master Cell Banks (MCB) of rodent cell lines. These methods emerged as a means of detecting specific rodent viruses in the early 1970s [2]. With 21 viruses recommended in ICH Q5A [3], the basic method has not changed for over 50 years.

Mice, hamsters or rats are inoculated with the test material and, after a period of 4 weeks, serum samples are collected and tested for virus-specific antibodies using enzyme-linked immunosorbent assay (ELISA) or immunofluorescence (IFA). Antibody production testing can be supplemented with an intracranial challenge to test for the presence of lymphocytic choriomeningitis virus (LCMV).

The Need for an Alternative to MAP/HAP/RAP

MAP/HAP/RAP testing has been one of the recommended assays in ICH Q5A for the detection of virus when characterizing rodent manufacturing cell lines such as CHO.

Methods for cell line characterization are performed under GMP and are required to be qualified or validated to demonstrate that they are suitable for the intended purpose. This is not the case for animal assays for ethical reasons, and only the serology endpoints of the MAP/HAP/RAP assays can be validated.

Animal-based testing presents a number of challenges, and is generally less robust than in vitro methods. This, combined with the risk of unspecific findings, can result in repeat testing being required. Given this assay requires 8 weeks, repeat testing can lead to an expensive 2 months delay in getting your product to market.

It is clear that both the industry and the regulators are pushing for a more ethical and robust testing package for cell line characterization. With the adoption of the new revision (R2) of ICH Q5A in November 2023, developers are asked to consider a suitable alternative that is fit for purpose.

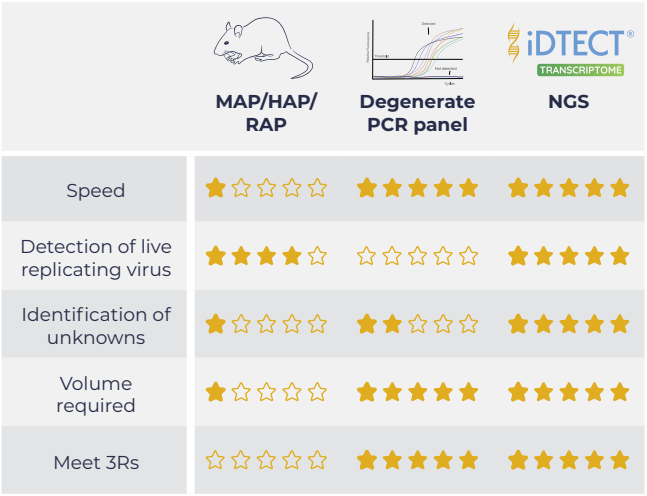

Figure 1 Comparison of iDTECT® Transcriptome, degenerate PCR panel and MAP/HAP/RAP testing

PCR as a MAP/HAP/RAP alternative

Sensitivity and robustness of these animal-based methods are of significant concern to the industry [4].

PCR has long been suggested as a potential alternative to *in vivo* MAP/HAP/RAP [5]. While the sensitivity, robustness and speed arguments for PCR are clear, developers have been reluctant to make the switch. One reason for this is that PCR only identifies very small loci of known and targeted viruses, i.e. (like MAP/HAP/RAP) PCR may miss variants of the virus species. Also, there is the broader consideration of discriminating between a live infectious virus which poses a contamination risk, and fragments of viral sequence which may be identified by PCR, which are not true infections and do not pose a risk to the manufacturing process.

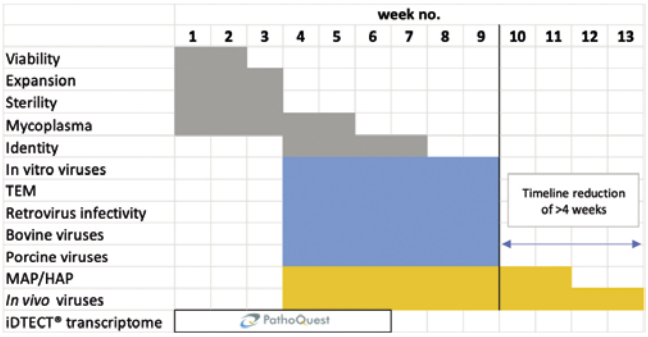

Figure 2 Reduced CHO MCB release timeline with iDTECT® Transcriptome

iDTECT® Transcriptome as a MAP/HAP/RAP alternative

In partnership with Charles River Laboratories, PathoQuest has previously demonstrated that its GMP validated iDTECT® Transcriptome assay is a suitable replacement for *in vivo* adventitious virus testing. Specifically, we have demonstrated that this method:

- Is a powerful agnostic method that can detect a full range of viruses.
- Can detect virus-infected cells with a sensitivity similar to that of (RT-)PCR [6].
- Can replace animal-based testing and significantly increase the probability of detecting viral contaminations in cell substrates like cell banks [7].

Now, Charles River Laboratories and PathoQuest have successfully performed a study which demonstrates that the iDTECT® Transcriptome assay detects all of the MAP/HAP/RAP viruses and, in addition, possible viral variants.

This was achieved by spiking synthetic transcripts for the 21 viruses listed in the ICHQ5A guideline into a CHO cell matrix then testing with the assay. Close and distant transcripts were used, with the distant transcripts selected to mimic distant viral strains within the International Committee on Taxonomy of Viruses (ICTV) species demarcation criteria.
All transcripts were successfully detected in the background of CHO cells.

We, therefore recommend that not only *in vivo* adventitious virus testing but also MAP/HAP/RAP assays should be replaced with the iDTECT® Transcriptome.

Data from this spiking study can be provided to clients for justifying the replacement of the two animal based assays by iDTECT® Transcriptome.

Figure 3 Benefits of replacing MAP/HAP/RAP testing with iDTECT® Transcriptome

Conclusion

In vivo testing was the best method available for the detection of viral threats in 1970.

With the launch of the new revision of ICH Q5A, now is the time to switch to the best method available today: GMP validated iDTECT® Transcriptome assay by PathoQuest.

iDTECT® TRANSCRIPTOME

References

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3. ICH Q5A (R2), https://database.ich.org/sites/default/files/ICH_Q5A%28R2%29_Guideline_2023_1101.pdf (accessed 23Nov23)
4. Gombold, J, et al. "Systematic evaluation of in vitro and *in vivo* adventitious virus assays for the detection of viral contamination of cell banks and biological products." Vaccine 32.24 (2014): 2916-2926.
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