Journal of Virological Methods 189 (2013) 189–195

Contents lists available at SciVerse ScienceDirect

Journal of Virological Methods

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

Rapid and accurate identification of poliovirus strains used for vaccine production

Olaf E.M. Nijst[a], Justin J. Mouthaan[a], Dirk R. Mekkes[b], Edin Jusic[a], Harrie G.A.M. van der Avoort[a], Bernard Metz[a,*]

[a] National Institute for Public Health and the Environment, Antonie van Leeuwenhoeklaan 9, 3720 BA Bilthoven, The Netherlands
[b] Bilthoven Biologicals, Antonie van Leeuwenhoeklaan 9-13, 3721 MA Bilthoven, The Netherlands

Article history:
Received 12 September 2012
Received in revised form 25 January 2013
Accepted 30 January 2013
Available online 19 February 2013

Keywords:
Poliovirus
Vaccine
Vaccine-related polioviruses
Real-time PCR
Sabin
Mahoney
MEF-1
Saukett

A B S T R A C T

In the context of eradication of poliomyelitis the World Health Organization stimulates the development of inactivated polio vaccines based on attenuated virus strains. In addition to vaccine development, tests have to be designed to assess the vaccine quality. An important test is the identification test for poliovirus strains that are used for the vaccine production. A rapid and accurate PCR method with fluorescent probes has been developed to identify unequivocally the vaccine-specific poliovirus strains, such as Mahoney, MEF-1, Saukett H, Sabin type 1, Sabin type 2 and Sabin type 3.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

The use of live-attenuated oral polio vaccine (OPV) and inactivated polio vaccine (IPV) has drastically reduced the number of cases and outbreaks of poliomyelitis (Crawford and Buttery, 2010; Dutta, 2008). The Global Polio Eradication Initiative of the World Health Organization (WHO) is now focusing on the elimination of the disease and eradication of wild poliovirus (Sutter et al., 2010; WHO, 2003). Despite considerable effort expanded in mass vaccinations, dozens of polio cases are still reported in the last years (Crawford and Buttery, 2010; Kew et al., 2005; WHO, 2012). Vaccination with OPV can cause the introduction of vaccine-derived polioviruses, resulting in outbreaks of poliomyelitis (Kew et al., 2005). Some of these vaccine-derived polioviruses are highly virulent and transmissible (Boot et al., 2004; Kew et al., 2005). IPV does obviously not have this disadvantage. However, the production capacity of IPV is currently very limited and increased use of IPV will require building more production facilities. These facilities will have to deal with stringent arrangements in containment, since IPV is produced using virulent poliovirus strains (WHO, 2003). Therefore, the WHO decided to stimulate the development of IPV based on attenuated polioviruses, *e.g.* the Sabin strains (Heymann et al., 2005, 2006). Thus, the risk of hazardous effects by virus escape from a production facility or accidental infection of manufacturing personnel can be minimised, although not completely eliminated (Kew et al., 2005).

During transition from IPV based on wild-type strains to Sabin strains, producers of IPV might use both wild-type and attenuated poliovirus strains. Wild-type poliovirus strains used for vaccine production are, *e.g.* Mahoney, MEF-1, Saukett H, and the attenuated strains used are Sabin type 1, 2 and 3. The unambiguous identification of these poliovirus strains used for IPV production will be an important quality control test for vaccine release. Currently, serological methods are used for the identification and quantification of the three poliovirus serotypes present in IPV, but in general they do not discriminate between wild-type and attenuated vaccine strains (Westdijk et al., 2011). Although serological assays are available that differentiate between wild-type and attenuated poliovirus strains, they require highly specific antisera (van der Avoort et al., 1995) and the preparation of the antisera is complex and laborious (van Wezel and Hazendonk, 1979). Alternatively, molecular methods are available for routine analysis of polioviruses in field isolates that can identify wild-type or attenuated poliovirus strains. The techniques are based on nucleic acid hybridisation (Kilpatrick et al., 1996), reverse transcription PCR (Boot et al., 2004; Kilpatrick et al., 1998, 2004, 2009), restriction fragment length polymorphism (Furione et al., 1993; Otelea et al., 1993) or sequencing (Neverov and Chumakov, 2010). Each technique has his own advantages and

* Corresponding author at: P.O. Box 1, Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands. Tel.: +31 30 27 433 73; fax: +31 30 27 444 26.
E-mail address: bernard.metz@rivm.nl (B. Metz).

0166-0934/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jviromet.2013.01.026

Table 1
Identification methods for polioviruses.

Method	Result	Advantage	Disadvantage
ELISA	Specific binding of monoclonal or polyclonal antiserum to poliovirus	Easy to perform	Low specificity. Production of specific antibodies is complex
Hybridisation	Sequence-specific interaction between poliovirus RNA and a probe	Accurate results	Test is laborious
PCR	Amplification of cDNA generated from poliovirus	Rapid and accurate results	Less detailed information than sequencing
Length polymorphism assays	Strain specific restriction profile	A marker to identify the origin of the poliovirus	Less detailed information than sequencing
Sequencing	Determination of the (partial) sequence of the poliovirus	Unambiguous identification of polio strain	Complicated data analyses; test is laborious

disadvantages (Table 1). However, these methods described in the literature have to be adapted before they can be used for the identification vaccine-specific poliovirus strains. Therefore, the decision was made to develop a rapid and accurate PCR method with fluorescent probes which can identify vaccine-specific poliovirus strains used for vaccine production, such as Mahoney, MEF-1, Saukett H, Sabin type 1, Sabin type 2 and Sabin type 3.

2. Materials and methods

2.1. Primers and probes

Primer and probes (Table 2) for identification of Mahoney and Sabin type 1 were custom made by Isogen Life Science (De Meern, Netherlands) and for identification of MEF-1, Saukett H, Sabin type 2 and type 3 by TIB Molbiol GmbH (Berlin, Germany).

2.2. Polioviruses

Poliovirus samples were obtained from different stages of the IPV production. IPV was produced with either wild-type strains or attenuated strains as described previously (van Wezel et al., 1978, 1979). Briefly, Vero cells were cultivated in bioreactors on micro carriers. Vero cells were infected with wild-type polio strains: Mahoney (type 1), MEF-1 (type 2) or Saukett H (type 3), or with attenuated strains: Sabin type 1 (LSc 2ab KP2), Sabin type 2 (P712 Ch2ab-KP2) or Sabin type 3 (Pfizer 457-III) strains. After three or four days of cultivation, poliovirus was purified from the culture supernatant by clarification, gel permeation chromatography and ion-exchange chromatography. Prior to inactivation the fluid was filtered to remove large virus aggregates and formaldehyde was added for inactivation. Samples taken prior to formaldehyde inactivation were heated for 30 min at 60 °C to inactivate the polioviruses.

2.3. RNA isolation

Viral RNA was extracted and purified from 200 μl of poliovirus samples using a fully automated procedure (MagNA Pure Compact System; Roche Applied Science, Almere, the Netherlands). Extracted RNA samples were stored at −20 °C prior to analysis.

2.4. Real-time PCR

Preparation and amplification of cDNA were performed in a real-time PCR apparatus (LightCycler 2.0; Roche). A reaction mixture was prepared by using LightCycler RNA Master HybProbe kit (Roche). The mixture contained 7.5 μl RNA master, 3.2 μl H_2O, 1.3 μl of a 50 mM $Mn(OAc)_2$ solution, 2 μl probe, 2 μl of 5.0 μM forward primer, 2 μl of 5.0 μM reverse primer and 2 μl isolated RNA, plasmid (positive control) or PCR grade water (negative control). The final concentration of probe for MEF-1 was 0.4 μM, and the concentration of the remaining probes was 0.2 μM. The sequences of primers and probes used are listed in Table 3. Design of experiments was used as an approach to optimise the reaction conditions, such as annealing temperature, annealing time and ramp rate. Software for Design of Experiments was used for modelling and visualisation of research data and for the calculation of optimal conditions (MODDE 8.0.2, Umetrics; Umeå, Sweden). For a rapid and accurate identification of the vaccine-specific polio strains, the reverse transcription step was executed in the PCR apparatus that was programmed to run for 20 min at 61 °C. For polioviruses of type 1, the amplification step was performed for 30 s 95 °C; [5 s 95 °C; 1 s 55 °C; 7 s 72 °C] × 35; 30 s 40 °C and ramp rate of 2 °C/s. For polioviruses

Table 2
Primer and probes.

Primer or probe	Sequence (5′–3′)	Orientation	Position	T_M (°C)	Specificity
MAHONEY-F	CCCTTTGACTTAAGTACCAC	Forward	1905-1924	55.3	Mahoney
SABIN1-F	TCCCTTTGACTTAAGTACAAA	Forward	1904-1924	52.0	Sabin type 1
POLIO1-R	GATCCTGCCCAGTGTGTGTAG	Reverse	2083-2063	56.9	Mahoney/Sabin type 1
POLIO1-TM	FAM-AGGGTTCGGTTAAGTGACAAACCACATAC-BBQ[a]	–	1950-1978	63.4	Mahoney/Sabin type 1
MEF-1-F	GGTTGTTGAGGGAGTCACGAGA	Forward	2505-2526	59.6	MEF-1
MEF-1-R	CCCTGTCTCTACGGCTGTTAGC	Reverse	2631-2610	59.5	MEF-1
MEF-1-TM	YAK-ACACCACTGACACCTGCCAACAACT-BBQ[a]	–	2536-2560	64.0	MEF-1
SAUKETT-F	GCAATTACGCCGCAAGC	Forward	2076-2092	57.8	Saukett H
SAUKETT-R	GTGTAGGTGCTCCTGGAGGT	Reverse	2227-2208	56.6	Saukett H
SAUKETT-TM	YAK-TTCGTGGTAACAGCCAACTTCACCA-BBQ	–	2134-2158	65.0	Saukett H
SABIN2-F	AAGGAATTGGTGACATGATTGAGG	Forward	2480-2503	58.7	Sabin type 2
SABIN2-R	CTCGGCTTTGTGTCAGGC	Reverse	2579-2562	57.4	Sabin type 2
SABIN2-TM	FAM-TGGAAGTCGGGGGAACCAATGC-BBQ	–	2551-2530	67.1	Sabin type 2
SABIN3-F	AATGACCAGATTGGTGATTCCTTG	Forward	3134-3157	58.7	Sabin type 3
SABIN3-R	GTAAATGCGGACTTTGGAGGTTACT	Reverse	3253-3229	59.9	Sabin type 3
SABIN3-TM	FAM-TGTGATCATTGACAACACGAACTGCCAA-BBQ	–	3218-3191	66.7	Sabin type 3

[a] *Abbreviations*: FAM, carboxyfluorescein; YAK, Yakima yellow; BBQ, BlackBerry Quencher.

Download English Version:

https://daneshyari.com/en/article/6134416

Download Persian Version:

https://daneshyari.com/article/6134416

Daneshyari.com