

Use of Whatman FTA Indicating Microcards (“FTA cards”) for the Transportation of Viral Lysates

Purpose

The following protocol is to be used to submit measles or rubella isolates to the Regional or Global Specialized Laboratory for sequence analysis and genotyping. Patient specimens may also be shipped on FTA cards; however, the efficiency of recovery of RNA from patient specimens is still under investigation. Modifications have to be made to the QiaAmp Viral RNA Mini kit extraction procedure (see RNA Extraction protocol) to extract RNA from FTA cards. Do not use FTA cards for shipment of serum samples.

Important: FTA cards are treated with a chemical to destroy viral infectivity, but retain viral RNA. Measles and rubella viruses are inactivated on the card by using the procedure below and the cards can be shipped at ambient temperature as non-infectious material. It is not possible to recover live virus from FTA cards.

Reagents and materials needed

- 10% Bleach
- 70% ethanol
- Desiccant packs
- Gloves
- Indicating FTA micro cards (Whatman, WB120311) with 25 mm diameter circles
- Lab coat
- RNase-free 1.5 ml microcentrifuge tubes
- RNaseZap (Sigma, # R2020-250ML)
- Scalpel
- Sealable plastic bag
- Virus lysate
- Nuclease-free water
- 1.5 ml microcentrifuge tubes

Equipment needed

- Class II biological safety cabinet (BSC) with UV light
- Forceps
- Micropipettors and sterile pipette tips with aerosol-resistant filters
- Paper punch (6 mm diameter)
- Refrigerator

Loading lysate on an FTA card

1. In a Class II BSC, add 125-200 μ l of viral lysate to one circle on the FTA card. Use a circular motion to distribute the lysate. Do not pool all the lysate in the middle of the card.
2. Allow to dry in the BSC for 1 hour with the airflow on.

Collection of discs from FTA cards for RNA extraction

1. Clean a 6 mm paper punch and forceps with 10% bleach for 1 minute, rinse with water, and then 70% ethanol for 1 minute and dry.

2. Use the paper punch to cut out disks from the circle on the FTA card. Up to 10 disks can be punched from one card.
3. Use forceps to place the discs from one circle in a 1.5 ml tube.
4. Before moving on to another lysate, clean the 6 mm paper punch and forceps with 10% bleach for 1 minute, rinse with nuclease-free water, and then 70% ethanol for 1 minute and dry. This prevents cross-contamination.
5. Store tubes at -20 °C in an airtight container (sealable plastic bag) with desiccant packs.

Storage of FTA cards or disks

Cards with viral lysate and disks punched out from FTA cards are stable at room temperature for up to two months; however, it is recommended to store them at -20 °C. It is important to store the cards or disks in airtight container with desiccant bags.

Shipment of FTA cards or disks

FTA cards can be shipped at ambient temperature. Enclose FTA cards in envelopes; avoid cross-contamination of cards loaded with different isolates by wrapping each card individually in plastic wrap. Disks punched out from FTA cards can be placed in 1.5 ml tubes and shipped at ambient temperature.

Use of FTA cards for patient specimens

Viral lysates contain large amounts of viral RNA; therefore sufficient quantities of RNA for amplification can be eluted from one 6 mm disk. The concentration of viral RNA in patient specimens is variable and can be very low. Limited studies at CDC indicate that RNA quantities sufficient for amplification can be extracted if several disks (3-4) are extracted together. FTA cards should only be used for shipment of patient specimens if a sufficient volume of the original specimen can be retained for diagnostic purposes.

Extraction of RNA from FTA disks

Please see the RNA extraction protocol for details.