

# **Collection of Dried Blood Spots from Infants for Diagnosis of HIV by DNA PCR**

MOH Regional Trainings March 2013

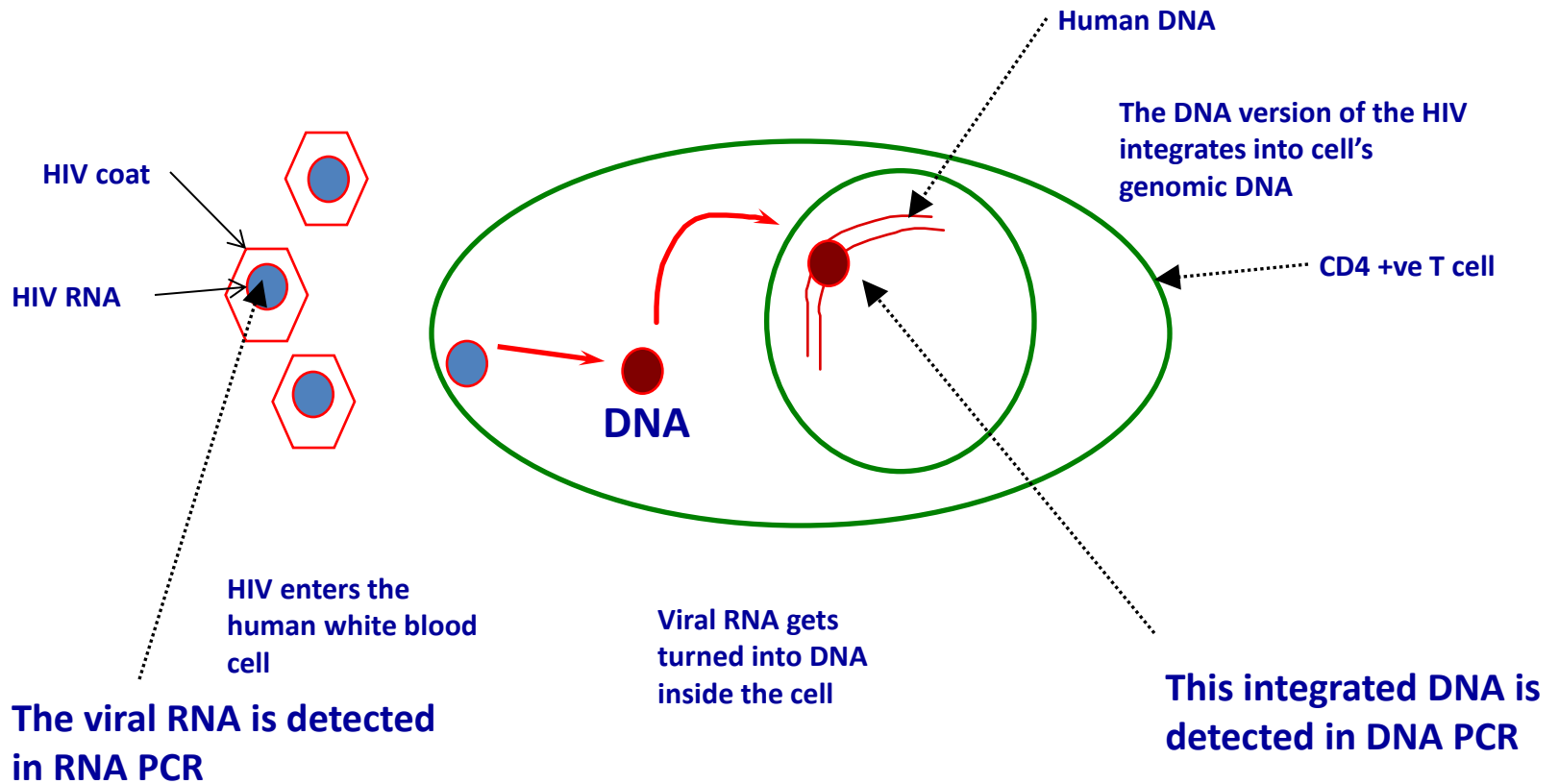
# Outline

- Introduce DBS testing
- Rationale
- Testing algorithm
- Sample collection, storage and transportation

# Challenges of laboratory diagnosis of HIV in infants

- Most HIV tests detect HIV antibodies
  - eg. Rapid tests, ELISA
- Infants have maternal antibodies for 1-2 yrs
- If HIV exposed, infants <18/12 will have positive/inconclusive antibody tests
- For definitive dx < 18 mos. virologic tests required
  - eg RNA PCR, DNA PCR, Viral load

# The Science of HIV RNA and DNA



**For RNA PCR, the sample MUST arrive at the testing lab within 6 hours of collection**

**For DNA PCR using DBS, samples are stable for many weeks**

# DNA PCR is the most accurate and widely accepted technology for early infant diagnosis

- DNA PCR testing is the gold standard for HIV diagnosis in infants
- Directly detects presence of HIV
  - Not subject to the weaknesses of serological tests
- Extremely accurate
- Most accurate after 4 weeks (>99% sensitivity)
- Not a new test
  - Used in USA and Europe for 15 years
  - Used extensively in Africa
  - Being implemented widely in Asia
- *Unlike RNA PCR, DNA PCR is highly suited to the DBS collection technique*

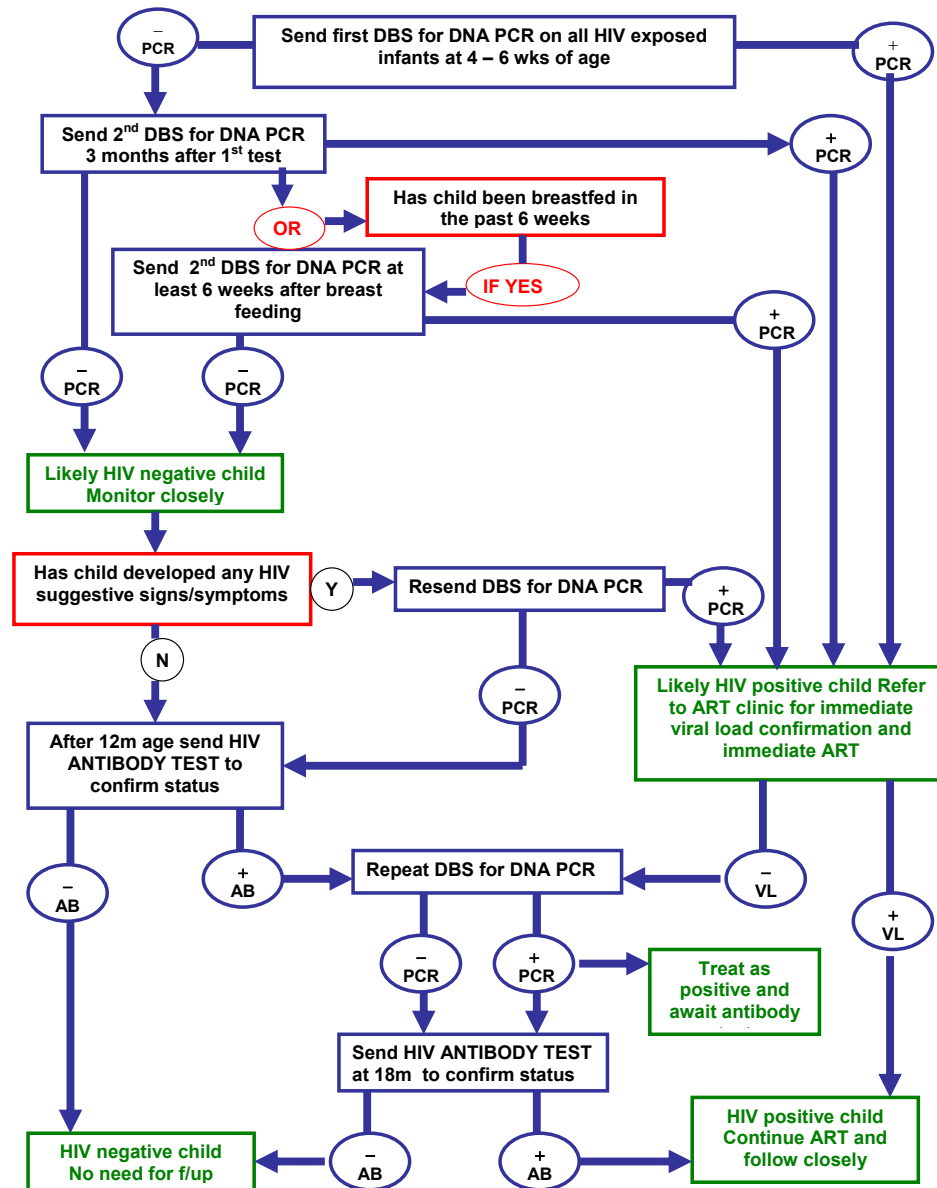
# DNA PCR testing using DBS

- Dried Blood Spots (DBS) are easier to collect from infants than whole blood samples
  - No need for venepuncture, only skin prick required
  - Requires only a very small amount of blood
  - Must be done carefully and properly to avoid contamination
  - DBS stable up to 15 weeks
  - DBS samples stable at room temperature
- Samples are highly stable, easy to store, and easy to transport
- The DBS protocol has the same sensitivity & specificity as whole blood
- DBS makes DNA PCR testing more feasible

# National Priorities

- HIV is a notifiable disease= mandatory reporting to Ministry of Health
- For every PCR done JaPPAAIDS in collaboration with the National HIV/STI Programme will need to know whether infants are HIV exposed or not
- This information is important for:
  - Accurate recording of National Statistics
  - Monitoring & evaluation of the PMTCT programme
  - Continuation of funding for treatment and care
  - Research to ensure evidence based management

# Testing algorithm for Jamaica



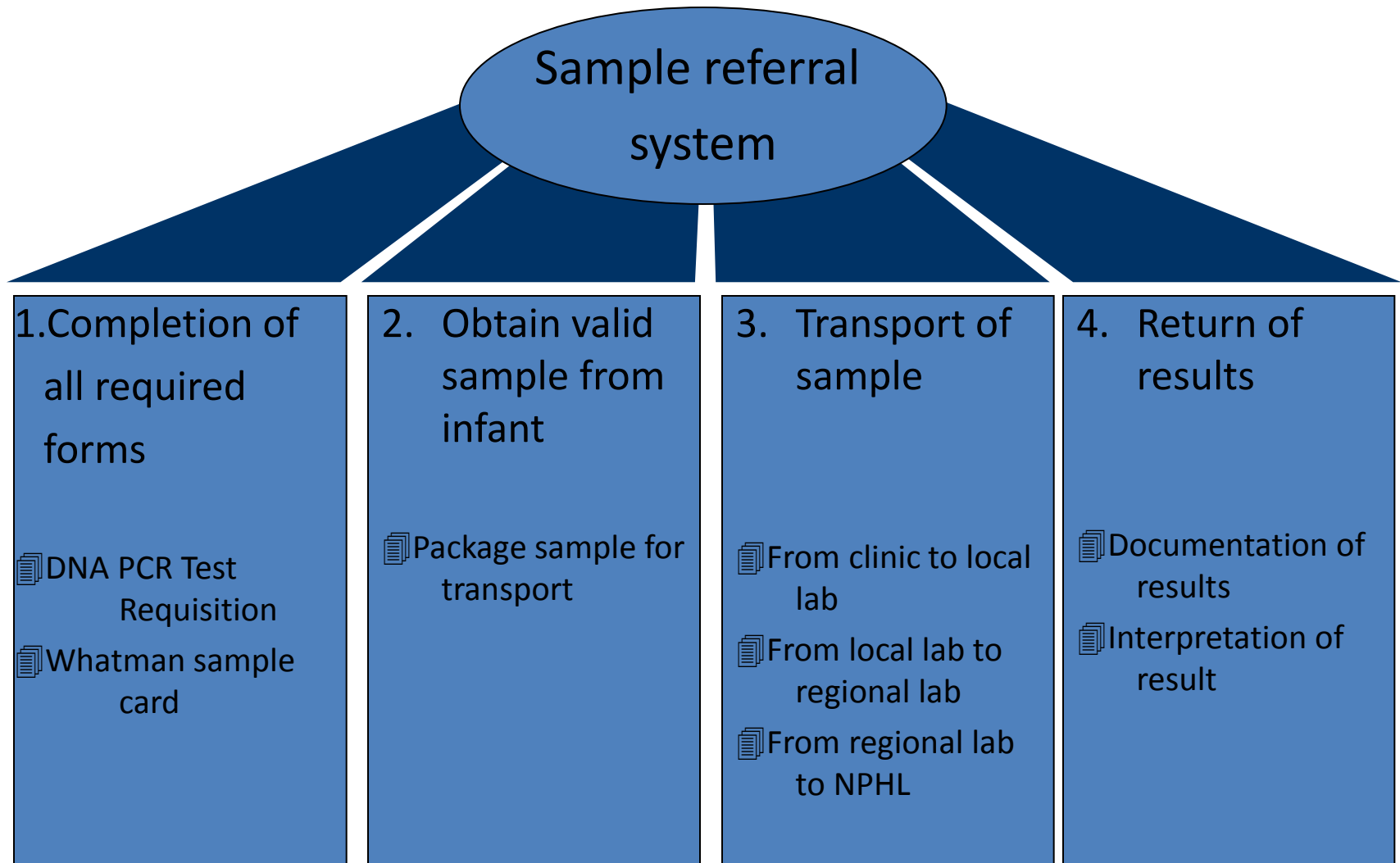


# What to do if DNA PCR is positive

- Document the result
  - Clinic: patient file, PMTCT register
  - Notify Paediatrician
  - Notify MOH
- Use local resources to get child to clinic
- Send urgent RNA Viral Load (whole blood sample) for confirmation
- Begin Antiretroviral Therapy immediately

*Treatment should be started while waiting for confirmation*

# Each step in the sample referral system is important



# Sample referral step 1.1: Completion of the DNA PCR Requisition Form: DBS Sample

Should record:

- Child name, and docket number
- Child's date of birth, age and sex
- Mother's name and (if possible) docket number
- Ordering consultant or physician
  - Collection facility
  - Sample details including date of collection and name and signature of person collecting the sample

# Sample referral step 1.2: Record patient information on the Whatman sample card

Spec #: \_\_\_\_\_

Collection Date: \_\_\_\_\_

Whatman 903® LOT W071 6279807 REF 10534737 2009-06

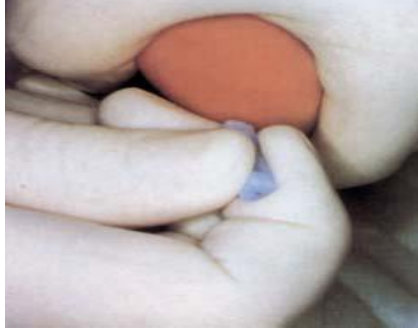
IMPORTANT:

DO NOT TOUCH COLLECTING CIRCLES  
WHEN FILLING OUT INFORMATION

SAMPLE WILL BE REJECTED WITHOUT  
DATE OF BIRTH OR DATE OF  
COLLECTION

Spec# <b>for lab use only</b>	Patient Name
Collection Date	Docket #                  Date of Birth
Initials of person taking sample	Mother's Name                  Docket #
	Physician's Name Clinic/Hospital

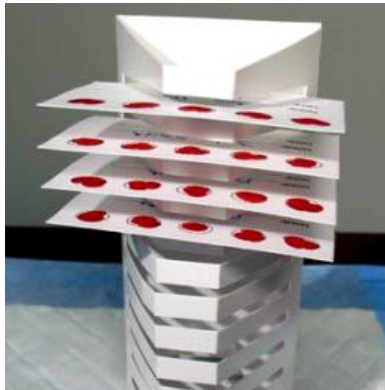
# Sample referral step 2.1: Obtain a valid sample



1. Sample collection site (usually the infant's heel) is punctured with a sterile lancet



2. Several drops are collected in circles on filter paper



3. Drops are dried in a rack or on a clean, flat, non-absorbent surface for a minimum of 4 hours



4. DBS can then be packaged with glassine paper, desiccant packs, and humidity indicator cards in a re-sealable plastic bag and stored

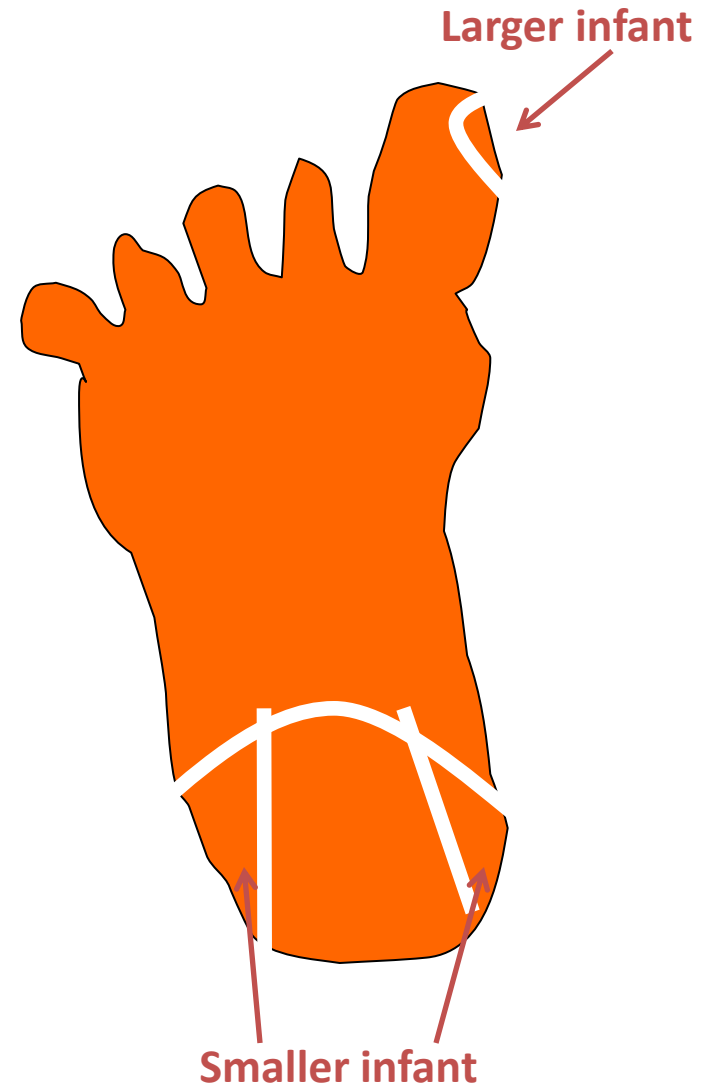
# Procedure for heel prick

## Site for pin prick

< 5 kg or 4 mths Heel

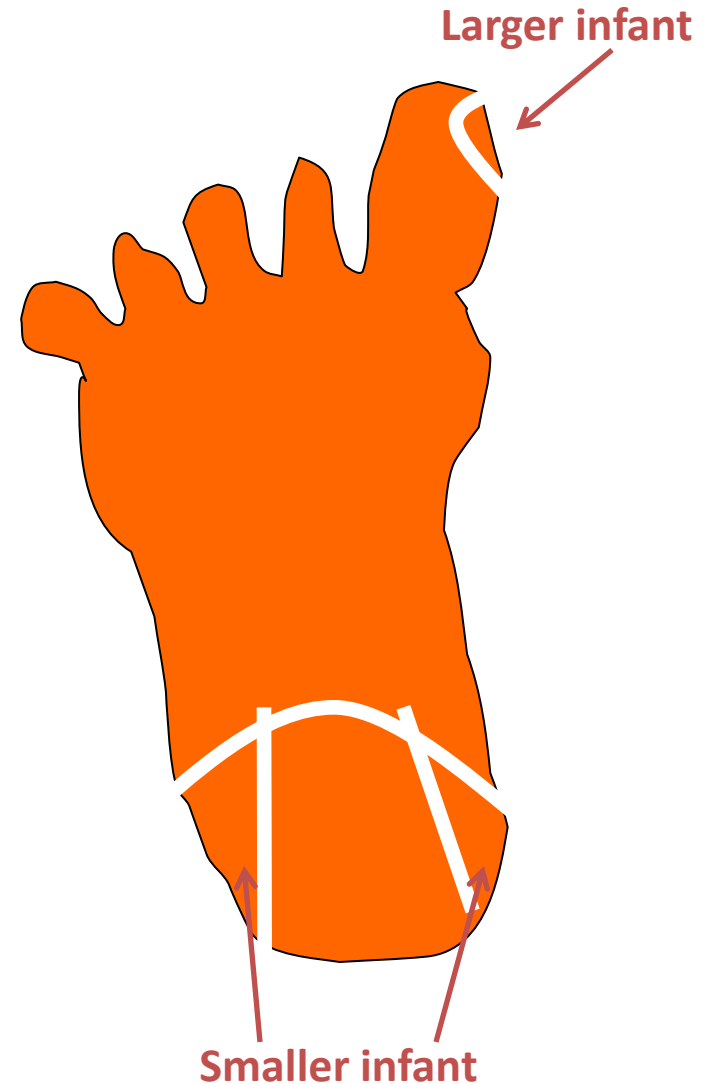
5-10 kg or <1 yr Toe

> 10 kg Finger



# Procedure for heel prick

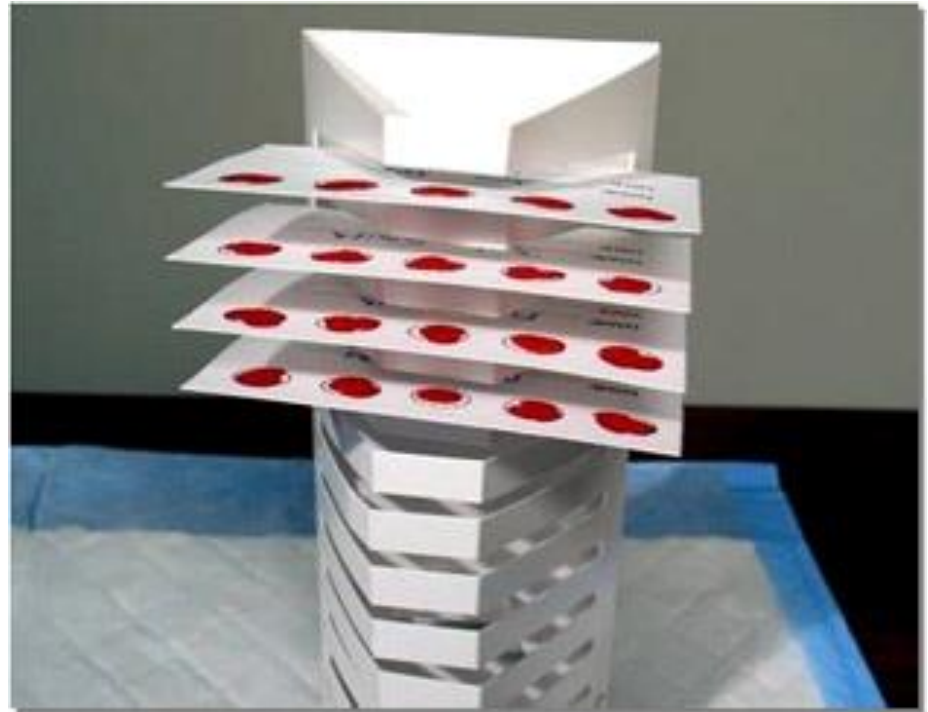
1. Clean the foot (if needed)
2. Warm the area
3. Put on gloves, wash hands
4. Position baby with foot down
5. Clean area, dry 30 sec
6. Press lancet into foot, prick skin
7. Wipe away first drop
8. Allow large drop to collect
9. Touch blood drop to card
10. Fill entire circle with drop
11. Fill at least 3 circles, try to fill 5
12. Clean foot, no bandage



# How to dry DBS

- Do not touch or smear the blood spots
- Can be dried in dedicated rack or on clean, flat, dry, non-absorbant surface
- Allow the specimen to air dry horizontally for at least 4 hours, can be left overnight
- Keep away from direct sunlight, dust, and insects
- Do not heat, stack or allow DBS to touch anything during the drying process (including other DBS cards)

**Dry completely before packaging**



**Keep requisitions with DBS cards at all times**



# How to package DBS for storage

**At end of each day, place the dry DBS in individual glassine paper envelopes  
(leave for following day if not dry after 4 hrs)**



**Insert into a special gas impermeable re-sealable plastic bag**

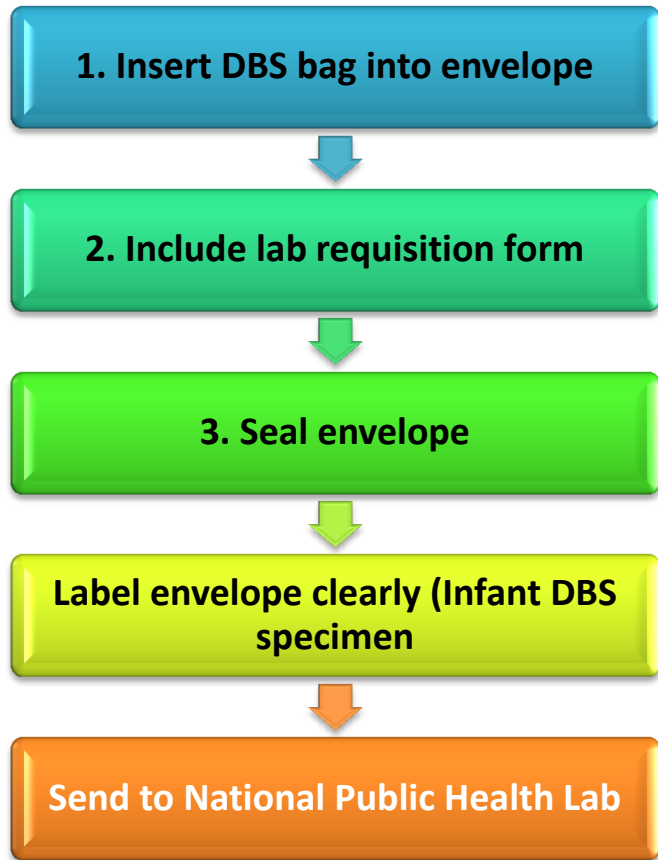


**Add minimum 5 desiccant packets & 1 humidity card  
Press air out of bag and seal**



**If not being transported to the Laboratory that day, store bag in dedicated area at  
room temperature with completed documentation until next lab transport day**

# How to Package DBS for Shipping



## Sample referral step 2.2: prepare samples for transport from collection site

- After properly drying the sample, each sample should be placed in an individual glassine envelope and placed in the special re-sealable plastic bag with humidity card and desiccants.
- Make sure that there is a lab requisition form for each sample
- Use of a large manila envelope for transport from the clinic will help ensure against loss of samples or requisitions

# What are your responsibilities?

It is the responsibility of the clinic to do the following:

- Label specimens correctly and LEGIBLY ( BLOCK LETTERS)
- Collect valid specimens
- Label and store samples appropriately until transported for testing
- Fill in lab forms and records correctly

***A test result is only as good as the specimen collected***

# Summary

- Early Infant Diagnosis (EID) reduces mortality and morbidity by enabling early treatment for HIV positive infants
- DNA PCR using DBS facilitates EID and is the preferred method
- HIV exposed infants should be tested at 4 to 6 weeks, at 3 months after the initial test or 6 weeks after cessation of breast feeding, and if symptomatic up to 18 months of age using DBS DNA PCR
- The confirmatory test for positive DNA PCR is an RNA viral load
- Commence antiretroviral therapy while awaiting confirmation
- HIV antibody testing at 12 to 18 months of age depending on feeding method will be the final confirmation of status
- HIV is a Class 1 Notifiable Disease - REPORT