

National Microbiology Laboratory (NML)

DRIED BLOOD SPOT (DBS) COLLECTION



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NOTE: Children under the age of 13, and individuals who have had a mastectomy performed, or are currently taking blood thinners, should not undergo a DBS card collection.



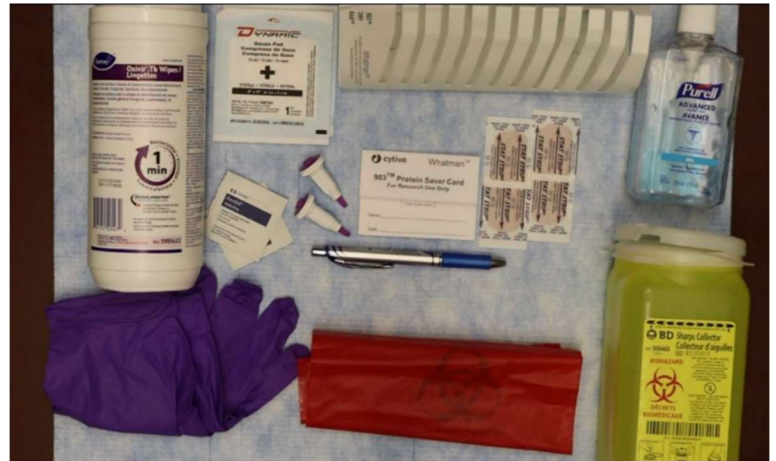
DRIED BLOOD SPOT (DBS) COLLECTION

1. Setting up your workstation

Before beginning any sample collection, please confirm you have all the necessary supplies.

You will need the following supplies:

- Surface decontaminant
- Absorbent material
- Hand-washing station/hand sanitizer
- Latex-free gloves
- Sharps container
- 2 adhesive bandages (such as Band-Aids®)
- 2 sterile gauze
- 2 alcohol swabs
- 2 lancets
- 1 dried blood spot (DBS) card
- Pen or pencil
- Waste bag
- Drying rack
- Carrying container (optional)



To prepare your workstation for a DBS sample collection, first wipe down the table with approved decontamination material (either an NLHRS-provided decontaminant, or any over-the-counter disinfectant wipes or spray, such as Lysol®).

Cover the surface with an absorbent material with a liquid proof barrier (if none are available, use any type of absorbent material, such as paper towels), then lay out the supplies listed above.

Here is some additional information on some of the required supplies:

LANCETS

The lancets provided for DBS collection are different from the lancets used for other procedures such as taking a blood-glucose measurement. Ensure that you use the lancets provided as they are designed to help collect more blood from the client. The lancets that can be provided by the NLHRS are:



The HTL-Strefa Haemolance Max Flow lancet is push-button operated. The button on the end needs to be depressed to engage the blade. Ensure that the lancet is held firmly against the client's finger when pressing the button.



The BD Microtainer® Contact Activated Lancet is contact operated. This lancet must be pressed firmly into the client's finger in order to activate the lancet.



The Owen Mumford Unistik Touch Lancet is contact-operated, but is meant for more calloused hands, and is provided upon request. This lancet must be pressed firmly into the client's finger in order to be activated.



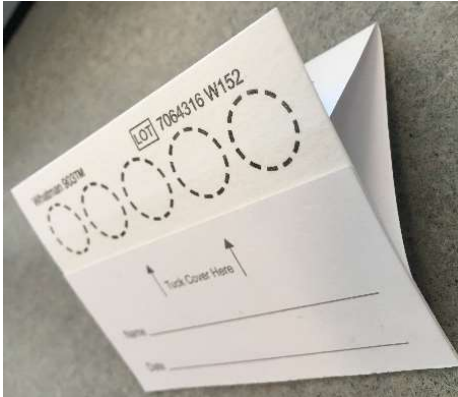
Lancets are single-use only. The blade automatically retracts into the plastic sheath after activation and cannot be reactivated after it has been used. Dispose of all used lancets into a sharps container.

DRIED BLOOD SPOT CARD (903™ Whatman Protein Saver Card)



The 903™ Whatman Protein Saver dried blood spot card, or “DBS” card, is a medical device. There are 5 dotted circles in the sample collection area as a guide to show how much blood to collect on the card.

Do not touch the sample collection area with your bare hands or lay on dirty surfaces as this can contaminate the sample area.



There is a cardboard flap on the card that protects the sample collection area where the five dotted circles are located. It also acts as a stand for the card.

Fold open the flap and bend it back so it can balance the card in the open position, which allows the sample collection area to be exposed, but not be touching anything.

2. Preparing the client:

GET WARM

While you are talking to the client about the testing procedure, have them warm up their hands to increase blood flow. You can do this by:

- having them rub their hands together quickly.
- putting their hands between their knees.
- doing some quick exercises like arm circles.
- or by giving them a warm drink (water/tea) or handwarmer to hold.

GET CLEAN

The client should sanitize their hands. If a station with warm water and soap is not available, please provide hand sanitizer, or wet wipes to ensure their hands are clean.

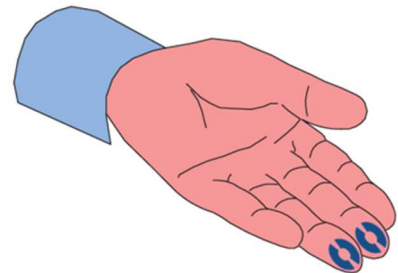
Once the client is ready, put on your gloves.

GET COMFORTABLE

Ensure your client is seated comfortably.

Choose a finger, either the middle finger or ring finger, preferably on their non-dominant hand (as it is usually less-calloused). Avoid using the thumb (pulse), the pointer/index finger (calloused) or the pinky finger (too small).

Have the client set their hand flat on the table.



GET CLEAN AGAIN

Check that the chosen finger is healthy and free from other wounds or infections (if so, choose another finger).

Sterilize the finger from the tip to the first knuckle with an alcohol swab.

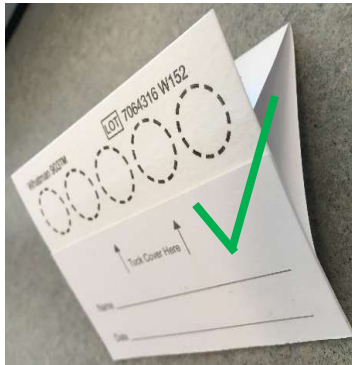
Allow the finger to air dry while you prepare for the collection, ensuring that they do not touch anything.



3. Prepare for the Collection:

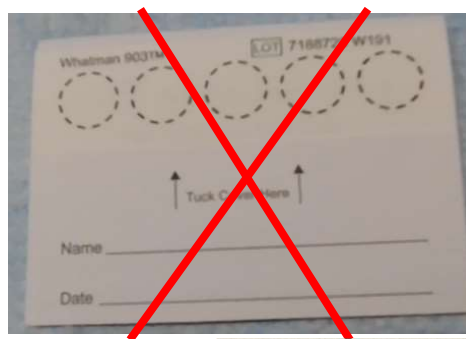
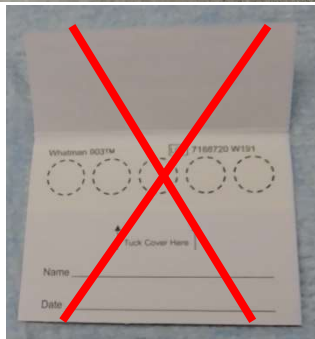
PREPARING THE CARD

Record a collection date, and the personal identifiers required by your provincial laboratory, on the front area of the DBS card, using either a pen or a premade sticker.



Fold back the flap of the card so that the card can sit on the work surface without anything touching the sample collection area.

Do not collect the sample with the card lying flat, or with the protective flap tucked behind, as blood can wick through onto the protective flap or onto the work surface.



Right before the collection, open a package of gauze and prepare a bandage.



PREPARING THE CLIENT

Prepare for the collection by making sure the client's hand is flat on the surface, and positioning yourself and your client in such a way that the collection won't be awkward. You want to make sure that you are not placing the client in an uncomfortable position when you are collecting.

It can help to choose the side of the client's finger that is closest to the collection card, so that their wrist/arm will not be twisted during the collection.





PREPARING THE LANCET

To uncap the lancet, twist off the cover and dispose of it in a sharps container.



Step 1: Twist

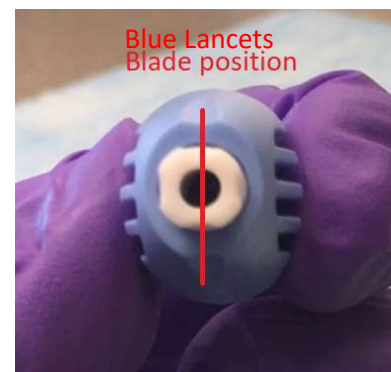
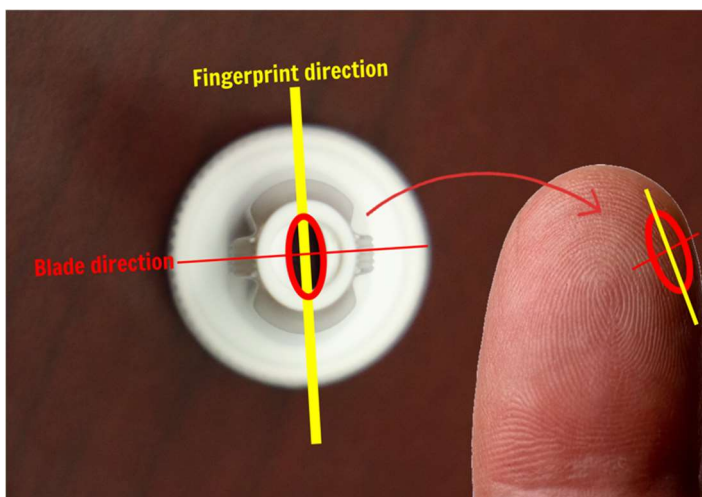
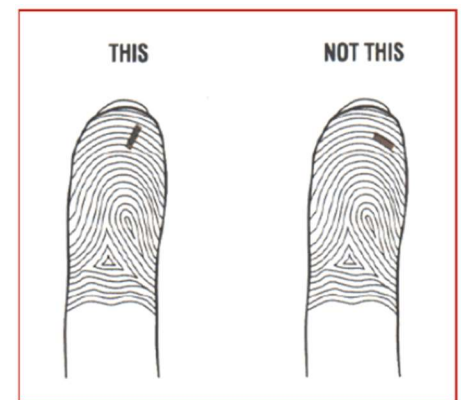


Step 2: Pull

As in this picture, try to use the lancet off-centre. Do not poke in the centre of the finger (the finger pad) or on at the tip.

Identify the direction of the fingerprint on the client's fingers.

Orient the lancet so that the lancet poke is across (or perpendicular to) the fingerprint.



This helps ensure the blood will form nice droplets, instead of spreading across the finger. Holding the poked finger downwards also helps the blood to pool into droplets.

You can now use the lancet and collect a DBS sample.



4. Using the lancet and Collecting a Dried Blood Spot sample

Ensure you have done the following as per Sections 2 and 3:

- Put on your gloves.
- Cleaned the client's finger with an alcohol swab.
- Recorded the client's identifier and the collection date on the card.
- Have a gauze pad and adhesive bandage ready.
- Removed the lancet cap.

Hold the client's hand and finger firmly on the table to prevent them from involuntarily jerking their hand away.

Position the lancet on the side of the finger, angled across the fingerprint and hold firmly against the finger.

For the HTL Strefa Haemolance Max Plus lancet (purple lancet), hold the lancet between your index and middle fingers, press lancet firmly into finger, and depress the button on the end of the lancet with your thumb until you hear a click (see photo below).



For the BD Microtainer Contact-activated lancet, and Owen Mumford Unistik Touch lancet; hold the lancet with your thumb and index finger, position it on the side of the finger, and activate the lancet by pushing it firmly against the finger until you hear a click (see photo below).

Discard the lancet immediately in the sharps container.





Using the prepared gauze, quickly wipe away the first drop of blood. This will remove any residual alcohol that may be left after cleaning the finger. Wiping with the gauze can also help stimulate blood flow for a better collection.

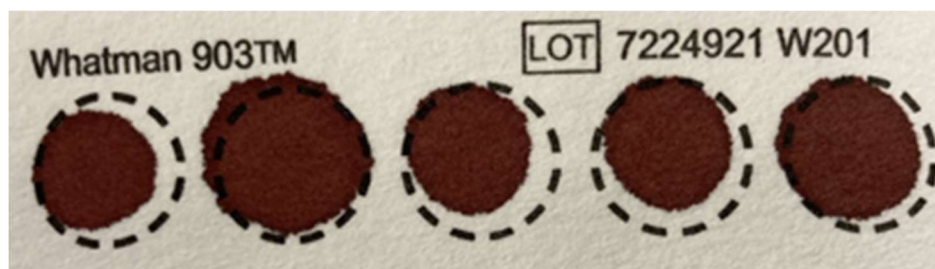


*Example of
sharps container*

Start collecting the blood droplets on the card.

Allow blood droplet to form on the finger, then touch the droplet to the card, allowing it to diffuse into the filter paper. Avoid touching the finger to the card (if a finger touches the card, simply continue the collection and try to avoid it in the future as it can increase the risk of contamination).

Ensure droplets are large enough to soak through (or saturate) the card, and fill the dotted circle, creating a blood "spot."

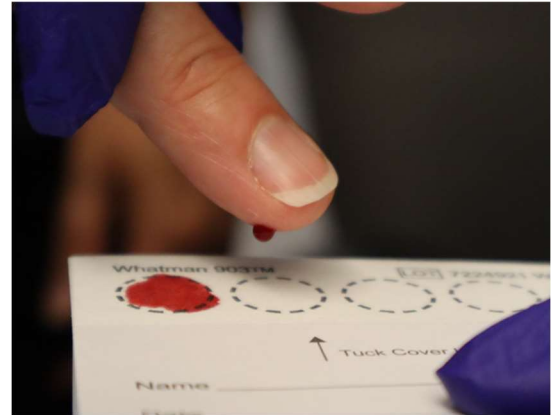
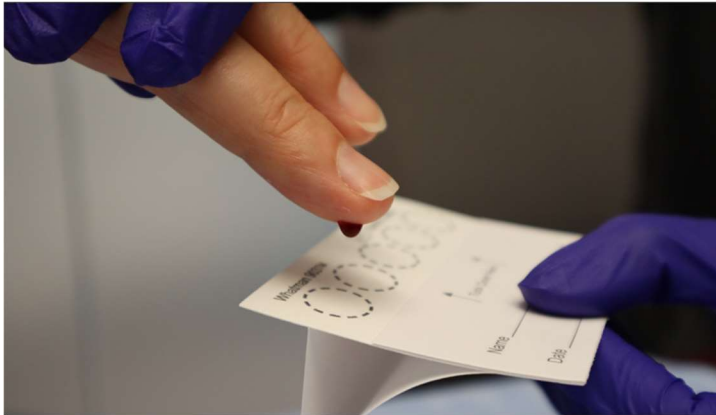


Example of grouped, saturated samples



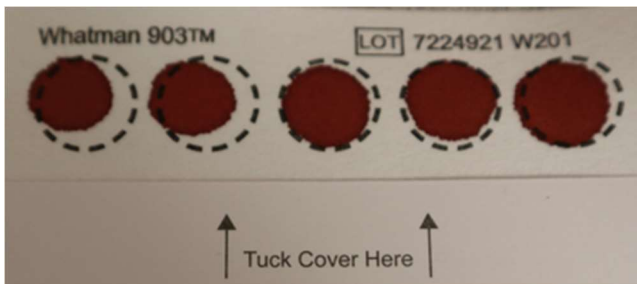
If one droplet does not fill the circle completely, place the next droplet directly beside the previous one in the remaining white space in that circle, in order to create a full “spot.”

NOTE: The droplets can soak into each other and overlap but DO NOT REPEATEDLY LAYER MULTIPLE DROPLETS directly on top of each other. This oversaturates the card and may make the sample invalid.

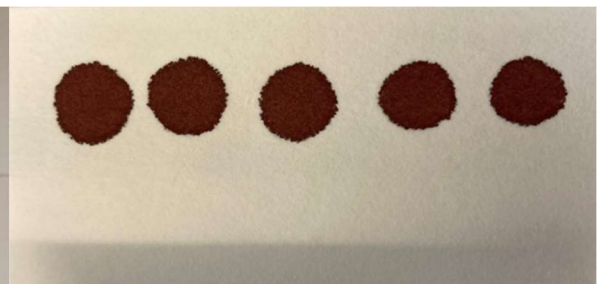


It is important to take the time to fill all of the circles if possible. Be patient, as the time and speed of collection can vary from person to person, as well as due to many other factors (temperature, etc.). We request all five circles to be filled and saturated (soaked through to the back of the collection area) in order for all the testing to be completed. However, if five full circles cannot be filled, please send the DBS card for testing anyway, and we will test what we can based on a provided priority list.

Front of Card:



Back of Card:



Any blood that spreads or falls outside of the collection circles can still be used for testing, as long as it is on the collection area (as seen in the photo here).



After collecting the sample, use a gauze pad to wipe away any remaining blood on the client's finger. The client can also apply pressure with the gauze pad to stop the bleeding. Help the client apply an adhesive bandage.

Clean up and appropriately dispose of any used materials. Refer back to the Section 1, “Setting up your collection station”, in order to prepare for the next client.



5. Troubleshooting difficult collections (stimulate blood flow)

If the client does not have a lot of blood flowing, there are a number of things you can do:

- Wipe the finger again with the gauze to encourage blood flow.

- Squeeze the finger gently. NOTE: do not milk/pull down on the finger, like a tube of toothpaste. Pressing too hard will cause hemolysis and introduce excess body fluids into the sample.



- Ask the client to stand up if they feel comfortable and do not get queasy at the sight of blood, and have their arm pointing downwards as gravity can help the blood flow. Additionally, if the client is unable to stand, you can also place some absorbent material on the floor, and bring their arms down to their sides, collecting blood on the DBS card over the absorbent material.



- You can also gently squeeze or massage the arm in a downward motion or ask the client to massage their own arm. This will help the blood to flow.



- If you have not collected enough circles and the blood has stopped flowing, ask the client if you can poke another finger. It is better to have a well-collected sample so all the testing can be completed and you don't have to collect another sample at a later date.



6. Good quality vs. bad quality samples

GOOD QUALITY SAMPLE



A good quality sample has 5 circles completely filled, with the blood fully saturated through the card.

Remember, it is OK if blood goes over the dotted line, as any part of the sample collection area can be used for testing.

Here are the hole punch sizes used for testing in the lab.

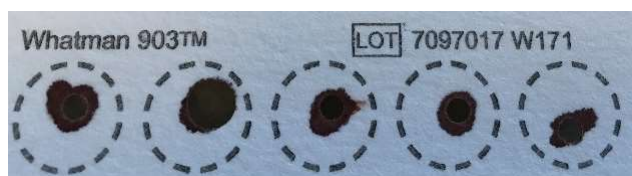


The smaller holes (1/4 inch) are the size required for screening of HIV, Syphilis, and Hepatitis B and C, and the larger, full circle (1 inch) punches are the size required for confirmatory testing of HIV and HCV exclusively (unfortunately, we cannot perform confirmatory testing for Syphilis).

A good quality sample gives the technician the ability to perform multiple tests. However, it is important to note that whatever the sample size or sample quality, please send all collection samples to the NLHRS, and we will test what we can. Priority lists should be included with or written directly on requisition forms in order to assure that testing is performed in the required priority.

BAD QUALITY SAMPLES

UNFILLED CIRCLES - SPOTS ARE TOO SMALL



The blood spots on this card are too small for testing. It would have been better to put the small drops of blood beside each other to completely fill one circle. It is OK for the droplets to flow into one another and overlap. If the blood stops flowing, ask the client if you can poke a different finger.



UNSATURATED CIRCLES – NOT SOAKED THROUGH



Although the front on this card looks OK (the circles appear filled), the blood did not soak all the way through to the back of the collection area (the circles are not saturated). This sample cannot be used for testing.

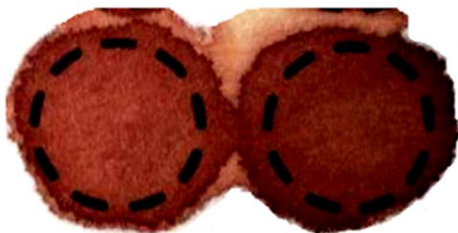
Five full circles should be filled and completely saturated to have enough sample for testing.

OVERSATURATED SAMPLE (LAYERED)



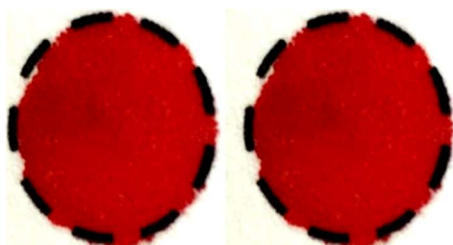
These circles are well filled, but oversaturated. Multiple blood droplets were layered one on top of each other repeatedly. This changes the concentration of blood in the circle so this sample is not useable for testing.

CONTAMINATED SAMPLE



This sample is contaminated. Either the finger was squeezed too hard and the blood was haemolyzed, causing excess bodily fluids to be introduced into the sample, or the sample was stored inappropriately and became contaminated (ex: from getting wet). This sample is not useable for testing and will have to be recollected.

WET SAMPLES - NOT DRIED PROPERLY



This sample was not dried sufficiently before packaging and shipping it to the lab. It is still bright red after a number of days of being in transit, indicating it is still wet. This sample cannot be used for testing.



7. Drying the samples

After the sample is collected, the card will need to be dried for at least 3 hours. This step is critical.

Dry the DBS cards in a secure location with good airflow to prevent samples from being tampered with, away from dust or direct sunlight.

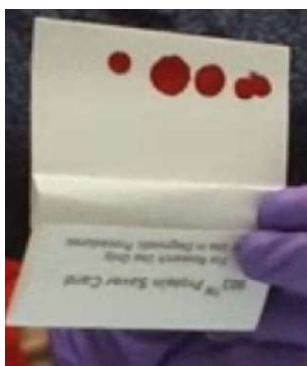


If you are collecting multiple cards, a drying rack can be used to minimize the amount of space you need.

The disposable cardboard racks provided can be folded to stand up, and can hold up to 11 cards (as seen to the left).

They can be used multiple times, but dispose of them if they become contaminated or dirty.

To put the card in the rack, fold the flap of the DBS card all the way back, completely exposing the sample collection area (as seen below).



This allows the card to slide into the rack without the sample area touching the rack, and keeps the sample area separate from the other cards beside it. This prevents samples from getting contaminated by the other cards.

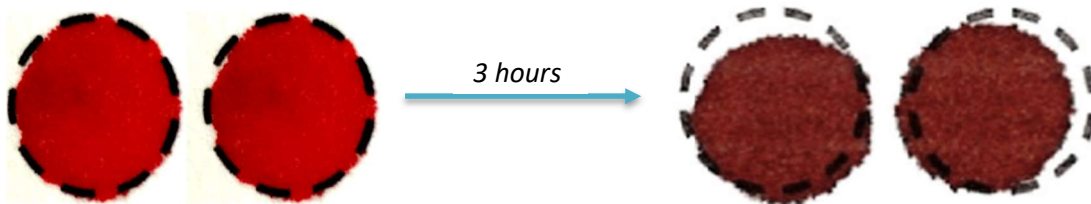


If cards need to be transported to a secure location to finish drying, a plastic carrying case can be used (these can be provided). Velcro straps are provided that can anchor the racks to the bottom of the case.

Put a lid on the case to transport the samples to a secure location. Once there, remove the lid to allow air flow to complete the drying process.



After the samples have dried for at least 3 hours, they should no longer be bright red. They should be a darker, reddish-brown colour.



Samples can be left out overnight to dry if they are unable to be packaged after three hours; however, they must be left in a secure, locked location, and must be packaged as soon as possible. Samples cannot be left to dry over an extended period of time, like a weekend.

Once the samples are completely dry, they can now be packaged for storage and shipment.

8. Packing the samples

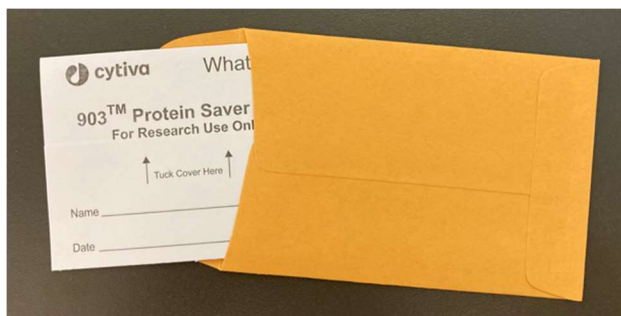
Fully dried samples need to be stored in a dry, cool environment, and should be packed as soon as possible.

DBS cards can be batched in groups of up to 10 cards. With each batch, you will require:

- One specialized gas-impermeable bag (Bitran bag).
- One yellow envelope per DBS card.
- One desiccant pack.
- One humidity indicator card.

NOTE: the Bitran bag provided is NOT a Ziplock bag. DO NOT make any substitutions. The Bitran bag is specially designed to prevent humidity from getting into the bag.

Ensure that you use the correct bag when packaging.

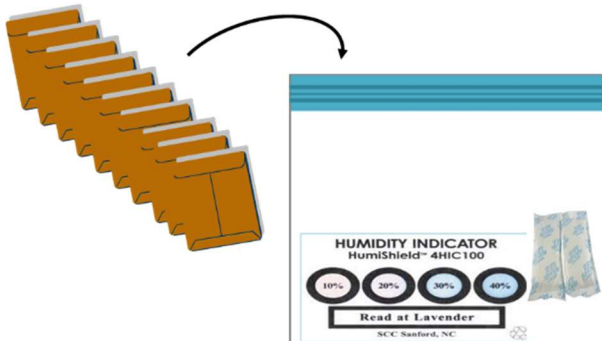


To pack a fully dried card, fold the protective flap over the sample collection area and tuck it in, then place it in a provided yellow envelope.

Do not glue or tape the envelope closed.



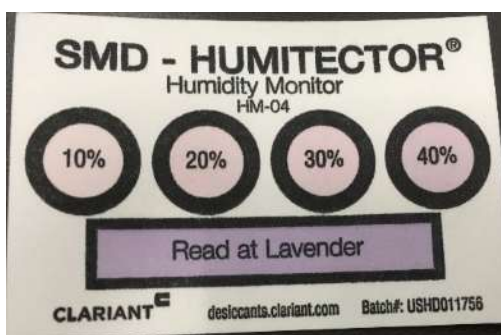
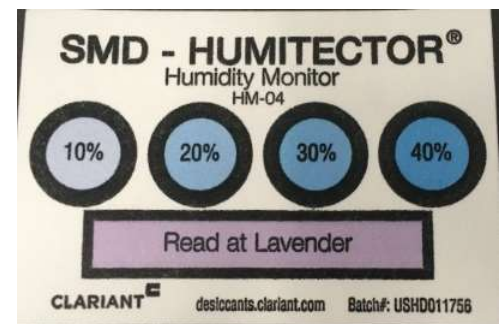
Place the enveloped card in the gas-impermeable bag with a desiccant pack, and a humidity indicator card.



You can have up to 10 enveloped cards per Bitran bag.

Remove all the air from the bag, and firmly press the strip at the top to seal.

The humidity indicator card will show if the humidity in the bag is getting high and the desiccant is saturated. If the circles are blue, the bag and desiccant are working and the humidity is low. Check the humidity frequently.



If the desiccant is saturated (full of moisture), the circles on the humidity indicator card will turn pink. This means that the desiccant is no longer keeping your samples dry. Replace both the desiccant and Bitran bag. After the replacements, the humidity indicator card should turn back to blue confirming your new desiccant and bag are working. Recheck the humidity frequently. If the humidity indicator doesn't recharge, then replace it.

If you are not sending the packaged samples right away to the lab, store them in a fridge or freezer. Do not keep the packaged samples at room temperature longer than one week.



9. FAQs

Sample Quality

Q - The sample is not collected very well (fewer than five circles filled), and the client did not want to do another lancet poke. Should I discard the sample?

A - Do not discard the sample. Please send it to the lab. The laboratory will assess the sample to see if there is enough for any of the testing requested. Priority lists of desired tests are requested to be provided by every site so that testing can be done according to the stated priority.

Q - Blood soaked through onto the flap of the card or dripped onto the cardboard covering of the DBS card. Is this OK?

A - Yes, please dry the card as usual, making sure that the contaminated parts of the card do not come in contact with anyone else's card, then send card for testing as usual.

Sample Packing

Q - I finished collecting samples at the end of the day and need to go home before they are finished drying. Do I need to wait to package them? How long can I let them dry for?

A - You can let the samples dry overnight if it is not convenient to package them within three hours. They are still valid if left 12-18 hours before packaging, but do not leave them longer than that.

Q - We are collecting a few samples over the course of a few days spanning two weeks. Do I need to pack and send them each day?

A - You do not have to send the samples each time you collect. If you have a fridge to store them in, and you pack them properly in a Bitran bag with desiccant each day, you can combine your samples over a few days and send them together.

Humidity indicator cards and desiccant

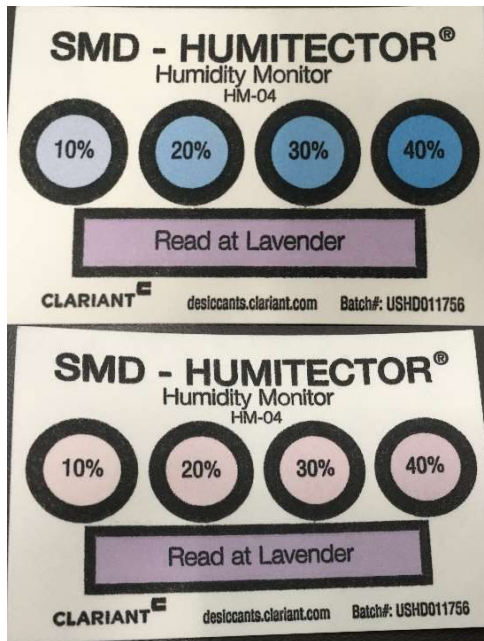
Q - I took the humidity indicator out of the bag before packing the samples, and it turned pink. Is it still OK to use?

A - Yes, the humidity indicator card will 'recharge' itself and turn back to blue when stored with a good desiccant pack and a working Bitran bag. If it doesn't return to blue, use a new indicator.



Q - The circles on the humidity indicator card stored in a bag with a desiccant have turned pink. What does that mean?

A - If the bag with a desiccant is no longer able to keep the humidity indicator card spots blue (i.e., humidity below 10%), then the desiccant has soaked up a lot of moisture and will no longer be able to keep the samples dry. Place the DBS cards in a new Bitran bag with a new desiccant, then request more Bitran bags and desiccant to be sent, and review your storage practices to make sure the desiccant is not exposed to humid air for very long.



Example of a humidity card in a dry environment. The spots are blue or shades of blue.

Example of a humidity card that has been exposed to humidity. All the circles are pink.

List of abbreviations/terminology

NLHRS: The National Laboratory for HIV Reference Services, a section of the Public Health Agency of Canada (PHAC) that runs the Community-based DBS program supporting this alternate form of testing.

DBS: Dried Blood Spots are a form of blood collection where blood is blotted and dried on filter paper.

Haemolyzed: When red blood cells burst and a substance called hemoglobin is released which can cause errors in testing of the blood samples.

Humidity: A measure of water vapor in the air. This greatly affects DBS cards, and the ability to accurately test them for infectious agents.

Lancet: A small medical device used for collecting small amounts of blood.

Desiccant: Material used to absorb the humidity and moisture in the air, usually available in pouch form.



NML Dried Blood Spot Collection

Blood Flow Tips and Tricks

This document is a information sheet on tips for assisting and potentially increasing blood flow during the collection of Dried Blood Spot (DBS) cards.

1. Before the DBS collection

a) Offer water to make sure the client is hydrated.

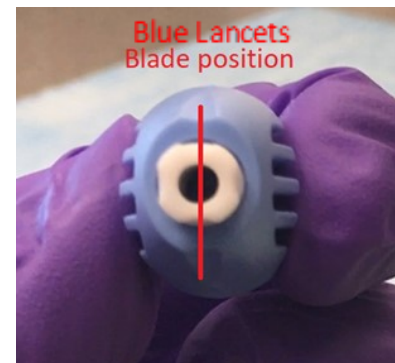
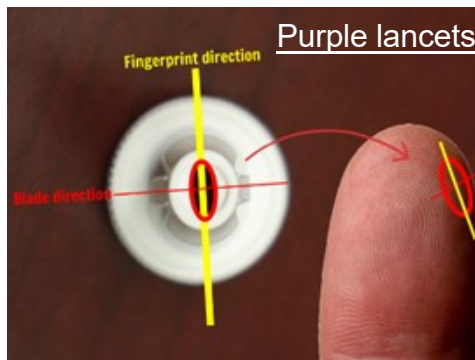
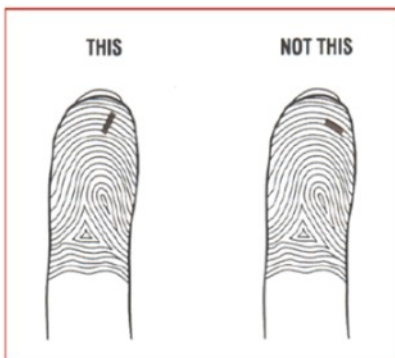
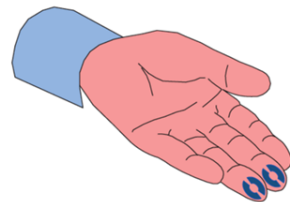
b) Have the client warm up their hands by:

- ▶ Rubbing their hands together.
- ▶ Putting their hands between their knees.
- ▶ Doing quick little exercises like arm circles.
- ▶ Giving them handwarmers or a hot drink to hold.



c) Choose either the ring-finger or middle finger on the non-dominant hand of the client.

- ▶ Avoid the thumb (pulse), index/pointer finger (calloused), and pinky (too small).
- ▶ Identify the direction of the fingerprint, and try to orient the lancet to poke across the fingerprint which helps the blood form into droplets .



2. During the DBS collection

- a) **Quickly wipe away the first drop of blood with gauze** – this removes clotting factors and residual alcohol.
- b) **Have the client hold their hand below heart level.**
- c) **Have the client stand if they are comfortable, with their arm pointing downwards.** If the client is unable to stand, have them hold their arm down by their sides and collect the sample kneeling.



3. If blood flow stops before filling the DBS card

- a) **Wipe the finger again to encourage blood flow.**
- b) **Ask the client if you can poke another finger.**



BE PATIENT. DBS collection can take time. We require a minimum of four filled circles to complete all testing. However, if enough circles can't be filled, please send the DBS card anyway, and we'll test what we can.



NML Dried Blood Spot Collection – Experienced User Protocol

This is an information sheet on the preparation, collection, drying, and packaging of Dried Blood Spot (DBS) cards. Additional information can be found in the DBS Collection training manual and video, or by contacting the NLHRS at: nml_dbs-lnm_gss@phac-aspc.gc.ca.

NOTE: Children under the age of 13, and individuals who've undergone a mastectomy or are currently on blood thinners, should not undergo a DBS card collection.

1. Preparing workstation

Please confirm you have the following supplies before starting:

- 2 alcohol swabs
- 2 sterile gauze pads
- 2 single-use lancets
- 1 DBS card
- Latex-free gloves
- Sharps container
- 1 gas-impermeable Bitran bag
- 1 coin envelope
- 1 humidity indicator
- 1 desiccant pack

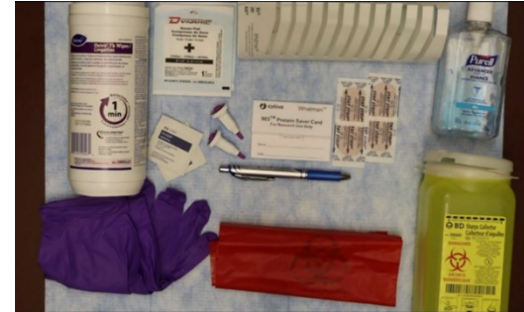


Figure 1

Your work surface should be wiped down between each collection with an appropriate surface decontaminant, then covered with an absorbent material (preferably with a liquid proof barrier). Set up work station (Fig. 1), then clean hands and put on gloves.

2. Preparing client

Record collection date and personal identifier required by your provincial laboratory on the DBS card while your client warms their hands by:

- ▶ Rubbing their hands together/putting their hands between their knees
- ▶ Holding a warm drink, or handwarmer.
- ▶ Doing quick exercise like arm circles.



Figure 2

Client must sanitize their hands, preferably with soap and warm water (or hand sanitizer). Have them sit comfortably. Fold the flap on the DBS card to create a stand (Fig. 2).

3. Preparing for collection

Choose either the ring-finger or middle finger on non-dominant hand of the client (Fig. 3):

- ▶ Avoid the thumb (pulse), index/pointer finger (calloused), and pinky (small).
- ▶ Confirm the finger is free of wounds/infections.
- ▶ Clean chosen finger with an alcohol swab to first knuckle, and allow to dry.

Make sure client's hand is flat on the work surface, and in a comfortable position. Choose the side of the tip of finger that is closer to you.

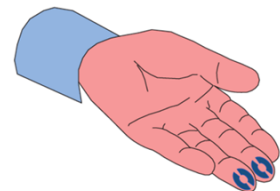


Figure 3

4. Using lancet

Twist off cover from the lancet, and position lancet off-centre of finger (less calloused). Try to orient lancet to cut across fingerprint (Fig. 4). Hold lancet firmly against finger then poke.



- ▶ HTL-Strefa Haemolance lancets (purple) are push-button activated (Fig. 5).
- ▶ BD Microtainer lancets (blue) are contact-activated (Fig 6.).
- ▶ Discard lancet in sharps container right after use.

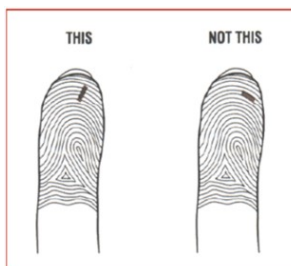


Figure 4

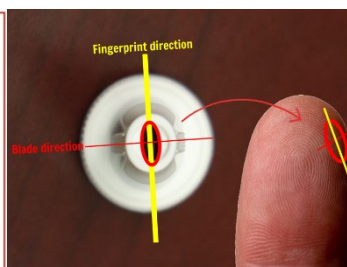


Figure 5

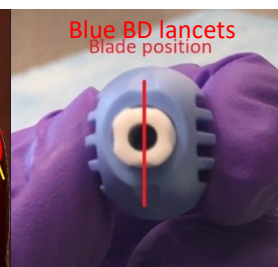


Figure 6

5. Collecting a Dried Blood Spot sample

Quickly wipe away first drop of blood with gauze, then begin collecting on the DBS card.

- ▶ Allow droplet to form, then touch droplet to DBS card without touching finger to card.
- ▶ Fill in full circle before moving onto next one. Place droplets beside each other, allow them to soak into each other and saturate card before moving on (do not layer).
- ▶ To aid blood flow, wipe finger again with gauze, or have client stand and massage arm (client can also stay seated and hang arm off to side while you collect below).

BE PATIENT. DBS collection can take time. We require all five circles to be filled in order to complete all testing (Fig. 7). However, if fewer circles are filled, submit the DBS card anyway along with required priority testing list.

After collection, wipe away remaining blood from finger then apply bandage once bleeding stops. Clean up work station, then reset as per section 1.

FRONT

BACK



Figure 7

6. Drying and packaging DBS cards

After the collection, the card must be dried for 3 hours minimum in a secure location with good airflow, away from dust and direct sunlight. Drying rack can be used to maximize space. Fold flap back to expose the collection area, then slide card into drying rack (Fig. 8).

- ▶ If cards must be moved before completely dried, a carrying case can be used to transport samples. However, the lid must be removed once cards arrive at secure location to allowing drying.
- ▶ Samples will turn dark reddish brown when dried.
- ▶ Samples can be left overnight for maximum of 18 hours.



Figure 8

Fully dried samples must be packed as soon as possible. Each DBS card goes into a coin envelope, and you can pack 10 envelopes per Bitran bag along with a desiccant pack and a humidity indicator. Be sure to remove all air from bag and seal correctly after packing (Fig. 9).

If circles on humidity indicator turn from blue to pink, replace Bitran bag and desiccant pack to protect from humidity. Check frequently. If not sending packaged samples right away, store in fridge or freezer.

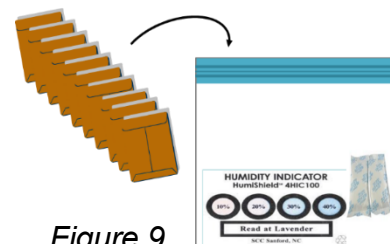


Figure 9



NML Dried Blood Spot Collection

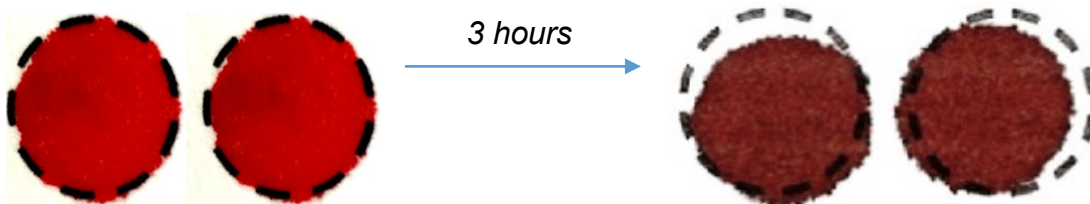
Packaging & Storage Information Sheet

This document is an information sheet on the proper packaging and storage of Dried Blood Spot (DBS) cards after a collection event.

1. Before packaging the DBS cards

a) Make sure the cards have dried for a minimum of three hours:

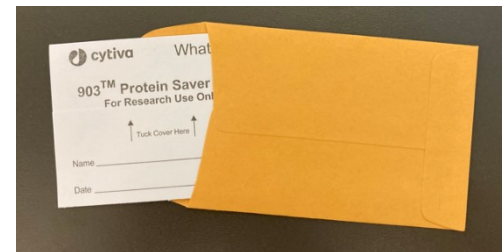
- ▶ The cards should be dried in a secure, locked location with good airflow, away from direct sunlight for a maximum of 18 hours.
- ▶ The colour of the dried blood spots should change from bright red to reddish brown.



2. Packaging the DBS cards

a) Fold the protective flap over the sample collection area, then place in a provided yellow envelope.

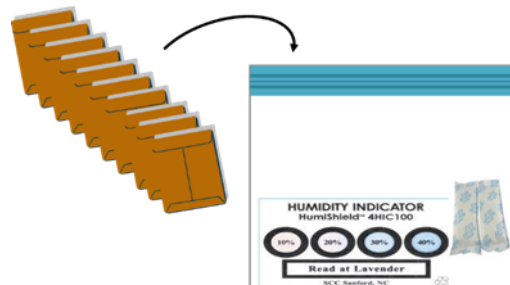
Do not glue or tape the envelope shut.



b) Place the enveloped card in a gas-impermeable Bitran bag with a desiccant pack and humidity indicator card. The Bitran bag CANNOT be substituted with a regular Ziploc bag.

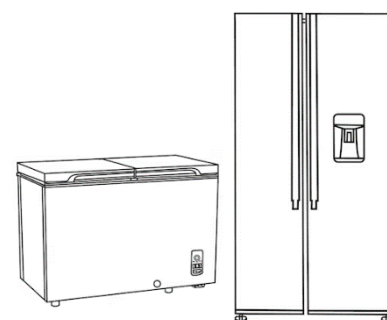


- c) **You can place up to 10 enveloped DBS cards per Bitran bag.** Remove all air from the bag, then firmly press the strip at the top to seal.



3. After packaging the DBS cards

- a) **Dried DBS cards can be stored at room temperature for up to one week.** If samples are being batched or will not be sent right away, store in a fridge or freezer.



- b) **Check the humidity indicator frequently.**

If the desiccant pack is saturated, it will turn from blue to pink. If this happens, please place the DBS cards in a new Bitran bag with a new desiccant pack and humidity card.

