



World Health Organization

**STANDARD OPERATING PROCEDURE:
Mutant Analysis by PCR and Restriction Enzyme Cleavage
(MAPREC) for Oral Poliovirus (Sabin) Vaccine Types 1, 2 or 3**

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Draft

1 **Part 1. Overview of the assay**
2

3 **1. Aim**

4 The MAPREC assay is a molecular biological method used to determine the proportion of a
5 single base mutation at a given point within the viral RNA. If the calculated value of the
6 mutation at this site is greater than acceptable values (see **the current WHO Recommendations**
7 **to Assure the Quality, Safety and Efficacy of Live Attenuated Poliomyelitis Vaccine (oral)**)
8 characterised (see Part 1 section 1.10. Reference I), the vaccine will fail the MAPREC test.
9

10 **2. Introduction**

11 Isolates of all three serotypes of poliovirus were passaged *in vivo* and *in vitro* to produce the
12 Sabin strains of poliovirus that make up Oral Poliovirus Vaccine (OPV). Although these
13 attenuated vaccine strains are stable, they can revert to full or partial virulence through
14 point mutations in the 5' non-coding region of the viral RNA, particularly if grown at
15 temperatures greater than the optimal 35°C. These mutations differ in each of the three
16 types of polio vaccine (1, 2 and 3) and are well characterised (see Part 1 section 1.10.
17 Reference II III and IV).
18

19 In type 3 poliovirus vaccine, the mutation of U to C at base position 472 in the viral RNA 5' NCR
20 is directly related to the neurovirulence of the virus in monkeys. As the proportion of C in the
21 viral population increases, so does the neurovirulence, such that if the C content rises above
22 0.9%, the vaccine will fail the standard monkey neurovirulence test (see part 1 section 1.10
23 references V and VI). In Type 1 and 2 OPV there are mutations within the 5' NCR which revert
24 rapidly when passaged in the human gut or in cell culture, in Type 1 base positions 480 G→A,
25 525 U→C and in Type 2 base position 481 A→G. These mutations can lead to increased
26 neurovirulence when present in high proportions in viral population, or possibly acting with
27 other mutations within the viral genome. However, no correlation with virulence in monkeys
28 has been established when these mutations are present at levels typically found in vaccine
29 batches. Therefore, the MAPREC test for Type 1 and 2 OPV has been developed to measure the
30 consistency of vaccine production.
31

32 **3. Special considerations**

33 MAPREC is a PCR based assay, which allows very small quantities of starting nucleic acid to
34 be amplified to provide large amounts of DNA for quantification. The ability to amplify very
35 small quantities of target material via the PCR reaction means that contamination with DNA
36 or RNA, either by cross-contamination of samples, or other nucleic acids in the laboratory
37 may present a serious problem.
38

39 One of the most important general considerations for PCR is the provision of clean areas in
40 which to prepare reagents and reaction mixes. It is important to separate the various
41 stages of the PCR reaction, such as RNA extraction and cDNA addition; so that there is no
42 possibility of contamination of PCR mixes by previously amplified DNA. Staff should change
43 laboratory coats and gloves when using the different laboratories and equipment should be
44 dedicated to each room. Local and whole-room UV-irradiation and other decontamination
45 measures could be used to reduce the possibility of PCR-amplified DNA contaminating
46 reagents and samples. Other measures such as employing dUTP PCR contamination
47 prevention protocols could be also considered.
48

49 Adequate controls should be included in the test to ensure that contamination will be
50 detected. A cDNA control of water, which is extracted at the same time as the viral RNA,
51 should be included as well as a PCR reaction control. The PCR control ensures that the
52 reagents are free of DNA contamination.

1
2 If radioisotopes are used, the laboratory should comply with all national regulations for
3 their use and disposal.
4

5 **4. The assay**

6 Each vaccine bulk is assayed individually, before the combination of the three serotypes into
7 the final vaccine product.
8

9 Each assay will include four standard preparations:

- 10 International Standard DNA (IS DNA)
 - 11 High Mutant Virus Reference (HMVR)
 - 12 Low Mutant Virus Reference (LMVR)
 - 13 100% DNA control
- 14

15 Firstly, the RNA in each vaccine bulk is extracted, together with appropriate control
16 materials (HMVR, LMVR, cDNA control). The extracted RNA is then reversed transcribed into
17 cDNA using a mixture of random hexanucleotide primers and reverse transcriptase.
18

19 For each serotype, specific PCR primers are used to amplify the segment of viral cDNA
20 containing the base to be quantified. DNA standards (IS DNA and 100% DNA control) are
21 included along with the previously prepared cDNA. One of the primers contains the
22 modifications required to create a unique restriction site for enzyme digestion. This primer is
23 included at 10 times the concentration of the second PCR primer and results in the
24 accumulation of a large amount of single stranded template. A low concentration radioisotope
25 or fluorescently labelled primer is subsequently used to prime a second-strand DNA synthesis
26 by a one-step DNA polymerase reaction. One half of this labelled double stranded product is
27 then digested with the specific restriction enzyme, and the other half is used as an undigested
28 control.
29

30 The radiolabelled/fluorescent products are separated on a polyacrylamide gel, and visualised
31 and quantified using a suitable detector (imager). The intensity for each band in each sample is
32 entered into a computer spreadsheet programme (or calculated manually) and the mutant
33 content of the samples is calculated.
34

35 Two separate PCR reactions are generated from one cDNA preparation and are used to
36 perform 5 individual determinations for each mutation.
37

38 **5. Calculation of revertant content**

39 Two lanes, one with enzyme digested DNA [D] and the other with undigested control [C],
40 are analysed for each sample.

41 In each of these lanes, areas containing both the undigested full length (upper) DNA band
42 [DU and CU] and the restriction fragment (lower) band [DL and CL] are quantified (For type
43 2 and type 3 MAPREC) see Figure 1.
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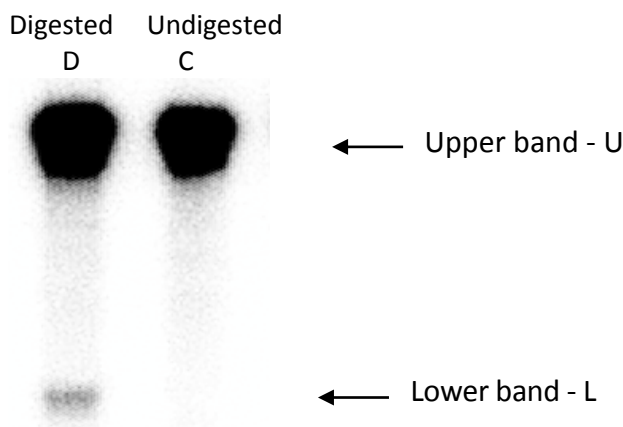


Figure 1. Appearance of Types 2 and 3 MAPREC gel

The fraction of radioisotope/fluorescence in the restriction fragment is compared to the total radioisotope/fluorescence (digested DNA + the full length DNA) for each lane:

$$FD = DL/[DU+DL]$$

$$FC = CL/[CU+CL]$$

FD represents the fraction of DNA molecules with the reversion plus non-specific background fluorescence.

$$\text{The \% of revertants} = FD - FC = (DL/[DU + DL] - CL/[CU + CL])100.$$

In the Type 1 MAPREC assay, there are two digested bands which are quantified, see Figure 2.

In this case the fraction of radioisotope/fluorescence in the restriction fragment compared to the total radioisotope/fluorescence for the specific DNA is calculated for each lane:

$$\text{The \% of revertants} = FD - FC = [(DL1+DL2)/(DU+DL1+DL2) - (CL1+CL2)/(CU+CL1+CL2)]100$$

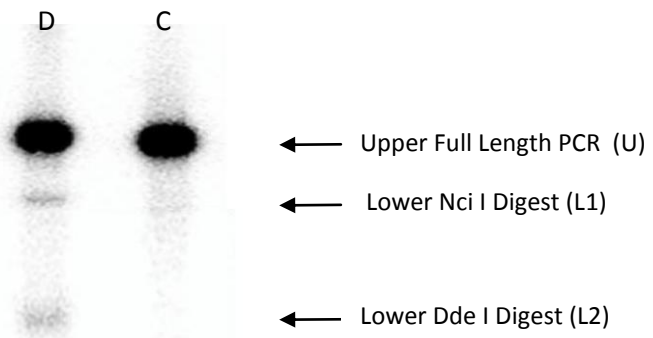


Figure 2. Appearance of type 1 MAPREC gel

6. Criteria for a valid determination:

Criteria 6.1 and 6.2 below apply to each determination, whilst criteria 6.3, to 6.7 apply to each set of five determinations which make up a complete assay.

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6.1 The cDNA and PCR controls should be negative (no full length PCR product detected).

Bands Appearing in the cDNA and PCR water controls

If DNA bands are detected in either of these controls, it is important to determine whether the DNA is a true contaminant or is an aberrant amplification product often referred to as "primer dimer".

To do this, the sample is digested with a mixture of enzymes, which will result in the complete digestion of a genuine DNA PCR product.

If DNA bands are resistant to both enzymes, they are primer-dimers and not contaminants. If the DNA band in blank samples is digested by a mixture Mbo I and Hinf I (in case of Type 3; Bsp1286I and Afl III in case of Type 2) giving DNA fragments of appropriate size, it is a contaminant. Detection of contamination will invalidate a MAPREC determination. Sources of contamination should be identified and eliminated.

Poliovirus	Nucleotide	Restriction enzyme
type 1	480-A (revertant)	Dde I
	480-G (vaccine)	BstX i
	525-C (revertant)	Nci I
	525-U (vaccine)	Mro i
type 2	481-G (revertant)	Bsp 1286I
	480-A (vaccine)	Afl III
type 3	472-C (revertant)	Mbo I
	472-U (vaccine)	Hinf I

6.2 The value for the 100% DNA control should be equal to or greater than a maximum feasible value established within the testing laboratory.

It is important to ensure that the restriction enzyme digestion conditions are optimal and give a maximum percentage of cut DNA. However, the apparent mutant content determined in MAPREC assay for 100% DNA control is always somewhat lower than 100%. This is because the efficiency of restriction enzymes is less than 100%, even when present in a large excess. For this reason, values determined for mutant content can vary from 90 to more than 95%, and can depend on the type and source of restriction enzyme. Therefore the level of digestion for the 100% DNA should be within previously established limits, and be monitored for consistency. To do this, all valid experimental values for this sample should be pooled and a mean (μ) and standard deviation (SD) calculated for all determinations. A 95% fiducial lower limit (FLL) on the experimental value for 100% DNA control is calculated as follows:

$$FLL = \mu - 1.96 * S.D.$$

or roughly two "sigmas" below the mean value.

If a particular determination falls short of the historically established 95% FLL, the determination is deemed invalid and should be repeated. If more than one determination in a series of five fails this criterion, further experiments must be performed to identify the problem that led to incomplete digestion of the DNA. This may be due to the quality of the enzyme, which should be replaced.

1 **6.3 All test samples and controls, other than 100% DNA control, should have a standard**
2 **deviation equal to or below 0.3.**

3
4 0.3 is a value based on the results of the WHO collaborative studies, where most
5 laboratories obtained values below this. This limits the variability allowed within a complete
6 MAPREC assay.

7
8 **6.4 The values obtained for the IS DNA should be consistent with previously obtained**
9 **values.**

10
11 % of mutation content in International Standard (IS) DNA should be consistent with previous
12 tests. (480-A and 525-C in 00/418 for Type 1 OPV, % 481-G in 97/758 for Type 2 OPV, %
13 472-C in 95/542 for Type 3 OPV).

14
15 Data on determinations of the IS DNA sample should be accumulated and a mean
16 (μ) and standard deviation (SD) calculated.

17 95% Fiducial lower and upper limits (FLL and FUL) on the values obtained for the IS
18 are calculated as:

19
20
$$\text{FLL} = \mu - 1.96 * \text{S.D.}$$

21
$$\text{FUL} = \mu + 1.96 * \text{S.D.}$$

22
23 or roughly two "sigmas" below or above the mean value. If a particular determination for
24 the sample falls beyond this range, it is rejected. If more than one determination in a series
25 of five is invalidated, further experiments must be performed.

26
27 **6.5 The ratio of the % of the duplicate DNA reference controls (A and B), should not differ**
28 **significantly from each other as determined by a paired t test of % mutation values.**

29
30 If the mean ratio of IS DNA duplicates A and B, in a particular set of five valid individual
31 determinations falls beyond the 95% confidence interval, it indicates that there may be
32 some systematic error within determinations, the results for the whole set should be
33 invalidated and the problems investigated and resolved.

34 The individual ratios of IS DNA duplicates A and B to each other in a set of five
35 determinations should be consistent with previously obtained data. Data from previous
36 experiments (four sets of five determinations) are accumulated, and the mean IS DNA ratio
37 (μ) and standard deviation (SD) are calculated. The 99% fiducial upper limit (FUL) on the ratio
38 variability is calculated as:

39
40
$$\text{FUL} = \text{S.D.} * \text{SQUARE ROOT} (X^2P, N-1 \div (N - 1))$$

41
42 where $X^2P, N-1$ is a value taken from a statistical table for a number of tests N and
43 probability P (0.01 for 99% confidence).

44
45 **6.6 The 'Failed' reference DNA should fail the MAPREC test.**

46
47 % of mutation content in International Standard (IS) DNA should be consistent with previous
48 tests. (480-A and 525-C in 00/422 for Type 1 OPV, % 481-G in 96/596 for Type 2 OPV, %
49 472-C in 96/578 for Type 3 OPV).

1 **6.7 The 'Passed' reference DNA should pass the MAPREC test.**

2
3 % of mutation content in International Standard (IS) DNA should be consistent with previous
4 tests. (480-A and 525-C in 00/416 for Type 1 OPV, % 481-G in 97/756 for Type 2 OPV, %
5 472-C in 95/572 for Type 3 OPV).

7 **7. Interpretation of MAPREC results and sample acceptability criteria**

8
9 The accumulation of 472-C mutations in the 5' NCR of type 3 OPV during vaccine
10 production, leads to the increased neurovirulence of vaccine batches as determined by the
11 Monkey Neurovirulence test (MNVT). When the level of 472-C mutations exceeds a certain
12 threshold, the OPV will fail the MNVT.

13
14 For Type 1 and Type 2 OPV, the accumulation of revertants in the 5'NCR during vaccine
15 production does not necessarily lead to increased neurovirulence in the MNVT. Therefore
16 the MAPREC assay should be regarded as a measure of production consistency.

17
18 All results should be expressed as ratios relative to the relevant type specific International
19 Standard (IS DNA) for the MAPREC analysis of poliovirus (Sabin). The acceptable variation of
20 mutant content from batch to batch should be agreed with the national regulatory
21 authority in the light of production experience.

22
23 The maximum level of revertants permissible is stated in the current WHO
24 Recommendations to Assure the Quality, Safety and Efficacy of Live Attenuated
25 Poliomyelitis Vaccine (oral) (see Part 1 section 1.10 reference I).

26
27 **8. Critical reagents**

28
29 The specific primers for PCR and International Standards detailed in the MAPREC test
30 procedure are critical to the assay. If fresh batches of primers are obtained they should be
31 validated in an assay with the international standards.

32
33 **9. Equipment**

34
35 Equipment should be maintained and calibrated according to the manufacturer's instructions
36 or *in house* guidelines. Equipment records should be kept and updated as appropriate.

37
38 **10. References**

- 39
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16 [65.pdf](http://www.who.int/biologicals/publications/meetings/areas/vaccines/polio/BS97.1865.pdf)
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Part 2. MAPREC test procedures

The reagents used in the MAPREC Standard Operating Procedures depend upon the serotype of the vaccine bulk, and the *base position to be analysed*.

These reagents are critical to the test and have been established by a WHO collaborative study as below (see Part 1 section 1.10. References VII, VIII, IX):

Poliovirus type 1 base 480 and base 525

PASSED VIRUS REFERENCE	IS STANDARD 00/416
FAILED VIRUS REFERENCE	IS STANDARD 00/422
100% REVERTANT CONTROL	IS STANDARD 00/410
1.0% REVERTANT CONTROL*	IS STANDARD 00/418

*two replicates of this samples should be included in each test, one labelled A, and one labelled B.

pS primer: pS-1/445 5' CTC CGG CCC CTG AAT GCG GCT AAT CCa AAC CTC tG 3'
Hplc purified, used at 3µg/ml

pA primer: pA-1/526 5' AAC ACG GAC ACC CAA AGT AGT CGG TTC CGC tcC GG 3'
Hplc purified, used at 30µg/ml

pS labeled primer: pS-1/445 5' CTC CGG CCC CTG AAT GCG GCT AAT CCa AAC CTC tG 3'
used at 3µg/ml

***Label:** - the label should be suitable for the detector used and can be added by the manufacturer or *in house* for radioisotope

‡**Restriction enzyme:** - Dde I at 1 unit/µl and Nci I at 1 unit/µl (diluted if appropriate in the buffer supplied).

Poliovirus type 2 base 481

PASSED VIRUS REFERENCE	IS STANDARD 97/756
FAILED VIRUS REFERENCE	IS STANDARD 98/596
100% REVERTANT CONTROL	IS STANDARD 98/524
1.0% REVERTANT CONTROL*	IS STANDARD 97/758

*two replicates of this samples should be included in each test, one labelled A, and one labelled B.

pS primer: pS-2/431 5' GCT ACA TAA GAG TCC TCC GGC CCC TGA ATG CGC CT 3'
Hplc purified, used at 3µg/ml

pA primer: pA-2/483 5' CGC GTT ACG ACA AGC CAG TCA CTG GTT CGC GAC CaC Gt 3'
Hplc purified, used at 30µg/ml

pS labeled primer: 5' GCT ACA TAA GAG TCC TCC GGC CCC TGA ATG CGC CT 3'
used at 3µg/ml

***Label:** - the label should be suitable for the detector used and can be added by the manufacturer or *in house* for radioisotope

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1 ‡**Restriction enzyme:** - Bsp 1286 I at 1 unit/µl (diluted if appropriate in the buffer supplied)

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3
4 **Poliovirus type 3 base 472**

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6 PASSED VIRUS REFERENCE IS STANDARD 96/572

7 FAILED VIRUS REFERENCE IS STANDARD 96/578

8 100% REVERTANT CONTROL IS STANDARD 94/790

9 1.0% REVERTANT CONTROL* IS STANDARD 95/542

10
11 *two replicates of this samples should be included in each test, one labelled A, and one labelled B.

12
13 **pA primer:** pA-3/484 5' CAG GCT GGC TGC TGG GTT GCA GCT GCC TGC 3'
14 Hplc purified, used at 3µg/ml

15
16 **pS primer:** pS-3/470 5' TGA GCT ACA TGA GAG TGC TCC GGC CCC TGA ATG CGG
17 CTG A 3'
18 Hplc purified, used at 30µg/ml

19
20 **pA labeled primer:**
21 pA-3/484 5' CAG GCT GGC TGC TGG GTT GCA GCT GCC TGC 3'
22 used at 3µg/ml

23
24 ***Label:** - the label should be suitable for the detector used and can be added by the manufacturer
25 or *in house* for radioisotope

26
27 ‡**Restriction enzyme:** - **Mbo I** at 1 unit/µl (If the enzyme is more concentrated, dilute to 1 unit /µl in
28 the buffer supplied with the enzyme)

29
30
31 ‡ Isoschizomers of the restriction enzymes may be used, however these enzymes must be validated
32 before use.

33 Examples of isoschizomers are:

34

<u>Enzyme</u>	<u>Isoschizomers</u>
Dde I	BstDE I, HypF3 I,
BstX I	-
Nci I	AsuC2 I, Bcn I, BpuM I
Mro I	Acc III, Aor13H I, BseA I, Bsp13 I, BspE 1, Kpn2 I.
Bsp 1286I	Mh1 I, Sdu I
Afl III	-
Mbo I	BfuC I, Bsp143 I, BssM I, BstKT I, BstMB I, Dpn II, Nde II, Sau3A I
Hinf I	-

35
36
37 **Detailed steps of the MAPREC assay procedure**

38
39 The RNA extraction and cDNA preparation is the same for all three types of OPV monovalent bulks.
40 The primers used for the PCR and labelling reactions differ for each of the three types, as do the
41 restriction enzymes used to digest the PCR products.

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1. Extraction of RNA

There are many procedures and kits available for the preparation of high quality RNA. The method described below requires that approximately 125µl of the original viral suspension is available for the subsequent reverse transcription reaction to cDNA.

If an alternative RNA extraction method is used, it should be ensured that it will yield sufficient RNA for the subsequent reactions.

Equipment

Microfuge
P1000, P200 Micropipettor,
P200, P1000 Filtered Micropipettor tips
Vortexer
-20°C freezer
Tube racks
Magnetic Tube Rack
-80°C freezer
Micro tubes

Materials

All materials are used within the manufactures expiry date.
500µl Nuclease free water
500µl LMVR*
500µl HMVR*
500µl each vaccine sample for testing*
Phenol/Chloroform/Isoamyl alcohol (ratio 25:24:1 Sigma # 77617 or equivalent) or pH 7.5 Buffer saturated Phenol
Propan-2-ol stored at -20°C
10% Sodium Dodecyl Sulphate (SDS)

	Type 1	Type 2	Type 3
HMVR	00/422	98/596	96/578
LMVR	00/416	97/756	96/572

* samples stored in at -80°C prior to testing

Method

- 1.1. Thaw 500µl of 96/572 and 96/578 virus reference and each test virus sample at room temperature.
- 1.2. Mark 3 x 1.5ml microtubes for each virus reference, sample and water, with appropriate reference numbers.
- 1.3. Take 450µl of each virus and nuclease free water and place in one of the appropriately marked tubes.
- 1.4. Add 50µl of 10% SDS to each of these tubes.
- 1.5. Add 500µl of phenol to each tube, cap, vortex and centrifuge at high speed for 5 minutes at room temperature. Remove the upper aqueous phase to a fresh tube.
- 1.6. Repeat step 1.5.
- 1.7. Add 500µl of chloroform to each tube, cap, vortex and centrifuge at high speed for 5 minutes at

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1 room temperature. Remove the upper aqueous phase to a fresh tube.

2
3 1.8. Add 1ml of Propan-2-ol (stored at -20°C) to each supernatant, mix thoroughly and place at -20°C
4 overnight.

5
6 1.9. Remove the RNA/Propan-2-ol suspensions from freezer and mix thoroughly using a vortexer.

7
8 1.10. Remove 300 μl of each sample to a fresh, labelled 1.5ml microtube. Close and centrifuge for 15
9 minutes at approximately 14,000 rpm at $+4^{\circ}\text{C}$.

10
11 1.11. Carefully remove and discard the supernatant. Add 300 μl of 70% ethanol and wash each pellet
12 in the ethanol, very gently.

13
14 1.12. Centrifuge at approximately 14,000 rpm at $+4^{\circ}\text{C}$ for five minutes, remove the supernatant with a
15 micropipette, and discard.

16
17 1.13. Dry the RNA pellet.

18 19 **2. Preparation of cDNA**

20
21 There are a number of kits available which can be used to prepare cDNA. The method below
22 describes a procedure based on the purchase of individual components and a dried RNA pellet. If
23 the RNA has been eluted into a buffer, then vary the amount of water used to make up the mix.

24 25 **Equipment**

26 Vortex mixer
27 P20, P200, P1000 Micropipettor
28 P20, P200, P1000 Micropipettor filtered tips
29 -20°C freezer
30 1.5ml microtubes
31 Tube rack
32 Micro centrifuge
33 Water bath set at 37°C
34 Refrigerated micro centrifuge
35 Vortex mixer
36 0.75ml microtubes
37 Tube rack
38 Heating block set at 94°C

39 40 **Materials**

41 Nuclease free water
42 MMLV reverse transcriptase (RTase) (200 units/ μl)
43 5 x MMLV reverse transcriptase buffer
44 10mM dNTP's
45 Random Primer (50 $\mu\text{g}/\text{ml}$)
46 0.1M DTT (Dithiothreitol)
47 RNA preparations from:
48 LMVR- Passed virus Reference
49 HMVR- Failed virus Reference
50 Poliovirus vaccine test samples
51 Nuclease free water (cDNA control)

52

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Method

- 2.1. Prepare sufficient cDNA mixture for the required number of reactions as set out in the table. The number of reactions will be 4 (LMVR, HMVR, water plus the number of samples to be tested) with extra mix to allow for the viscosity of the mix. Add all the components, except MMLV RTase, in any order, mix thoroughly and spin in microfuge, briefly.
- 2.2. Label the tube appropriately and store at +4°C (on ice) until required.
- 2.3. The appropriate amount of MMLV Reverse Transcriptase is added immediately before use.

<u>Reagent</u>	<u>For 1 test sample</u> 5 reactions	<u>For 2 test samples</u> 6 reactions
5 x RTase Buffer	20.0 µl	24.0 µl
10mM dNTP	5.0 µl	6.0 µl
DTT	2.5 µl	3.0 µl
Random Primer (50µg/ml)	2.5 µl	3.0 µl
Nuclease free water	65.0µl	78.0µl
*MMLV RTase (200 units/µl)	5.0 µl	6.0 µl
Total	<u>100.0 µl</u>	<u>120.0 µl</u>

*Add immediately before use

- 2.4. Add 20.0µl of the cDNA mix to the appropriate RNA pellet and mix thoroughly. Dissolve the RNA completely in the cDNA mix, taking care not to cross-contaminate the samples.
- 2.5. Incubate the samples in the water bath set at 37°C for 1 hour.
- 2.6. Inactivate the RTase by heating in the heating block set at 94°C for approximately five minutes. (The reverse transcriptase will be inactivated at temperatures above 75°C).
- 2.7. Briefly centrifuge each tube, and store in -20°C freezer. The cDNA may be stored for up to one year.

3. Preparation of reaction mixtures for the determination of optimal cDNA concentrations

Equipment

- Vortex mixer
P20, P200, P1000 Micropipettor
P20, P200, P1000 Micropipettor filtered tips
-20°C trend monitored freezer
0.75ml microtubes
Tube rack

**WHO SOP for Mutant Analysis by PCR and Restriction Enzyme Cleavage
(MAPREC) for Oral Poliovirus (Sabin) Vaccine, version 5**

1 **Materials**

2 PCR Primers:

Type	pS primer	pA primer
1	pS-1/445 (3µg/ml)	pA-1/526 (30µg/ml)
2	pS-2/431 (3µg/ml)	pA-2/483 (30µg/ml)
3	pS-3/470 (30µg/ml)	pA-3/484 (3µg/ml)

3 Nuclease free H₂O

4 Components for PCR Mix:

5 100mM dATP

6 100mM dGTP

7 100mM dCTP

8 100mM dTTP

9 H₂O

10 Taq DNA polymerase (5units/µl)

11 Or use a PCR Master mix: 2 x PCR Master Mix (eg. Reddy-mix Thermo-Fisher Scientific AB-
12 0575/DC/LC/B or equivalent)

13

14 **Method**

15 Prepare the PCR mix for one sample and references as follows:

16

17 From separate components:

18 Take: 10µl 100mM dATP,

19 10µl 100mM dGTP,

20 10µl 100mM dCTP

21 10µl 100mM dTTP

22 and place into a 1.5ml microtube with 960µl H₂O to give 1mM dNTP mix.

23

24 Make a PCR mix containing the following components:

25

Reagent	1 sample + references	2 samples + references
10 x PCR Buffer	168.0 µl	214.0 µl
1mM dNTP	168.0 µl	214.0 µl
Antisense primer (pA)	168.0 µl	214.0 µl
Sense primer (pS)	168.0 µl	214.0 µl
Taq DNA polymerase 5units/µl	6.7 µl	8.6 µl
H ₂ O	833.3 µl	1061.4 µl
TOTAL	1512.0 µl	1926.0 µl

26

27 Where each additional sample would require:

46.00µl	10x PCR buffer
46.00µl	1mM dNTPs
46.00µl	antisense primer
46.00µl	sense primer
1.84µl	Taq DNA polymerase 5u/µl
<u>228.16µl</u>	H ₂ O
<u>414.00µl</u>	Total

**WHO SOP for Mutant Analysis by PCR and Restriction Enzyme Cleavage
(MAPREC) for Oral Poliovirus (Sabin) Vaccine, version 5**

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Using the 2x PCR Master mix:

Reagent	1 sample + references	2 samples + references
2 x PCR Master mix	756.0µl	963.0µl
pA	168.0µl	214.0µl
pS	168.0µl	214.0µl
H ₂ O	420.0µl	535.0µl
TOTAL	1512.0µl	1926.0µl

Each additional sample would require:

207.0µl	2x PCR Master Mix
46.0µl	pA
46.0µl	pS
<u>115.0µl</u>	H ₂ O
<u>414.0µl</u>	Total

Keep on ice and use the mix within 2 hours.

3.1. Label 8 x 0.75ml microtubes for each test sample cDNA, HMVR cDNA and LMVR cDNA, 1-8.

3.2. To tube '1' in each series add 56.25µl* of PCR mix.

** If the limit of accuracy of the micropipettor does not allow for this measurement, pipette 56.2µl.*

3.3. Add 50.0µl of PCR mix to all the other tubes labelled '2-8'.

3.4. Dispense 45µl of PCR mix into each of 5 control tubes labelled appropriately:

	For Type 1	For Type 2	For Type 3
100% DNA Control	00/410	98/542	94/790
IS DNA (A)	00/418 A	97/758 A	95/542 A
IS DNA (B)	00/418 B	97/758 B	95/542 B
Assay controls 1 2	cDNA control	cDNA control	cDNA control
	PCR control	PCR control	PCR control

Equipment

- Vortex mixer
- P20, P200, P1000 Micropipettor
- P20, P200, P1000 Micropipettor filtered tips
- 20°C trend monitored freezer
- 0.75ml microtubes
- Tube rack
- Thermal Cycler

**WHO SOP for Mutant Analysis by PCR and Restriction Enzyme Cleavage
(MAPREC) for Oral Poliovirus (Sabin) Vaccine, version 5**

1 Materials

- 2 cDNA preparations (see section 2)
3 PCR mix (see section 3.1)
4 100% (DNA)*
5 DNA IS (A) (DNA)*
6 DNA IS B (DNA)**see below
7 Mineral oil

8
9 3.5. Vortex the vaccine test sample, LMVR, HMVR and control cDNA preparations and then
10 microfuge them briefly.

11
12 3.6. For each cDNA preparation:
13 Add 6.25µl of the cDNA to tube number '1'. Discard the tip. Mix using a fresh tip and transfer
14 12.5µl to tube '2'. Discard the tip. Mix, then transfer 12.5µl to tube '3', proceed in this manner
15 until the last tube. After mixing, discard 12.5µl.

16
17 3.7. Add 5.0µl of cDNA control to the cDNA control tube.

18
19 3.8. Add 5.0µl of H₂O to the PCR control tube.

20
21 3.9. Add 5µl of each reference samples to the appropriately labelled PCR reaction tube.*

22
23 3.10. Add 50µl of mineral oil to all the tubes, including controls: 94/790, 95/542A and B, cDNA
24 control and PCR control.
25 *this is not necessary if the thermal cycler has a heated lid.*

26
27 ***To prepare International Standard (IS) DNA A and B and 100% standard DNA.**

28
29 Resuspend freeze dried International Standard (IS) DNA, (A and B) control in 100µl H₂O.
30 Transfer the solution into appropriately marked 1.5ml microtubes.

31
32 Resuspend freeze dried reference DNA sample, 100% control in 100µl H₂O and transfer the
33 solution into a marked 1.5ml microtube.

34
35 Reconstituted reference material is stored at -20°C after use, until required for the second PCR.
36 This material may be stored for up to one year.

37
38 3.11. Cap, and microfuge the tubes briefly.

39
40 3.12. Incubate the samples as follows8:-
41 94°C for 10 minutes
42 94°C for 30 seconds } 40 cycles
43 55°C for 15 seconds
44 65°C for 3 minutes

45
46 * or other suitable cycling programme
47 Temperatures and times may be varied depending on the thermal cycler used and are as
48 indicated on the cycler.

49
50 3.13 After incubation is complete, the samples are stored in the -20°C freezer for up to one year.

51
52

**WHO SOP for Mutant Analysis by PCR and Restriction Enzyme Cleavage
(MAPREC) for Oral Poliovirus (Sabin) Vaccine, version 5**

4 MAPREC labelling primer preparation

The primer that was used at the lowest concentration for the PCR, should be used for labelling.
Either use a commercially prepared labelled primer or add a radiolabel using polynucleotide kinase.

Equipment

- P10, P20, P200 Micropipettor and filtered tips
- 20°C freezer
- 0.75ml microtubes
- Tube racks

Materials [All materials are used within the manufacturers' expiry date.]

Labelling primers:

Type	Primers for radiolabelling	Fluorescent primer	
1	pS-1/445	pS-1/ 445	used at 3µg/ml
2	pS-2/431	pS-2/431	used at 3µg/ml
3	pA-3/484	pA-3/484	used at 3µg/ml

Nuclease free water

Primer (3 µg/ml)

Gamma [³²P] ATP, 10 µCi/µl

10x PNK buffer

T4 PNK, 1 u/µl

} For radiolabelling

Method

4.1. Making the radiolabelled primer:

Add the following components together in a 0.75 ml microtube:-

Labelling primer (3 µg/ml)	17.6 µl
gamma [³² P] ATP, 10 µCi/µl	4.0 µl
10x PNK buffer	2.7 µl
T4 PNK, 1 u/µl	<u>2.7 µl</u>
Total	<u>27.00 µl</u>

Incubate at 37°C for 30 minutes.

Then add:

10mM dNTP Mix	50.5µl
Taq DNA Polymerase	<u>2.5µl</u>
Total	<u>80.0µl</u>

4.2. For the fluorescently labelled primers:

Add the following components together in a 0.75 ml microtube:-

Labelled primer at 3µg/ml)	16.3µl
2 x PCR Master Mix	40.0µl
H ₂ O	<u>23.7µl</u>
	<u>80.0µl</u>

4.3. Place the mix on ice and use within 5 hours. This is the labelled primer mix.

1 **5 Labelling of PCR bands of cDNA optimisation**

2

3 **Equipment**

4 P10, P200 Micropipettor Pipettes

5 P10, P200 Micropipettor filtered tips

6 Heating block

7 Microfuge

8 High voltage power pack

9 Electrophoresis tank

10 0.75ml microtubes

11 Tube racks

12

Materials

PCR products from the dilutions of cDNA for each virus sample as described in section 3.

PCR products from the standard references and PCR + cDNA control samples prepared as described in section 3

Labelled primer mix, prepared as described in section 4

Loading buffer: 10x Bromophenol blue or equivalent (eg 10x Orange Gel Loading dye (Licor # 927-10100))

10% polyacrylamide gel(s), prepared as described in Annex 1

0.75ml microtubes

Gel loading tips

Blue/Orange Loading dye for gel tracking (Promega # G1881)

13

14

Method

5.1. Label 0.75ml microtubes appropriately and add 5µl of each PCR product to the tube.

5.2. Add 1µl of labelled primer mix to each PCR product, microfuge briefly and incubate at 72°C (as indicated on the machine) for 10 minutes.

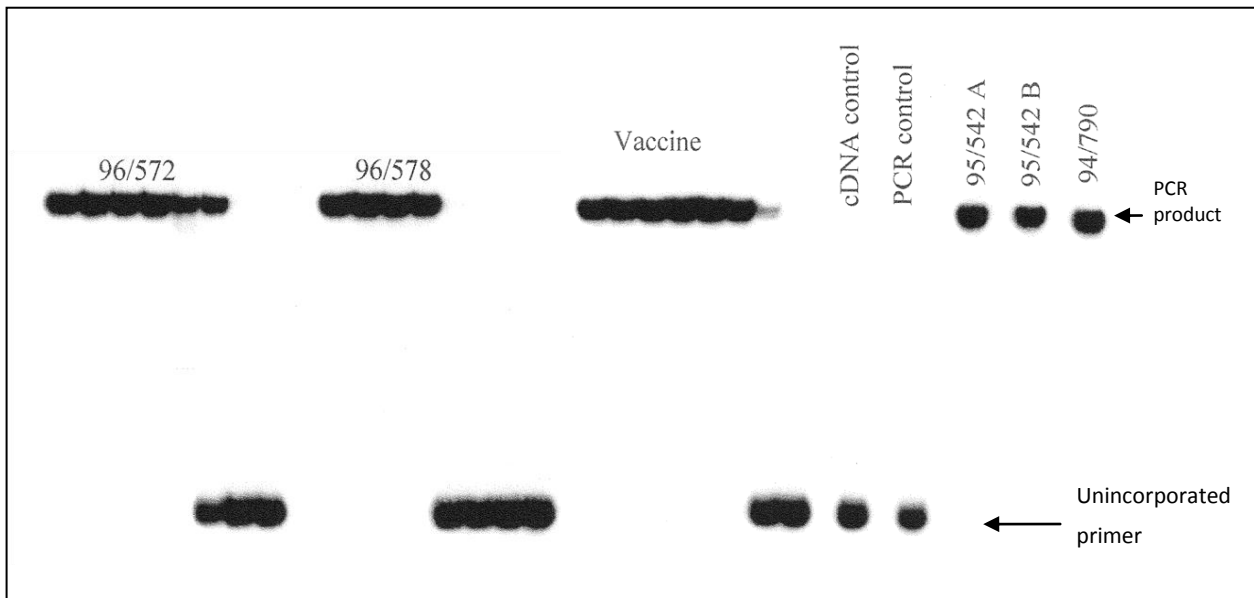
5.3. Microfuge all the tubes, then add 2µl of loading dye mix to each tube. Microfuge all the tubes briefly.

5.4. The samples are loaded onto a 10% polyacrylamide gel, leaving an empty well between each sample set, and between each standard and control.

5.5. Run the gel at a constant 40 watts until the orange dye front has travelled at least 6 cm.

5.6. Remove the gel from the electrophoresis tank. Rinse the glass plates briefly in water to remove buffer. Visualise the bands using a suitable detection system.

1



2 Example of a calibration gel (Type 3)

3

4

5 **6 Assessment of Optimisation**

6

7

I. There should be no primer dimers.

8

9

II. There should be PCR product in the 1 in 125 dilution of each cDNA titration for the test sample, LMVR and HMVR.

10

11

ie. There should be PCR products in the first 4 wells of each dilution set.

12

13

III. There should be PCR product in the DNA controls IS A, IS B and the 100% DNA.

14

15

IV. The cDNA and PCR controls should be negative

16

17

18 **7 Quantification of base mutation**

19

20 **Equipment**

21 P10, P200 Micropipettor Pipettes

22 P10, P200 Micropipettor filtered tips

23 Heating block

24 Microfuge

25 High voltage power pack

26 Electrophoresis tank

27 0.75ml microtubes

28 Tube racks

29

WHO SOP for Mutant Analysis by PCR and Restriction Enzyme Cleavage (MAPREC) for Oral Poliovirus (Sabin) Vaccine, version 5

1

Materials

PCR products from the **lowest** appropriate dilutions of cDNA for each virus sample as described in section 2.

PCR products from the standard references and PCR + cDNA control samples prepared as described in section 2

Labelled primer mix, prepared as described in section 4

Restriction enzyme :

Polio Type	Type 1	Type 2	Type 3
Restriction enzyme (1u/μl)	DdeI and Nci I	Bsp1286	Mbo I

Note: restriction enzymes that were isolated from different microorganisms, but have the same substrate specificity (isoschizomers) can also be used after appropriate validation as a replacement for the above enzymes.

Proteinase K (10mg/ml) □

10x Loading dye (eg Bromophenol Blue or Orange Gel Loading dye Licor # 927-10100)

10% polyacrylamide gel, prepared as described in Annex 1

0.75ml microtubes

Blue/Orange Loading dye mix (Promega G1881) – optional tracking dye*

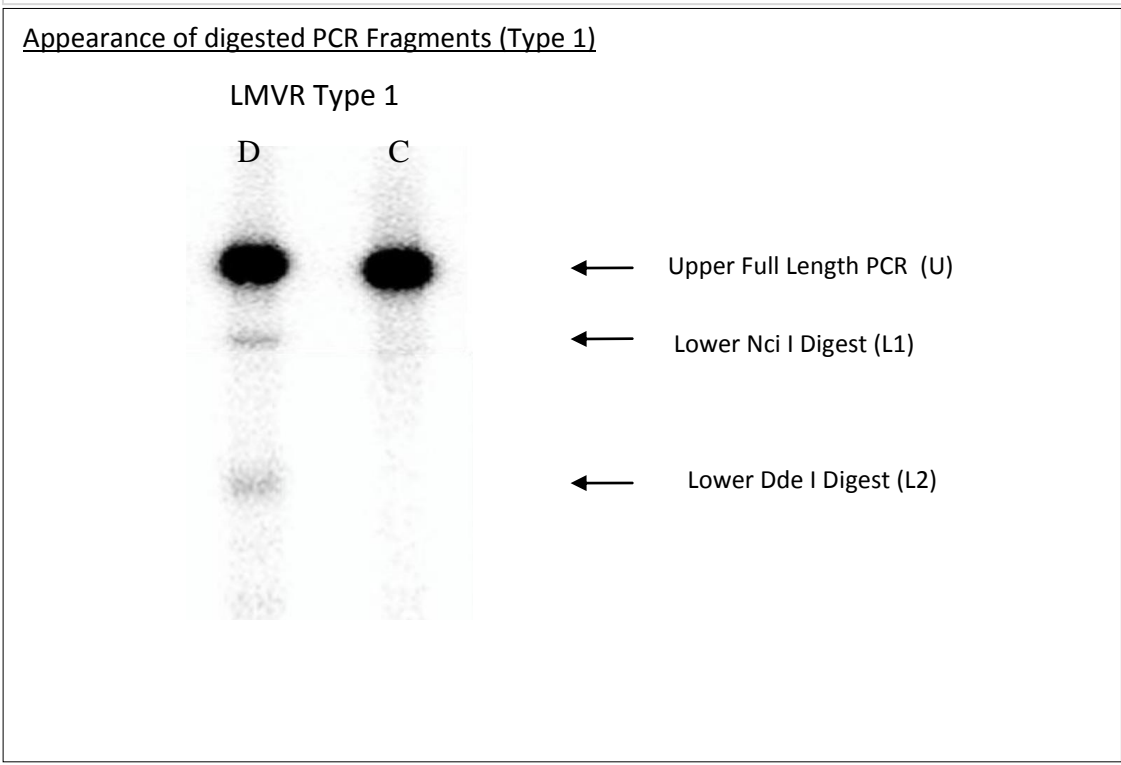
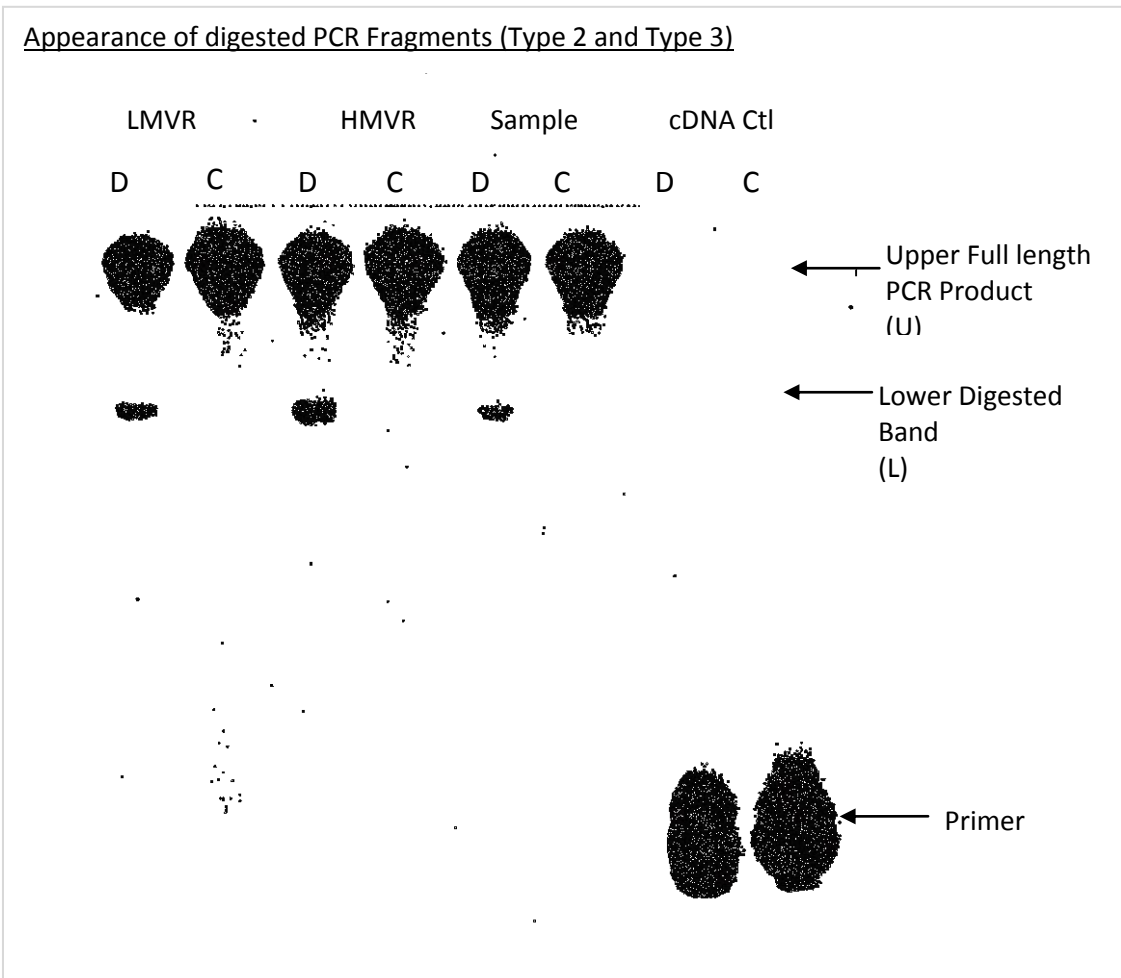
Method

- 7.1 Label 8 x 0.75ml microtubes appropriately and add 10μl of PCR product to the tube.
- 7.2 Add 2μl of labelled primer mix to each PCR product, microfuge briefly and incubate at 72°C (as indicated on the machine) for 10 minutes.
- 7.3 Microfuge tubes. Label two sets of 0.75μl tubes for each labelled product and put 6μl of the labelled product into each tube.
- 7.4 Add 1μl of restriction enzyme to one tube of each pair (For type 1 add 1μl of both restriction enzymes). Cap all tubes and microfuge briefly.
- 7.5 Incubate all tubes at 37°C (as indicated on the machine) on the heating block for 1 hour.

In some instances, the enzymes will remain bound to the PCR product. To remove these enzymes it may necessary to digest the enzyme/PCR mix with Proteinase K: Microfuge all tubes. Add 1μl of Proteinase K (10mg/ml) to each tube and incubate at 55°C for 30 minutes.
- 7.6 Microfuge all the tubes, then add 2μl of loading dye mix to each tube. Microfuge all the tubes briefly.
- 7.7 The samples are loaded onto a 10% polyacrylamide gel, leaving an empty well between each sample, and with digested and undigested samples adjacent to each other. See Annex 1 for an example of a gel loading scheme
- 7.8 The gels are run at a constant 40 watts, in 1 x TBE buffer, until the orange dye front has migrated about 15- 16cm (approximately 40 minutes to an hour).
- 7.9 The gel plates removed from the electrophoresis tank and rinsed briefly in tap water, then patted dry. The gels are identified by the blue/orange tracking dye and each gel is analysed using a suitable detection system.
- 7.10 All waste gel and electrophoresis buffer is discarded according to local regulations
Quantify the bands using the instructions provided by the manufacturer of the detector.

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**WHO SOP for Mutant Analysis by PCR and Restriction Enzyme Cleavage
(MAPREC) for Oral Poliovirus (Sabin) Vaccine, version 5**

Calculation of % base content.

Two lanes, one with enzyme–digested DNA (D) and the other with undigested control (C), are analyzed for each sample. In each of these lanes, areas containing both the full–length (upper) DNA band (DU and CU) and the restriction fragment (lower) band (DL and CL) are quantified. (see Fig. 4).

The fraction of radioactivity/fluorescence in the restriction fragment compared to the total radioactivity/fluorescence in the specific DNA is calculated for each lane:

	Type 1 OPV	Type 2 and Type 3 OPV
FD =	$(DL1+DL2)/(DU+DL1+DL2)$	or $DL/(DU+DL)$
FC =	$(CL1+CL2)/(CU+CL1+CL2)$	or $CL/(CU+CL)$

FD represents the fraction of DNA molecules with reversion plus some nonspecific background.

FC represents nonspecific background caused by uniform smearing of radioactive/fluorescent materials along the electrophoresis track, as well as artifactual DNA products that may have formed.

Therefore:

$$\% \text{ Revertants} = FD - FC = DL/(DU+DL) - CL/(CU+CL) * 100$$
$$\text{or } (DL1+DL2)/(DU+DL1+DL2) - (CL1+CL2)/(CU+CL1+CL2)*100$$

It is recommended that computer worksheets be used (e.g. Microsoft Excel or Lotus 1–2–3) to perform these calculations, as well as further statistical treatment, validation of the test, and for making pass/fail decisions.

Validation criteria for each individual determination:

- I. No contamination in water blank or mock cDNA controls should be detected, see Overview 6.1
- II. There should be no primer dimer, but see Overview 6.1.
- III. Digestion of the 100% DNA sample (00/410 for Type 1, 98/524 for Type 2, 94/790 for Type 3) should be within previously established limits. eg 90% or above, see Overview 6.2.
- IV. The % of mutant content in International Standard (IS) DNA is consistent with previous tests. See Overview 6.4.

A complete MAPREC test consists of 5 quantitative determinations following the steps outlined in sections 4 to 9.

The 5 determinations should be made using at least 2 separate PCR amplifications. Additional PCR reactions are performed as described in section 8 below. Once the five determinations have been completed the Mean and Standard Deviations for each vaccine is calculated.

**WHO SOP for Mutant Analysis by PCR and Restriction Enzyme Cleavage
(MAPREC) for Oral Poliovirus (Sabin) Vaccine, version 5**

8 Additional PCR reactions for the quantification of the base mutation.

Equipment

- P10, P20, P200, P1000 Micropipettor
- P10, P20, P200, P1000 Micropipettor filtered tips
- 1.5ml and 0.75ml microtubes
- 20°C freezer
- Microfuge
- Thermal cycler

Materials

- All materials are used within the manufacturer's expiry dates:
- 2 x PCR Master mix (eg. Reddy-Mix Thermo-Fisher Scientific AB-0575/DC/LC/B)
- PCR primers:

Type	pS primer	pA primer
1	pS-1/445 (3µg/ml)	pA-1/526 (30µg/ml)
2	pS-2/431 (3µg/ml)	pA-2/483 (30µg/ml)
3	pS-3/470 (30µg/ml)	pA-3/484 (3µg/ml)

- Nuclease free water
- 100% DNA control
- IS-DNA A
- IS-DNA B

	For Type 1	For Type 2	For Type 3
100% DNA Control	00/410	98/542	94/790
IS DNA (A)	00/418 A	97/758 A	95/542 A
IS DNA (B)	00/418 B	97/758 B	95/542 B
Assay controls 1 2	cDNA control	cDNA control	cDNA control
	PCR control	PCR control	PCR control

- cDNA's from section 2:
- Control,
- Test sample,
- LMVR,
- HMVR
- H₂O
- Mineral Oil

Method

- 8.1. Prepare the PCR mix as follows:
For 3 cDNA's and 5 controls:

2x PCR Master mix	225.00µl
pA (30µg/ml for type 1 and 2, 3µg/ml for type 3)	45.00µl
pS (3µg/ml for type 1 and 2, 30µg/ml for type 3)	45.00µl
H ₂ O	90.00µl
	<hr/> 405.00µl

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(MAPREC) for Oral Poliovirus (Sabin) Vaccine, version 5**

- 1 8.2. Transfer 45µl of PCR mix into each of 8 tubes labelled as follows:-
2 cDNA control, LMVR, HMVR, test sample, IS DNA- A, IS DNA- B, PCR control.
3
4 8.3. Thaw all reagents and microfuge briefly.
5
6 8.4. Add 5µl of DNA/cDNA to each appropriately labelled tube. Add 5µl of water to the PCR control.
7 Add 50µl of mineral oil to each tube. Close all tubes and microfuge briefly.
8 8.5. Place all the samples in the PCR thermal cycler and incubate as follows*.
9
10 94°C for 10 minutes
11 94°C for 30 seconds } 40 cycles
12 55°C for 15 seconds }
13 65°C for 3 minutes }
14
15 Temperature and times are as indicated on the machine
16 * or other suitable cycling programme
17
18 8.6. After incubation is complete, store the PCR products at -20°C for up to 8 weeks.
19
20

21 **Validation criteria for a set of five individual determinations:**

- 22
23 I. The ratio of the IS DNA duplicates should not be significantly different from 1.0.
24
25 II. The mean ratio of the IS DNA, for the five determinations should be consistent with previous
26 results, see Overview 6.4.
27
28 III. The LMVR should have a mutant content less than the concurrently tested IS.
29
30 IV. The HMVR should have a mutant content higher than the concurrently tested IS.
31
32
33

1 **Annex 1. Preparation of 10% Polyacrylamide gel**

2
3
4 **Gel thickness and well spacing will affect the running conditions and migration of the PCR**
5 **fragments. If alternative gel systems are used the parameters described below may not be**
6 **applicable.**

7
8 **Equipment**

9 A suitable gel electrophoresis system (eg Hoefer™ SE 600 Chroma System using 0.75mm spacers and
10 combs)

11 0.75mm x 0.5mm x 15wells for the quantitation gel

12 Or 0.75mm x 0.4mm x 20 wells for the cDNA optimisation gel

13 Prepare the 10% polyacrylamide gel according to the manufacturer's instructions.

14 100ml measuring cylinder

15 P200 Micropipettor

16 P200 Micropipettor tips

17 Gloves

18 50ml syringe

19
20 **Materials**

21 Ethanol

22 Siliconising solution

23 10 x TBE Buffer [eg.Sigma]

24 40% Stock solution of 19:1 Acrylamide: Bis-acrylamide [eg Sigma]

25 10% Ammonium persulphate [APS]

26 TEMED [eg Sigma]

27
28 **Method**

29 **Wear gloves throughout.**

30
31 A.1 Wash the glass plates thoroughly in hot water, and dry.

32 A.2 Apply 2ml of siliconising solution to each plate and spread over the surface of the plate with a
33 tissue, until the solution has dried. Remove excess siliconising solution by applying ethanol to
34 each plate from a wash bottle and polishing the plate with a tissue (prevents gel sticking to
35 glass surface).

36 A.3 Place the plastic spacers along each short side of the plain glass plate and cover with eared
37 plate, siliconised sides facing inwards. Remove gloves and tape both sides and the bottom of
38 the gel together, with electrophoresis tape, or clamp.

39 A.4 Put on gloves and pour 25 ml of 40% acrylamide into a 100 ml measuring cylinder and add 75
40 ml of 1 x TBE. Add 1.2 ml of 10% APS to the solution.

41 A.5 Pour 50 ml of the solution into a 50 ml syringe and add 50 µl of TEMED. Mix thoroughly and
42 pour between the glass plates immediately.

43 A.6 Place the comb into the top of the gel, centrally, and clamp the top of the plate with bulldog
44 clips if required. Leave the gel to set, approximately 10 minutes.

45 A.7 Pour 1 x TBE buffer into the bottom buffer chamber of the electrophoresis tank. Remove the
46 electrophoresis tape from the bottom of the gel plates.

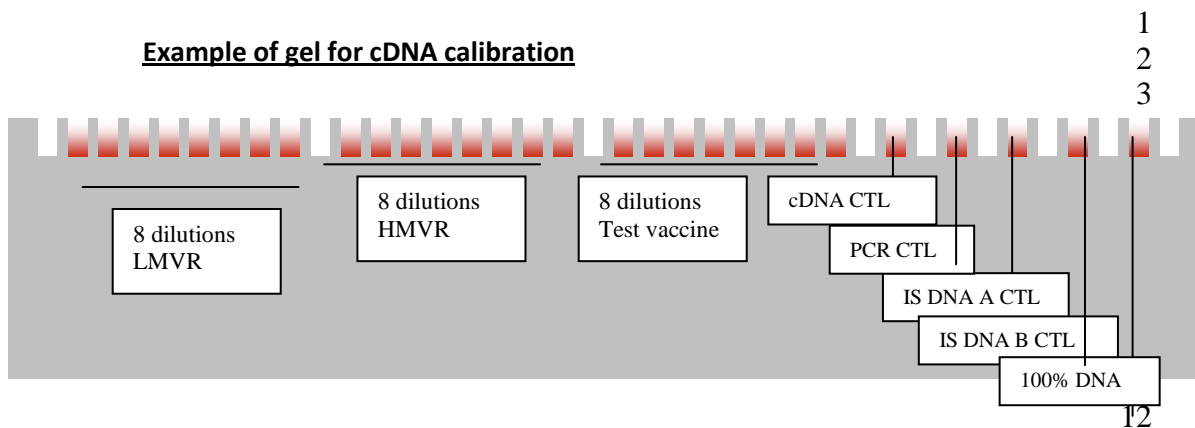
47 A.8 Place the gel/plates into the electrophoresis tank, eared plates facing the perspex. Remove the
48 bulldog clips and reuse to clip the plates to the tank along the side spacers.

49 A.9 Pour 1 x TBE buffer into the top buffer chamber of the electrophoresis tank, and remove the
50 comb. Rinse out the wells in the gel with buffer using a liquipette.

51 A.10 The gels can be kept for up to 1 week in buffer. Any remaining solution should be stored at
52 +4°C for up to one week.

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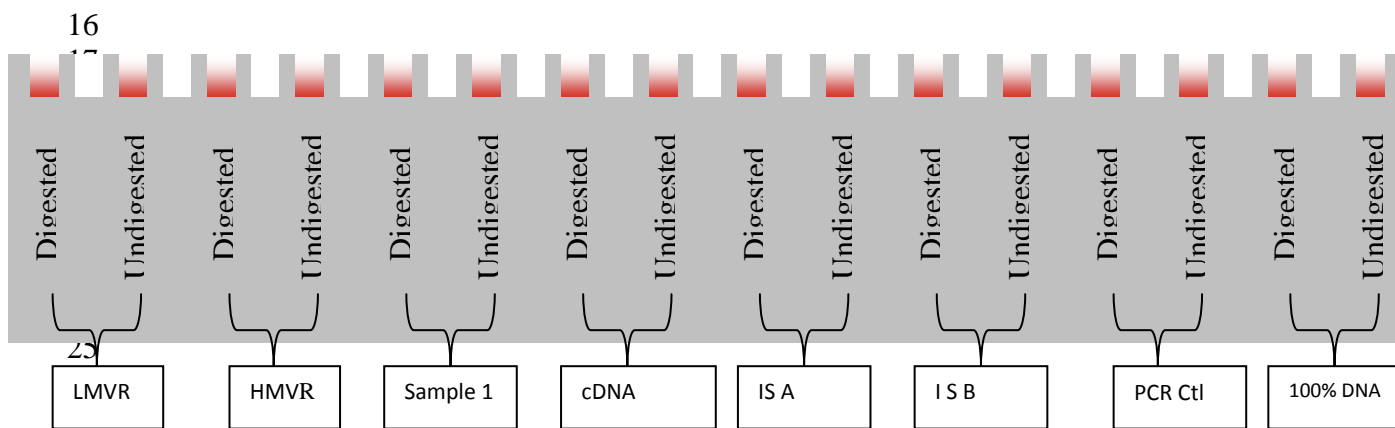
Example of gel for cDNA calibration



13
14
15

Load the samples onto the gel(s) in the following order:

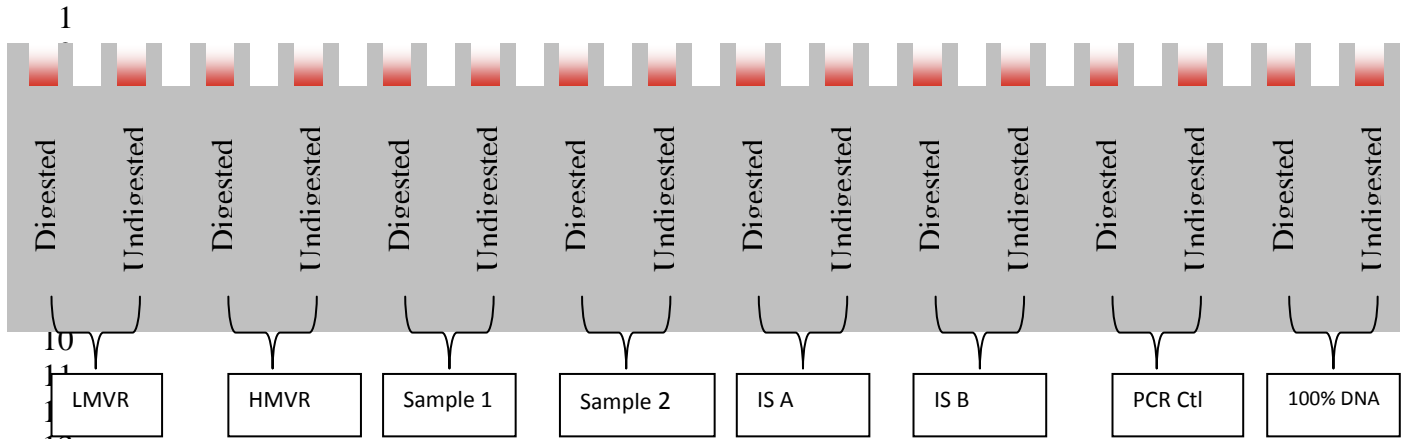
1	LMVR control	Digested
2	LMVRvirus control	undigested
3	HMVR virus control	Digested
4	HMVR virus control	undigested
5	Test sample	Digested
6	Test sample	undigested
7	cDNA control	Digested
8	cDNA control	undigested
9	IS DNA control A	Digested
10	IS DNA control A	undigested
11	IS DNA control B	Digested
12	IS DNA control B	undigested
13	PCR control	Digested
14	PCR control	undigested
15	100% DNA control	Digested
16	100% DNA control	Undigested



28
29
30
31

Diagram of a Gel Loading Scheme for Single Sample – may use one or two gels*

**WHO SOP for Mutant Analysis by PCR and Restriction Enzyme Cleavage
(MAPREC) for Oral Poliovirus (Sabin) Vaccine, version 5**



14 Diagram of a Gel Loading Scheme for two Samples – may use one or two gels*

17 **Tracking dye - If more than one gel is used, identify the gels by loading:
18 one extra lane of blue/orange loading dye mix for gel 1, and
19 two extra lanes of blue/orange loading dye mix for gel 2.*

1 **Annex 2. PCR fragments generated during MAPREC assay**

2
3 **Type 1 MAPREC primers**

4
5 **pS1- 445** 5' CTC CGG CCC CTG AAT GCG GCT AAT CCa AAC CTC tG 3' (35 bp)

6
7 **pA1- 526** 5' AAC ACG GAC ACC CAA AGT AGT CGG TTC CGC TcC GG 3' (35 bp)

8
9 **Type 1 MAPREC fragments**

10 Dde I

11 **C'TNAG**

12 *CTCCGGCCCCTGAATGCGGCTAATCCAAACCTC TGAGCAGGTGGTCACAAACCAGTGATTG
13 CTCCGGCCCCTGAATGCGGCTAATCCAAACCTC GGGCAGGTGGTCACAAACCAGTGATTG

14
15 Nci I

16 **CC'CGG**

17 GCCTGTCGTAACGCGCAAGCC CGGAGCGGAACCGATACTTTGGGTGTCCGTGT

18
19 GCCTGTCGTAACGCGCAAGTC CGTG GCGGAACCGATACTTTGGGTGTCCGTGT

20
21 **Fragments generated:**

22 Base Pairs

23
24 Full length = 115

25
26 pS + Nci I = 82

27
28 pS +Dde I = 33

29
30
31 **Type 2 MAPREC primers**

32
33 **pS-2/431** 5' GCT ACA TAA GAG TCC TCC GGC CCC TGA ATG CGc CT 3'

34
35 **pA-2/483** 5' CGC GTT ACG ACA AGC CAG TCA CTG GTT CGC GAC CaC Gt 3'

36
37 **Type 2 MAPREC Fragments**

38 Bsp 1286I

39 **GDGCH'C**

40 *GCTACATAAG AGTCCTCCGG CCCCTGAATG CGCCTAATCC TAACCACGGA GCA GGC GGTC
41 GCTACATAAG AGTCCTCCGG CCCCTGAATG CGGCTAATCC TAACCACGGA ACA CGTGGTC

42
43 GCGAACCAGT GACTGGCTTG TCGTAACGCG

44 GCGAACCAGT GACTGGCTTG TCGTAACGCG

45
46 **Fragments generated:**

47 Base Pairs

48
49 Full length = 90

50
51 pS + Bsp 1286I = 53

52

WHO SOP for Mutant Analysis by PCR and Restriction Enzyme Cleavage
(MAPREC) for Oral Poliovirus (Sabin) Vaccine, version 5

1 Type 3 MAPREC primers

2
3 **pS-3/470** 5' TGA GCT ACA TGA GAG TgC TCC GGC CCC TGA ATG CGG CTG A 3'

4
5 **pA-3/484** 5' CAG GCT GGC TGC TGG GTT GCA GCT GCC TGC 3'

6
7 Type 3 MAPREC Fragments

8
9 MboI

10 'GA TC

11 TGAGCTACAT GAGAGTgCTC CGGCCCTGA ATGCGGCTgA TcCTAACCAT GGAGCAGGCA

12 TGAGCTACAT GAGAGTCTC CGGCCCTGA ATGCGGCTAATTCTAACCAT GGAGCAGGCA

13
14 GCTGCAACCC AGCAGCCAGC CTG *

15 GCTGCAACCC AGCAGCCAGC CTG

16
17 Fragments generated:

18 Base Pairs

19
20 Full length = 83

21
22 pA + Mbo I = 43