

Dried Blood Spot Cards Best Practices



Recommendations and Guidelines for Collection,
Drying, Storage and Shipping of DBS Cards

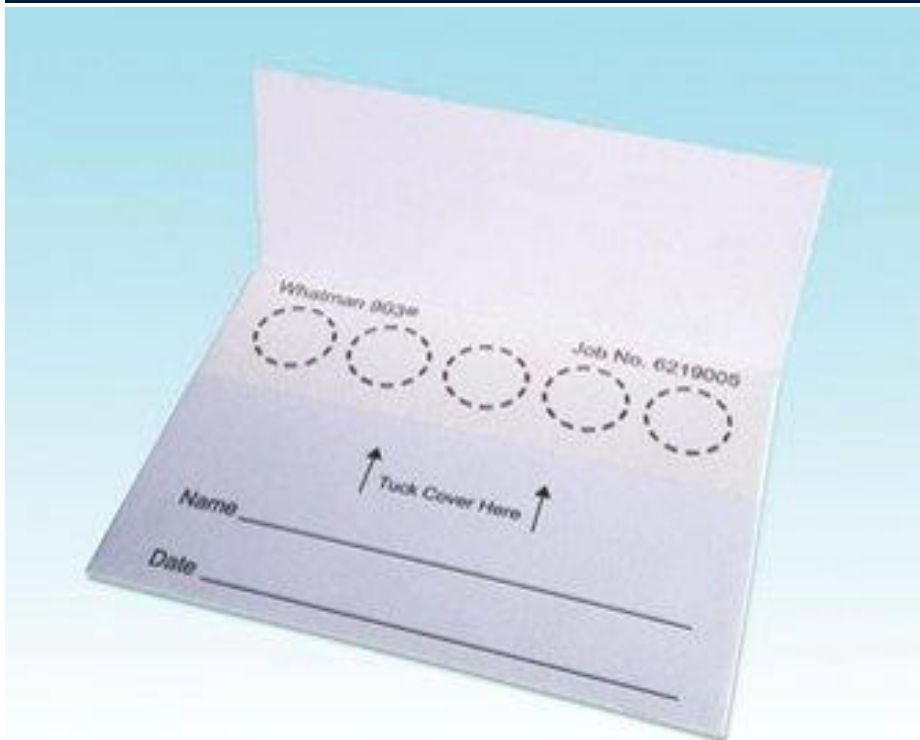
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Sample Requirements

Dried Blood Spot Cards



Whatman™ 903 Protein Saver Cards



- ▶ **Two Spots:** Must be large enough to take one 6 mm punch from each* (recommend sending ≥ 4 spots)
- ▶ **Spot Size:** Recommend ~ 9 mm ID (~ 40.0 uL)
 - min = > 7 mm ID; “pencil eraser” (~ 25 uL)
 - max = ~ 12 mm ID; fills circle (~ 55 uL)
- ▶ **Dry Time:** 3-5 hours at room temp (4hrs recommended)
- ▶ **Seal in gas impermeable bags w/ color-changing desiccant**
- ▶ **Store and ship frozen** at -80°C , or as cold as possible.
- ▶ If possible, provide hematocrit (HCT) for each sample, measured at the time of sample collection
- ▶ If possible, provide blank cards from collection site (1 card/40 samples)

Spot Appearance

A “Specimen Check” is performed for every sample at Metabolon



Valid Specimen



- Uniform brown/red color
- > 7 mm diameter








Invalid Specimens

- Insufficient volume
- More than one drop applied to spot
- Card scratched with pipette tip or capillary
- Specimen not dry before packaging
- Too much blood applied, or applied to both sides of card
- Finger touched the card while spotting
- Alcohol on finger was not dry before puncture
- Finger squeezed (“milked”) to encourage blood flow
- Desiccant wasn’t used (moisture accumulation on card)

Critical Success Factors

Common Issues with invalid specimens and potential solutions



Invalid Specimen	Issue	Solutions
	Insufficient volume	Read Tips to Increase Blood Flow <ul style="list-style-type: none"> ✓prepare finger to encourage blood flow ✓ensure a <i>large</i> droplet forms on finger <i>before</i> spotting
	More than one drop applied to spot	Only apply one drop per spot <ul style="list-style-type: none"> ✓move to the next spot, <i>even if the first is too small</i>. ✓prick a different finger and spot remaining areas.
	Specimen not dry before packaging	Carefully observe each spot for complete dryness before packaging <ul style="list-style-type: none"> ✓high HCT samples take longer to dry ✓spots in the middle of the card may take longer to dry than others.
	Finger touched the card while spotting	Be careful not to touch the spots <ul style="list-style-type: none"> ✓use the “drip” technique if possible (read “Tips for Blood Flow” for success).
	Alcohol on finger was not dry before puncture	Ensure alcohol is completely dry before puncture <ul style="list-style-type: none"> ✓wipe off first drop of blood with gauze
	Finger squeezed (“milked”)	Do not squeeze the finger to encourage blood flow (causes hemolysis, releasing interstitial fluid) <ul style="list-style-type: none"> ✓read “Tips for Blood Flow” to avoid “milking”
	Desiccant wasn’t used	Avoid moisture accumulation on cards <ul style="list-style-type: none"> ✓dry cards in a humidity-controlled environment ✓seal card in a <i>gas-impermeable</i> zip-top bag with <i>color-changing</i> desiccant
	Specimen scratched with pipette tip or capillary	Do not touch pipette tip or capillary tube to paper.
	Too much blood was applied, or applied to both sides of the card	Avoid spotting extra-large droplets <ul style="list-style-type: none"> ✓for high-flow bleeders, use a medium-flow lancet (pink) rather than high-flow (blue) ✓spot quickly rather than waiting for droplet to form.

Collection Guidelines

Dried Blood Spot Cards



- ✓ Only one sample per card (5 spots/sample)
 - Cards may be cut *prior to spotting* if > 1 subject per card is desired.
 - ✓ Puncture just off-center on the finger pad.
 - Recommend middle or ring finger of non-dominant hand
 - Prick 2nd finger if needed to collect remaining spots
 - ✓ Only apply one droplet of blood per spot
 - even if insufficient volume was collected.
 - allow a large droplet to pool at puncture site *before* spotting on the card.
 - ✓ Prepare within *5 minutes* of puncture
 - < 2 minutes recommended to avoid coagulation
 - ✓ View “Tips for Improving Blood Flow”
- ✗ **Do not** apply more than one drop of blood per spot, *even if the spot appears too small.*
 - ✗ **Do not** “milk” the finger to encourage blood flow.
 - ✗ **Do not** touch or smear the spot.
 - ✗ **Do not** puncture the tip of the finger near the nail, or directly in the center of the pad.
 - ✗ **Avoid** puncturing the pointer or pinky fingers of dominant hand.



Consistency of sample collection is critical to study success and the best biological conclusions.

Tips to Increase Blood Flow

Dried Blood Spot Cards



Prepare puncture site:

- ▶ **Warm the puncture site** (warm water rinse; hold a cup of hot liquid; use hand-warmers)
- ▶ **Gently massage** the area in the direction of puncture site (palm to fingertip)
- ▶ **Use “windmill technique”** to encourage blood flow to the fingers:
 - Straighten arm and rapidly rotate at the shoulder to create large circles, like a windmill.
 - Emphasize the downward portion of each rotation.
- ▶ **Use a “high flow” lancet** (16.5g x 2mm depth)



BD Microtainer®
366594


During Collection:

- ▶ **Maintain flow** by standing during collection, keeping puncture site lower than heart
- ▶ **Gently massage** from palm to fingertip, **DO NOT** “milk” the finger

Capillary Blood Collection & Spotting Protocol

Dried Blood Spot Cards



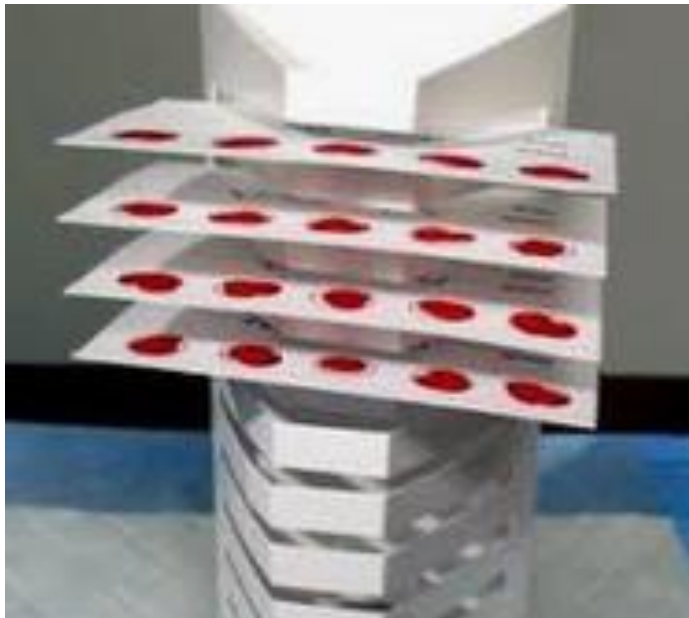
1. Gather the Materials Needed and prepare them for use 
2. Read DBS Collection Guidelines & Tips to Improve Blood Flow
3. Clean finger-tip with alcohol wipe
4. **STOP!** and ensure alcohol has fully dried or wipe dry with gauze
5. Press lancet **firmly** against finger until it “clicks”, **hold for ~1 second** then release
6. Wipe off first drop of blood with gauze
7. **STOP!** Allow a **large droplet to pool on the finger** before proceeding
8. Once large droplet forms, prepare the spot using either technique:
 - **Drip:** Hover finger over outlined area until droplet falls freely onto card
 - **Wick:** Carefully touch the surface of the droplet (not the finger) to the card until the paper wicks a sufficient volume of blood from the puncture site
9. Repeat Step 8 until remaining spots are prepared (at least two spots required per analysis). **Prepare all spots within 5 minutes** (<2 minutes for best results). Prick an additional finger if blood flow becomes too slow.
10. Stop the blood flow with gauze and apply bandage.
11. Wait ~ 2 minutes, then proceed to **drying steps**.

Materials Needed:

- alcohol wipe, *opened*
- gauze pad, *opened*
- Lancet, *cap unscrewed*
- bandage, *opened*
- DBS card, *fold back cover to expose spots*
- Sharps container for lancets
- drying rack, *set up*

Drying Steps

Dried Blood Spot Cards



- ▶ Passively dry spots for 4 hours (± 1) at room temperature.
 - Keep away from direct air flow, sunlight and heat
- ▶ Place cards face up (spots exposed; cover tucked behind spots), in a horizontal position as shown on Whatman drying rack or in a similar manner
- ▶ Since Hematocrit (HCT) influences dry time, be sure to **visually check each spot for dryness** before packaging
 - Higher HCT = longer dry time



▶ **After drying, package each sample** (including blank cards):

- Secure flap to cover spots.
- Seal in a **gas-impermeable**, zip-top bag.
- Add 1 – 2 packs of color-changing desiccant (1-2 grams each) to each bagged sample.
- Sporadically place a small humidity card inside the sample bags (~1/20 samples should contain a humidity card). *This is only required if desiccant is not humidity-indicating.*

▶ **Store samples as cold as possible** (-80°C preferred).

- Contain all bagged samples in a secondary vessel (large zip-top bag, or box).
- For best stability results, all samples should be stored for at least 3 weeks after collection is complete.

▶ **Ship samples as cold as possible** (dry ice is preferred).



Consistency in how your samples are stored and shipped is critical to study success and the best biological conclusions.

Thank you!

For any additional questions on our **Dried Blood Spot** card global metabolomic analysis and how to empower your study for success, please contact your Metabolon representative or study director.



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