DBS Sample collection for Early Infant Diagnosis (EID) by PCR

Laboratory Services Division
December 2015
I. Revised EID Algorithm
Background

- HIV-infected babies are the most vulnerable of all patients with ~ mortality > 50% by age 2 in untreated patients

- These patients would benefit the most from ART, but diagnosis is difficult due to the presence of maternal HIV antibodies transferred from mother to child during pregnancy, childbirth and breastfeeding

- Most infants born to HIV+ mothers would test positive using standard HIV antibody tests such as ELISA or rapid tests until the level of maternal antibody falls below limit of detection at 18 months

- Thus, in infants below 18 months of age, direct detection tests for the virus have to be conducted, and the current test of choice is the HIV-1 PCR which detects HIV pro-viral DNA & RNA
• HIV-1 PCR testing involves amplification of **target viral nucleic acid**

• The HIV-1 PCR test is:
  – Sensitive: 99.0%
  – Specific: 98%
  which means that a confirmatory test is critical to eliminate the rare cases of false positives

• **Window period** for HIV-1 PCR is typically **6 weeks** after last exposure

• Dried Blood Spots (DBS)/ Plasma are the **specimens** that can be used to perform HIV-1 PCR testing
Two types of infants who will need HIV diagnostic testing

- Infants who are **HIV-exposed** (mother known HIV positive from ICTC)
- Infants who are **sick with signs and symptoms of HIV**, even if unknown HIV exposure status (referred by MO / Paediatrician)
Objectives

1. Infant HIV testing algorithm to be universally followed and implemented on every HIV exposed infant to ensure equal and routine access

2. Linkage of the Exposed and infected infants to appropriate referral and care and treatment services to ensure timely intervention to reduce infant morbidity and mortality due to HIV infection
NATIONAL TESTING ALGORITHM FOR HIV-1 EXPOSED INFANTS AND CHILDREN <18 MONTHS: 2015

Follow Advisory 1

HIV exposed baby (born to HIV positive mother) or baby referred from Medical Officer with unknown exposure status presents at the ICTC (at least 6 weeks old)

Collect Dried Blood Spot (DBS) for HIV-1 PCR Test (at ICTC)

6 weeks to less than 6 months

6 months old or more

DETERMINE AGE OF THE BABY

Send the DBS sample for HIV-1 PCR Test

Collect blood and test for HIV antibodies using All 3 Serological tests. Also prepare a Dried Blood Spot (DBS) for HIV-1 PCR Test (at ICTC) at the same time.

Antibody Reactive (on all 3, any 2 or any 1 of the 3 tests)

HIV-1 Not Detected

Baby does not need HIV-1 PCR Test

Antibody Non-reactive (on All of the 3 tests)

HIV-1 Detected

Baby is HIV-1 infected

Refer to ART centre

Follow Advisory 2

Collect and send another DBS sample of the baby for Confirmatory HIV-1 PCR Test

HIV-1 Not Detected

Discordance: Lab will request for another DBS sample for second confirmatory HIV-1 PCR test from ICTC and rely on the result of this test for final diagnosis

HIV-1 Detected

A

B

Advisory 1

• Start cotrimoxazole if not already started
• Encourage exclusive breastfeeding for all babies till 6 months of age and avoid mix feeding
• If <6 months of age initiate complementary food along with breastfeeding

Advisory 2

• Continue cotrimoxazole till 5 years of age
• Manage OI, if any
• Start LPV/r based ART irrespective of CD4 count
• If breastfed, continue breastfeeding till 2 years of age for ‘HIV-1 Detected’ baby, avoid mix feeding before 6 months of age and initiate complementary food after 6 months of age
• Test for HIV Antibody for definitive diagnosis using all three serological tests at 18 months of age at the ICTC

Advisory 3

• Repeat testing from A at 6 months of age OR 12 months of age OR 6 weeks after last breast milk feeding, whichever is earlier
• If baby develops signs and symptoms of HIV infection at < 6 months of age, repeat HIV-1 PCR test from A
• If baby develops signs and symptoms of HIV infection at greater than or equal to 6 months of age, repeat test from B
• Continue cotrimoxazole until proven negative by all three antibody tests at 18 months of age or later
• If breastfed, continue breastfeeding till 1 year of age for ‘HIV-1 Not Detected’ baby, avoid mix feeding before 6 months of age and initiate complementary food after 6 months of age
• Test for HIV Antibody for definitive diagnosis using all three serological tests at 18 months of age at the ICTC

HIV-1 Not Detected

Baby is probably not infected, but is at risk

Schedule a follow-up visit

Follow Advisory 3

Universal Advisory

• Serological test not recommended
• If baby is < 6 weeks of age: HIV-1 PCR Test not recommended
• If baby is >6 weeks and above is the optimal age for a routine first HIV-1 PCR Test

1. Cotrimoxazole Preventive Therapy (CPT) to be initiated for all HIV exposed babies from 6 weeks of age and continued until proven HIV negative on all three serological tests at 18 months of age or later. In case the baby is found to be HIV infected by final confirmatory diagnosis on all three serological tests, LPV/r based ART should be initiated and CPT should be continued until 5 years of age.

2. Initiate babies on exclusive breastfeeding till 6 months of age, avoid mix feeding (any other milk products) before 6 months of age and add complementary food after 6 months of age. If the baby has been started on exclusive replacement feeding, continue the same and add complementary food after 6 months of age.

3. In rare cases of Serological-Discordance, i.e., the baby tests negative on any one or any two or all three serological tests after having been confirmed positive by HIV-1 PCR Test, continue ART and verify HIV-1 infection status through PCR testing. Refer the chart on ‘18 month antibody testing algorithm for all babies’. 
Baby presents at the ICTC at 18 months of age or later

WHAT IS THE HIV-1 INFECTION STATUS BY PREVIOUSLY DONE PCR TESTS?

HIV-1 Detected by PCR
Test for HIV antibody for a definitive diagnosis using all three serological tests at the ICTC

- Antibody reactive on all 3 tests
- Antibody reactive on 2 tests
- Antibody reactive on 1 test
- Antibody Non-reactive on all three tests

SEROLOGICAL-DISCORDANCE
- Collect two fresh DBS samples on two separate cards on the same visit for Verification PCR tests & send them to the testing lab
- DO NOT STOP ART

- Baby is HIV-1 infected
- Continue lifelong ART

- HIV-1 Detected on any one or both DBS Samples
- Contact NACO and email the baby’s details at serodiscordance@gmail.com
- DO NOT STOP ART

HIV-1 Not Detected by PCR
Test for HIV antibody for a definitive diagnosis using all three serological tests at the ICTC

- Antibody reactive on all 3 tests
- Antibody reactive on 2 tests
- Antibody reactive on 1 test
- Antibody Non-reactive on all three tests

- Baby is HIV-1 infected
- Initiate lifelong ART

- Indeterminate: Repeat antibody testing after two weeks

- HIV-1 Not Detected on both DBS Samples
- Contact NACO and email the baby’s details at serodiscordance@gmail.com
- DO NOT STOP ART

- Antibody reactive on any one or both DBS Samples

- No detected by PCR
- Never tested by PCR
At ICTC level: Collect & send Dried Blood Spot (DBS) of babies between 6 weeks to < 6 months of age for HIV-1 PCR test to the PCR testing laboratory

- **HIV-1 Detected**: Infant is probably HIV-1 infected. Lab will ask for another DBS sample.
  - If repeat sample is positive then baby is infected. Refer baby to ARTC for treatment.
  - If repeat sample is negative, then the lab will ask for another DBS sample and will rely on the result of this sample for establishing diagnosis.

- **HIV-1 Not Detected**: Ask baby to visit at 6 months age for testing again or if the baby develops signs and symptoms of HIV (whichever is earlier).
Algorithm for diagnosis of HIV infection at < 6 months of age (cont.)

• NOTE:

  - Rapid antibody test is not recommended

  - If baby is < 6 weeks old then PCR test is not recommended; 6 weeks and above is the optimal age for a routine first PCR test

  - If a DBS tests positive then another sample collected at a different time (not two samples spotted at same time) should be sent for confirming diagnosis
Algorithm for diagnosis of HIV infection at 6-18 months of age

- At ICTC level: Collect blood and test for HIV antibodies using all three serological tests. Also prepare a Dried blood spot (DBS) for HIV-1 PCR test simultaneously.

- If all three or any 2 or any one serological test is positive
  - Send Dried Blood Spot (DBS) of child for HIV-1 PCR test and follow the EID testing algorithm

- If all three rapid serological tests are negative
  - Baby does not need HIV-1 PCR test at this point
  - Ask baby to come back for testing at 6 weeks after last breast milk feeding OR at 12 months OR if baby develops symptoms of HIV infection, whichever is earlier
II. Overview of EID tests
Benefits of Early ART in Children

- Could mitigate negative effect of HIV on growth and development

- May help in immune recovery and reduce effects of HIV-induced chronic immune activation
Tests for Early Infant Diagnosis (EID) of HIV Infection

- Serology unreliable in infants (maternal antibodies cross placenta; can give a positive HIV antibody test in baby)
- Testing for EID is by a qualitative nucleic acid test (NAT)
- NACO EID referral laboratories tested with the Roche Amplicor HIV-1 DNA test (version 1.5) based on conventional PCR and hybridization for proviral DNA, until it was phased out by the manufacturer this year
- New tests detect total nucleic acid (TNA). The instrument can also be used for quantitative testing (viral load assay)
Tests for HIV Total Nucleic Acid

• Detect both RNA and DNA
• Examples
  – Abbott Real Time HIV-1
  – Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 (ver. 2)
• Real-time PCR
  – Quantitative test is FDA approved for determination of viral load on plasma
  – Qualitative test has CE-mark and is WHO pre-qualified for testing DBS samples
• Limit of RNA detection in DBS (claimed by manufacturer)
  – Roche: 300 copies/ml, Abbott: 2500 copies/ml (for hit-rate ≥95%); excellent correlation between the two tests
Abbott Real Time HIV-1

- **Fully automated** (sample preparation and RNA extraction on \textit{m2000sp}, test on \textit{m2000rt}) and manual extraction options

- **Target**: Highly conserved 172 nucleotide region of \textit{pol} (\textit{integrase}) gene

- **Internal control (IC)**
  - Unrelated to HIV-1 target; incorporated for each assay

- **Qualitative detection of HIV nucleic acid in DBS**
Target 2020: UNAIDS

90% diagnosed
90% on treatment
90% virally suppressed
III. Dried Blood Spot (DBS) Sample collection at ICTC
This presentation describes a procedure for collection of a dried blood spot (DBS) specimen from an infant below 18 months of age for the purpose of performing HIV-1 PCR testing.

Correct performance of the DBS collection using the aseptic technique is critical to ensure the safety of the procedure and to assure the quality of the test results obtained thereof.

Optimal specimen collections contribute significantly to the comfort and satisfaction of the donors thus encouraging retesting.
What is a dried blood spot (DBS)?

- Whole blood dried on filter paper provided (do not use ordinary filter paper)
- Usually obtained from pricking skin, not from phlebotomy
- Requires only a small amount of blood
- Easy to store
- Easy to transport
- Low biohazard
- A widely used method of specimen collection for HIV-1 PCR testing
- Performs comparably with whole blood collection methods in terms of sensitivity and specificity for HIV using PCR assay
- Facilitate wider accessibility to the testing service
Responsibility

Dried blood spots (DBS) should be made only by persons who have been appropriately trained in both the making of dried blood spots and in standard work precautions:

- MO/Pediatrician
- Nurse on duty
- Lab technician
Attend to exposed infants on a priority for collection of Dried Blood Spot (DBS) specimens everyday between 10 am and 1 pm

Check if the baby is born to an HIV positive mother or is carrying the referral for HIV-1 PCR testing from MO/Paediatrician

Obtain consent from the parent/guardian after pre-test counselling

Ensure availability of materials for dried blood spot collection

Label DBS card and fill TRRF just before taking the specimen

Store DBS samples at 2-8°C until transported and package appropriately

Ensure packaging appropriate specimens after verifying the TRRF and delivery checklist

Ensure all required documents (TRRF, delivery checklist, self addressed envelopes) are present

Ensure transportation of samples to the PCR testing laboratory through post / registered mail / courier on every Tuesday of the month
Every Infant will be allocated a **15 digit Unique Infant code**, to be allocated on the following basis:

- **First 3 digits:** DNA
- **Next 2 digits:** State code
- **Next 3 digits:** district code
- **Next 2 digits:** ICTC centre number
- **Next 2 digits:** Year
- **Next 3 digits:** Serial number of the Infant at the ICTC (001 onwards)
Material for ICTCs

- Guidelines
- Chart for testing algorithm
- TRRF (Test Requisition and Result Form) pads
- Delivery checklist pads
- Consent form pad
- Algorithm poster
- Labels
- Envelopes for mailing DBS cards to respective linkage lab
- Stickers of respective linkage laboratory address
- Dried blood spot collection kit
Materials required for DBS collection

- Sterile disposable lancet
- Sterile gauze and clean water
- Sterile alcohol preparation (70% isopropanolol)
- Gloves (powderless)
- Test requisition cum result form (TRRF)
- DBS card [specially formulated commercially available absorbent filter paper]
- Discard jar with 1% Sodium hypochlorite
- Drying rack
How to use the lancets provided

1. After cleaning the puncture site, TWIST OR PULL protective tab and place in disposal.

2. Position the puncture site facing downwards towards the card.

3. Gently squeeze and release the area to be pricked until it is ready to be bled, then prick the infant in the selected spot with the 2mm lancet.

4. After the first drop of blood is formed, wipe it away with sterile gauze and discard the gauze into the discard jar containing 1% sodium hypochlorite solution.

Please note that the pictures are for illustration and are in no way indicative of endorsement by NACO of any particular company or product. The same holds true for any commonly used terminology which is/are company specific.
Clearly label the DBS card with:

- The patient’s name and ID number
- The date and time of specimen collection
- Other information such as age, center name
Choosing the area to prick

- There are age specific recommendations for sites from where specimens are to be collected:
  - Children aged 6 weeks to 4 months: heel
  - Children aged 4+ to 10 months: big toe
  - Children aged 10+ to 18 months: finger prick- 3rd or 4th finger
- Use 2mm lancets
- Take care not to hit the bone of the child when pricking

*Areas shown by the arrows are the right areas to prick*
Preparation for collection

- Ensure that the baby has been born to an HIV positive mother/referred by MO/Pediatrician with strong suspicion of HIV infection
- Ensure completion of all lab records and proceed as below
- Introduce yourself and identify the patient (this is a critical step in specimen collection). Be warm and reassuring though honesty in your manner
- Explain the procedure and obtain proper written informed consent from the parent/ guardian with appropriate pre test counseling. Explain that if the child holds still, you are more likely to be successful
- Encourage parent involvement
- Obtain proper written informed consent from the parent/ guardian with appropriate pre test counseling
- Observe Standard work precautions at all times by wearing gloves and laboratory coat
- Put down a clean paper towel and lay out all the material you will need
- Complete the test requisition form with the details of the patient
- Label the DBS card (Patient Name / ID, date and time of specimen collection)
- Warm the area
- Position the baby
- Put on gloves, wash hands
Common DBS sample collection steps

- After pricking discard the lancet safely into the discard jar for sharps containing 1% freshly prepared Sodium hypochlorite solution.
- Wipe away first blood drop with sterile gauze pad
- Allow another LARGE blood drop to form.
- Lightly touch filter paper to LARGE blood drop. Apply blood to one side of filter paper only (the side with printing).
- Allow blood to soak through and completely fill circle with SINGLE application to LARGE blood drop. (To enhance blood flow, VERY GENTLE intermittent pressure may be applied to area surrounding puncture site).
Common collection steps after prick has been made - 2

- Do not contaminate filter paper circles by allowing the circles to come in contact with the body part, spillage or by touching before or after blood collection.
- Fill remaining circles in the same manner with successive blood drops.
- Take at least 3 to 4 good drops so that we have 3 to 4 complete circles filled with the sample.
- If blood flow is diminished, repeat from beginning with cleansing again.
- Once the required amount of specimen has been collected, pressure must be applied gently at the puncture site with a gauze to ensure that there is no further bleeding from the site.
Hold the whole foot. If necessary, apply moderate pressure to help form large drops of blood.
1. Position the hand palm side up. Select one of the 3rd or 4th fingers. Select the site on the - medial /lateral upper quadrant of the selected finger.

2. Massage finger to increase blood flow.

3. Clean the fingertip with alcohol. Work from the middle out to reduce contamination. Allow the area to dry.

4. Grasp the finger and place a new sterile lancet on the side of the fingertip.
Drying the DBS Cards

- Dry blood spots on a drying rack if available or on a dry, clean, flat non-absorbent surface for a minimum of four hours - the part of the card carrying the DBS should overhang the edge of the surface (as shown in the next slide).

- Do not touch the part of the card where blood has been applied to see if the blood has dried.

- As the blood dries on the filter paper it will change from a bright red color to a darker red-brown color, also the paper will buckle slightly.

Acceptable Sample:

Unacceptable Sample:

NOTE: At least 3-4 printed circle should have been completely filled with blood – otherwise the sample will be rejected by the lab.
Dry completely before packaging

- Place the DBS cards in the rack with the blood spots oriented outwards
- Dry horizontally
Invalid DBS specimens

Circles not filled well

Specimen not dry before mailing
Invalid DBS specimens (cont.)

- Specimen exhibits serum rings
- Alcohol not allowed to dry completely before puncture to draw blood
Documentation

- Document and maintain all requisition, delivery checklist and consent forms
- All necessary documentation and information of every specimen collected must be made in the designated register
- Record of failed attempts at taking a DBS specimen must be recorded.

Accountability of documentation, sample collection, storage, packaging, labeling and handing over to courier in time will lie with the MO/lab technician/nurse (person who collected the sample)
IV. Packaging & Transportation of DBS Samples
Materials required for DBS packaging

- Gloves (powderless)
- Glassine paper
- Plastic zip lock packets
- Desiccant sachets
- Humidity indicator cards
- Biohazard labels
- Blank label
- Plain paper envelopes
- Self addressed envelopes
- Padded paper envelopes
- Stapler
- Pen
DBS Packaging and Storage

• If specimen is not to be immediately transported, keep DBS packaged in sealable plastic bags refrigerated at 2-8° C until transported to testing lab (sent on every Tuesday of the month). **Do not freeze.**

• Each such ziplock bag carrying the specimens must have pasted on it:
  – a **biohazard sticker**
  – a **sticker specifying the identity of the specimens enclosed in that ziplock bag** - this information may be used to tally with the TRRF’s at the time of packaging before handing over to the courier. **This would also enable the compilation of the delivery checklist and ensure that no specimen/TRRF is missed out in final mailing/courier.**

• File the TRRF’s (3 copies per specimen) separately until required to be packaged in an envelope to go along with the specimens at the time of handing over the specimens to the courier. **2 copies of the TRRF will accompany the specimens to the testing lab and one copy will be retained at the centre for records**
• Check the color of the **humidity indicator** card twice a week. Anytime during storage or before packaging for handing over to the courier/mailing, if you notice a pinkish tinge in the blue circles on the humidity indicator card, change the card and replace the moist silica sachets with fresh dry silica sachets.

  65°C (hot air oven)

  **PINK** (High humidity)  **BLUE** (Dry)

• In case the specimen is to be handed over to the courier or mailed, **pull out the stored package from the refrigerator 1 hour before** the next steps of final packaging

• When contents have come to air conditioned room temp (20-25°C), place the zip-lock bag containing the DBS inside an envelope.
Transport of DBS specimens

- Use a reliable and tested courier/ mailing system for transportation of the specimen packages
- Centre must dispatch these collected specimens to the testing laboratory on every Tuesday of the month
- Take care to keep the DBS free from moisture and humidity.
- Postal and local transport regulations must be followed if using the postal or courier services.
## State & Testing Laboratory linkage

<table>
<thead>
<tr>
<th>State</th>
<th>Testing Lab</th>
<th>Testing Lab location</th>
<th>Lab in-charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP, Telangana, Tamil Nadu, Kerala, Pondicherry, Andaman and Nicobar</td>
<td>NIIRT</td>
<td>Chennai</td>
<td>Dr. Luke Hanna</td>
</tr>
<tr>
<td>Karnataka</td>
<td>NIMHANS</td>
<td>Bengaluru</td>
<td>Dr. Anita Desai</td>
</tr>
<tr>
<td>Mumbai, MP, Gujrat, Goa, Dadra &amp; Nagar Haveli</td>
<td>Kasturba Hospital</td>
<td>Mumbai</td>
<td>Dr. Jayanti Shashtri</td>
</tr>
<tr>
<td>Maharashtra</td>
<td>NARI</td>
<td>Pune</td>
<td>Dr. Sonali Kurle</td>
</tr>
<tr>
<td>J&amp;K, Delhi, Chandigarh, UP, Haryana, Uttaranchal, Rajasthan, Punjab, Himachal Pradesh</td>
<td>AIIMS</td>
<td>New Delhi</td>
<td>Dr. Lalit Dar</td>
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<tr>
<td>West Bengal, Assam, Manipur, Nagaland, Mizoram, Orissa, Bihar, Jharkhand, Chhattisgarh, Arunachal Pradesh, Meghalaya, Sikkim</td>
<td>NICED</td>
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RECAP: Dried Blood Spot Specimen Collection

Step 1: Counseling done and Consent form filled
Step 2: Make the child comfortable
Step 3: Fill out records
Step 4: Ensure availability of materials required for collection
Step 5: Preparation for collection (wash hands, wear gloves etc.)
Step 6: Collect the Dried Blood Spot (DBS) specimen
Step 7: Drying and packaging
Step 8: Storage and transportation
Step 9: Send to the testing laboratory
V. Supply Chain Management
Materials being supplied by NACO for the program

**Recurring supplies provided by SACS**
- DBS cards
- Gloves
- Lancets
- Humidity indicators
- Glassine envelopes
- Ziplock bags
- Drying Rack
- Labels
- Padded envelope for mailing
- Consent forms
- TRRF’s (Test Requisition and Referral Forms)
- Delivery checklists

**NACO supply**
- Guideline
- Poster showing EID algorithm
Inventory Management Leads to High Quality Testing

**Inventory management**

- Ensures availability of materials and kits when needed
- Avoids the use of expired items
- Minimizes wasting and relocations
Inventory Management Involves Knowing…

- What and how much stock you have
- When to order fresh supplies
- What and how much has been ordered, when it was ordered
- Where all supplies are stored
- When and how much fresh stock was received, and by whom
Inventory Management Involves …

- Performing a “stock count”
- Maintaining proper inventory records
- Determining when to re-order
- Determining how much to re-order
- Placing orders properly
- Inspecting delivery of new orders
- Ensuring proper storage of inventory
Inspect Delivery of New Orders

Upon receipt

- Verify contents of order received with requisition
- Check integrity of received supplies
- Date each item
- Note expiry date
- Store new supplies behind existing supplies
- Create or update records
Key Messages

• Maintain an adequate inventory at all time to ensure uninterrupted service

• Don’t let any item run out before re-order.

• Never order more than your storage space can hold. Never order more supplies than you can use before they are expired.

• All items in the inventory must be accounted for and recorded.

• Always inspect new shipment before accepting.
DBS card MONTHLY STOCK REPORT- SITE LEVEL (to be sent to SACS)

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### DRIED BLOOD SPOT (DBS) KIT MONTHLY STOCK REPORT

<table>
<thead>
<tr>
<th>Name of Center</th>
</tr>
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<tbody>
<tr>
<td>Unique Center ID</td>
</tr>
<tr>
<td>Reporting Month and Year</td>
</tr>
</tbody>
</table>

**Note:** For the Unique Center ID, please enter the first 10 digits of the Unique Infant ID assigned to your center (e.g. DNA MH THN 01 for GH Ulhasnagar, Thane)

For the Reporting Month and Year, please enter the month and year in full (e.g. September 2010, October 2012 etc.)

<table>
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<th>Opening Balance</th>
<th>Stock Received</th>
<th>Stock Transferred Out</th>
<th>Consumption</th>
<th>Wastage</th>
<th>Number of Tests Performed</th>
<th>Closing Balance</th>
</tr>
</thead>
</table>

Please enter the balance at the start of the month here

Please enter the number of DBS kits received in the month (from NACO or from other site)

Please enter the number of DBS kits transferred out to other centers during the month

Please enter the number of DBS kits consumed in this month

Please enter the number of DBS kits lost to wastage in this month

Please enter the total number of tests performed in this month

Please enter the closing balance at the end of the month

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Thank You

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