



# **Newborn Screening Quality Assurance Program**

## **Use of Dried Blood Spots for anti-HIV-1 Testing**

**Joanne Mei, Ph.D.  
Lead Research Chemist  
Centers for Disease Control and Prevention**

# Newborn Screening Collection Devices



# **Advantages of Dried Blood Spot Samples**

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

# **Disadvantages of Dried Blood Spot Samples**

- **Skin puncture required**
- **Small sample volume**
- **Dilution for analysis**
- **Suitability for confirmatory method**
- **Clinical Sanction of data**

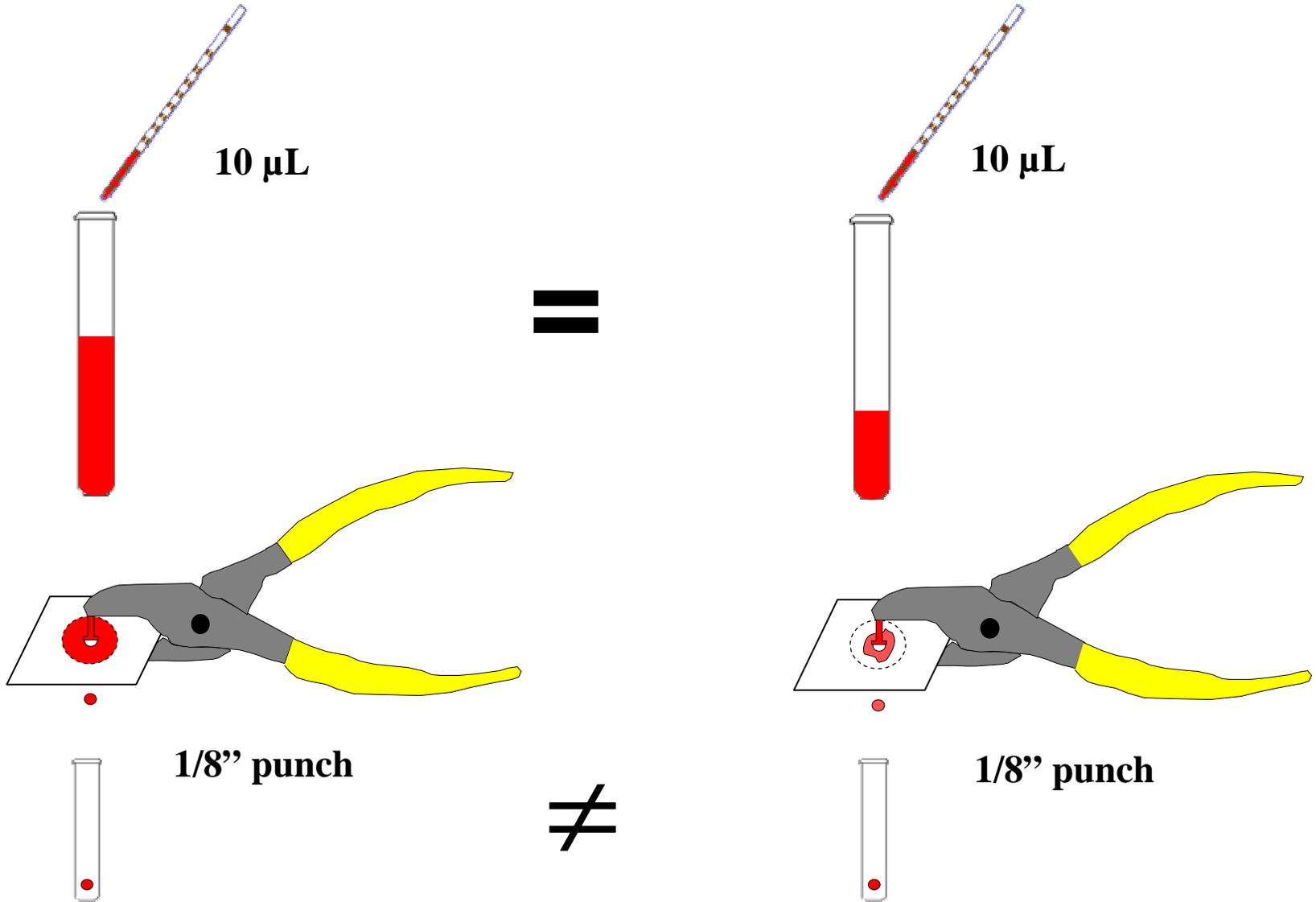
# **Variable Affecting Measurements for Specimens Collected on Filter Paper**

- **Homogeneity within a production lot**
- **Homogeneity among production lots**
- **Variance among manufacturers**
- **Variance within a collection card**
- **Cutting and printing process**

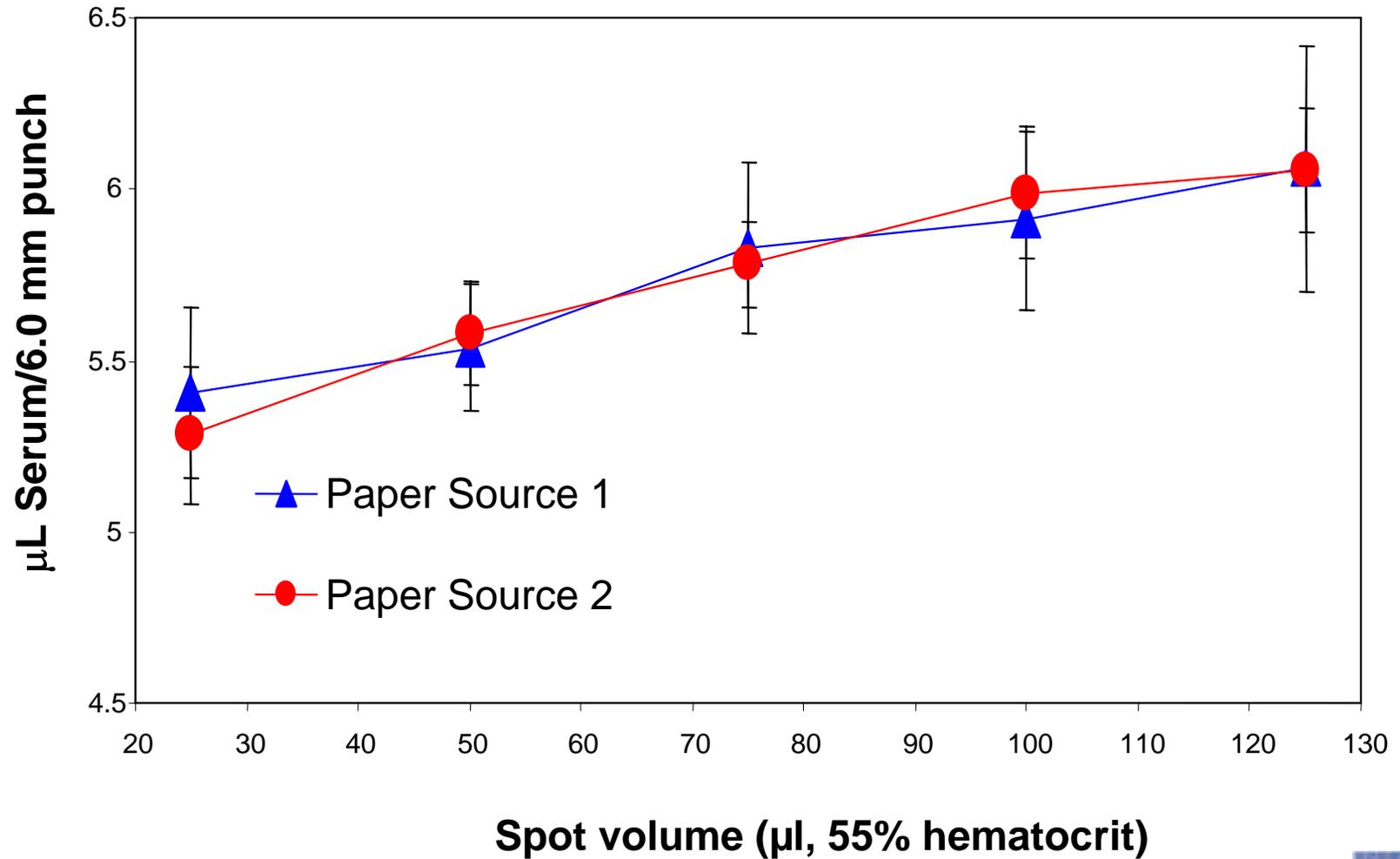
# **Variable Affecting Measurements for Specimens Collected on Filter Paper**

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

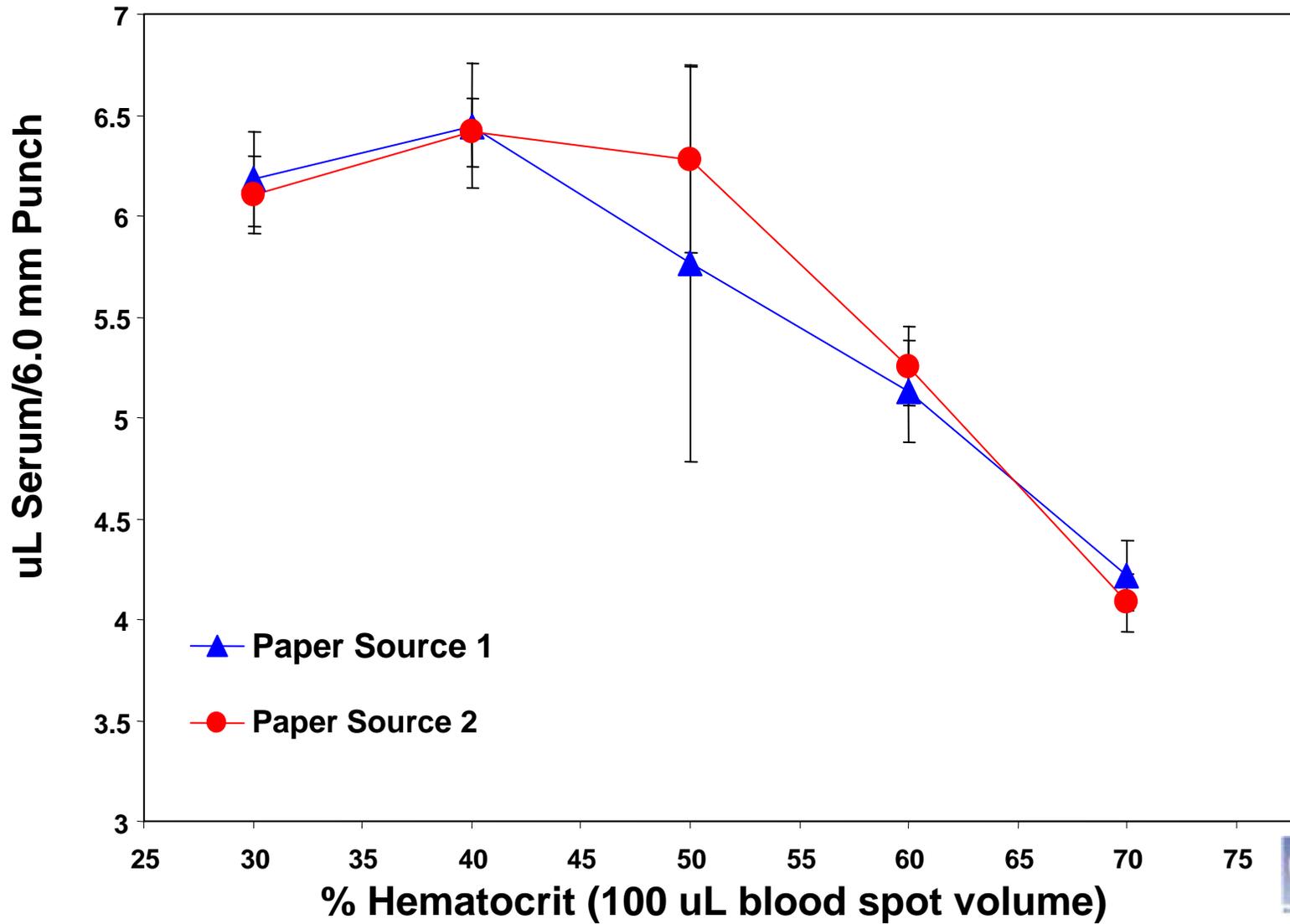
# POTENTIAL ALIQUOT VARIABILITY WITH DRIED-BLOOD SPOT SPECIMENS



# Spot Volume



# Hematocrit Effect



# FDA-Approved Filter Papers

- Whatman
  - BFC 180
- Scheicher and Schuell
  - Grade 903

# Current NCCLS Standard

LA4-A3 Volume 17 No. 16

Authors:

W. Harry Hannon, Ph.D.

James Boyle

Brad Davin

Anne Marsden

Edward R.B. McCabe, M.D., Ph.D.

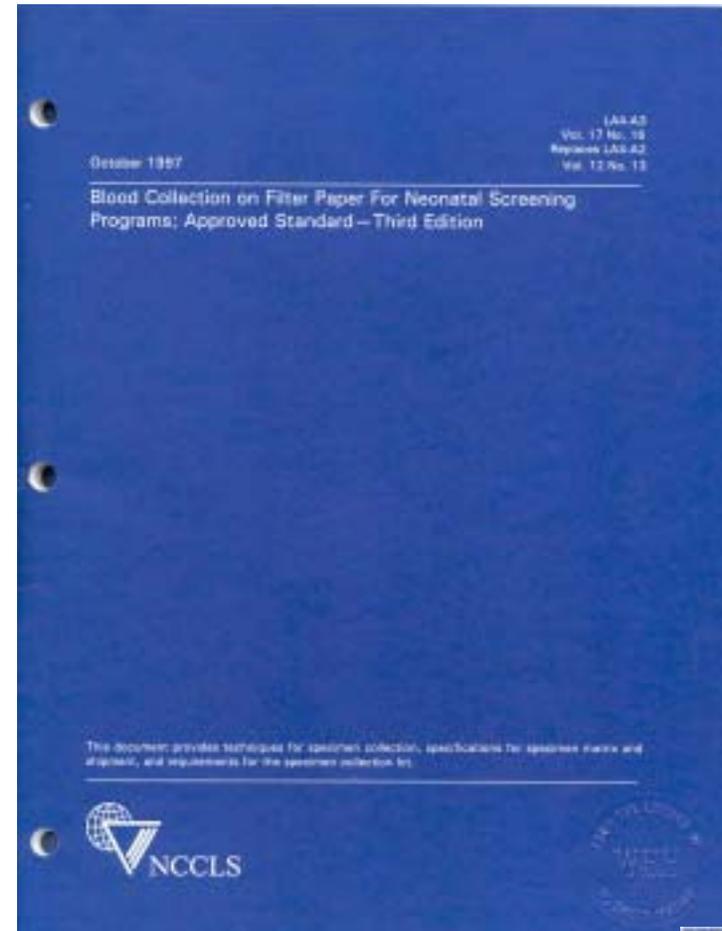
Marion Schwartz, R.N., M.S.N.

George Scholl

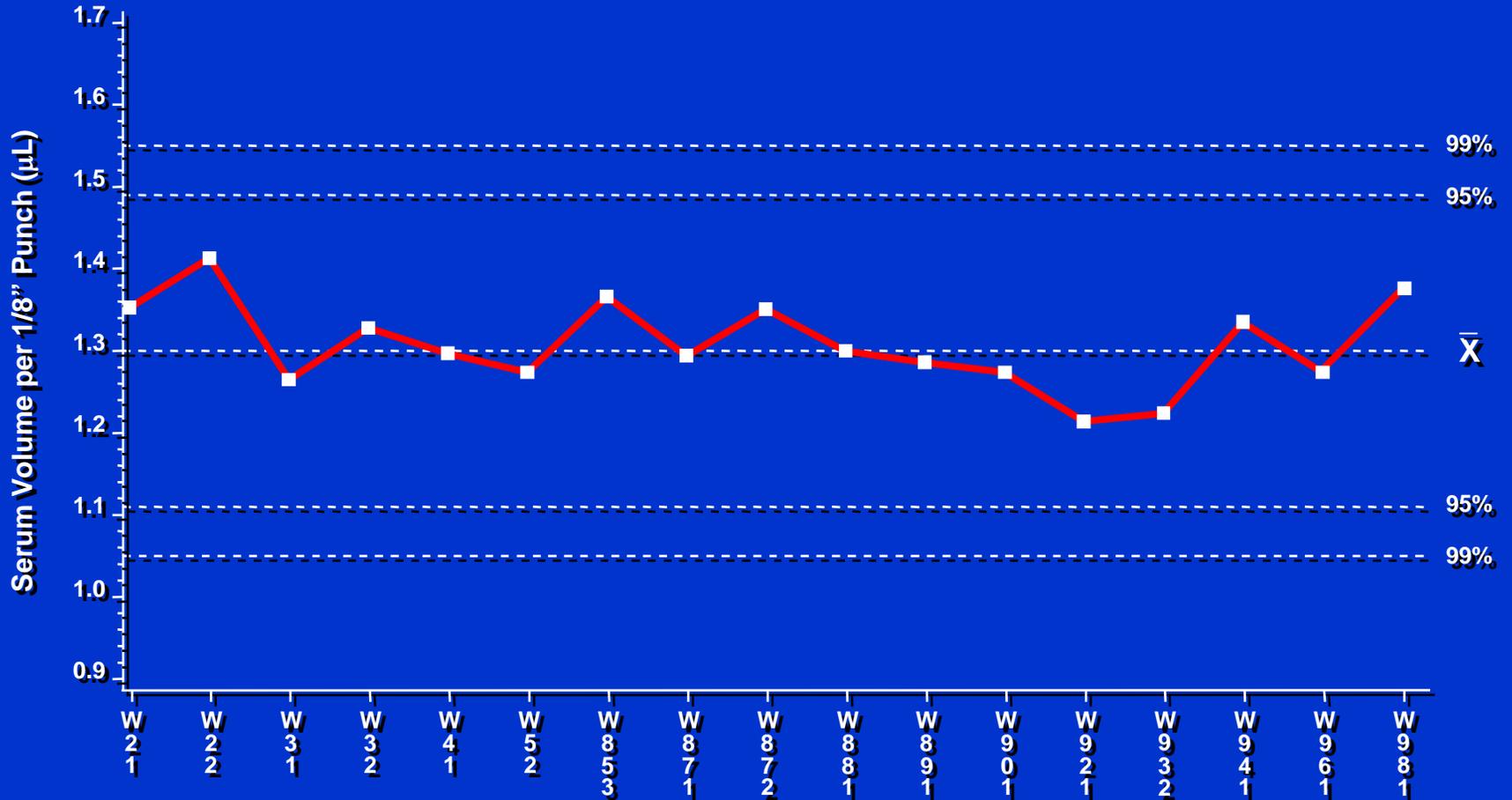
Bradford L. Therrell, Jr., Ph.D.

Martin Wolfson

Freda Yoder



# Schleicher and Schuell Grade 903 Filter Paper Lysed Red Blood Cells



Lot Numbers In Chronological Order





NAME \_\_\_\_\_

DATE \_\_\_\_\_

SAS® 903™ Lot #W981 A01659



NAME \_\_\_\_\_

DATE \_\_\_\_\_

SAS® 903™ Lot #W981 A01659

READ INSTRUCTIONS ON REVERSE BEFORE COMPLETING FORM OR COLLECTING SPECIMEN

NEWBORN SCREENING SPECIMEN COLLECTION FORM		DO NOT TOUCH THE COLLECTION AREA
BABY'S NAME MOTHER'S NAME MOTHER'S ADDRESS  PHYSICIAN'S NAME TELEPHONE  HOSPITAL/SUBMITTER NAME ADDRESS  TELEPHONE	BABY'S MEDICAL RECORD # SEX:                      ETHNICITY/RACE BIRTHWEIGHT FEEDING STATUS: BREAST FORMULA SOY NONE (SHOW ONE) DATE OF BIRTH:        /        /        AM/PM DATE OF SPECIMEN:    /        /        AM/PM  TESTING FACILITY NAME ADDRESS  <div style="text-align: center; color: red; font-weight: bold;">SUBMITTER COPY</div>	<input type="checkbox"/> FIRST SPECIMEN <input type="checkbox"/> REPEAT TEST RESULTS: Phenylketonuria _____ Hypothyroidism _____ Galactosemia _____ Maple Syrup Urine Dis. _____ Homocystinuria _____ Biotinidase _____ Hemoglobinopathy _____ Other (specify) _____  <input type="checkbox"/> All results normal <input type="checkbox"/> One or more unsatisfactory

MINOR SCREENING RESULTS ONLY  
 READ INSTRUCTIONS ON REVERSE FOR  
 USE FULLY FOR PRINT CLEARLY COMPLETE ALL INFORMATION  
**Whatman**  
 Form No. 100-100-100-100

WHATMAN SPEC 100  
 LOT #216411  
 EXPIRATION DATE:  
 END OCT 1998

NEONATAL SCREENING BLOOD TEST (SKN897) Rev. 87  
 BABY'S ID No.

SURNAMES FORENAMES ADDRESS  POST CODE CHS or Area Code G.P. NAME & INITIALS  G.P. ADDRESS & TEL. No.	DATE OF BIRTH  ETHNIC GROUP CODE  SEX (✓) BIRTH WEIGHT (g) M    F G.P. CODE No. MOTHER'S DOB  MOTHER'S FULL NAME ALTERNATIVE SURNAME	DATE OF SPECIMEN Is Pk in report (✓)                      YES    NO Days on Milk Protein Feed Has baby had a blood transfusion (✓) If yes state of last transfusion Is the baby in hospital (✓) If YES, Current Residence Professional Hospital/Block  COMMENTS (Type of feeding, antibodies, drug therapy, family history, temporary address)  Name of MWHV taking sample (PRINT)	<div style="text-align: center; color: red; font-size: 24px; font-weight: bold;">970848</div> <div style="text-align: center; color: red; font-size: 18px; font-weight: bold;">S&amp;S<sup>®</sup> 903<sup>™</sup> LOT # W-961</div> <div style="text-align: center;">DO NOT DETACH</div> <div style="text-align: center; color: red; font-size: 24px; font-weight: bold;">970848</div>
--	---	--	---

LABORATORY USE ONLY

Phenylketonuria (✓)	Normal result <input type="checkbox"/>	Further test required <input type="checkbox"/>
Hypothyroidism (✓)	Normal result <input type="checkbox"/>	Further test required <input type="checkbox"/>

\*Complete if required by local laboratory



# 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



# Hand Washing

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



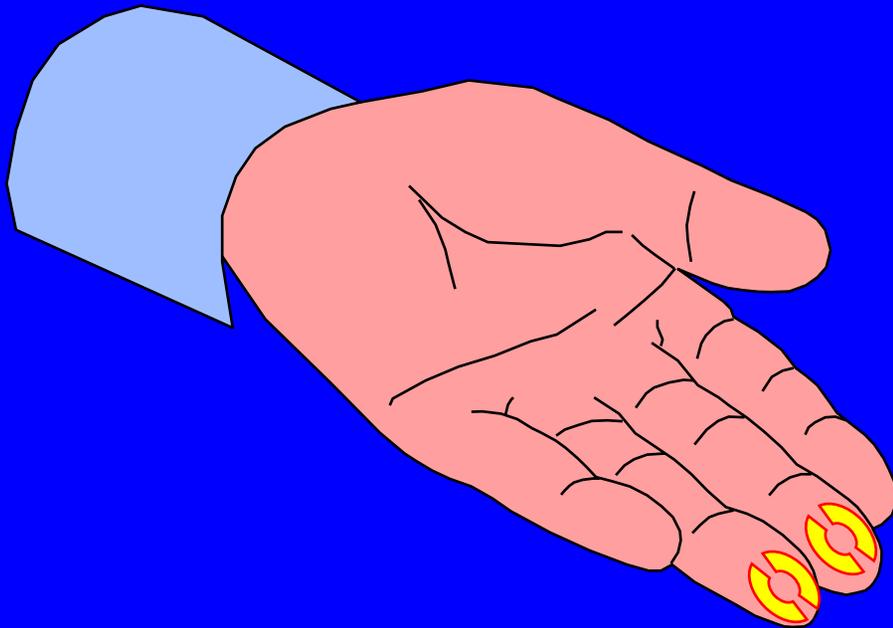
# **Universal Precautions must be observed!**

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

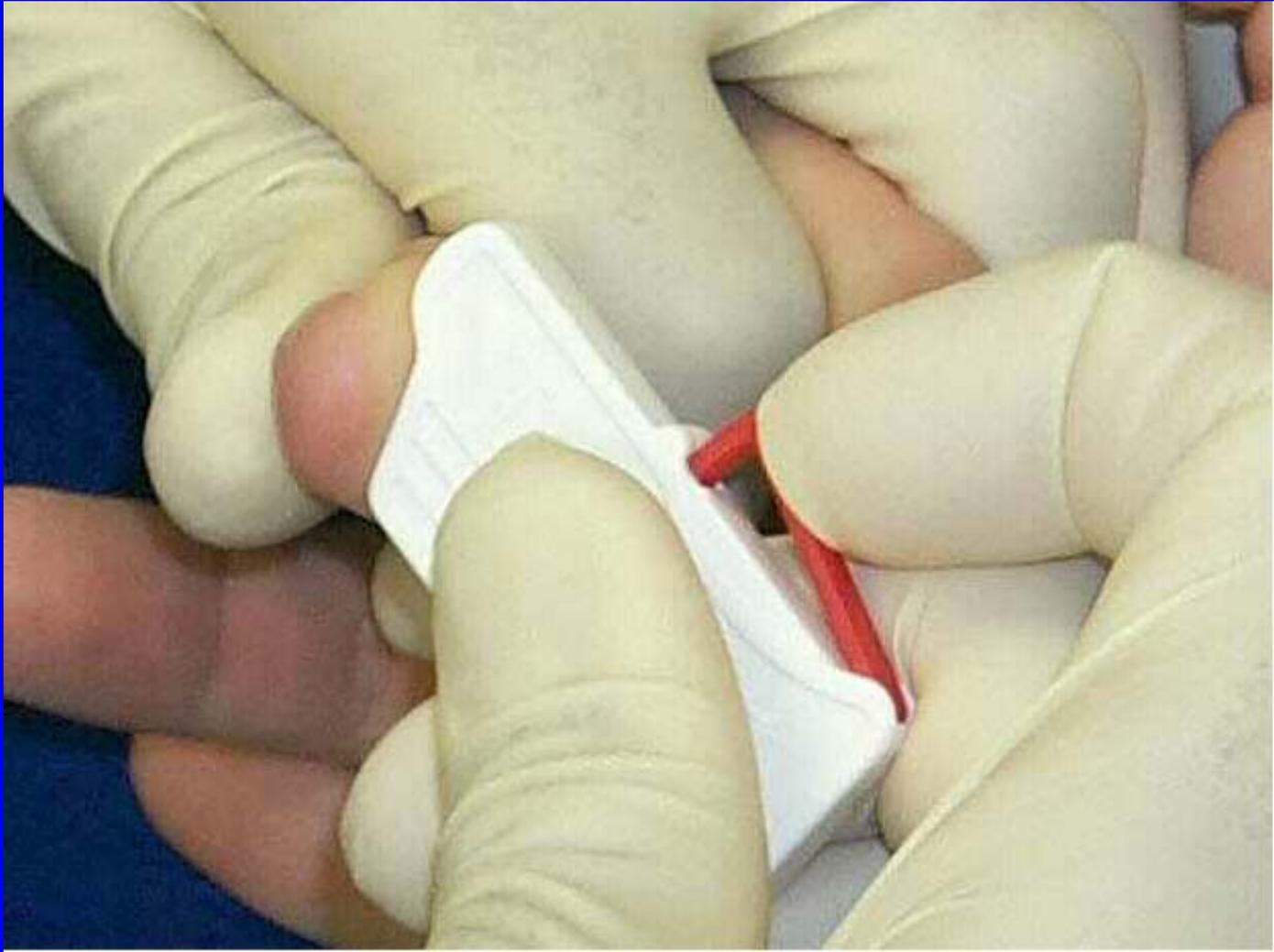
# Sample Collection

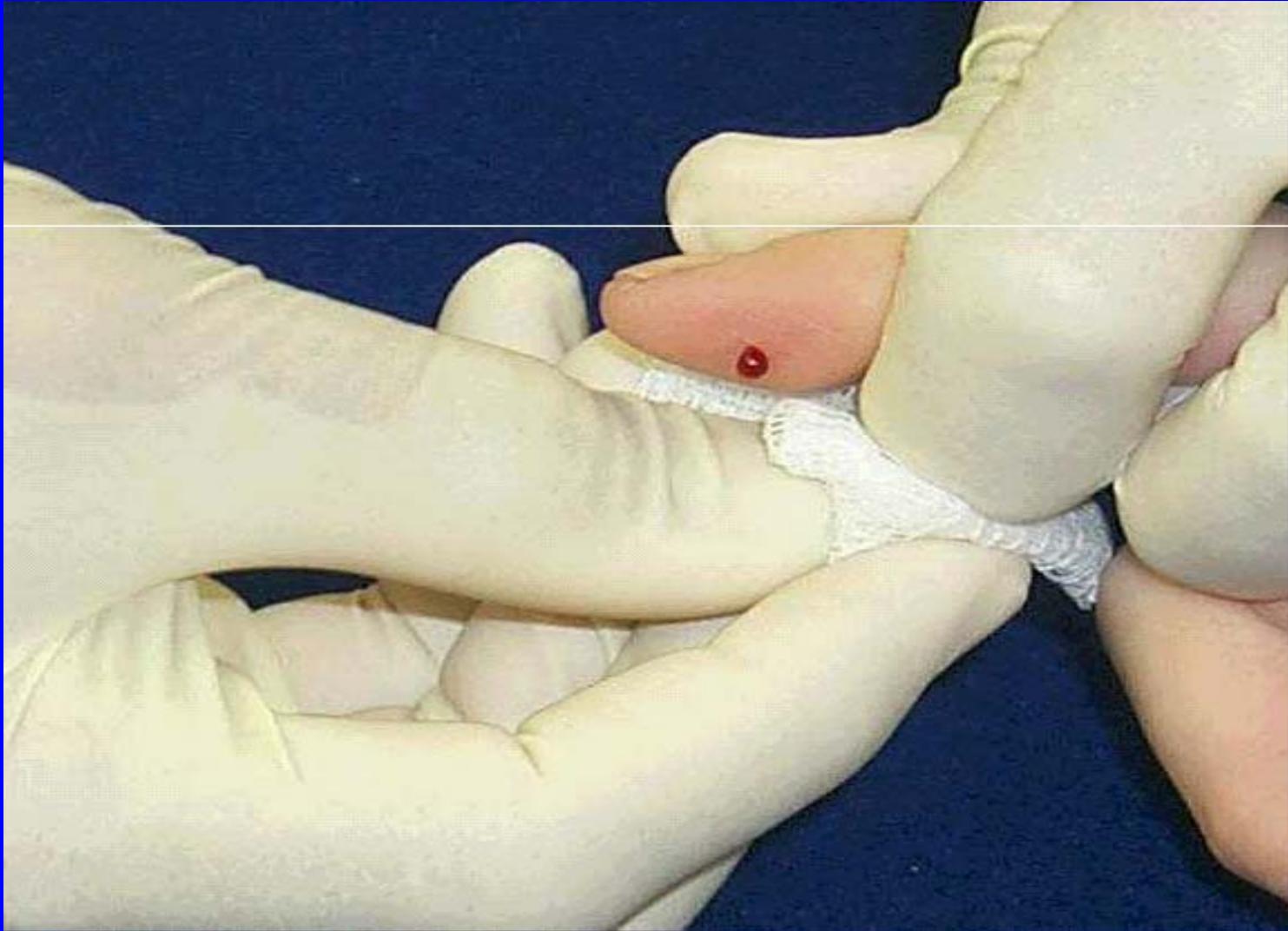
- Use “Universal Precautions”
- Venous versus Fingertick
- Proper cleaning of blood collection site
- Only sterile, disposable supplies are to be used
- All new supplies are used for each patient

# Skin Puncture Site on the Fingers of Adults or Children Over 1 Year Old



- Ring or middle finger
- palmer surface-not side or tip







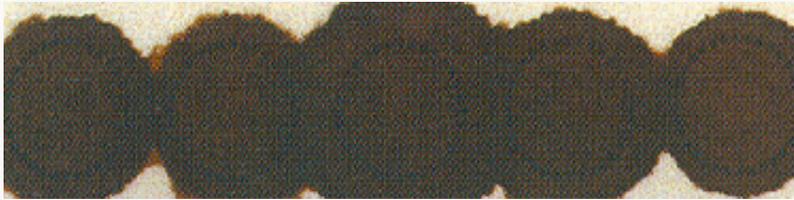
# Capillary Collection Device



- Fill capillary to the mark
- Be sure there are no air bubbles
- Wipe the outside of the capillary collector with a gauze pad in a downward motion
- Do not keep blood in capillary for more than one minute

# Unsatisfactory Specimens

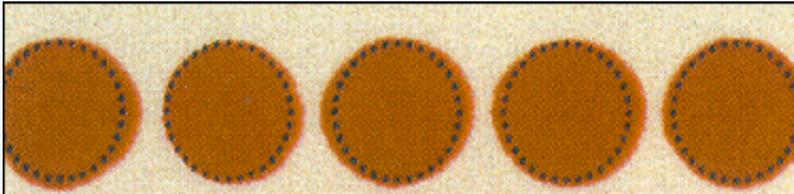
(Provided by the New York State Department of Health)



**Supersaturated**



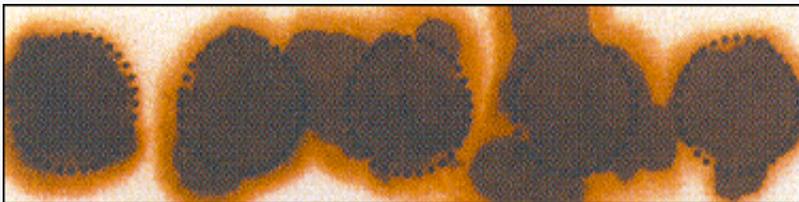
**Quantity Insufficient for Testing**



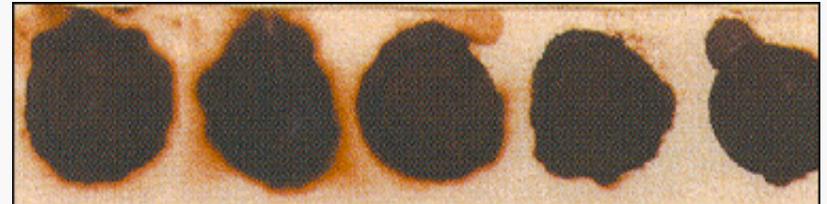
**Specimen Not Dried Before Mailing**



**Scratched or Abraded**



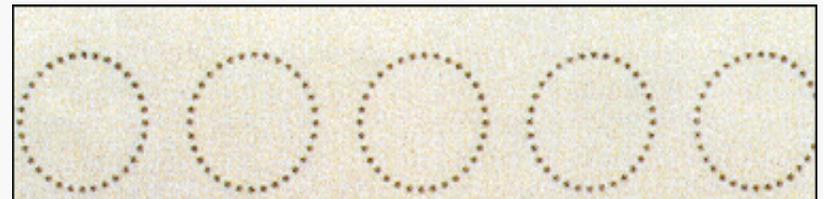
**Serum Rings**



**Diluted, Discolored, or Contaminated**



**Clotted or Layered**



**No Blood**

# Blood Spots drying at CDC



# Blood spots drying in Nepal





# FDA-Licensed DBS HIV-1 Kits

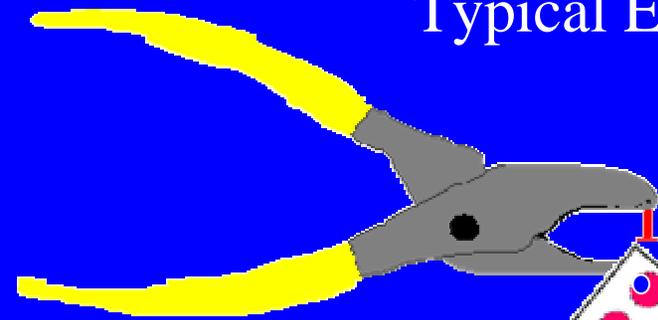
- Screening Method
  - Genetic Systems *rLAV* EIA (BioRad)
  - Vironostika HIV-1 Microelisa System (bioMerieux)
- Confirmatory Method
  - Genetic Systems HIV-1 WB (BioRad)
  - Fluorognost HIV-1 IFA
  - Cambridge Biotech HIV-1 WB (Calypte?)

# DBS Quality Control Materials are only available from CDC

- Commercial kits do not have DBS controls
- CDC Provides:
  - Negative DBS
  - HIV Low Positive
  - HIV High Positive
- Newborn Screening Quality Assurance Program
  - Contact: Carol Bell, [CBell@cdc.gov](mailto:CBell@cdc.gov)



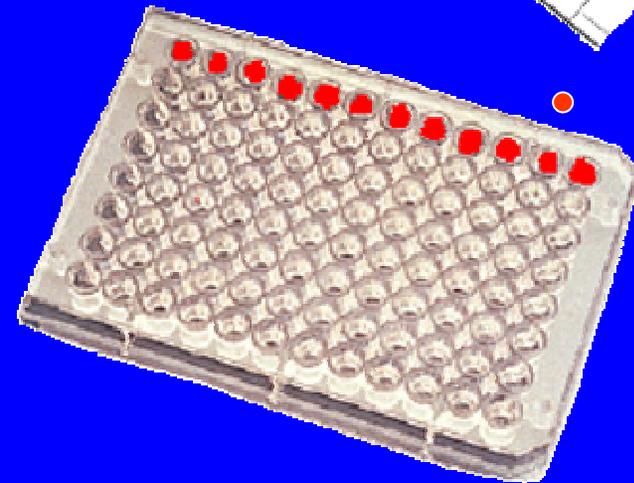
# Typical EIA Assay Procedure for Dried Blood Spots



Punch 3 or 6 mm disks into microwell plate

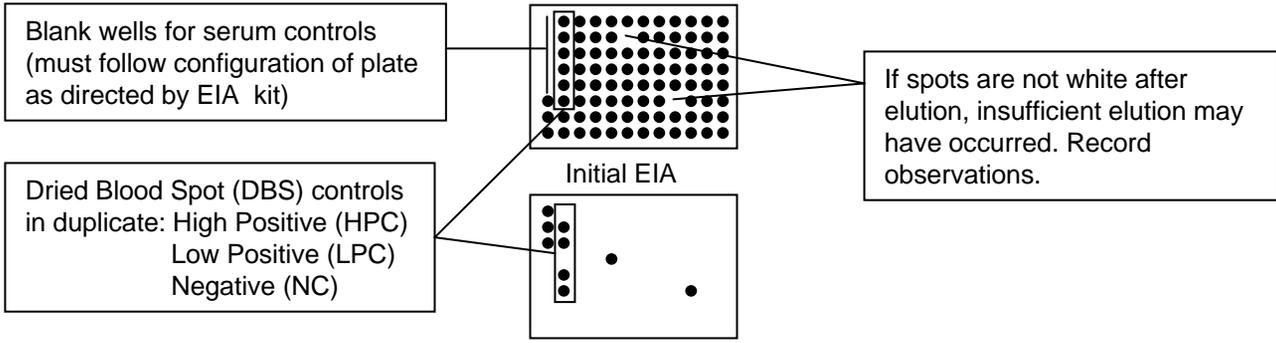
Elution Plate

Assay Plate

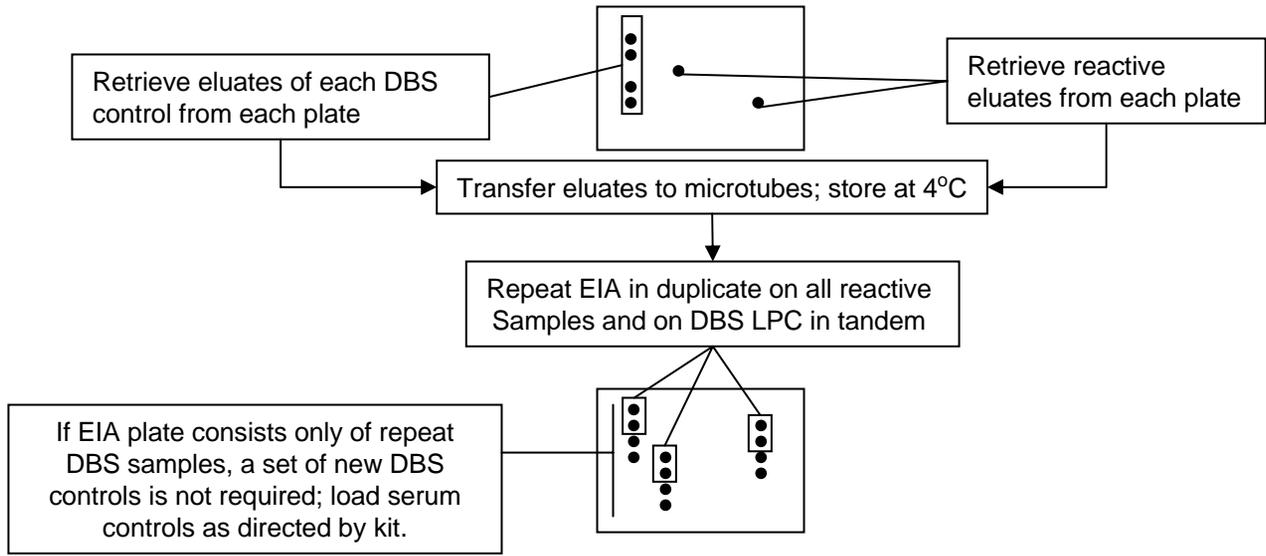


1. Add Kit Diluent (150  $\mu$ L, 1:30)
2. Cover plate, incubate overnight at 4°C
3. Shake plate gently to mix
4. Add Diluent to assay plate (125  $\mu$ L)
5. **Transfer DBS eluate (25  $\mu$ L) to assay plate (1:150 final serum dilution)**

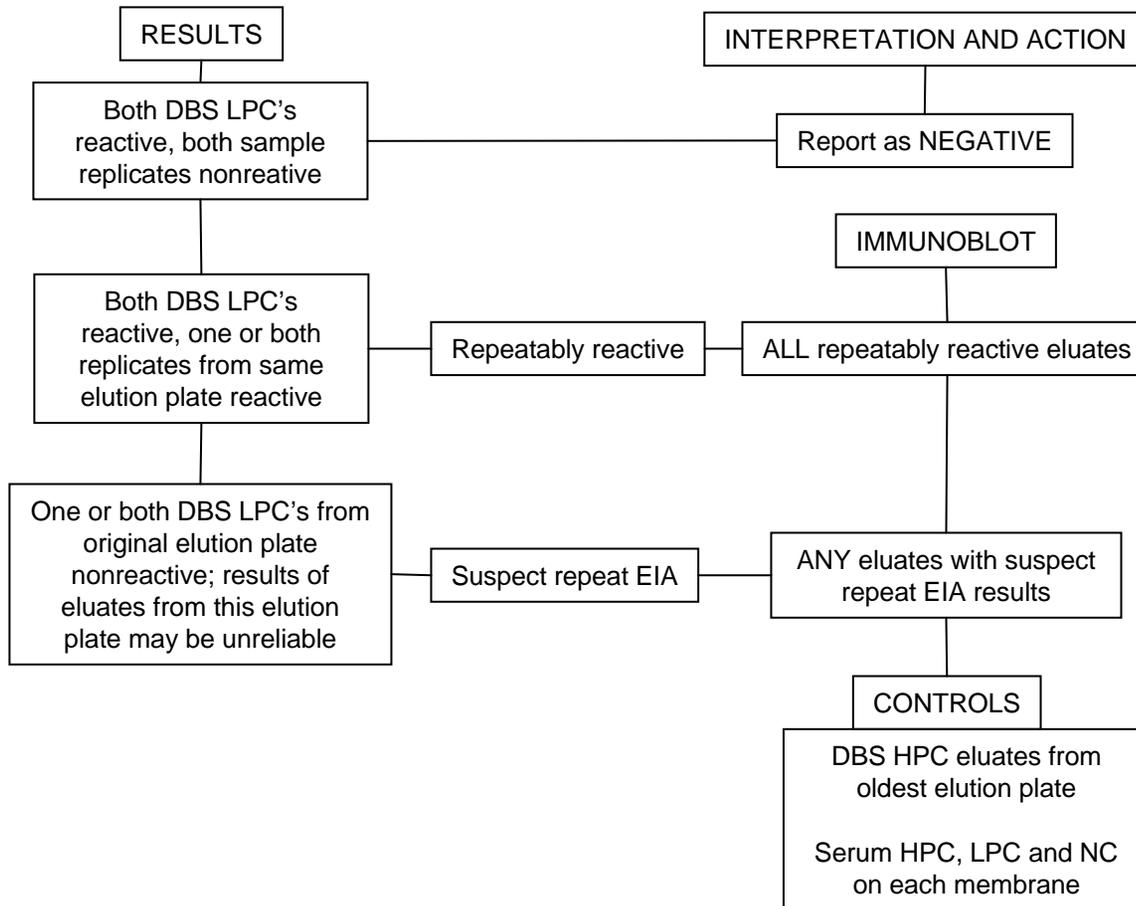
1. Cover plate, incubate plate 90 min at 37°C
2. Wash plate 4x
3. Add IgG-Enzyme Conjugate
4. Cover plate, incubate 30 min at 37°C
5. Add Substrate (150  $\mu$ L)
6. Incubate 10 min at 25°C
7. Add Stop Solution (150  $\mu$ L)
8. Read plate at 405 nm



**Elution Plate with Initially  
REACTIVE EIA Samples**



# REPEAT EIA



**SEROLOGIC ASSAYS FOR  
HUMAN IMMUNODEFICIENCY VIRUS  
ANTIBODY IN DRIED-BLOOD SPECIMENS  
COLLECTED ON FILTER PAPER  
FROM NEONATES**



**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Public Health Service  
Centers for Disease Control  
Atlanta, Georgia 30333**

**National Institute of Child Health and Human Development  
National Institutes of Health  
Bethesda, Maryland 20892**



# Changing the Algorithm with Rapid Tests

- Test Procedure
  - Fingertick done at remote site
    - Rapid test – report result to patient
    - DBS – save for confirmation
  - Send DBS to centralized lab for EIA testing
    - Return EIA result to site
    - Compare Rapid Test and EIA result
    - Tie breaker (Immunoblot)???

# Screening vs. Confirmatory test

- DBS have traditionally been used for HIV screening using EIA
- Western Blot has traditionally been used for confirming DBS EIA screening result
- NOW – a screening test is being proposed as the confirmatory test
- Quality Assurance A MUST!!!!

# Programs that provide blinded panels for proficiency testing:

- CDC Newborn Screening Quality Assurance Program
  - DBS only – QC and blinded PT materials
- Wisconsin State Laboratory of Hygiene (WSLH):
  - DBS – PT only for all matrices
  - Urine
  - Oral Fluid
  - Anti-HIV 1 and 2
  - Approved by HCFA, COLA, CAP, participation meets CLIA '88 requirements

