Genetic characterization

Detection of adventitious agents

Custom solutions

Detection of Adventitious Agents

Clinical molecular diagnostics of viral pathogens using Next Generation Sequencing

Viral pathogens remain a major public health threat, causing significant morbidity and mortality globally. Accurate and sensitive detection of viruses in clinical samples is crucial for timely diagnosis, effective treatment, and containment of infectious diseases. Over the past decade, Next-Generation Sequencing (NGS) has emerged as a powerful tool for high-throughput viral metagenomic analyses, revolutionizing the field of clinical virology. NGS enables simultaneous detection and quantification of multiple viruses in a single sample, providing a comprehensive and sensitive analysis of viral infections. This technology can also provide valuable information on viral load, diversity, and evolution, which is useful for monitoring infectious disease progression and the effectiveness of antiviral treatments.

The use of NGS offers several advantages over traditional molecular methods:

1. High Sensitivity
NGS allows for the detection of ultra-low levels of viral particles, which may be missed by traditional.

2. Multiplexing
NGS can simultaneously detect multiple viruses in a single reaction, reducing the time and resources required. Importantly, multiplexing also allows for testing large number of viruses with only a small aliquot of a sample.

3. Broad Viral Diversity Detection
NGS can detect a wide range of viruses, both known and novel strains, which allows the identification of new or emerging viruses in a timely manner.

4. Superior Specificity
Utilizing NGS methods minimizes the risk of false-positive results.

5. Superior Accuracy
NGS provides high resolution information on the genetic diversity and evolution of viruses, which allows Superior accuracy while identifying and classifying the viruses.

6. Rapid Turnaround
NGS can generate large amounts of sequencing data in a relatively short period of time, providing faster results in comparison to traditional methods that can take several days or even weeks per test.

7. Cost-Effective
NGS is becoming increasingly cost-effective, making it more accessible for large-scale or routine viral detection in various settings, such as public health surveillance, clinical diagnosis, and food and water testing.

In clinical settings, NGS-based solutions for the detection of viruses can be either agnostic or targeted. With agnostic approaches, all genetic material present in a sample is processed, and no prior knowledge of viral sequence is required. This approach allows for unbiased detection of any viruses that are present in a sample, including discovery of unknown or novel viruses. Alternatively, targeted NGS approaches use primers or probes to specifically enrich for viral nucleic acids. This method is typically more cost-effective, has increased sensitivity and requires less computational resources compared to agnostic approaches. Ultimately, the choice between agnostic and targeted NGS approaches will depend on the specific aim of the testing.

Here, we present validation of our hybridization-based target enrichment solution for viral detection in clinical samples, capable of screening for > 3,000 different viruses, including > 15,000 viral strains, in a single reaction. This workflow can detect viruses of all nucleic acid genome types: single-stranded RNA, double-stranded RNA, single-stranded DNA, and double-stranded DNA.

Method

Our viral pathogen detection workflow is based on the Twist Comprehensive Viral Research Panel, which uses capture enrichment to detect a wide range of pathogenic viruses with incredible sensitivity. In short, cDNA synthesis is performed on the total nucleic acid (TNA), after which library preparation and hybridization-capture enrich the sample for viral nucleic acid. By including unique molecular identifiers (UMIs) to the workflow, we are able to remove any sequencing artifacts and PCR amplification bias that might occur during library prep. The library is then sequenced, and the data analyzed using a proprietary data analysis pipeline that identifies any viruses that are present. This pipeline compares the sequencing reads to a database, evaluates the similarity of the sequencing data to the virus genome (% identity) and the completeness of the viral genome (% genome covered).

To determine the sensitivity and specificity of our viral pathogen detection workflow we performed a thorough experimental validation using 11 different spike-in controls including ssRNA, dsRNA and dsDNA viruses. We tested sensitivity by varying the absolute number of viral genome copies and increasing the amount of background nucleic acid (NA), as shown in Table 1. To assess the specificity, we used mixed viral spike-in samples as shown in Table 2.

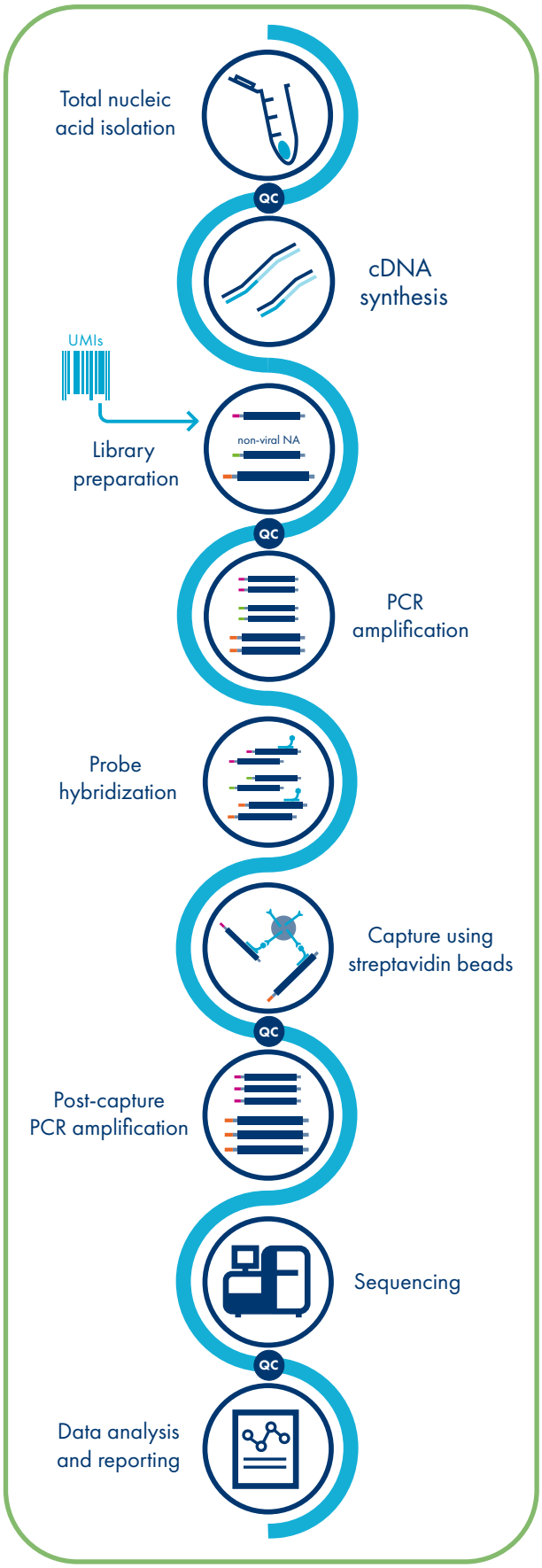


Figure 1: Schematic overview of the viral pathogen detection workflow.

Results

Sensitivity & specificity

Here, we demonstrate that our comprehensive viral panel reliably detects as little as 250 viral genome copies. Moreover, despite lowering the proportion of viral NA to < 0.001% of total NA in the reaction, the viruses were correctly identified, without any false-positive findings. Notably, when multiple viruses are present in a single sample, the identity of the spike-ins remains above 88%, illustrating the exceptionally high sensitivity and specificity of the workflow.

Virus	Genome type	Copies/ reaction	Viral NA	Identity (%)	Genome covered (%)
Measles virus	ssRNA	20,000	10%	99.63%	95.63%
		250	10%	98.47%	86.04%
		250	1%	99.60%	94.76%
Enterovirus	ssRNA	75,000	10%	99.75%	100%
		1000	10%	99.75%	100%
		1000	1%	98.24%	100%
Bocavirus	dsDNA	75,000	10%	98.50%	95.01%
		1000	10%	98.67%	91.78%
		1000	1%	98.67%	92.82%
Influenza A virus	ssRNA	2500	< 0.001%	96.604%	98.25%

Table 1: Sensitivity of GenomeScan's viral pathogen detection workflow

Sample	Virus	Genome type	Copies/ reaction	Viral NA	Identity (%)	Genome covered (%)
Influenza A virus	Influenza A virus	ssRNA	250	10%	95.96%	82.65%
SARS-CoV-2	SARS-CoV-2	ssRNA	250	10%	99.85%	97.51%
Flurona mix	Influenza A virus	ssRNA	25,000	10%	96.50%	98.72%
	SARS-CoV-2	ssRNA	250	0.2%	99.81%	97.55%
Flurona mix	Influenza A virus	ssRNA	250	0.2%	96.53%	98.56%
	SARS-CoV-2	ssRNA	25,000	10%	99.87%	97.64%
Virome mix	Mastadeno virus F	dsDNA		16.67%	99.88%	99.82%
	Herpesvirus 5	dsDNA		16.67%	99.86%	97.09%
	Respiratory Syncytial virus	ssRNA		16.67%	96.44%	67.71%
	Influenza B virus	ssRNA		16.67%	98.96%	100%
	Orthoreovirus 3	dsDNA		16.67%	98.27%	98.02%
	Zika virus	ssRNA		16.67%	88.31%	88.40%

Table 2: Specificity of GenomeScan's viral pathogen detection workflow

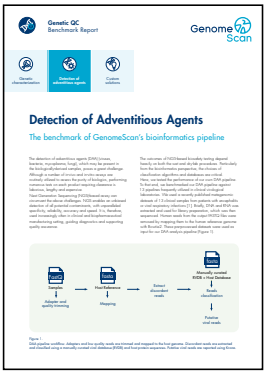
The results presented herein were generated in collaboration with the department of Medical Microbiology, Leiden University Center for Infectious Diseases, Leiden University Medical Center1.

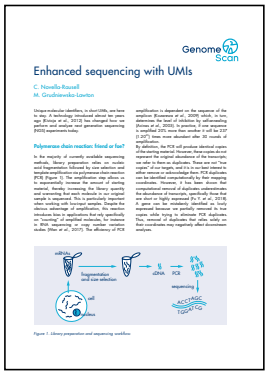
1 Mourik, K. et al: DOI:10.1101/2023.08.23.23294459

Contact us!

Are you looking for a reliable and cutting-edge solution for the detection of viruses in clinical samples? Do not settle for outdated or unreliable methods. Choose NGS and stay ahead in the fight against viral pathogens. Let us help you take your clinical virology to the next level with our state-of-the-art NGS technology!

Explore related resources





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