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High resolution identity testing of inactivated poliovirus vaccines

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ABSTRACT

Background: Definitive identification of poliovirus strains in vaccines is essential for quality control, particularly where multiple wild-type and Sabin strains are produced in the same facility. Sequence-based identification provides the ultimate in identity testing and would offer several advantages over serological methods.
Methods: We employed random RT-PCR and high throughput sequencing to recover full-length genome sequences from monovalent and trivalent poliovirus vaccine products at various stages of the manufacturing process.
Results: All expected strains were detected in previously characterised products and the method permitted identification of strains comprising as little as 0.1% of sequence reads. Highly similar Mahoney and Sabin 1 strains were readily discriminated on the basis of specific variant positions. Analysis of a product known to contain incorrect strains demonstrated that the method correctly identified the contaminants.
Conclusion: Random RT-PCR and shotgun sequencing provided high resolution identification of vaccine components. In addition to the recovery of full-length genome sequences, the method could also be easily adapted to the characterisation of minor variant frequencies and distinction of closely related products on the basis of distinguishing consensus and low frequency polymorphisms.

1. Introduction

Rapid and accurate identification of poliovirus strains present in vaccines is important to ensure correct antigenic profile and limit the risk of an incorrect virus strain being included in a final product. The latter is a particular concern in cases where both attenuated (Sabin) poliovirus strains destined for oral and/or inactivated polio vaccine and wild-type strains destined only for inactivated vaccines or used as controls for quality control testing are handled on the same site.

Serological identification of vaccine components has been used effectively [1] and is the identification method approved in the European Pharmacopoeia [2], however this approach may be limited by the availability of appropriate reagents and inter-lab variation, may not distinguish between wild-type and attenuated strains of the same serotype and will not distinguish between virus strains that differ by a small number of nucleotides and/or amino acids. Resolution to the nucleotide level is useful not only in a prospective monitoring setting, but may also assist in tracing the source should a contaminant be detected. It also has the advantage that the nucleotide sequence of a given sample will be invariant regardless of the reagents and methods used for its determination. Several methods have been developed for identification of poliovirus strains at the molecular level, the most advanced of which was recently described by Nijst et al. [3]. This method, based on quantitative reverse transcriptase (RT)-PCR offers several advantages over serological testing including high specificity. It provides, however, information about only a small region of the genome (typically the 60–80 nucleotides constituting the primer and probe sequences), requires multiple reactions per sample to cover all possible strains and serotypes and has lower sensitivity for serotype 1 than for serotypes 2 and 3 [3].

Genome sequencing would provide the ultimate identity test. The availability of rapid, high-throughput benchtop sequencers makes full genome sequencing of multivalent vaccine samples feasible, with a tiny fraction of the time, effort and cost previously required. We evaluated a sequence-independent RT-PCR [4] and shotgun sequencing approach for the unambiguous identification of poliovirus strains present in a variety of inactivated poliovirus vaccines. All expected strains were detected in live and inactivated monovalent intermediates and inactivated trivalent final products. Retrospective testing demonstrated that the sequencing approach

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Table 1
Vaccine material.

Vaccine	Trivalent product	Monovalent product	Live/inactivated	Extraction
A	sIPV A		Inactivated	MinElute
		A-s1	Live	vRNA
		A-s2	Live	vRNA
		A-s3	Live	vRNA
		A-s1IPV	Inactivated	vRNA
		A-s2IPV	Inactivated	vRNA
		A-s3IPV	Inactivated	vRNA
B		B-s1	Live	vRNA
		B-s1IPV	Inactivated	MinElute
		B-s2	Live	vRNA
		B-s2IPV	Inactivated	MinElute
		B-s3	Live	vRNA
		B-s3IPV	Inactivated	MinElute
C	cIPV C		Inactivated	MinElute
		C-c1IPV	Inactivated	MinElute
		C-c2IPV	Inactivated	MinElute
		C-c3IPV	Inactivated	MinElute
D	cIPV D		Inactivated	MinElute
		D-c1	Live	vRNA
		D-c1IPV	Inactivated	MinElute
		D-c2	Live	vRNA
		D-c2IPV	Inactivated	MinElute
		D-c3	Live	vRNA
		D-c3IPV	Inactivated	MinElute
E	cIPV E		Inactivated	MagNA Pure
F	sIPVsample		Inactivated	MinElute

s Sabin; c conventional; IPV inactivated polio vaccine; vRNA QIAMP viral RNA Mini Spin Kit; MinElute Qiagen MinElute Virus Spin Kit; MagNAPure MagNA Pure LC total RNA isolation kit.

allowed for detection and characterisation of contaminating strains in an IPV product. This technique has the advantages of requiring only a single assay per sample, distinguishing between closely related strains and providing information on the full genome sequence of virus strains in a vaccine.

2. Methods

2.1. Vaccines

Samples were commercial vaccine components from different manufacturers, sampled at various stages of manufacturing: live monovalent samples, fomaldehyde-inactivated monovalent samples and trivalent formaldehyde-inactivated pools equivalent to the final vaccine product. Vaccine characteristics are described in Table 1.

2.2. Nucleic acid extraction

RNA was extracted using QIAMP vRNA Mini Spin columns or QIAMP MinElute Virus Spin Kit (Qiagen) without the addition of carrier RNA. One sample was extracted using the Kingfisher automated particle processor (Thermo Electron Corporation) and a MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche), incorporating proteinase K digestion, following the manufacturer's instructions. Water only controls were extracted, amplified and sequenced in parallel with each set of samples.

2.3. Sequence-independent RT-PCR

Sequence-independent amplification was performed following the method of Victoria et al. [5]. cDNA was synthesised using the SuperScript III 1st strand cDNA synthesis kit (Life Technologies), with 8 μl RNA and 100 pmol primer K-N8 (5′-GAC CAT CTA GCG ACC TCC CAN NNN NNN N-3′), generating randomly primed first strand cDNA with an arbitrary 5′ tagging sequence. 10 μl of the first strand reaction was added to 50 μl second strand mix containing 100 pmol primer K-N8 (to randomly prime second strand synthesis and add the same arbitrary 5′ tagging sequence), 150 μM dNTPs (Roche) and 5 U Large (Klenow) Fragment DNA Polymerase in 1× REACT2 buffer (Life Technologies). The reaction was incubated at 37 °C for 1 h followed by 75 °C for 20 min, resulting in double-stranded cDNA with the tagging sequence 'K' at both ends. PCR was performed using 2.5 μl of cDNA in a 25 μl reaction containing 0.7 μM primer K (the arbitrary tag introduced during cDNA synthesis, 5′-GAC CAT CTA GCG ACC TCC CA-3′) and Kapa HiFi HotStart Ready Mix (Anachem). Amplification conditions were: 98 °C for 60 s; 30 cycles of 98 °C for 20 s, 60 °C for 30 s, 72 °C for 30 s; 72 °C for 5 min. Amplified products, comprising fragments of variable length and theoretically representing an unbiased sample of all nucleic acid in the sample, were visualised by ethidium bromide staining on agarose gels. A representative gel is shown in Supplemental Fig. S1. Products werepurified using AMPure XP magnetic beads (Beckman Coulter), quantified using Qubit High Sensitivity dsDNA assay (Life Technologies), analysed on an Agilent High Sensitivity DNA chip (Agilent) and diluted to 0.2 ng/μl in molecular grade Tris–EDTA, pH8.0.

2.4. Sequencing

Sequencing libraries were prepared using Nextera XT reagents (Illumina) and the manufacturer's protocol, and sequenced on a MiSeq using a 2 × 251 paired-end v2 Flow Cell (Illumina). Six to 10 vaccine samples were pooled for each run.

2.5. Primer, quality trimming and assembly

Raw sequence data were imported into Genious R7 (Biomatters) and paired end reads combined. Data were filtered and aligned using a custom workflow with the following parameters: shotgun primer K and Nextera adaptor/index sequences were trimmed from 5 and 3′ ends with a minimum 5 bp overlap; reads were trimmed to have an average error rate <1%, no more than one base