



Global Polio Laboratory Network

Guidance Paper 3

Poliovirus antibody testing for GPLN personnel using Dried Blood Spot

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1. Background

The rationale for implementing poliovirus antibody testing in Global Polio Laboratory Network (GPLN) facilities is to document the immune status of polio laboratory staff against all three types of poliovirus (PV), and to determine their potential need for immunization. It will also allow meeting GAP III requirements for PV antibody testing and vaccine during the Containment of PV2 in Phase II and Final Containment of PV in Phase III. Reference can be made to the Biorisk Management Standards described in GAP III Annex 2, 3 and 6, Sub-element 9.1.2 (Worker Health Programme) and Sub-element 9.2.1 (Vaccination of Personnel). Polio seroimmunity can be assessed using either serum or dried blood spots (DBS).

While serum is relatively easy to collect and process, countries that face difficulties in shipping liquid specimens may prefer to use DBS. DBS are prepared by applying a small amount of whole blood onto filter paper cards and dried. Specimens can be shipped ambient as non-dangerous goods. Care must be taken, however, to properly collect, dry, store and ship DBS to ensure valid and accurate results.

2. Objective

To describe the procedure for collecting, packaging, storing and shipping samples collected as dried blood spots (DBS) for poliovirus antibody testing of personnel in GPLN facilities.

3. Scope

This document applies to personnel of GPLN facilities (and other institutional personnel considered to have significant risk of exposure to poliovirus materials) who cannot be tested locally in a certified Polio-Essential Facility (PEF), or whose serum samples cannot be shipped to a PEF of their choice. The determination of PV antibody titres shall be done annually.

DBS specimens will not be used for testing parameters other than polio antibody at designated GPLN reference laboratory and the testing laboratory will not receive personal information related to any specimens.

4. Responsibility

The Head of GPLN polio laboratory is responsible for the implementation of this guidance document. He/she is responsible for :

- collecting valid specimens, timely shipment to designated reference laboratory, and obtaining feedback of results;
- maintaining complete documentation of specimens and confidentiality of results and records;
- implementing measures according to institutional policy for seronegative staff or those with low-antibody titer (below protective level) by providing booster vaccine, e.g. IPV vaccination if type 2 or bOPV vaccination if type 1 or 3, and follow-up antibody response evaluation.

5. Documentation

A linelist of all specimens collected and referred should be maintained containing the required identifiers such as Code of staff, Specimen ID, Date of Collection, Date of last OPV or IPV dose, No. years of exposure to Poliovirus materials, Referral Lab, Date referred, Result of antibody testing, Date Results Received, and Remarks (e.g. reimmunization, date, vaccine type, condition of DBS). An example of the linelist is provided in Section 15.

6. Biosafety Precautions

It is essential that Universal Precautions be taken while working with or collecting DBS specimens. DBS on filter paper are not considered to be a biohazard, but whole blood handled

during specimen collection may be hazardous. Appropriate personal protective equipment (PPE), including gloves and lab coat, should be worn to ensure safe handling of specimens.

Precaution should be taken to avoid needle injury. If a needle puncture should occur, the institutional policy for handling work-related injuries should be followed. Wash hands after handling DBS samples. Sharps and other contaminated materials should be disposed according to the laboratory's biosafety procedures.

7. Materials needed

7.1. The following materials will be supplied through the WHO Regional Office (Fig 1):

- Whatman Protein Saver Card #903* (Whatman 10531018) - 5 spots
- Cardboard drying rack, 10 cards slots (Whatman 10537173)
- Desiccant packs (Whatman 10548234)
- Humidity Indicator Cards 5%, 10%, 60% RH (DESCO 13859)
- Sealable (Zip-lock) plastic bags

Important:

Whatman Protein Saver Card #903, Desiccant packs and Humidity Indicator Cards should be kept securely sealed in a dry area of the laboratory that is protected from heat (e.g. direct sunlight, motor, etc..) and other adverse conditions during storage.

7.2. The following materials will be provided/prepared by the laboratory:

- Supplies for blood collection, e.g. single-use blood lancets for Fingerprick or syringe with needle and EDTA Vacutainer tube (purple top) for venipuncture collection.
- Alcohol pad
- Bandage
- Gloves for specimen collection and handling of filter paper cards
- Discard bins
- Waterproof marker for labelling

Fig. 1. Materials to be supplied by WHO for DBS specimen collection



8. Collection of DBS from Fingerprick

- 8.1. Clearly label each Whatman Protein Saver Card #903 with Specimen ID and Date of collection. Do not include personal identifiers other than Specimen ID.
- 8.2. Follow the Fingerprick procedure and the additional steps below (Fig. 2):
 - Before skin puncture, massage finger to enrich the blood flow towards the puncture site.
 - Clean the puncture area with an alcohol pad (or a suitable disinfectant) and allow to dry. Do not touch the puncture site or allow to come into contact with any non-sterile item or surface.
 - Puncture the skin with a single-use blood lancet.
 - Apply gentle pressure to the finger and allow a large drop of free-flowing blood to collect in the puncture site. Do not press the filter card against the puncture site.
 - Working quickly, hold the filter paper by the edges and touch the filter paper gently against the large drop of blood and in one step allow a sufficient quantity of blood to uniformly soak through until the circle is full or saturated. A completely filled or saturated spot will contain 100 µl of blood.
 - Repeat the procedure 4 times to fill all 5 circles of the card.
 - Do not touch the DBS circle once blood is applied.
 - Proceed to Section 10: Drying of DBS specimens.

Important:

- Do not squeeze or “milk” the finger/puncture site excessively as this might cause hemolysis of the specimen or result in collection of tissue fluids with the specimen.
- Perform a second puncture on another finger if more blood is needed to fill all the required circles.
- Do not layer successive drops of blood in the same collection circle.
- See example of a valid DBS specimen in Fig. 4 and bad spots in Section 13.

Fig. 2. Collection of DBS from Fingerprick



9. Collection of DBS from whole blood collected in EDTA Vacutainer tube

- 9.1. Clearly label each Whatman Protein Saver Card #903 with Specimen ID and Date of collection. Do not include personal identifiers other than Specimen ID.
- 9.2. Follow the procedure for venipuncture and the additional steps below:
 - Collect blood in EDTA tube to the recommended volume so that the anticoagulant is at the proper dilution.
 - Gently invert the tube several times to mix the blood and anticoagulant thoroughly.

- Using a pipette, gently apply 100 µl of blood to the center of one circle of the filter card until the circle is fully saturated (Fig. 3). Caution: Do not touch the surface of the card with the pipette tip.
- Repeat the procedure 4 times to fill all 5 circles of the card.
- Proceed to Section 10: Drying of DBS specimens.

Important:

- Do not apply blood more than once in the same circle.
- Do not touch the DBS circle once blood is applied.
- See example of a valid DBS specimen in Fig. 4 and bad spots in Section 13.

Fig. 3. Collection of DBS from a Vacutainer tube

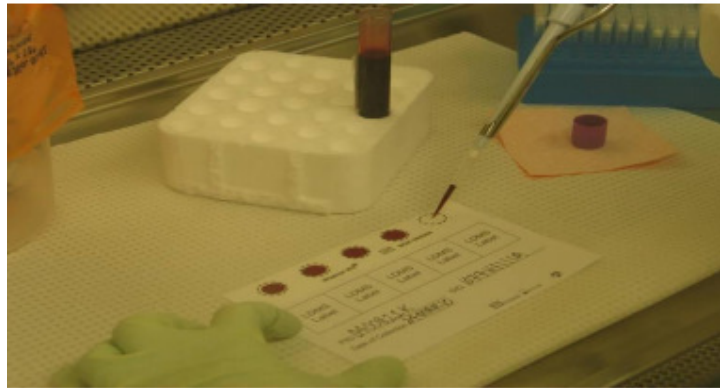


Fig. 4. Example of a valid DBS specimen



10. Drying of DBS Specimens

- 10.1. Allow the blood spot to fully air dry without the card flap covering the spots, in a clean, dry place, e.g. Drying rack (Fig 5) or biosafety cabinet, that is protected from insects, rodents, and direct sunlight for at least 4 hours or overnight (in areas with higher humidity). DBS change from bright red to dark red or brownish colour as they dry (Fig. 4).
- 10.2. When drying is complete (i.e., no red areas are visible), tuck in the flap of the Whatman Protein Saver Card #903 as indicated on the card.
- 10.3. Seal the card in a zip-lock bag containing at least two desiccant packs and one Humidity Indicator Card (HIC). Store only one specimen card in a single bag (Fig. 5).

Important:

- Do not heat, stack or allow DBS to touch other surfaces during the drying process.
- Do not use electric dryer or oven to dry the cards.
- Keep away from direct sunlight. DBS should not be dried near an open window as sunlight, dust, and in some cases, flying insects may come in contact with the DBS during the drying process.
- Care must be taken to avoid exposing DBS to environmental conditions that may compromise the integrity of the specimens.

Additional tip:

- Ensure that the bag is properly sealed to prevent moisture from entering.
- HIC and desiccant packs can be re-charged before use. If the HIC is pink at 10% level, recharge card and desiccant packs by heating at 50-60°C for 3-4 hours in a drying oven. Heat only the HIC and desiccant packs, and not the DBS. Cool for 10 minutes and IMMEDIATELY RETURN card and desiccant packs to sealable plastic bag.

Fig. 5 Example of drying DBS specimens in Whatman Cardboard drying rack and storage of DBS



11.Storage of DBS Specimens

- 11.1. DBS cards in sealed bag (with desiccant and HIC) may be stored in a refrigerator (2-8°C) or at ambient temperature (15 - <30°C) for up to 14 days. However, for optimal preservation, long term storage (>90 days) at ≤ 20 °C is recommended.
- 11.2. Store DBS cards (preferably in a Styrofoam box) in a cool, dry place that has no exposure to direct sunlight or dripping water (e.g., malfunctioning refrigerator, ceiling), free of insects and rodents, and where ambient temperature will not exceed 30°C until shipped to a reference laboratory.
- 11.3. Check HIC in bags weekly while DBS are stored at ambient temperature or in a refrigerator. Change (or recharge) desiccant packs and HIC, if HIC colour changes from blue to pink and document date.
- 11.4. Make sure the zip-lock bags are sealed tightly.

12.Shipping of DBS Specimens

- 12.1. Ship DBS specimens to Centers for Disease Control and Prevention (CDC) or other polio serology reference laboratory of choice, as soon as possible.
- 12.2. DBS cards that are stored at room temperature may be shipped at ambient temperature.
- 12.3. DBS cards stored in a refrigerator or freezer must be equilibrated to room temperature (minimum of 30 minutes) prior to shipping under ambient conditions.

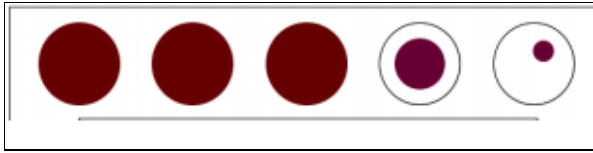
- 12.4. Inspect DBS specimens for contamination or humidity change, and replace or add desiccants prior to shipping. Record any observation (good or bad condition).
- 12.5. DBS are exempt biological specimens according to ICAO and IATA . They can be shipped via courier (or EMS Postal Service) as non-dangerous goods, avoiding arrival on weekend or holidays.
- Use the basic triple-packaging system where the primary container is the filter paper that contains the DBS, the sealable (zip-lock) bag enclosing the individual DBS card (with biohazard marking) as the secondary container . The individual DBS card in zip-lock bag may be placed in a bigger sealable plastic bag with additional desiccant packs (5-10 pcs. depending on the number of cards) and HIC to provide additional protection against humidity before placing in tertiary container..
 - Place in a tertiary container that is sturdy and of high quality paper, e.g. shipping envelope supplied by courier.
 - Enclose a linelist of DBS specimens containing the following information: *Laboratory name, Specimen ID and Date of collection*. Ensure that the list matches the specimens in the package.
 - No biohazard sign or content marking is required.
 - Clearly indicate delivery and return address.
 - Refer to Polio Lab Manual 4th Ed. for other relevant shipping arrangements.
- 12.6. Email an electronic copy of the linelist at least 2 weeks in advance of shipping date.
- For shipments to CDC, send email to Dr William Weldon <wiw4@cdc.gov> and Dr Steve Oberste <mbo2@cdc.gov>.
Address the package to:

Dr William Weldon
Centers for Disease Control and Prevention
Division of Viral Diseases
Polio and Picornavirus Laboratory Branch
1600 Clifton Rd., N.E., Mailstop G17
Atlanta, Georgia 30329
Email address: wiw4@cdc.gov
Tel. No: +1-404-639-5485

- For all shipments, copy the following in email communications:
WHO Regional Laboratory Coordinator and WHO HQ (Dr Ousmane Diop <diopo@who.int> and Dr Fem Paladin <paladinf@who.int>)

13. Examples of Invalid Spots and Invalid Collection of DBS

- Sample with variable volume, the last two circles are not usable.

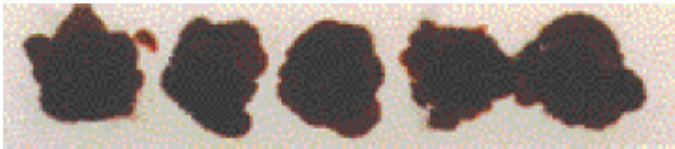


- Spot with incomplete absorption resulting to insufficient blood volume.



Possible causes:

- Removing the filter paper before blood has completely filled the circle or before blood has soaked through the other side.
 - Applying blood to the DBS card with a capillary tube.
 - DBS paper coming in contact with gloved hands or substances such as hand lotion or powder, either before or after blood specimen collection.
- DBS specimen appears scratched or abraded



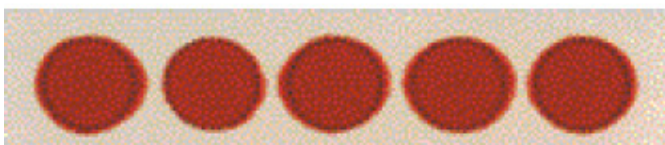
Possible causes:

- Blood sample was potentially rubbed against the finger
 - Applying blood with a capillary tube or other device.
- DBS specimen appears supersaturated



Possible causes:

- Applying excess blood to filter paper
 - Applying blood on both sides of filter paper
- DBS specimen not dry before shipment.



Possible cause:

- DBS not allowed to air dry completely before placing in zip-lock bag or before mailing. DBS must dry for a minimum of 4 hours or overnight, especially in areas with high humidity before packaging.
- DBS specimen appears clotted or layered. The volume of blood will not be uniform between spots resulting in errors during the testing process.



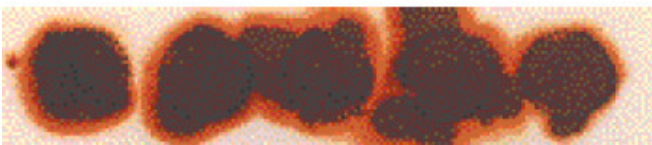
Possible causes:

- Touching the same circle on the filter paper to blood drop several times
 - Filling circle on both sides of filter paper
- DBS with diluted, hemolyzed or contaminated blood



Possible causes:

- Squeezing or “milking” the area surrounding the puncture site
 - Allowing filter paper to come in contact with gloved or ungloved hands or substances such as alcohol, water, hand lotion, or powder, etc. before or after blood specimen collection
 - Exposing blood spots to direct heat,
- DBS specimen exhibits serum rings



Possible causes:

- Not allowing alcohol to dry at puncture site before making the puncture
 - Allowing filter paper to come in contact with alcohol, hand lotion, etc...
 - Squeezing area surrounding puncture site excessively
 - Drying specimen improperly
 - Applying blood to filter paper with a capillary tube
- DBS card where no blood was added



14. References

- WHO Global Action Plan (GAP III) to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use. WHO/POLIO/15.05 .
http://polioeradication.org/Portals/0/Document/Resources/PostEradication/GAPIII_2014_CH.pdf
- ACTG/IMPAACT Lab Tech Committee, Processing of Dried Blood Spots Standard Operating Procedure (LTC-SOP-55 v2.0), Users of the ACTG/IMPAACT Laboratory Manual, 19Mar2012.
- Module 14: Blood Collection and Handling.
cdrwww.who.int/diagnostics_laboratory/documents/guidance/pm_module14.pdf
- Nico Grüner¹, Oumaima Stambouli¹, R. Stefan Ross. Dried Blood Spots - Preparing and Processing for Use in Immunoassays and in Molecular Techniques. *J. Vis. Exp.* (97), e52619, doi:10.3791/52619 (2015).
- GE Healthcare Life Sciences. Simple Spot check. Oct 15, 2016.https://www.gelifesciences.com/gehcls_images/GELS/Related%20Content/Files/1392818611307/litdoc28984392_20161015134119.pdf
- Centers for Disease Control and Prevention. Shipping Guidelines for Dried-Blood Spot Specimens.
[https://www.cdc.gov/labstandards/pdf/nsqap/Bloodspot Transportation Guidelines.pdf](https://www.cdc.gov/labstandards/pdf/nsqap/Bloodspot_Transportation_Guidelines.pdf)

15. Example of Linelist for Documentation of DBS samples

No.	Staff Code	Specimen ID	Date of Collection	Collected by/at	Date of last OPV (O) or IPV (I)	Polio Staff (PL) or Others (O) and no. yrs. exposed to PV material	Date Shipped	Referral Lab	PV1 Titer	PV2 Titer	PV3 Titer	Remarks	Date Results Received	Reviewed by