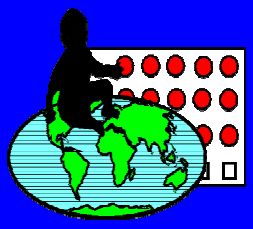


Newborn Screening
Quality Assurance Program

External Quality Assurance for HIV Rapid Tests Using Dried Blood Spots

Joanne Mei
Lead Research Chemist
Newborn Screening Quality Assurance Program
Centers for Disease Control and Prevention

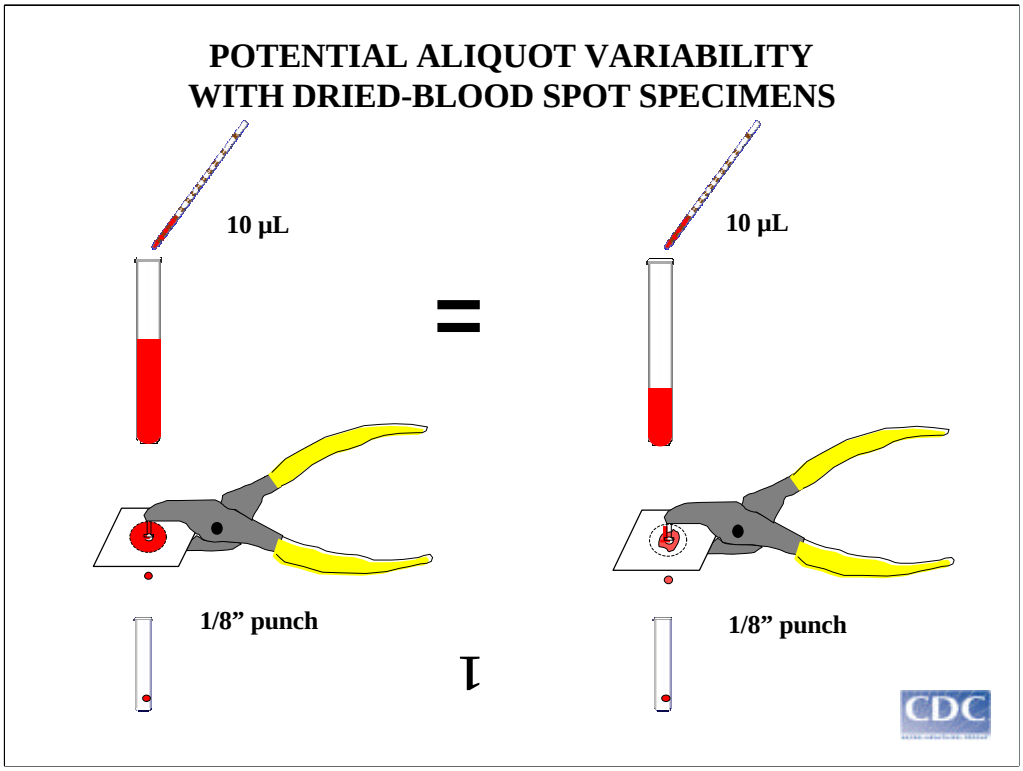
CDC

Advantages of Dried Blood Spots

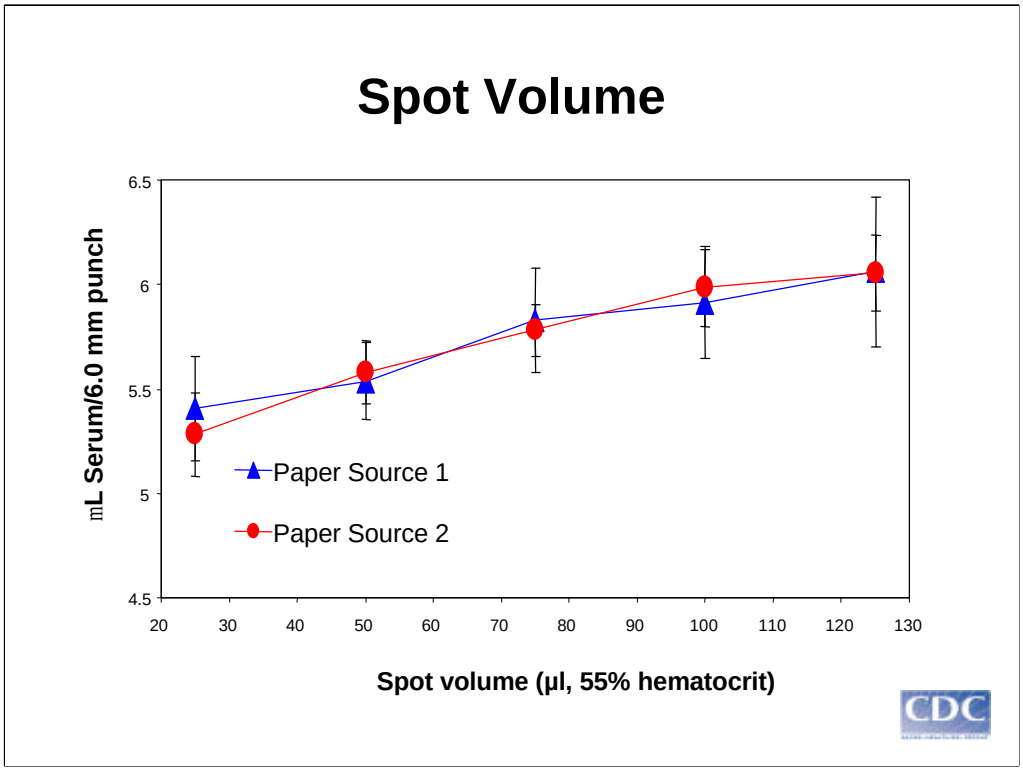
- Collection simple
- Most analytes stable
- Transportation simple
- Storage easy/compact
- Whole blood matrix
- Safety/handling exposure
- Centralized technology/laboratory

Variables Affecting Measurements for Specimens Collected on Filter Paper

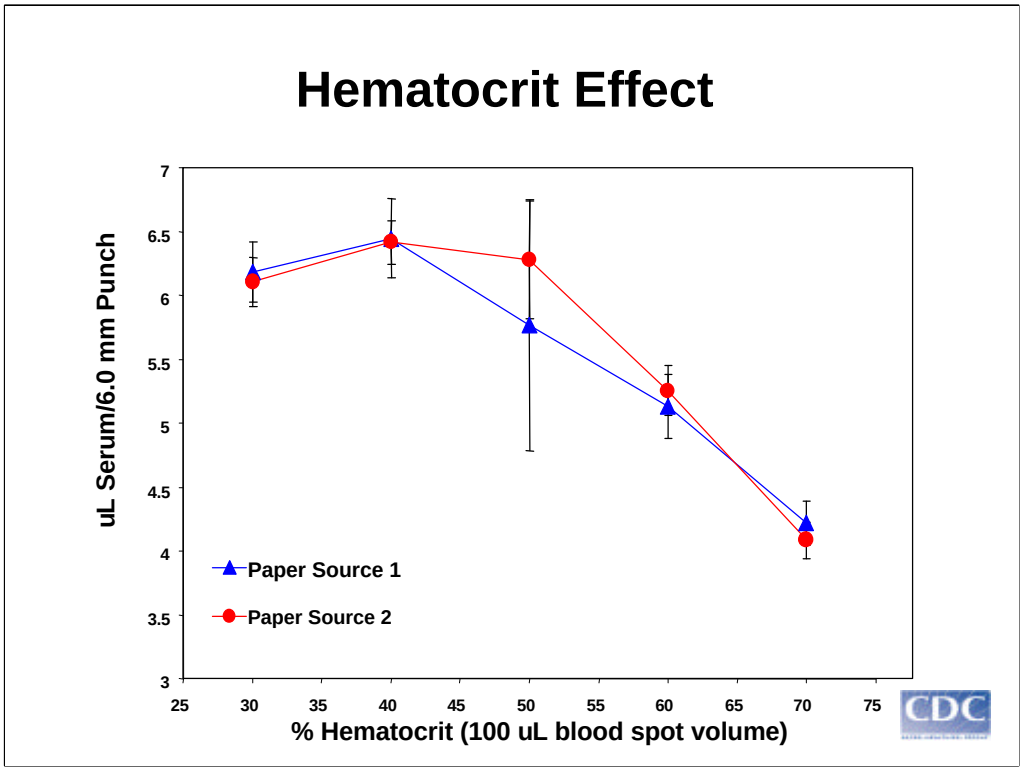
- Handling and storage of paper
- Humidity condition of paper
- Volume of blood collected
- Hematocrit level of blood donor
- Absorption time for blood



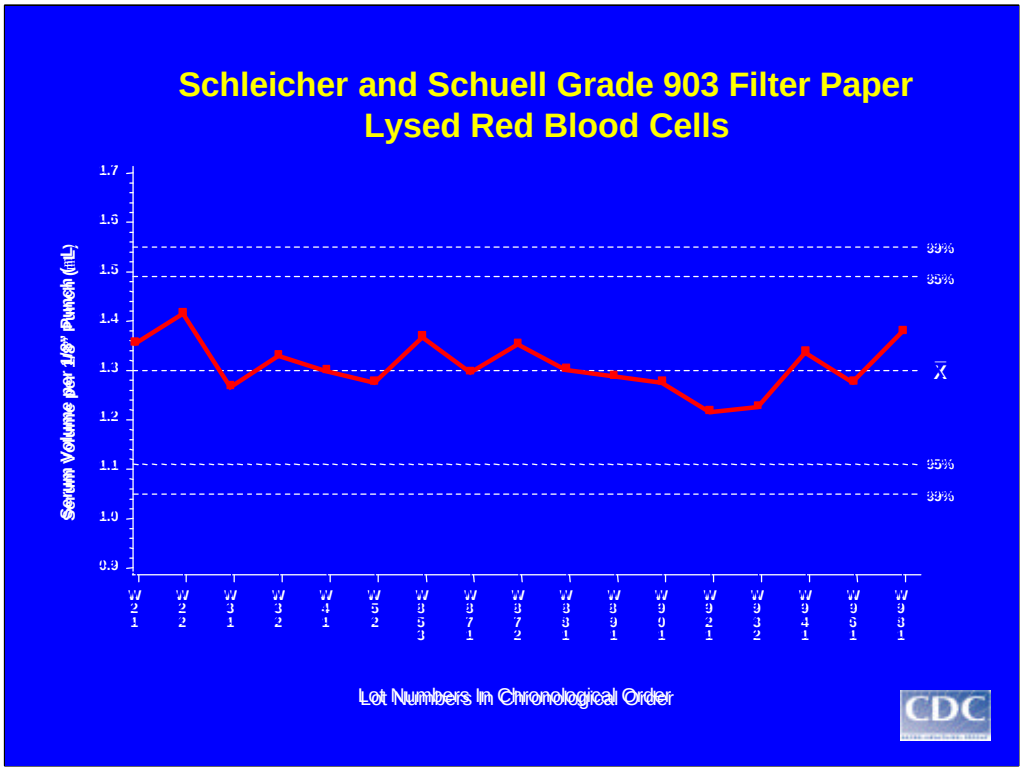
We have found that it is very important to keep the volume of blood that is applied to the filter paper constant. Unlike liquid measurements, a 1/8” punch (3 mm) from a 100 uL spot does not equal the same volume of blood as a 1/8” punch from a spot containing less blood.



You can see here that the volume of blood in a punch varies greatly from small blood spots to large blood spots, while keeping the hematocrit constant.



We also see large changes in punch volume If we keep the volume of blood in the spot constant and vary the hematocrit.



At the Newborn Screening Quality Assurance Program, we monitor the performance of new lots of filter paper for homogeneity and reproducibility. Both the volume of blood applied to the filter paper and the hematocrit are kept constant, as is the size of the punch taken for analysis. By doing this we can observe changes from lot to lot over time.

Current NCCLS Standard

LA4-A3 Volume 17 No. 16

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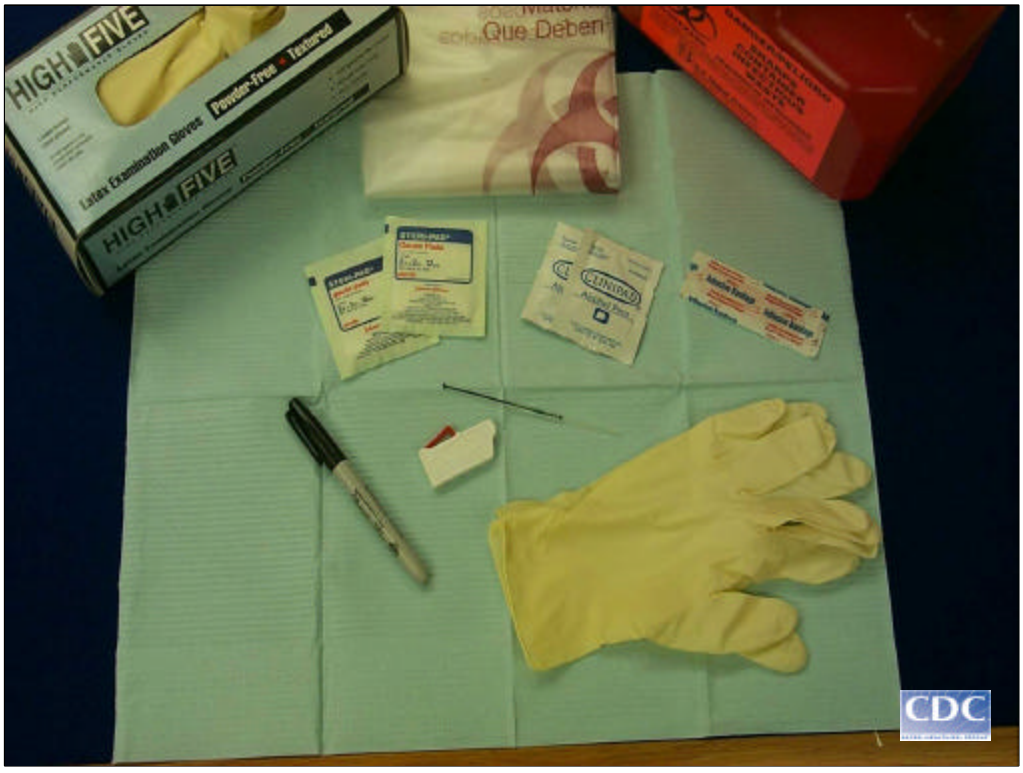
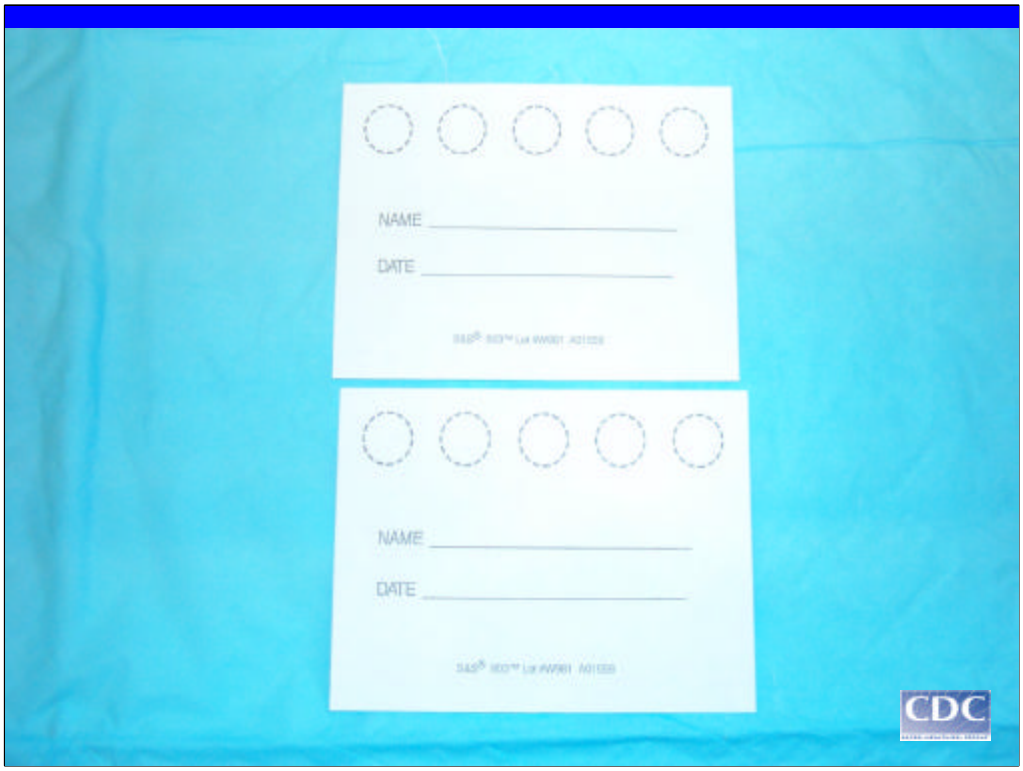
CDC

The performance characteristics of blood collection filter paper have been published as a National Clinical Laboratory Standard.

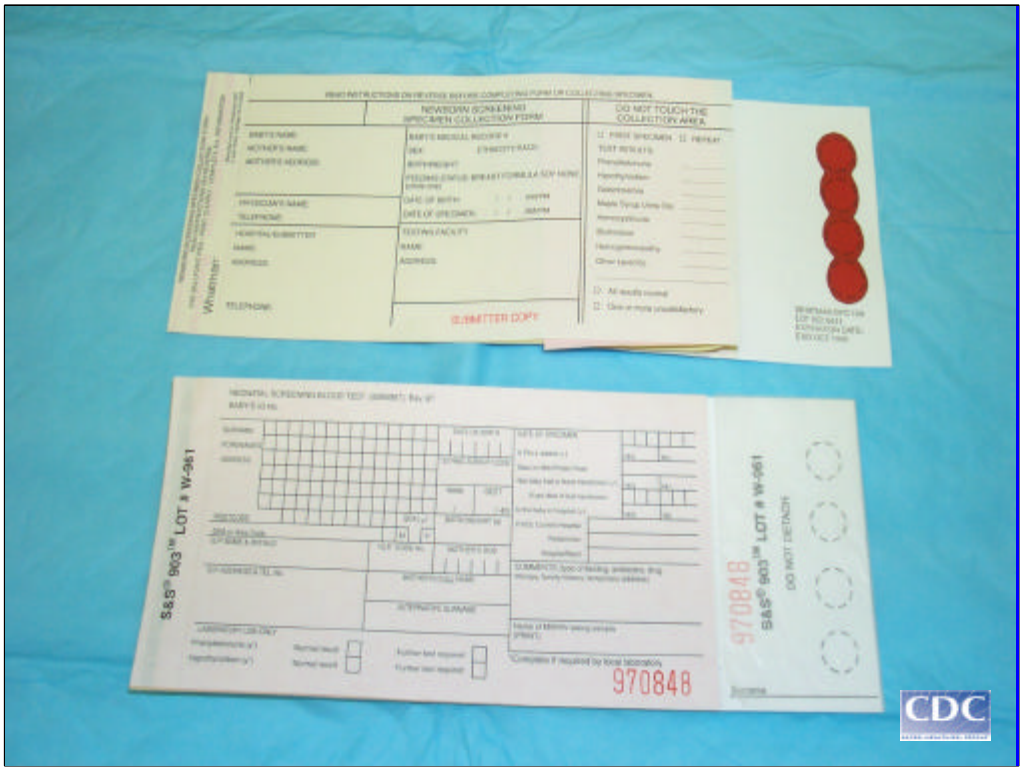
Fingerstick Screening Supplies

- Sterile Lancets or Autolets
- Filter paper collection device
- Sterile gauze pads
- Pen/marker
- Lab coat
- Sterile disposable powder-free gloves
- Alcohol wipes
- Adhesive bandages
- Biohazard disposal bags
- Sharps container
- Disinfectant


HIGH FIVE
Latex Examination Gloves
Powder-Free
Textured
HIGH FIVE
Que Deben
CDC



The device is for a single individual with space for identifying information.



There are many types of filter paper collection devices. These are 2 examples of newborn screening collection devices.

Hand Washing

- Wash with soap
- Rinse
- Dry (don’t use recycled paper towels)

Universal Precautions must be observed!

These precautions require that you assume that all human blood is potentially infectious for HIV, HBV and other bloodborne pathogens

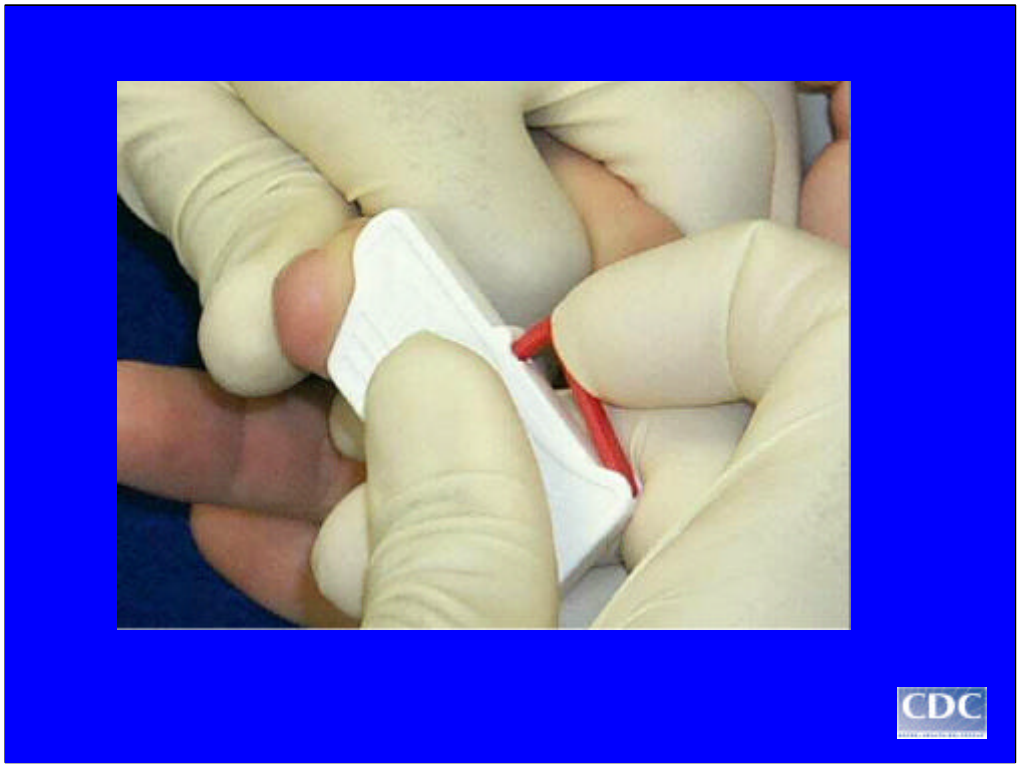
Specimen Collection: Dried Blood Spots

- Do not touch any of the filter paper circle before or after collection.
- Select puncture site and cleanse with 70% isopropanol.
- Use a sterile, disposable lancet with 2.0 mm, or less, point
- Wipe away first blood drop.
- Use second LARGE blood drop to apply to surface of FDA-approved filter paper circle.
- If not completely filled, add a second LARGE drop immediately.
- FILL all required circles completely. FILL from only one side of the filter paper.
- Dry specimen at room temperature 3-4 hours in HORIZONTAL position.
- See NCCLS LA4-A3, 1997. Blood collection on filter paper for neonatal screening programs; Approved standard – Third edition.

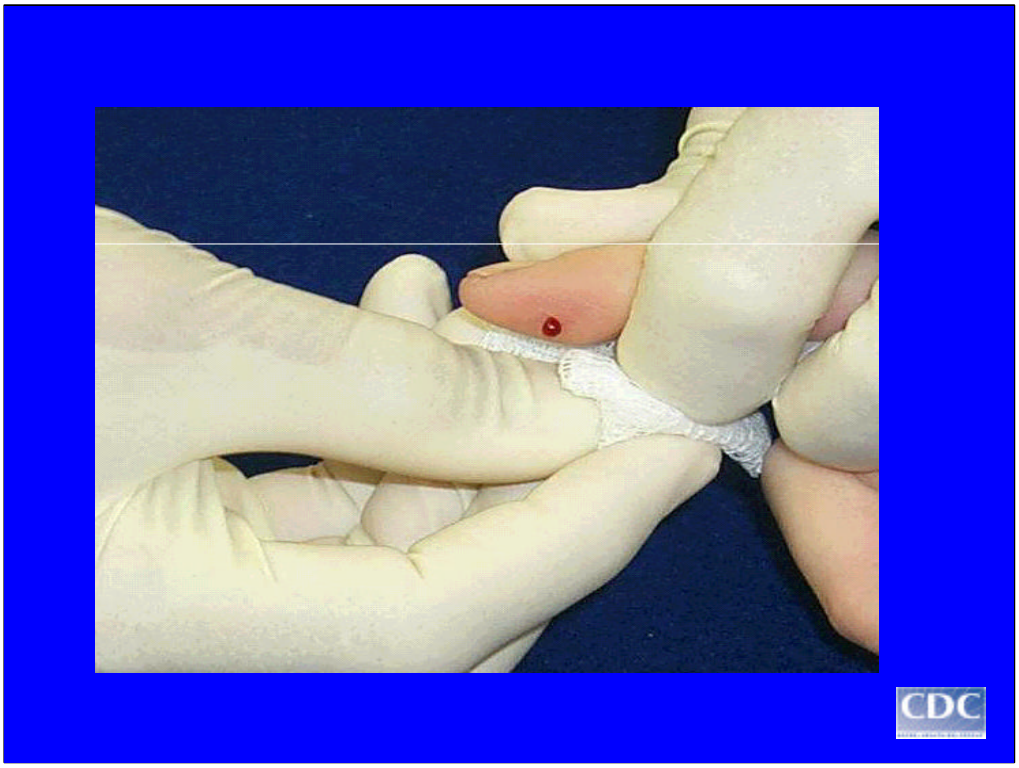
Puncture Ring or middle finger palmer surface- approximately half way between the center of finger and side or tip, with a sterile lancet.

Tenderlett® Lancet for Finger Sticks

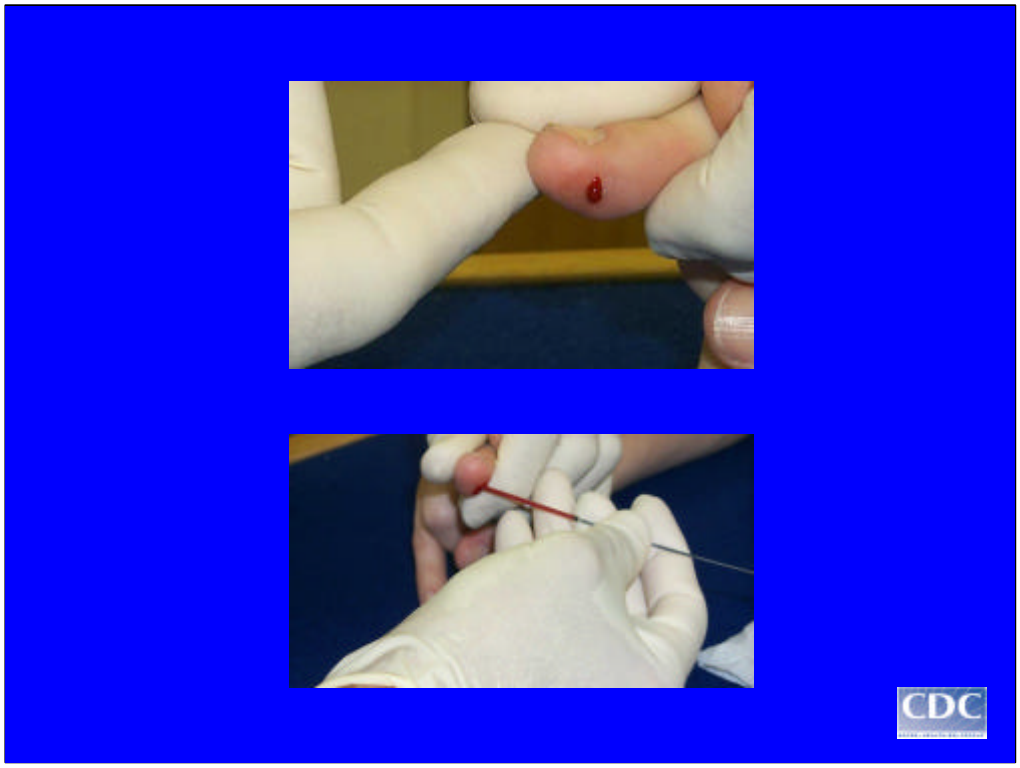
- Incision device with blade that cuts to a controlled, standardized depth.
- Shallow incision created which cuts more of the capillary bed without cutting too deeply.
- Blood flows more freely providing a higher quality blood specimen.
- Blade permanently retracts after use for safety
- Available in three depths for the appropriate patient population.



Puncture the patients finger with a sterile lancet or autolet. Dispose of the used autolet in the appropriately labeled sharp's container.

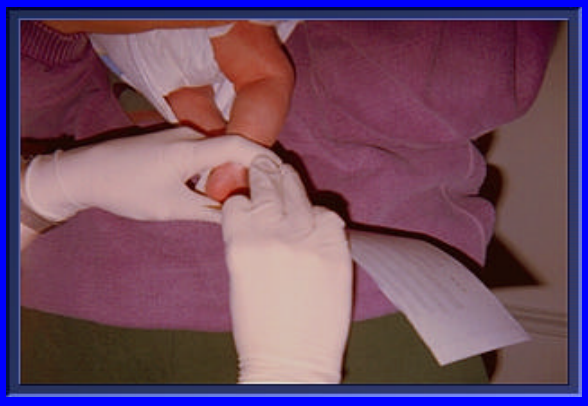


Wipe away the first drop of blood with a clean gauze pad. The first drop of blood often contains excess tissue fluids which can alter the results of the test. Gently massage the patients finger to establish the flow of blood and to create a small bubble of blood to sample. Do NOT squeeze the patients finger as tissue fluid may dilute the sample.



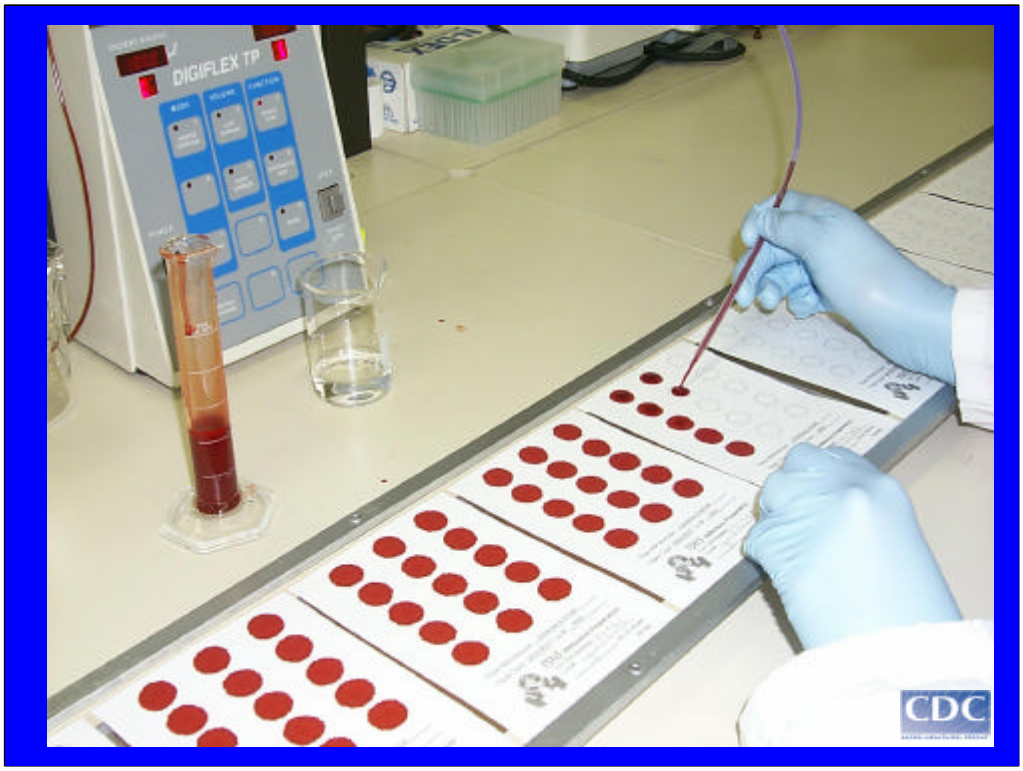
For collection into a capillary tube, turn the patient's hand over so that the blood drop forms toward the floor. Establish a bubble of blood for sampling. Holding the collection tube at an angle of about 10 ⊂⊃⊇⊕⊃⊃⊖ below the collection site, touch the tapered end of the tube into the bubble of blood. Capillary action will cause the blood to fill the tube. As the blood starts entering the tube be careful not to allow air bubbles to enter. Avoid collecting smeared blood and do not collect blood that has run into the cuticle or nail area.

Collecting Newborn Screening Sample from a Heel Stick



For collection on filter paper, lightly touch the paper to a large blood drop. Allow the blood to the blood to soak through and completely fill the circle with a single application of blood. Apply blood to one side of the paper only. Fill remaining circles in the same manner.


Completed Newborn Screening Collection Devices
CDC



Quality control materials are produced in large quantities at CDC, including dried blood controls for HIV antibody testing.

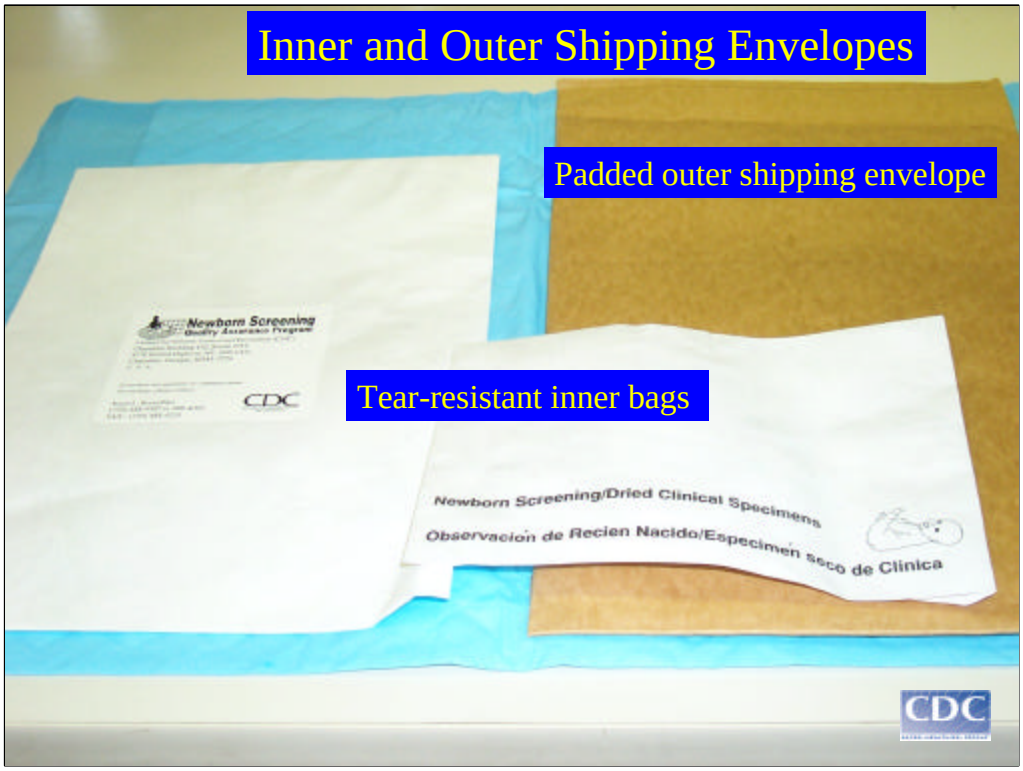
Blood spots are air-dried overnight. Humidity levels in CDC labs can range from very dry (20% to very humid >80%). Drying overnight ensures complete drying. Spots can often dry within 3-4 hours, depending on humidity conditions.

Blood spots drying in Nepal

Spots can be dried in any number of ways. Filter papers should be kept as horizontal as possible.

Biological markers in dried blood spots are very, including HIV antibodies. To maintain the integrity of dried blood spots, they should be stored in low gas permeable bags with desiccant, at –20oC. However, spots can remain at room temperature or in the refrigerator for up to one month without a decrease in HIV antibody response.

It is important to keep the spots dry. Humid conditions will accelerate blood spot degradation.



For shipping blood spots, choose tear-resistant inner and outer envelopes. These envelopes ensure that blood spots are protected within and that mail handlers are not exposed to biological specimens.

Problems with Blood Spots

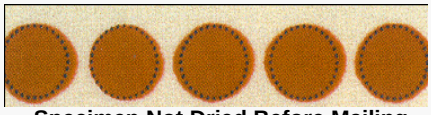
- Improper collection
  - Blood caked on paper
  - Blood applied to both sides of paper
  - Blood did not absorb through paper completely
- Improper drying
  - Serum separated from cells – forms halos
  - Spots placed in bags before completely dry
- Identifying information form not completed or filled out incorrectly.

Unsatisfactory Specimens

(Provided by the New York State Department of Health)

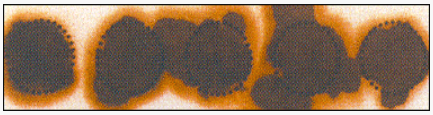
Supersaturated

Quantity Insufficient for Testing

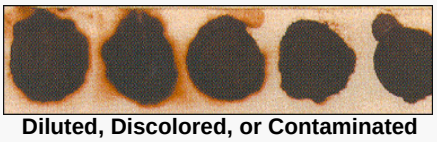


Specimen Not Dried Before Mailing

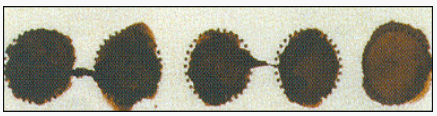
Scratched or Abraded



Serum Rings



Diluted, Discolored, or Contaminated



Clotted or Layered

No Blood

CDC

Proficiency Testing – External Quality Assessment

- Provides an external measure of the total testing system
- Uses blind coded samples that represent “normal” and “disease”
- Evaluates performance at that moment only
- Meets requirements for certification

EQA for HIV Rapid Tests

- HIV Rapid Tests done at clinics providing various levels of health care and counseling
- Plasma/serum/whole blood collected from finger stick or venipuncture
- Varying levels of complexity – Should use a test that provides HIV controls

Why Use DBS for Rapid Test EQA?

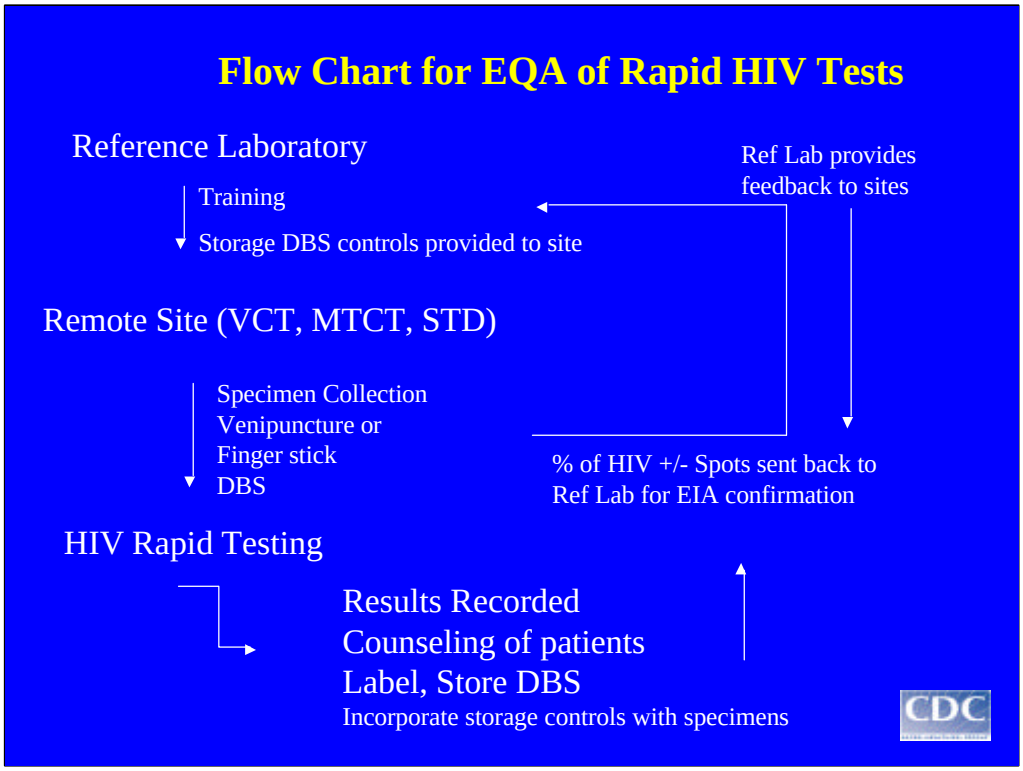
- HIV antibodies in DBS are stable (keep spots dry with desiccant)
- Remote sites collect DBS at the same time as samples are collected for HIV rapid testing
- DBS are easy to transport
- Reference labs test DBS specimens by EIA
- EIA and rapid test results are compared
- Results are provided back to remote clinic

EQA of Rapid Tests Cont.

Sites receive storage spot controls to be added to bags as patient spots are collected

- Sites collect dried blood spots from individuals at the time of finger stick or venipuncture
- Spots are stored, then sent to a centralized Reference lab for EIA/WB testing
- Reference lab tests spots, calculates correlation between rapid test results and matching EIA results, provides feedback to sites

Flow Chart for EQA of Rapid HIV Tests



How many DBS should be collected for EQA?

- Statistical sample based on HIV prevalence of the population
- May be difficult to obtain of enough specimens/site

Number of Specimens for EQA -- continued

Proposed frequencies

- Collect DBS on all patients in one day -- once or twice per month
- Collect DBS every 10th, 20th, … client
- Decrease as site gains experience

Role of the Reference Lab EQA of Rapid Testing

- In-country reference lab(s) will be responsible for providing EQA for remote testing sites
- Reference lab(s) will be trained to test DBS by EIA
- Reference labs will aid and guide remote site HIV rapid testing
- Reference labs will test DBS, compare to HIV rapid test results, and give feedback to sites
- CDC will provide EQA for reference lab(s)

Role of Testing Sites
EQA of Rapid Testing

- Sites receive storage spot controls to be added to bags as patient spots are collected
- Sites collect dried blood spots from individuals at the time of finger stick or venipuncture
- Spots are stored, then sent to a centralized Reference lab for testing

Role of CDC: HIV EQA for DBS – Program Description

- NSQAP distributes panels (4/yr) of dried spot specimens
- Subtype B
- 12 spots/panel
- Pre-tested in 2 FDA-approved EIA kits for blood spots and 1 approved WB
- Results returned and reports are distributed

EQA Program for DBS cont.

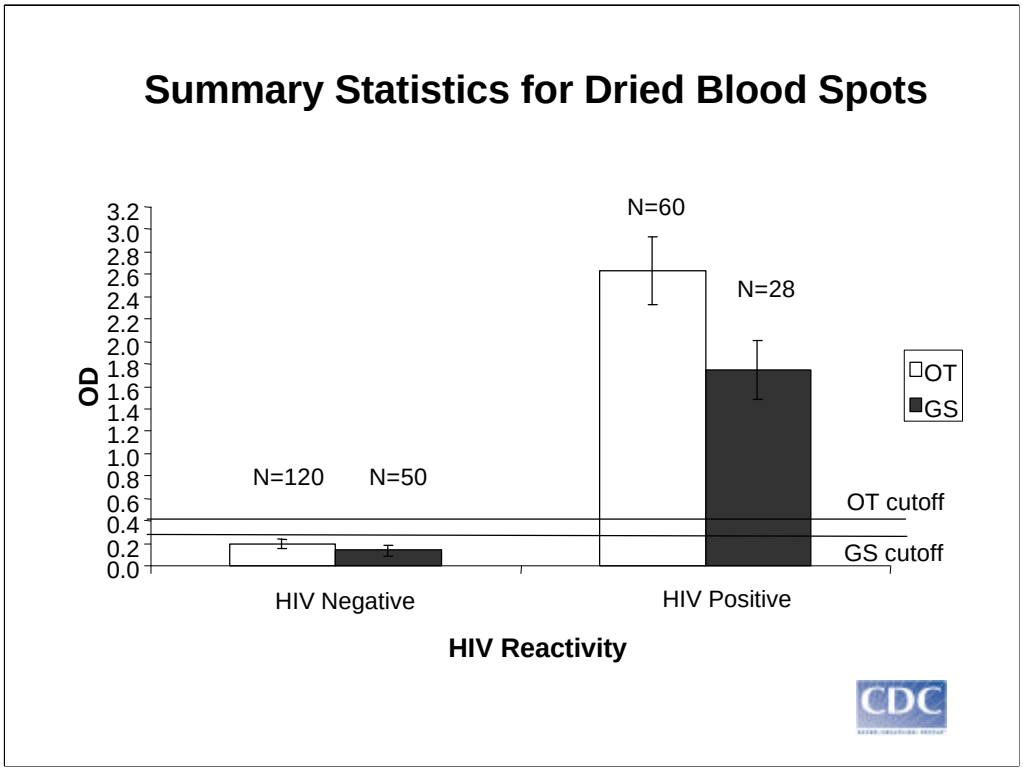
- Participants receive lab-specific report
  - Summary statistics
    - Range (min and max) and mean OD, and cutoff ranges and means for EIA methods
    - WB summary data for HIV positive specimens
    - Number of labs participating and methods used
- Report is used by lab to provide remedial action, if needed

EQA for HIV in DBS

- Laboratory Performance Evaluation Program for Anti-HIV-1 in Dried Blood Spots
- Administered by NSQAP, CDC
- Contact: Ms. Carol Bell

CBell@cdc.gov

770-488-4023



The graph summarizes EIA results from labs participating in our blood spot EQA program. The majority of participants use either of two manufacturers that have claims for dried blood spot testing. Here you see the differences in performance of both the kits and of the labs. The error bars refer to the spread of optical density results obtained for the blinded specimens.

Conclusions

- Filter paper: ideal method for collecting whole blood
- Stability of HIV antibodies
- Ease of transport: no refrigeration
- EQA for HIV rapid tests: reference labs can easily monitor remote site performance