



Biologics Testing Solutions


charles river

GMP Validated Next Generation Sequencing Services

Viral Safety Testing & Genetic Characterization





Next Generation Sequencing (NGS)

Comprehensive and robust quality control testing, with the assurance of accuracy, efficiency, and shorter turnaround times, are critical for manufacturing safe biologics while also reducing time to market and improving patient outcomes.

Through our partnership with PathoQuest, we have expanded our comprehensive testing portfolio to include NGS testing services to biopharmaceutical and biotech companies, including viral contamination testing and genetic characterization of cell lines, bulk harvests, virus seeds, and raw materials used for production of biologics including cell and gene therapy products.

We provide comprehensive testing services for a wide range of modalities:

- mAbs and recombinant proteins: including mAb characterization, CHO cell line characterization, and mAb and recombinant testing
- Viral Vectors: viral safety testing and viral vector

characterization are available to ensure your therapeutics are free of contaminants, high in quality, and effective

- Cell Therapies: NGS services include cell therapy quality control, iPSC characterization, stem cell characterization, and CAR-T release testing
- Viral Vaccines: From established recombinant and inactivated vaccine technologies to the emerging RNA and viral vector vaccine platforms, we can provide testing, characterization, release, and sequencing
- RNA Based Therapeutics: With its ability to identify potentially problematic ultra-rare sequence variants, NGS is uniquely suited to enable characterization and identification of RNA products



The NGS Advantage

Testing with NGS provides a sensitivity that can discriminate between live virus and inactivated virus or viral fragments.

The method combines the benefits of PCR with the agnostic capabilities of traditional culture or animal models. With its high throughput, scalability, and speed, NGS has transformed the landscape of genetic analysis and pathogen detection.

Unlike most other NGS service providers, we offer long-term experiences across broad study topics under quality assured (GLP/GMP) conditions including fully validated assays.

Ethical Standards Supporting Animal Welfare

- 3Rs compliant
- In accordance with Directive 2010/63/EU

Extensive Expertise

- Proven experience in testing industry standard cell lines, virus seeds, and ATMPs
- Comprehensive reports that meet regulatory standards
- Highest breadth of virus detection

Accelerated, Streamlined Processes

- Fast-track reporting in 2-3 weeks
- Quicker release to manufacturing
- Faster delivery to patients

A Simplified Solution

- Replace multiple tests for an all-in-one solution
- Available head-to-head comparison data for *in vivo* assays



NGS Services

Adventitious viruses can contaminate biopharmaceutical production systems and go undetected with consequent risks to the final product. Next Generation Sequencing (NGS) testing is a technique that does not require targeted primers to look for viral contamination; instead, it is able to scan millions of RNA/DNA sequences concurrently.

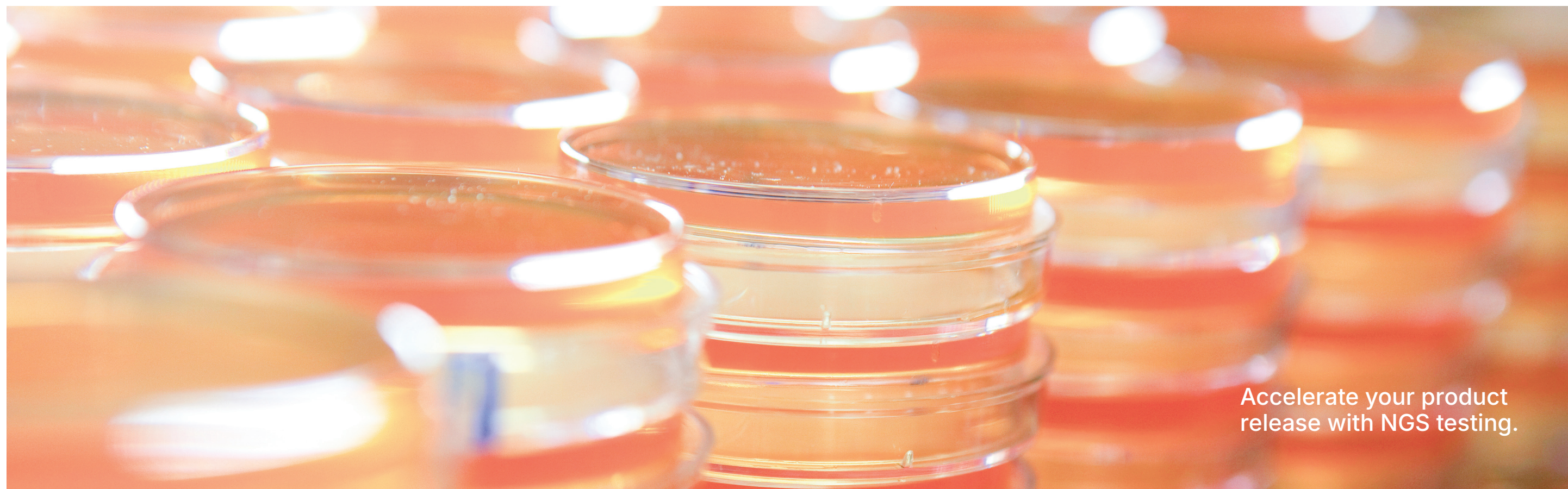
Bioinformatics are used to scan through the sequences to determine matches to known viruses and potentially to identify new, unknown viruses. Confirmation testing for NGS “hits” can then be done via targeted PCR approaches or other functional assays like cell based *in vitro* assays.

Our NGS-based adventitious virus testing approach uses a process called metagenomics to provide a more sensitive and comprehensive method to the identification of pathogens. This advanced approach permits the identification necessary to address the specificities of each sample type such as:

- Eukaryotic cell banks
- Bulk harvests
- Cell culture intermediates

- Unprocessed bulk harvest (recombinant proteins, e.g., mAb)
- Master or working viral seeds/vectors
- Drug substance or drug product

PathoQuest’s iDTECT® platform of NGS assays for Biologics quality control is faster, safer, animal free viral safety testing of raw materials, cell banks and bulk harvests.



Accelerate your product release with NGS testing.

A Powerful Replacement for Traditional Tests

NGS testing provides timely, sustainable results that completely remove animal testing from your product release requirements, helping you meet the 3Rs initiative (Replacement, Reduction, and Refinement).

The method has been used for agnostic detection of unknown pathogens in clinical settings and commercial biopharmaceutical manufacturing, especially in contamination investigations.

Considering both its detecting power and potential to reduce animal testing, NGS has been accepted as an alternative to *in vivo* adventitious virus testing strategies, depending on a risk assessment (ICH Q5A(R2) 3.2.3). It has the potential to replace many of the traditional assays, including standard *in vivo* tests, antibody productions tests (MAP/HAP/RAP), dedicated tests such as the 9CRF for bovine- and porcine-derived viruses, as well specific virus tests such as PCRs for MVM or calicivirus detection on CHO cells.

For human cell lines the agnostic NGS test could eliminate the need for testing a panel of human/ simian viruses by PCR, as the transcriptomic approach will detect these as well as any variants and potential unknown human viruses.

Other advantages NGS has over traditional methods include:

- Handling samples that may be difficult to test with traditional cell-based methods
- Using less sample for testing, making more available for patients
- Reducing false positives from PCR detection of inactivated virus
- Identifying both known and unknown contaminants, informing mitigation strategies
- Allowing manufacturing to start faster
- Discriminating between live versus inactivated viruses

A New Era for Characterization and Manufacturing

Next Generation Sequencing permits parallel sequencing of millions of DNA fragments and is now recognized as one of the most powerful and reliable tools for full genome sequencing.

The combination of Charles River and PathoQuest's expertise in microbiology and virology, with Illumina's and Oxford Nanopore's state-of-the-art NGS sequencing platforms, makes us a unique solution provider to genetically characterize cell lines and virus stocks.

While common in a research setting, the use of animal-derived materials has long been known to be of specific concern for GMP manufacturing. Implementing NGS in product release testing can streamline the detection process, providing faster and more accurate results.

This is especially critical in cell-based production, where the presence of active viral contaminants can compromise the entire manufacturing process. By integrating NGS into lot release procedures, manufacturers can ensure that only safe, uncontaminated materials are used, thus maintaining the high standards required in cell manufacturing.



Genetic Characterization

Regulations require manufacturers provide confirmation of the identity of their drug product. For viral vectors containing genetic material, identity testing should include a determination of the nucleic acid sequence.

Raw material plasmids should be tested with the same superior NGS technology as the final product to ensure consistent quality in manufacturing.

Next generation sequencing (NGS) is a vastly improved approach thanks to its much higher throughput, scalability, and speed when compared to traditional Sanger sequencing and it provides deeper insights into genetic variations within the product, specifically rare and ultra-rare variants.

Our newly validated Integration Site Analysis (ISA) method characterizes genetic modifications for clone selection, genetic

stability, and lot release testing. Modalities tested include cell banks for mAbs and recombinant biologics, viral vectors, cell therapies, vaccines, cell therapy lots, gene therapies, and cultured meat cell banks.

Benefits of NGS long-read ISA include:

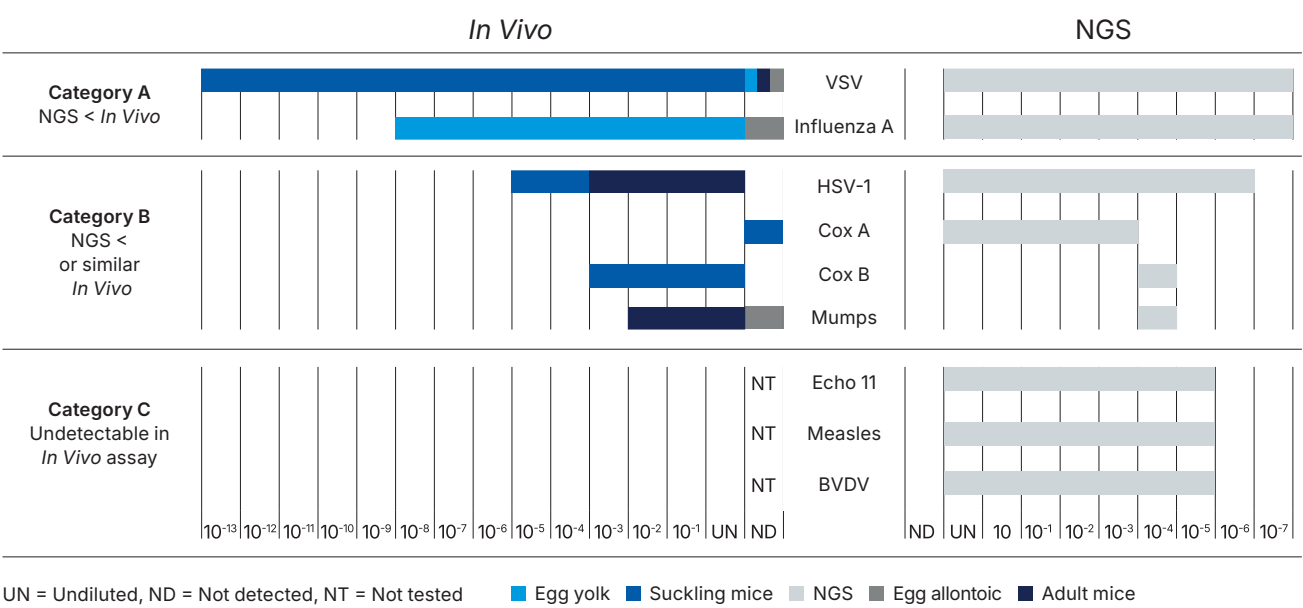
- Validated method
- Single nucleotide precision
- Resolve concatemers, truncations, and inverted repeats more easily than short-read ISA

For faster, simpler, more certain genetic characterization of cell banks, plasmids and virus lots we use PathoQuest iDTECT® Identity and iDTECT® Integration Site Analysis.



Ensure consistent quality and regulatory compliance.

NGS Solutions



Source: Evaluation of a viral transcriptome Next Generation Sequencing assay as an alternative to animal assays for viral safety testing of cell substrates, Vaccine, Volume 41, Issue 37, 23 August 2023, Pages 5383-5391

Different approaches to your testing are available depending on the source material and the desired outputs.

Our Next Generation Sequencing (NGS) services provided in partnership with PathoQuest include faster, safer, animal-free viral safety testing of raw materials, cell banks, and bulk harvests with iDETECT® Transcriptome and iDETECT® Virome. For genetic characterization we offer a simpler, more certain genetic characterization of cell banks, plasmids, and virus lots with iDETECT® Identity and iDETECT® Integration Site Analysis.

Our GMP NGS services include assays and bioinformatic approaches designed to accelerate adventitious agent detection and bioprocess characterization enabling safe, high-quality production of biopharmaceuticals.

As demonstrated by a comparative analysis, PathoQuest's iDETECT® Transcriptome is well suited to replace traditional *in vivo* testing strategies and provide enhanced safety assurance.

The NGS transcriptome assay detected 1 infected cell in the background of 103 to 107 virus-free cells.

For *in vivo* testing of highly sensitive (Category A) viruses, NGS has a high detection efficiency, ensuring high sensitivity.

For low sensitivity (Category B) or undetectable (Category C) viruses detected by *in vivo* methods, NGS exhibits better sensitivity and detection breadth than *in vivo* methods, thus ensuring better sensitivity.

***Note:** The differentiation of Category A, B, and C viruses is based on previously published data

GMP-compliant solutions are available to help ensure acceptance by regulatory agencies.



A MAP/HAP/RAP Alternative

Regulations require manufacturers provide confirmation of the identity of their drug product. For viral vectors containing genetic material, identity testing should include a determination of the nucleic acid sequence.

Charles River Laboratories and PathoQuest have successfully performed a study demonstrating that the iDTECT® Transcriptome assay detects all the MAP/HAP/RAP viruses as well as possible viral variants.

Spiking study data can be provided for justifying the replacement of the animal-based assays by iDTECT® Transcriptome, 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 tests.

The iDTECT® Transcriptome NGS assay detects both close and distant strains at 0.1 copy per cell for all viruses tested, achieving an analytical sensitivity comparable to PCR technology.

	MAP/HAP/RAP	Degenerate PCR panel	NGS
Speed	● ○ ○ ○ ○	● ● ● ● ●	● ● ● ● ●
Detection of live replicating virus	● ● ● ● ○	○ ○ ○ ○ ○	● ● ● ● ●
Identification of unknowns	● ○ ○ ○ ○	● ● ○ ○ ○	● ● ● ● ●
Volume required	● ○ ○ ○ ○	● ● ● ● ●	● ● ● ● ●
Meet 3Rs	○ ○ ○ ○ ○	● ● ● ● ●	● ● ● ● ●



Regulatory Support

The greatest perceived challenge to replacing an existing assay with NGS is regulatory acceptance.

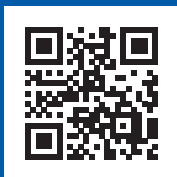
ICH Q5A(R2), the International Council for Harmonisation's guidance on viral safety testing of biological products, recognizes the broader detection capabilities and advantages of NGS over traditional methods like PCR-based nucleic acid amplification tests (NATs) and has begun to advocate that targeted or non-targeted NGS be used to replace the large number of PCR assays for virus-specific detection.

We can help you overcome any roadblocks with regulators starting at the R&D stage and going all the way to the batch release of marketed products with full GLP and GMP-integrated

services. From sample preparation to actionable reports, including review by an expert virologist or geneticist, we meet the highest requirements in quality control testing.

We can provide GMP compliant solutions including adventitious virus testing and specific virus testing, identity testing for viral vectors and plasmids, integration site analysis ensuring high acceptance by regulatory agencies.





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