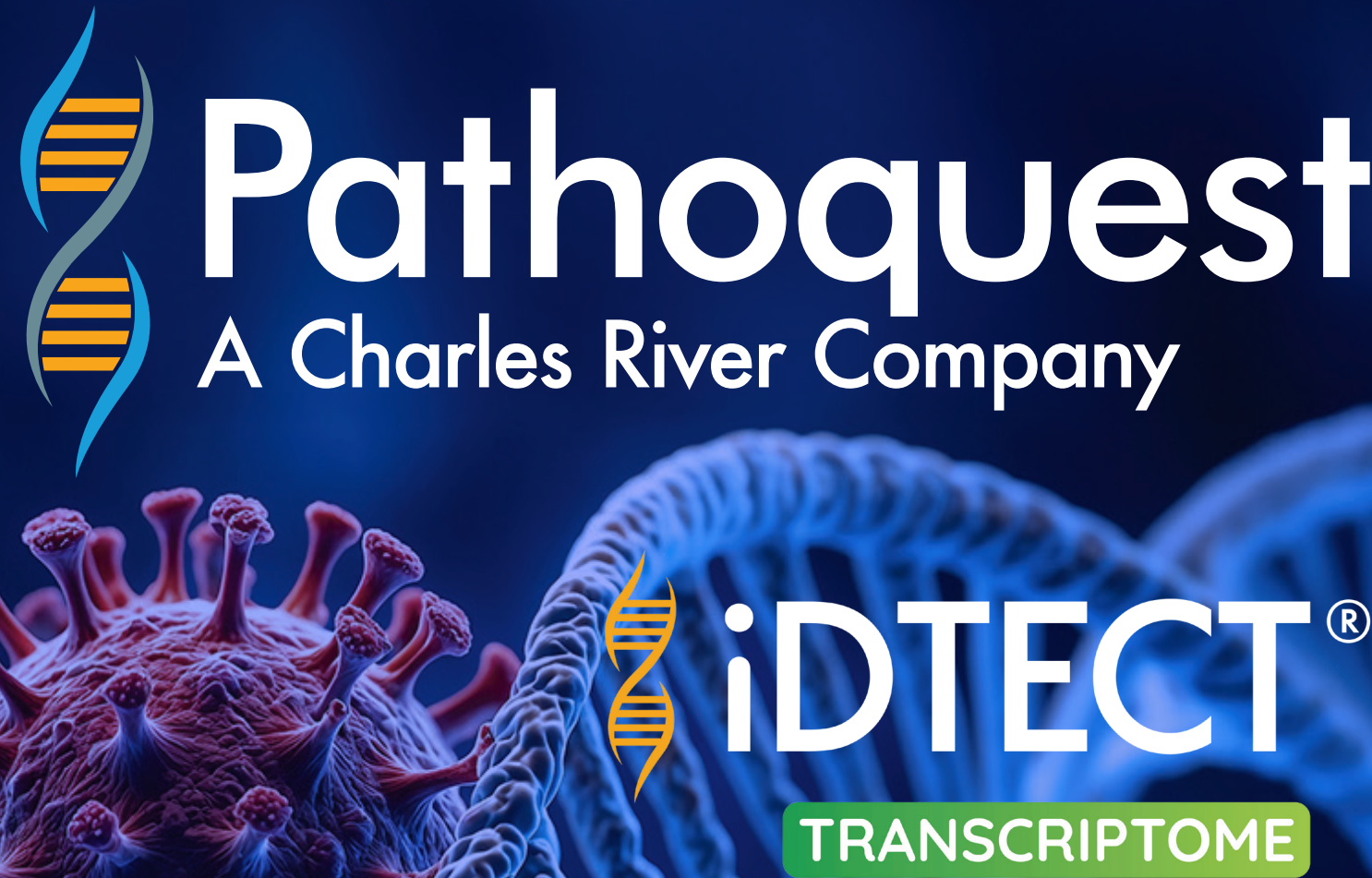


HAVE WE FINALLY REACHED THE END OF *IN VITRO* TESTING? POSITIONING NGS IN YOUR BIOSAFETY TESTING STRATEGY

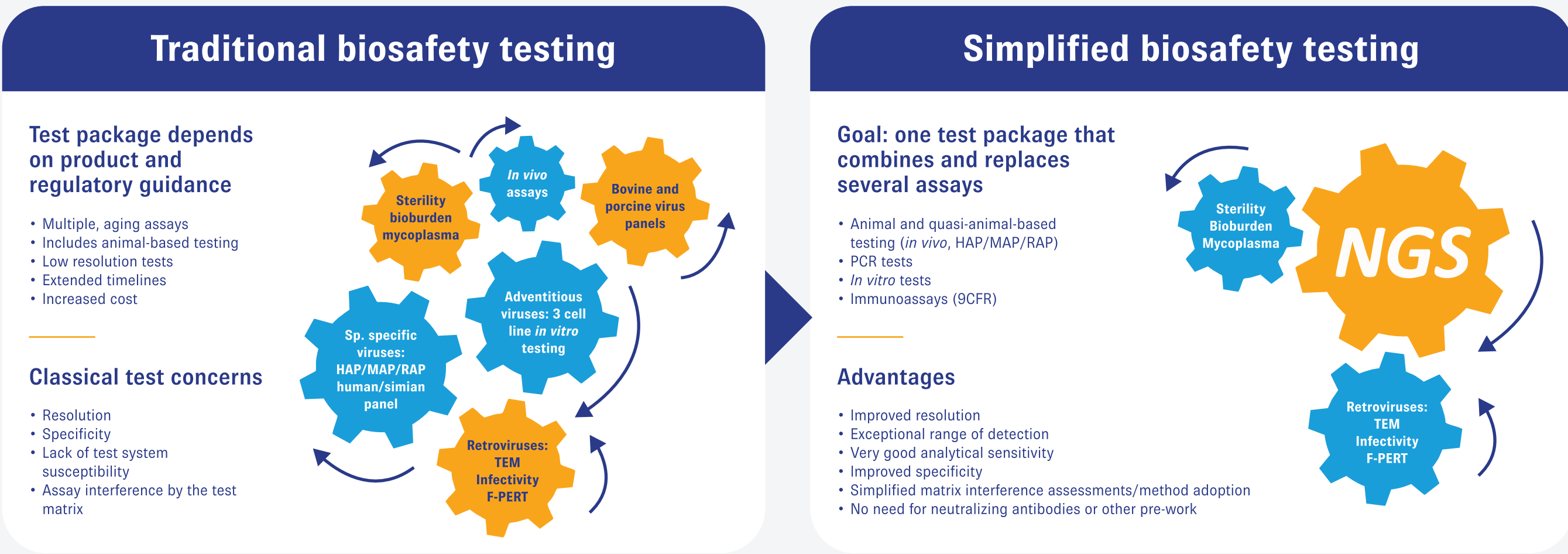


Delphine CHAUSSEC¹, Angéline DUCHÉ¹, Meissa BARBOUCHE¹, Deborah MANZA MIANZA¹, Celef ABOUDAGGA¹, Emeline SARRY¹, Guillaume DEPLAINE¹, Sandrine MOREIRA¹, Amina CHERIF LOUZANI¹, Marc ELOIT¹, Horst RUPPACH² and Colette CÔTÉ¹

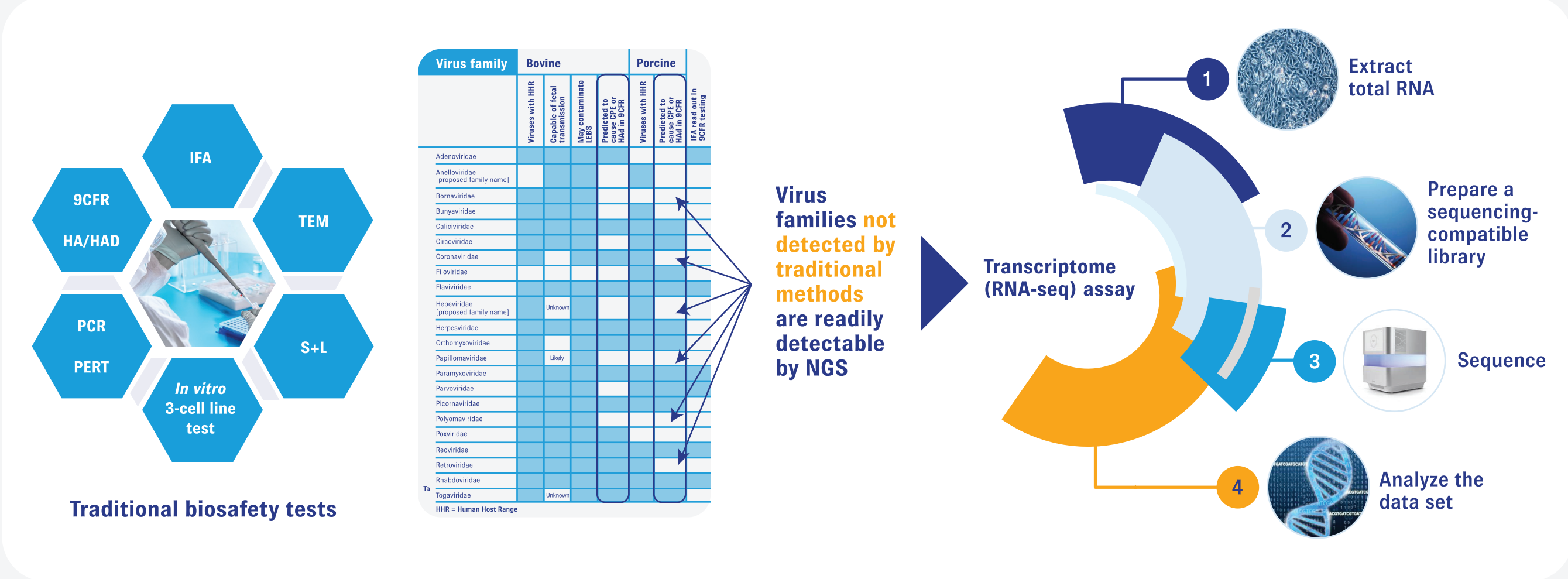
¹PathoQuest, Paris, France; ²Charles River Laboratories, Ekrath, Germany

Introduction

For decades, traditional biosafety testing packages for biologics have consisted of a complex collection of aging, low resolution assays with poor specificity and extended timelines. These assays have struggled to keep pace with the complexity and speed of development (and subsequently, the race to market) of today's biologics. Recent revisions to the guidance documents by global regulatory authorities have made great strides in encouraging the replacement of many of these legacy assays with next-generation sequencing (NGS)-based strategies. For example, ICH Q5A (R2) clearly endorses the use of NGS to replace both animal and quasi-animal-based tests in support of a faster, safer and more ethical testing package. This endorsement has two distinct advantages: 1) it overcomes many of the known shortfalls in animal-based testing, including limited susceptibility of the test system to viral agents and poor resolution/sensitivity of these assays, and 2) it supports the 3Rs principles of refinement, reduction and the replacement of animal model systems. Despite similar shortcomings in the specificity and susceptibility of *in vitro*, 9CFR and PCR-based biosafety tests, full acceptance by both the industry and, to some extent, regulatory authorities of NGS to comprehensively replace this latter collection of tests continues to lag.



NGS advances viral safety testing by providing a broad, unbiased method of detecting all viral contaminants in biologics. NGS is unmatched in terms of streamlined, accelerated testing that's not encumbered by target specificity requirements or test system susceptibility constraints as existing assays experience.



So why the hesitation to use NGS? A lack of publicly available use cases is the most common response. To overcome this perceived limitation, PathoQuest undertook a joint study with Charles River Laboratories to directly assess the use of NGS, specifically PathoQuest's iDTECT® Transcriptome Assay platform, as a direct replacement for the HAP/MAP/RAP, 9CFR bovine/porcine virus tests and human virus PCR panels.

Methodology

To assess the ability of NGS to detect the virus families targeted by traditional tests (detailed below), synthetic RNA transcripts (each 600 base pairs in length) corresponding to both close and distantly related viral sequences were spiked at decreasing concentrations into representative cell lysates shortly after cell disruption and prior to nucleic acid purification (illustrated below). Libraries were prepared from each test sample and sequenced using an Illumina platform. The data was then analyzed using PathoQuest's proprietary data analysis pipelines for adventitious agent detection.

HAP/MAP/RAP panels (rodent viruses)

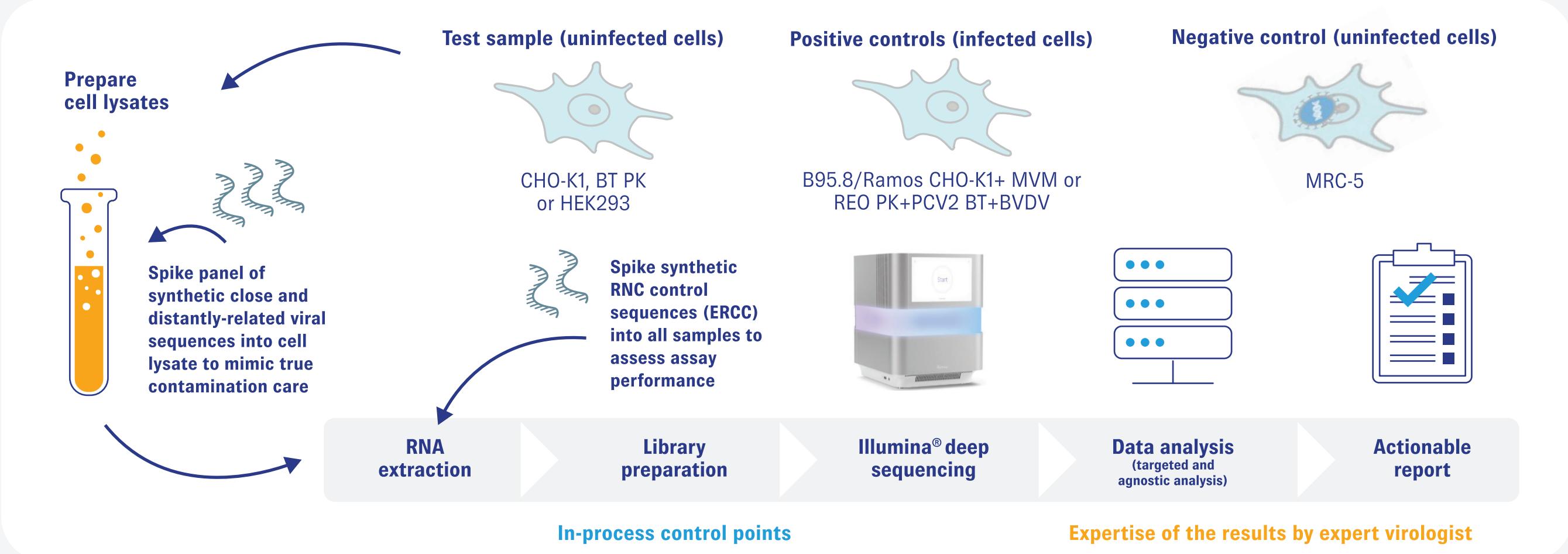
Virus	Family	Species
Adenoviridae	Mouse Adenovirus MAV	MAV/1
	Mouse Adenovirus MAV	MAV/2
	Lymphocytic choriomeningitis virus (LCMV)	
Arteriviridae	Lactate Dehydrogenase elevating virus (LDV)	
Coronaviridae	Mouse Hepatitis virus (MHV)	
	Sialodactylenitis virus (SDAV)	
Hantaviridae	Hantaanvirus (HANT)	
	Prospect Hill virus	
Herpesviridae	Mouse Cytomegalovirus (MCMV)	
Paramyxoviridae	Mouse thymic virus (MTV)	
	Simian virus 5 (SV5)	
Parvoviridae	Minute virus of mice (MVM)	
	Kilham's rat virus (KRV)	
Picornaviridae	Mouse Parvovirus (MPV)	
	Tobacco et H1 virus (H1)	
Picornaviridae	Theiler's murine encephalomyelitis virus (TMEV)	
Polyomaviridae	Mouse K virus	
	Estimotele (ECTRO)	
Reoviridae	Reovirus type 3	
Rotaviridae	Reovirus type 1	
	Mouse Rotavirus (A/EDM)	

9 CFR 113 panels (bovine/porcine viruses)

Virus	Family	Species
Flaviviridae	Bovine Viral Diarrhea virus	
	Reovirus	
	Rhabdovirus	
Adenoviridae	Bovine Adenovirus	
	Porcine Adenovirus A	
Sedoviridae	Bluetongue virus	
Paramyxoviridae	Bovine respiratory Syncytial virus	
	Parainfluenza virus type 3	
Herpesviridae	Infectious Bovine Rhinotracheitis (IBRHV)	
	Pseudorabies virus	
Polyomaviridae	Bovine Polyomavirus	
	Transmissible Gastroenteritis virus	
Picornaviridae	Porcine Hemagglutinating Encephalomyelitis virus	
	Porcine Reproductive and Respiratory Syndrome virus	
Circoviridae	Porcine Circovirus - 1	
	Porcine Circovirus - 2	
Parvoviridae	Porcine Parvovirus	
	Hepatitis E virus Genotype 3a	
Hepatitis E virus Genotype 4a		

Human/simian virus panels

Virus	Family	Species
Adenoviridae	Human Adenovirus (broad range A-G)	
	Human Immunodeficiency Virus type 1 (HIV-1)	
	Human Immunodeficiency Virus type 2 (HIV-2)	
Rotaviridae	Human Rotavirus	
	Human Foamy virus	
Papillomaviridae	Human Papillomavirus types 16 and 18 (HPV-16/18)	
	Epstein Barr virus (EBV/HRV4)	
Herpesviridae	Human Cytomegalovirus (HCMV/HHV5)	
	Human Herpesvirus 6 (HHV-6)	
Polyomaviridae	Human Polyomavirus JCV	
	Human Polyomavirus BK	
Parvoviridae	Human Parvovirus B19	
	Adeno Associated Virus	
Picornaviridae	Hepatitis A virus	
	Hepatitis B virus	
Flaviviridae	Hepatitis C virus	
	West Nile virus	
Hepatitis E virus		
	Severe Acute Respiratory Syndrome Virus 2 (SARS-CoV-2)	
Simian viruses	Simian Immunodeficiency Virus (SIV)	
	Simian T-lymphotropic virus (STLV)	
Reoviridae	Squirrel Monkey Rotavirus (SMRV)	
	Simian Foamy virus	
Polyomaviridae	Simian Virus 40 (SV-40)	



Results

Comprehensive screening for replication-competent viruses is crucial. Transcriptome-based NGS offers a broad, agnostic method that detects both known and novel viral variants that traditional assays may miss.

HAP/MAP/RAP tests

HAP/MAP/RAP assays have been the standard for rodent cell line characterization for over 50 years, but their sensitivity and validation have never been rigorously established, mainly due to ethical constraints.

In this study, PathoQuest's transcriptome-based NGS assay detected not only all 19 ICH Q5A (R2) rodent viruses, but also six (6) additional viruses, including both close and distant sequence variants. These results clearly demonstrate the wide range of detection by NGS of distant viral strains within the species listed by regulatory guidance, therefore ensuring an exceptional range of detection.

9CFR 113 tests

While the 9CFR test is designed to detect specific bovine and porcine viruses, it has a limited scope (screens for 11 bovine/porcine viruses) and therefore does not guarantee detection of virus variants or other porcine or bovine viruses. To mitigate this, it is often supplemented with PCR tests to broaden detection.

In this study, PathoQuest's transcriptome-based NGS assay detected not only the full 9CFR panel of bovine and porcine viruses, but also 13 additional bovine and porcine viruses beyond those required by regulatory guidance.

The 9 CFR 113 test (bovine/porcine viruses)		
Virus transcripts spiked into BT cell lysate		
Virus	Family	Species
Flaviviridae	Bovine Viral Diarrhea virus	
	Reovirus	
	Rhabdovirus	
Adenoviridae	Bovine Adenovirus	
	Porcine Adenovirus A	
	Porcine Adenovirus B	
Sedoviridae	Bluetongue virus	
	Porcine Respiratory Syncytial virus	
Paramyxoviridae	Bovine Parainfluenza virus type 3	
	Infectious Bovine Rhinotracheitis (IBRHV)	
Herpesviridae	Pseudorabies virus	
	Bovine Polyomavirus	
Picornaviridae	Transmissible Gastroenteritis virus	
	Porcine Hemagglutinating Encephalomyelitis virus	
Arteriviridae	Porcine encephalomyelitis virus	
	Porcine Reproductive and Respiratory Syndrome virus	
Circoviridae	Porcine Circovirus - 1	
	Porcine Circovirus - 2	
Parvoviridae	Porcine Parvovirus	
	Hepatitis E virus Genotype 3a	
Hepatitis E virus Genotype 4a		

Human/simian virus PCR panels

PCR in general is both rapid and robust, but it detects only known sequences and might not detect viral variants. Additionally, PCR assays may register non-infectious fragments as positives. In contrast, NGS targets viral RNAs, enabling detection of both known and novel viral species, thereby facilitating the differentiation of active infections from inert nucleic acids.

In this study, PathoQuest's transcriptome-based NGS assay detected all simian and human viruses, including 13 viruses requested by regulatory agencies and 15 additional human and simian viruses.

Conclusion

This study leveraged PathoQuest's iDTECT® Transcriptome assay – an agnostic, animal-free and regulatory-aligned NGS platform – to benchmark its performance against traditional viral safety assays, including panel-based HAP/MAP/RAP, PCR and 9CFR tests.

A minimal sensitivity of one (1) RNA copy per 10 cells, which can be translated into a sensitivity of roughly one (1) infected cell per 1,000 non-infected cells, was demonstrated for a total of 78 analyzed viruses covering a broad range of virus families and viral genome types. This sensitivity can be regarded as a 'worst-case' scenario as, in real contamination cases (based on published data for different virus families), the detection sensitivity will likely be higher, due to higher viral copy load, as well as the ability of NGS to detect broadly across the viral genome. Given the broad detection range demonstrated here, one final question to ask is 'what is the likelihood that a specific virus contaminant will only be detected by *in vitro* cell assays and not the iDTECT® Transcriptome Assay?' Conversely, what is the likelihood that a virus will not be detected by the *in vitro* cell assay but by this transcriptomic approach? In either case, NGS is the clear winner.

In all, these results illustrate that not only does NGS have comparable or superior detection sensitivity, but also has broader viral detection capabilities than traditional assays, enabling confident detection of known as well as novel or emerging viruses.

Contact us

PathoQuest's GMP-compliant NGS solutions are designed to meet and exceed regulatory expectations and accelerate adventitious agent detection as part of your biosafety testing strategy to help ensure safe manufacturing.

Contact our experts to hear more about how PathoQuest's GMP-compliant NGS testing portfolio can simplify and secure your virus safety testing strategy and accelerate your path to market... compliant, faster and animal-free.

contact@pathoquest.com | www.pathoquest.com