**Annex C10** **Agarose gel electrophoresis with measles and rubella synthetic positive controls**

**Agarose Gel Electrophoresis**

CDC protocols for the molecular epidemiology of measles virus and rubella virus; version of 04/17/2019

**Purpose**

The following protocol is to be used for the visualization of PCR products. This protocol can be used for the products of measles genotyping RT-PCR, rubella genotyping RT-PCR and rubella diagnostic RT-PCR. Many different gel casting and gel electrophoresis systems are commercially available. The protocol must be adapted to the materials available in the laboratory. Other protocols for gel electrophoresis are acceptable if they provide adequate resolution of bands in the range of 150-800 base pairs.

**Reagents and materials needed**

* 1X TBE (Tris-borate EDTA) buffer (e.g. Life Technolgies, catalog number 15581-044 for 10X buffer) or 1X TAE (Tris-acetate EDTA) buffer (e.g. Life Technologies, catalog number 15558-042 for 10X buffer)
* Agarose (e.g. Life Technologies Ultra-Pure, catalog number 16500-500)
* De-ionized water
* Gloves
* Lab coat
* GelRed (Biotium, # 41003)
* Loading dye containing bromophenol blue (e.g. Promega, catalog number G190A)
* Molecular weight marker (e.g. 100 bp ladder, Life Technologies, catalog number 15628-019)

**Equipment needed**

* Agarose gel casting tray and comb(s)
* Agarose gel electrophoresis box
* Camera
* Graduated cylinder
* Heat-resistant glove
* Micropipettors and sterile pipette tips with aerosol-resistant filters
* Microwave
* Microwave-safe glass beaker or flask
* Parafilm
* Power supply
* Scale
* UV transilluminator

**Precautions**

* Gel electrophoresis is a post amplification procedure and should not be performed in the same room where the RT-PCR reactions are set up. Do not share equipment (including lab coats) between pre-PCR and post-PCR rooms.
* Ethidium Bromide (EthBr) is a carcinogen. To avoid working with EtBr replace with GelRed. GelRed is a non-carcinogenic dye which is at least as sensitive as EtBr for visualizing DNA bands in agarose gels. It can be used with the same UV transilluminators and cameras used for gels stained with EtBr.

**Assay Protocol**

There are many different gel casting systems and gel electrophoresis boxes available. The volume of gel solution as well as voltage and running time must be determined based on the available equipment.

1. Weigh appropriate amount of agarose. For PCR products of measles and rubella genotyping RT-PCR and for products of rubella diagnostic RT-PCR 2% agarose gels will work well. Add 1 X TBE buffer (TAE is also acceptable).
2. Melt agarose in a microwave for laboratory use only. Avoid over-heating, as the boiling agarose will bubble may overflow its container.
3. Add 0.2 µl GelRed and swirl gently.
4. Pour into casting tray until agarose is half way up the tines of the comb and allow gel to solidify.
5. Remove gel from casting tray. Place in electrophoresis box with enough 1X TBE (or TAE) to cover gel. Gently remove comb.
6. Load the appropriate amount of a molecular weight marker into the first well (e.g. 5 µl Life Technologies 100 base pair ladder premixed with loading dye).
7. Place 2 µl of loading dye onto parafilm. Mix 5 µl of sample with the 2 µl of loading dye, pipet up and down until they are thoroughly mixed. Place all into an empty well of the gel. Repeat for all samples.
8. Set electrophoresis for 5-10 V/cm if using TBE buffer (1-5V/cm for TAE). Run gel until examination by UV light shows that the marker bands are well separated.
9. Visualize DNA by UV light. It is best to use a UV transilluminator. Photograph gel to record results.

**Analysis of results**

* The bands of the marker should be clearly visible.
* The negative controls (water control, RNA from uninfected cells) should not show any DNA bands. If there are bands in the negative controls, this indicates contamination of reagents with measles RNA. In this case, the RT-PCR must be repeated with fresh reagents.
* The positive control should show one band of the expected size (see below). If there is no band in the positive control, the RT-PCR did not work and must be repeated.
* The samples either show no bands (negative result) or a band of the expected size (see below, positive result).
* If the desired PCR products are present, purify PCR products from the remaining reaction.

**Example gel for Measles Genotyping RT-PCR**

1% agarose gel stained with GelRed and visualized by ultraviolet illumination. The positive control is synthetic measles RNA with insert (see below). Primers MeV214 and MeV216 amplify an 854 base pair (nt) amplicon from the synthetic control RNA. The PCR product for a patient sample is 634 base pairs (nt) in size.

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Lane 1: 100 base pair ladder molecular weight marker (Invitrogen)

Lane 2: Synthetic MeV RNA (MeV-N3in) with insert = 854 base pair fragment

Lane 3: wild-type Measles RNA = 634 base pair fragment

Lane 4: negative (water) control

**Schematic of measles positive control RNA with insert**

The location of the genotyping RT-PCR product is indicated. The real-time RT-PCR product is amplified from a different part of the synthetic RNA and is therefore not affected by the insert.

**220 nt insert**

**Measles N gene**

**genotyping PCR product of control RNA**

**real-time RT-PCR product**

**nt 426**

**nt 1764**

**nt 1522**

**nt 1521**

**Example gel for Rubella Genotyping RT-PCR**

1.5% agarose gel stained with GelRed and visualized by ultraviolet illumination. The positive control is synthetic rubella RNA (see below).

Examples of the 2 RT-PCR genotyping fragments and the diagnostic fragment made from wild-type and synthetic RNA are shown below. The wild-type size for fragment 1 should be 450 nucleotides (nt) while the control product should be 370 nt. The wild-type size for fragment 2 should be 633 nt while the control product should be 553. The wild-type diagnostic fragment should be 185 nt while the control product should be 215 nt.



**Schematic of rubella positive control RNA with insertion and deletion**

Synthetic Rubella E1 RNA showing the locations of the 30 nt insertion and 2 deletions.

 