Journal of Clinical Virology 86 (2017) 20–26

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Application of next generation sequencing for the detection of human viral pathogens in clinical specimens

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ARTICLE INFO

Article history:
Received 9 August 2016
Received in revised form 2 November 2016
Accepted 21 November 2016

Keywords:
Next-generation sequencing (NGS)
Infectious disease diagnostics
Human pathogen
Public health laboratory
Virus detection

ABSTRACT

Background: Next generation sequencing (NGS) is a new technology that can be used for broad detection of infectious pathogens and is rapidly becoming an essential platform in clinical laboratories. It is not known how NGS will displace or enhance gold standard methodologies in infectious disease diagnosis.
Objectives: To investigate the feasibility and application of NGS technology in public health laboratories and compare NGS technology with conventional methods.
Study design: Illumina MiSeq system was used to detect viral pathogens alongside other conventional virology methods using typical clinical specimen matrices. Sixteen clinical specimens and two CDC proficiency panels containing seventeen specimens were analyzed.
Results: Known pathogenic viral nucleic acid was positively identified in all clinical specimens, correlating and building upon results obtained by more conventional laboratory methods. Sequencing depths ranged from 0.008X to 319 and genome coverage ranged from 0.6% to 99.9%. To substantiate the described methods used to analyze data derived from clinical specimens, the results of a clinical proficiency panel are also presented.
Discussion: Our results reveal true scarcity of known pathogenic viral nucleic acids in clinical specimens. NGS outperforms more conventional detection methods in this study by turnaround time as well as the improved depth of knowledge in regards to serotyping and drug resistance.

1. Background

Methodologies to detect pathogenic viruses in clinical specimens have transitioned from classic cell culture and antibody-antigen techniques to more sensitive molecular methods such as polymerase chain reaction (PCR). The targeted nature of these methodologies inhibit their ability to accommodate the true diversity of human pathogens in a clinical specimen, especially viruses [1]. Next generation sequencing (NGS) technologies are quickly demonstrating their ability to provide broad detection of infectious agents in a target-independent manner [2–7]. NGS has many advantages beyond the improved detection of all suspected, unsuspected, or even novel pathogens in a clinical specimen [8]. Familiarization with pathogen genomic sequences within clinical specimens enhances our understanding of infectious disease through further discovery of pathogen variability and genotyping [9–13], drug resistance or response to therapy [14–16], vaccine development and efficacy monitoring [17], and further characterization of the metagenome [18,19]. The use of NGS for routine use in clinical diagnostics is emerging with its own set of limitations and challenges [13,20]. Focusing on viruses of public health importance, we compared the performance of NGS alongside other more common viral detection methodologies.

2. Objectives

- To investigate the feasibility and application of NGS technology in public health laboratories and compare NGS technology with conventional methods
- To examine genome coverage and read depth of viral nucleic acid in various types of clinical specimens.
- To address the need for acceptability standards when using NGS due to the true scarcity of pathogenic viral nucleic acids in some clinical specimens.

Abbreviations: NGS, next generation sequencing; CPE, cytopathic effect; TAT, turnaround time.

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http://dx.doi.org/10.1016/j.jcv.2016.11.010
1386-6532/

Comparison of NGS and traditional virology assays in detection of adenovirus infection

Specimen Type	Origination	Culture	Staining	Serum Neutralization Assay	NGS
Nasopharyngeal Swab	2yo M	Hep#2[a] CPE at Day 2 (3+)[b]	DFA[c], Adenovirus	Adenovirus (type 2) TAT: 14 days	Human adenovirus C strain human/ARG/A15932/2002/2 TAT: 4 days
Nasal Swab	29yo F	Hep#2[a] CPE at Day 8 (4+)[b]	DFA[c], Adenovirus	Adenovirus (type 3) TAT: 18 days	Human adenovirus B strain human/USA/UFL_Adv3a51/2007/3 TAT: 4 days

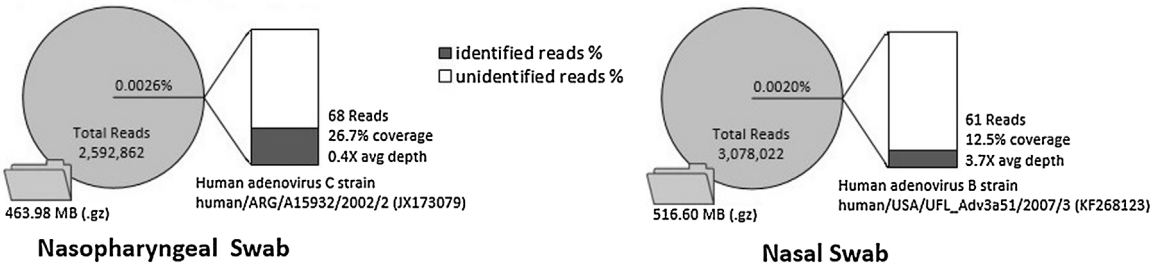

Fig. 1. Comparison of NGS and traditional virology assays in detection of adenovirus infection. Pie charts further describe sequence data as well as storage needed as compressed files (.gz). Percentage refers to the proportion of the total reads represented by the isolate reads. Exploding bar graph shows the percentage of the full genome that was identified by the reported number of reads.

3. Study design

3.1. Specimens

Sixteen previously tested clinical specimens, swab and serum specimens, were provided by the Alaska State Virology Laboratory in Fairbanks, Alaska (Supplement Table 1). Tests performed in addition to NGS on the serum specimens included an enzyme-linked immunosorbent assay (ELISA) to determine the presence of HCV antibodies (Roche) [21]. Two proficiency panels with a combined seventeen specimens for detecting antiviral resistance markers in the neuraminidase gene of influenza A virus were also tested as a quality indicator of our process. Proficiency specimens consisted of cultured Madin-Darby Canine Kidney Epithelial (MDCK) cells infected with influenza A virus.

3.2. Construction of sequencing library

Protocols regarding nucleic acids (DNA and/or RNA) extraction and purification were previously described [22,23]. Briefly, nucleic acid was isolated from 500 μL of the original clinical specimen using phenol/chloroform followed by ethanol precipitation. DNA or RNA molecules were selected for by using DNase I (serum and proficiency specimens) or RNAse (swab specimens, with the exception of the influenza specimens). Quantity was evaluated using the Qubit instrument (Thermo Fisher Scientific) and the Agilent Bioanalyzer. The Nextera DNA Specimen Preparation protocol (Illumina) and the NEBNext Ultra RNA Library Prep Kit protocol (New England Biolab) were followed to prepare sequencing libraries.

3.3. Sequencing and data analysis

Libraries underwent paired-end sequencing on the Illumina MiSeq using a v.2 500-cycle kit. Read files were imported into Illumina Virome app for reference genome identification. Alignments to the identified viral genome sequence(s) were performed by Sequencher (v5.1) in addition to an external tool, Genomic Short-read Nucleotide Alignment Program (GSNAP) [24]. Read depth and genome coverage was established using Tablet (v.1.13.12.17, [25]).

4. Results

4.1. NGS for detecting clinical adenovirus infections

Adenoviruses are important to characterize in the laboratory since some serotypes are more commonly associated with outbreaks, severe pneumonia, and possibly cancer such as serotypes 14, 55, and 12 [26]. Routine detection of adenoviruses in the clinical laboratory is largely done by either PCR or viral culture. Serotyping often requires that detected adenoviruses are propagated in large numbers in a live cell culture system to be further challenged with neutralizing antibodies produced against specific serotypes. These types of serum neutralization assays are not always definitive since they depend on the viability of the virus in the clinical specimen as well as the availability of continually depleting serotype-specific antibodies from vendors. In our experiment, two infections were able to be diagnosed and further characterized rapidly and definitively using NGS (Fig. 1).

4.2. NGS for detecting clinical herpesvirus infection

The results of three specimens are compared, two are clinical specimens and one is a cultured clinical isolate (Fig. 2). As expected, the overall proportion of viral reads is much higher when sequencing clinical isolates (>15%) when compared to raw clinical materials (<1%).

Results were concurrent amongst all methods; however, the result obtained by conventional methods for the nasopharyngeal swab were not definitively concurrent. Sequence analysis identified human herpesvirus 5 strain HAN2 (JX512200), same as conventional methods, but by only 10 reads. Torque teno virus isolate US32 (AF122921) was more definitively identified. Surveillance for torque teno viruses is not common since they are thought to be ubiquitous in humans and lack concrete disease association [27]. Nevertheless, NGS alone could not definitively identify actively replicating human herpesvirus 5, like viral culture could over 14 days, due to such low representation of viral total nucleic acid overall in the original clinical specimen (0.0005%).