Annex A4 Dried blood spot (DBS) protocols

Following the protocol below, there are additional extraction protocols using Serion and Schleicher and Schull 903 paper for rubella IgM (pg6).

DETECTION OF MEASLES-SPECIFIC IgM ANTIBODY IN DRIED BLOOD SAMPLES (DBS) USING EUROIMMUN ANTI-MEASLES VIRUS NP ELISA IgM ASSAY
V1: 7 Jan 2020

The following method has been validated by Euroimmun on PerkinElmer 226 (Ahlstrom 226) filter paper. Other filter-paper types such as S&S Whatman 903 are in the process of being validated.

Dried blood collection, storage and shipment and extraction and testing procedures

A: Collection and preparation procedure

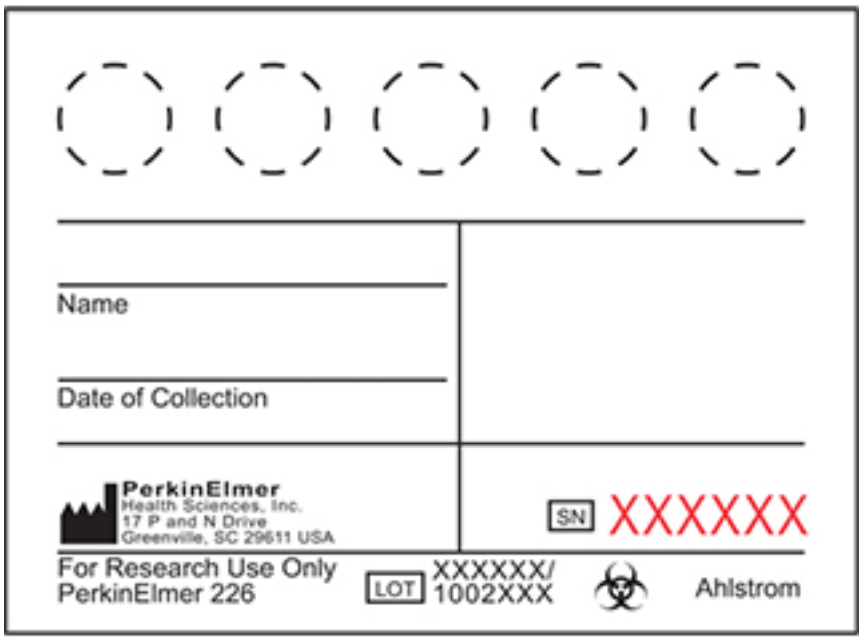
A.1: Preparation of filter paper

PerkinElmer filter paper suitable for dried blood collection is available in the following formats:

- PerkinElmer 226 Spot Saver Card™
- PerkinElmer 226 Five-Spot Card
- Custom Collection Cards

The PerkinElmer 226 Five-Spot Card (see figure 1, next page) will be used to illustrate the procedures of this protocol but any of the above three products are suitable for this purpose.

Figure 1. PerkinElmer 226 Five-Spot Card, example of filter paper for dried blood



A.2: Collecting capillary blood sample using sterile, single use lancet (HemoCue or similar)

Materials

1. Pencil/pen
2. Blood collection filter paper (PerkinElmer 226)
3. Alcohol skin swab
4. Safety Lancet (HemoCue AB or similar), sterile, single use
5. Sharps collection boxes
6. Laboratory/ case investigation form
7. Latex gloves for person performing bleeding
8. Ziploc bags (sealable plastic bags)
9. Silica gel desiccant (one ml adsorption), preferably with colour indicator

A.3: Capillary blood collection and drying method

A skin puncture may be performed on the finger or heel. The finger is preferred except in infants and children whose fingers are too small. The puncture site of the finger or heel affects the blood flow.

For the finger, the area with optimal vasculation and lowest sensitivity is the side of the fingertip about 3 mm from the nail bed (see figure 2). The middle and ring finger are best. The pulp on the tip of the finger should be avoided as it is very sensitive.

For the heel the puncture should be performed on the lateral or medial edges of the heel rather than the centre of the heel (see figure 3)

Figure 2. Recommended sites for finger puncture (arrows)

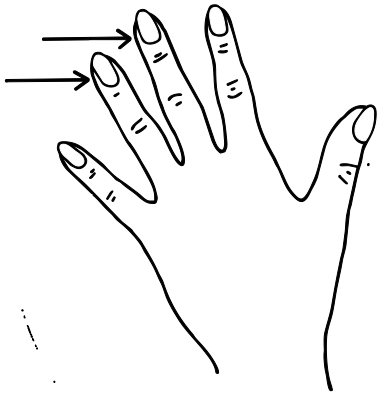
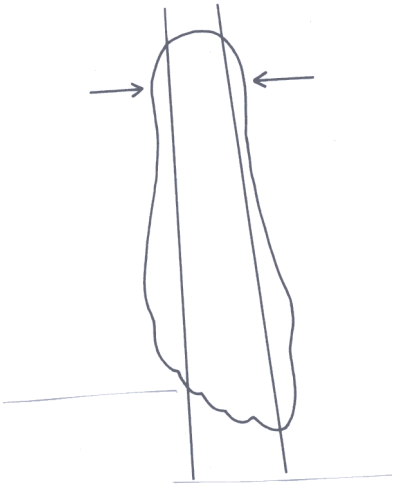


Figure 3. Recommended sites for heel puncture (arrows)



1. Label the DBS filter paper with necessary information for identification –the information must correspond with that included on the case investigation form.
2. Make sure the patient sits comfortably. A baby should be held gently but firmly by the parent.

3. For Finger: The hand should be warm and relaxed. The patient's fingers should be straight but not tense, to avoid stopping blood flow. For best results use the middle finger or "ring" finger for sampling.
4. Clean the puncture site with an alcohol wipe and allow to dry.
5. Twist off the protective tab of the safety lancet.
6. For the finger, use thumb to lightly press the finger from the top of the knuckle to the tip. This stimulates the blood flow towards the sampling point.
7. With the thumb's gentle pressure at the tip of the finger, place the lancet at the side of the fingertip. The flow is best at this point and is also the least painful site.
8. Press the lancet firmly against the finger or heel and push the plunger/trigger. The plunger will activate a needle that penetrates the skin by 2.25 mm and retracts back into the holder. After use, dispose of the device into a sharp's container.
9. The puncture site may be held facing down, below the level of the heart. If necessary, apply light squeezing pressure, until a drop appears.
10. Wipe away the first drop and collect subsequent drops of blood.
11. Allow one drop to fall onto each circle of the filter paper. Collect at least three circles and four or five if possible. **Ensure that the blood soaks completely through the paper**. Do not hold the filter paper against the puncture site.
12. Allow the filter paper to dry thoroughly (at least 60 mins) before enclosing in a bag with silica gel desiccant.

Note: Depending on humidity, drying may require up to 4 hours. The process is completed at room temperature, in a well-ventilated area and out of direct heat or sun.

• DBS stability: minimum is 2 weeks in airtight bags with desiccant bag at maximum 25°C or without desiccant in open bags

Drying stabilizes the IgM and reduces the chance of microbiological contamination. For drying, filter paper cards may be placed in a filter paper holder or similar device to keep cards separated from each other and avoiding direct contact with any surface.

13. Place dried blotting paper in Ziploc bag or similar, sealable plastic bag, preferably with active silica gel desiccant to prevent possible cross contamination and to keep desiccated. Each filter paper should be kept out of sunlight and stored in a cool place if possible until transported to the laboratory, as quickly as possible. It is preferable but not mandatory to use the reverse cold chain.
14. Remember safety (gloves for collector, correct disposal of sharps, single-use lancet, care with blood samples contacting handler).

B: Storage of DBS specimens

1. On arrival in the laboratory store dried blood spots in labelled individual Ziploc plastic bags or other airtight containers at 4°C until tested. Long-term storage should be at –20°C. One of the dried blood spots may be used for RNA extraction so ensure that each patient's blood spots are kept well separated.
2. For safety reasons handle blood spots only with gloved hands.

C: Extraction and detection of measles-specific IgM in dried blood specimens using the Euroimmun anti-measles virus NP ELISA IgM assay

Items needed:

Euroimmun anti-measles virus NP ELISA IgM assay. Pre-heat the ELISA sample buffer to 37°C.

a. Microplate 96-well (uncoated). U shaped, crystal clear preferred, (Greiner type PS 650101 or similar)
b. Hole punch, 4.76mm (3/16 inch)
c. Forceps
d. Micropipettes and tips

C.1. Extraction of dried blood spot

a. Punch out one (1) disc from one dried blood spot per case using a 4.76mm (3/16 inch) hole punch.

   Be sure to switch or clean the hole puncher between samples.

b. Transfer punched disc, using forceps, into a labelled, empty, uncoated microplate well and add 250 µl Euroimmun IgM sample buffer (from IgM kit) per sample (corresponding to a 1:101 diluted sample).
c. Ensure the disc is completely covered with sample buffer.
d. Incubate for 1 hour at 37°C.
e. Mix DBS disc and sample buffer by gently pipetting until completely homogenous and immediately transfer100 µl of the extract to the ELISA microplate for testing.

ELISA method: Test the 100 µl extracted DBS sample exactly the same as a normal serum sample in the Euroimmun anti-measles virus NP ELISA IgM assay, following manufacturer's instructions.

Annex A4 Dried Blood Spot (DBS) Protocols, additional protocols

Serion DBS Extraction

Note: Method was validated on filter paper Whatman Schleicher and Schull 903 for rubella virus. Other types would need to be validated.

• 50 - 100µl blood (venous or capillary) is dropped on filter paper

• Drying minimum 4 hours at RT, well ventilated and without direct heat or sun

• DBS stability: minimum is 2 weeks in airtight bags with desiccant bag at maximum 25°C or without desiccant in open bags

Directions for direct method (overnight extraction option below)

1. Using sterile hole puncher, punch one DBS into one well of the microtiter plate. Be sure to switch or clean the hole puncher between samples.

2. Prepare dilution buffer mixture by using 1 part RF solution and 4 parts dilution buffer solution.

3. Add 100µl dilution buffer mixture to each well containing a DBS. Be sure the DBS is completely covered by buffer.

4. Add all controls to the microtiter plate as needed.

5. Incubate at 37°C for 1 hour (no shaking necessary).

6. Prior to the first washing procedure, the wells have to be emptied. Tap the microtiter plate onto a stack of paper towels at which time, the DBS should be removed completely from the wells.

7. Proceed to the washing step following the standard ELISA protocol.

Directions for extraction of DBS overnight at 4°C

1. Using sterile hole puncher, punch two DBS into one well of a deep well plate or tube. Be sure to switch or clean the hole puncher between samples.

2. Prepare dilution buffer mixture by using 1 part RF solution and 4 parts dilution buffer solution.

3. Add 200µl dilution buffer mixture to each well or tube containing a DBS. Be sure the DBS is completely covered by buffer.

4. Incubate overnight at 4°C.

5. Following incubation, process the extracted DBS following the standard ELISA protocol by transferring 100µl of the extraction solution into the microtiter plates (after bringing all components to room temperature).