

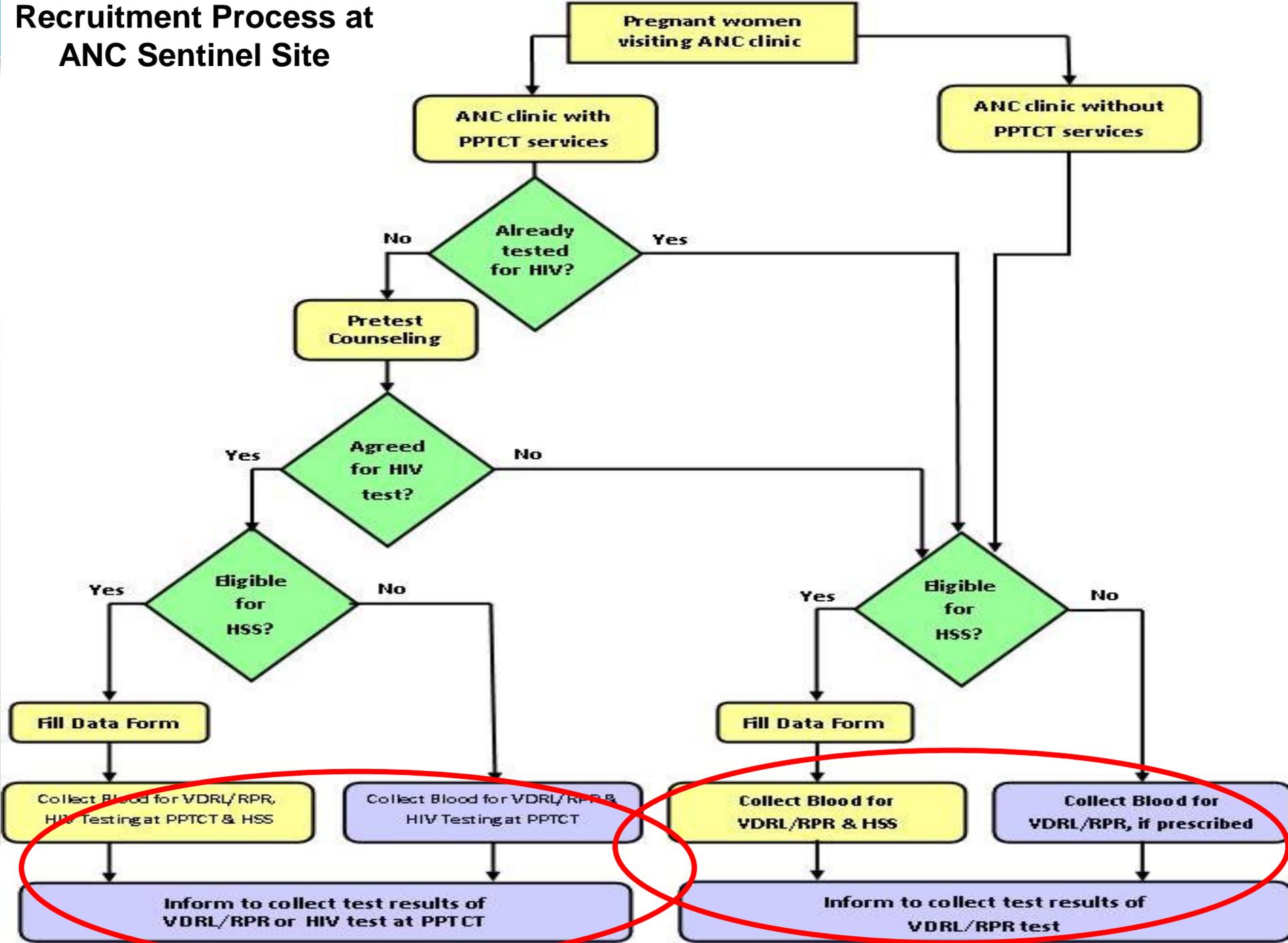
Session 5: Laboratory Procedures



Session Objectives

- ▶ At the end of this session, participants should be able to understand:
 - ▶ The basic requirements for blood collection and processing
 - ▶ The process of blood collection, serum separation, aliquoting and labeling
 - ▶ The procedures involved at site in preparing for transportation of specimens
 - ▶ The documentation involved with transportation of specimens
 - ▶ The basics of bio-waste management and management of needle stick injuries

Recruitment Process at ANC Sentinel Site



Blood Specimen Collection

Consumables Required for Blood Collection

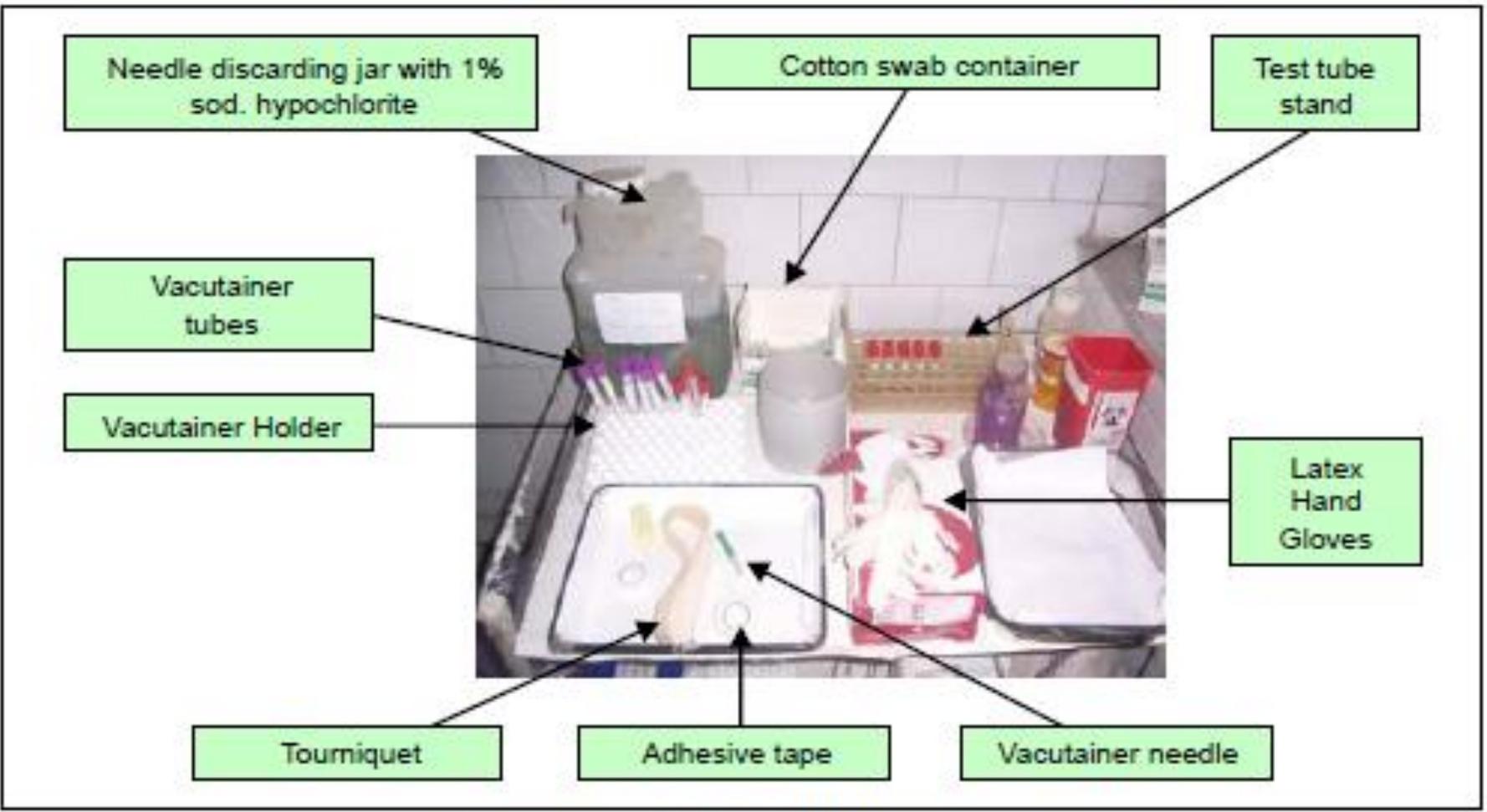
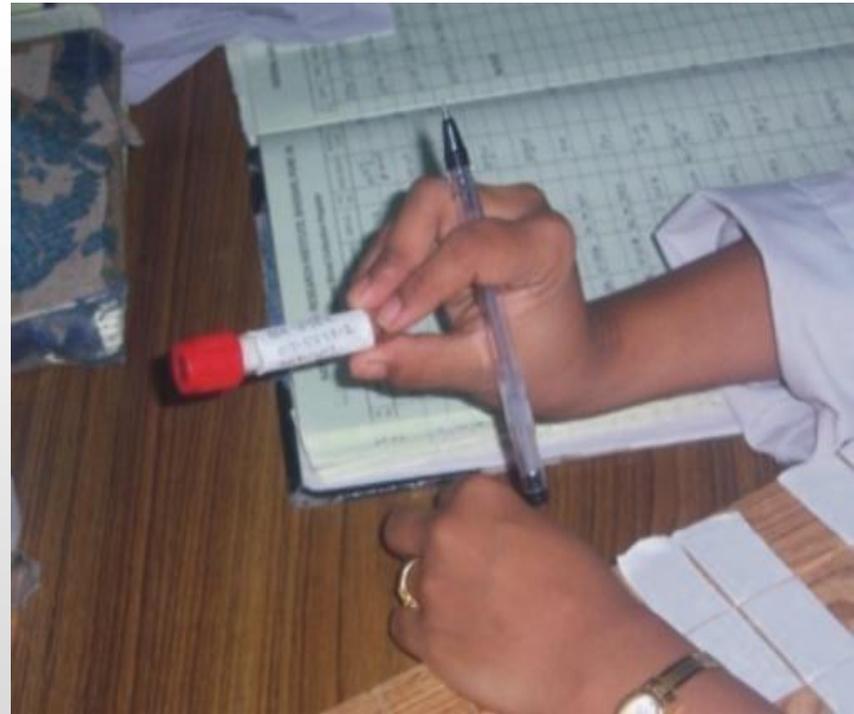


Fig 7 : Consumables required for blood collection

Blood Specimen Collection (1)

- ▶ Observe all universal precautions at all times by wearing gloves, apron & safety glasses
- ▶ Collect 5 ml blood by venipuncture in pre-labeled vacutainer/centrifuge tubes
- ▶ Prepare and label the tube for blood collection with Name, Age, ANC/PPTCT reg. no., Date and HSS sample number
- ▶ Keep this single labeled tube in the test tube rack to avoid picking up the wrong tube for specimen collection



Blood Specimen Collection (2)

Step 2 :

- Remove the rear protective cover (white) of the needle.
- Fix the rear end of the needle to the holder.
- Remove the forward / front protective cover of the needle (green).
- If blood is collected using needle & syringe, take a sterile disposable syringe & needle.



Fig 9 : Assembling the vacutainer system

Blood Specimen Collection (3)

Step 3 :

- The respondent made to sit on the chair and asked to Incline the arm in a downward position.
- Ask the respondent to clench and unclench the fist.
- Lightly tap the vein.
- Apply tourniquet.

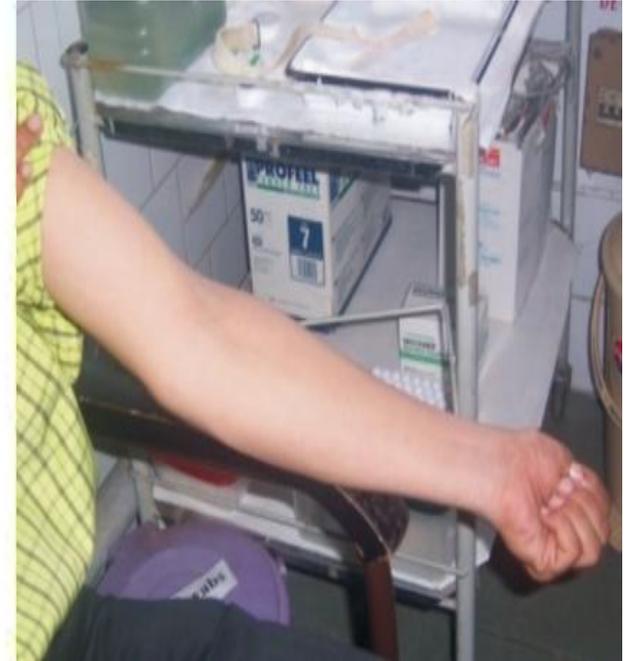


Fig 10 : suggested position of the arm for blood specimen collection

Blood Specimen Collection (4)

Step 4 :

- Disinfect the puncture site carefully and thoroughly.
- Wipe the skin surface with a cotton swab containing spirit or alcohol solution.
- Wipe in an outward moving circular motion. When dry, collect blood specimen.



Fig 11 : Disinfecting the puncture site for blood specimen collection

Blood Specimen Collection (5)

Step 5:

- Slowly insert the needle with the holder/syringe into the lumen of the vein.
- Hold the puncture device/syringe firmly to avoid any jerking movement with the needle in place to avoid unnecessary pain for the patient.



Fig 12 : Inserting needle into the vein

Blood Specimen Collection (6)

Step 6 :

- Hold the needle holder firmly and gently insert the vacutainer tube into the holder.
- Press the tube gently into the rear end of the needle in the holder so that the rear end of the needle penetrates the rubber top of the tube.
- Now the blood will flow into the tube.
- Holding the puncture device firmly gently remove the tube from the holder.
- If needle & syringe are used, gently pull the piston of the syringe to draw 5 ml blood into the syringe barrel.
- Placing cotton on the punctured site, gently remove the needle from the vein.
- Holding the puncture device/syringe in one hand, release the tourniquet completely.



Fig 13 : Inserting vacutainer tube into needle holder



Fig 14 : Removing vacutainer tube from needle holder

Blood Specimen Collection (7)

Step 7 :

- Place the vacutainer tube with blood specimen in the test tube rack.
- If needle & syringe are used, remove the needle and transfer the blood into the pre-labeled centrifuge tube from the syringe. Place the centrifuge tube with blood specimen in the test tube rack.



Fig 15 : Place vacutainer/centrifuge tube with blood specimen in the rack

Blood Specimen Collection (8)

Step 8 :

- Cover the puncture site with a sterile adhesive bandage. (Fig 16)
- Destroy the needle using the needle-cutter and discard it into the puncture proof discarding jar/sharps disposal container having 1% sodium hypochlorite solution. (Fig 17)
- Discard the gloves, cotton swab and gauze piece into the waste bucket with the yellow bag. (Fig 18)



Fig 16 : Apply adhesive tape over puncture site



Fig 17 : Discard needle in puncture proof container



Fig 18 : Use appropriate waste basket

Sample Processing: Serum Separation, Labeling, Aliquoting & Storage

Sample Processing (1)

Step 1:

- The blood specimen is allowed to stand for at least 20-30 minutes until the formation of clot before centrifugation.
- The blood specimen is centrifuged to separate the serum. Care must be taken to balance the vacutainer/ centrifuge tubes in the centrifuge, in order to prevent agitation and there by hemolysis.



Fig 19 : Blood specimen is allowed to stand before centrifugation

Sample Processing (2)

Step 2:

- The specimen should be centrifuged at 1,200 to 1,500 RPM for 10 minutes.
- Meanwhile, label the cryovials/serum vials into which serum will be transferred after centrifugation and keep them ready.
- Do not use glass tubes for storing specimens. Use only plastic vials.
- Determine the number of aliquots to be prepared from each blood specimen and prepare the labels accordingly.



Fig 20 : Centrifugation of blood specimens



Fig 21 : Blood specimen after centrifugation

Flow chart for activities at ANC sentinel site for blood Specimen Collection

Label tube with Name
Age, ANC/PPTCT
reg. no., Date and
HSS sample no.



Determining Number of Aliquots to be Prepared

- ▶ If Pregnant woman is not tested earlier under PPTCT but agrees to HIV testing during pre-test counseling and eligible for HSS, 3 aliquots (PPTCT, HSS, Routine) will be prepared
- ▶ If Pregnant woman is not tested earlier under PPTCT but refuses to HIV testing during pre-test counseling and eligible for HSS, 2 aliquots (HSS, Routine) will be prepared
- ▶ If Pregnant woman is tested earlier under PPTCT and eligible for HSS, 2 aliquots (HSS, Routine) will be prepared
- ▶ In a hospital where there are no PPTCT services, if pregnant woman is eligible for HSS, 2 aliquots (HSS, Routine) will be prepared
- ▶ In all other cases where pregnant woman is not eligible for HSS, follow the procedures for routine testing & PPTCT

Determining Number of Aliquots to be Prepared

Scenario	PPTCT Services Available	Already tested for HIV under PPTCT	Agreed for HIV test under PPTCT Now	Eligible for HSS	Carries Prescription/ Requisition form for lab tests	No. of Aliquots to be prepared
1	No	NA	NA	No	Routine tests	1
2	No	NA	NA	Yes	Routine tests & HSS data form	2
3	Yes	Yes	NA	No	Routine tests	1
4	Yes	Yes	NA	Yes	Routine tests & HSS data form	2
5	Yes	No	No	No	Routine tests	1
6	Yes	No	No	Yes	Routine tests & HSS data form	2
7	Yes	No	Yes	No	Routine tests & PPTCT	2
8	Yes	No	Yes	Yes	Routine tests, PPTCT & HSS	3

Sample Processing (3)

Step 3 : (Refer Flow Charts 2 & 3)

- Aliquot for routine testing (VDRL/RPR) and aliquot for HIV test under PPTCT should be labelled with personal identifiers (Name, Reg.No., Age, Sex, Date etc.) as per the routine practice.
- ALIQUOT FOR HSS SHOULD BE LABELED WITH HSS SITE CODE, SAMPLE NUMBER, SUB-SITE NUMBER AND DATE OF COLLECTION. No personal identifiers should be mentioned on HSS specimen, to ensure Unlinked Anonymous Testing.
- Make sure that the label is placed on the side of the tube, not on the cap.
- Only water resistant markers or lead pencil only should be used for labeling. Avoid use of ink or gel pens.
- Ensure that the HSS sample number is written only on the designated vial and the data collection form. It should not be recorded in the logbook or in any other place where it could be traced back to the patient.

Sample Processing (4)

Step 4 :

- After the specimen is centrifuged, transfer 0.5 ml of serum to the required number of sterile labeled serum vials (plastic, not glass) or cryovial (2.0 ml with screw cap) using a clean pipette (disposable plastic pipettes or micropipette with disposable tips).
- **DO NOT POUR** the serum from one tube to another. **USE** a pipette.
- Use separate pipette tips for each specimen.
- Make sure that the screw cap is tightly closed on the labeled cryovial or serum vial.
- After serum separation, the centrifuge tube with the clot should be decontaminated by autoclaving. Subsequently, tubes can be washed, cleaned & re-used.

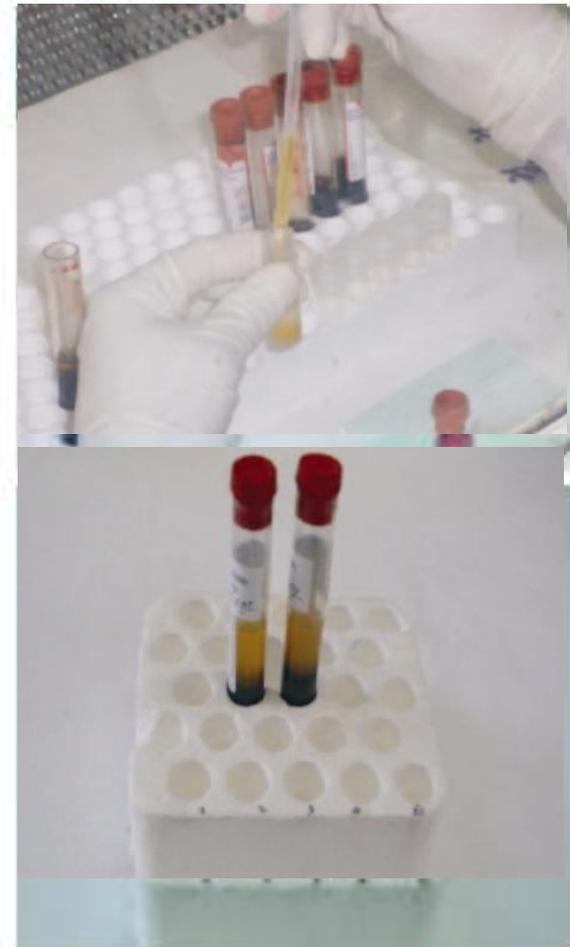


Fig 22 & 23 : Aliquoting the serum

Sample Processing (5)

Step 5 :

- Send the vial for routine testing to the concerned testing lab at the facility and return test results to the respondent subsequently.
- Send the vial for PPTCT to ICTC laboratory and return the test result subsequently.
- Store the vial for HSS at 4°C in the refrigerator **UPTO A MAXIMUM OF SEVEN DAYS.**
- Do not freeze. Do not de-frost the refrigerator when specimens are stored.



Fig 24 : Storage of HSS serum specimens

Wrong Practices in Sample Processing (1)



Fig 32 : Wrong practice of recapping the needle; Wrong practice of allowing blood to clot in the syringe itself. After collection, blood should immediately be transferred to the centrifuge tube and the tube should be allowed to stand for 20-30 minutes for clot formation, before centrifugation.

Wrong Practices in Sample Processing (2)



Fig 34 : Varying Quality of Sera at the Sentinel Site

Packaging of specimens and preparation for transportation

Packaging and Transportation of Specimens (1)

Step 1 :

- Check that each vial is tightly closed and sealed.
- Seal each vial with 'parafilm', just before transportation.
- The surface should be dried to ensure proper sticking of the film.
- Tightly wrap the parafilm on the junction of the cap & vial.



Fig 25 & 26 : Securing serum vials

Packaging and Transportation of Specimens (2)

Step 2:

- Sealed vials are packed in a proper sample transportation box with numbered lid so that the serum specimens remain upright during transportation.
- Do not transport any other material in this box.
- This container should be placed in a double plastic bag and sealed well.



Fig 21 : Proper Sample Transportation Box



Fig 22 : Numbered Lid of Sample Transportation Box

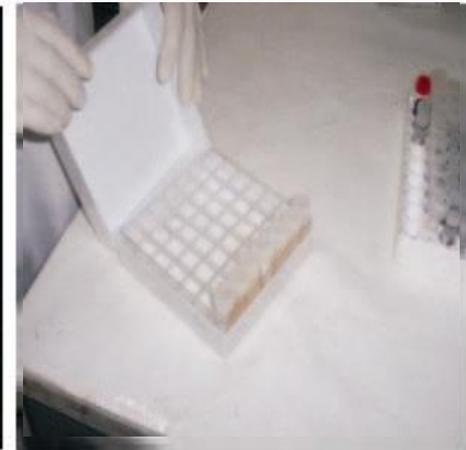


Fig 23 & 24 : Proper way of transporting serum specimens

Packaging and Transportation of Specimens (3)

Step 3:

- Place the sample transportation box in a vaccine carrier/ice box containing adequate number of pre-chilled cold packs to produce an ambient temperature of 4°C within the box for the duration of the journey.



Fig 31 : Placing sample containers in bigger box

Packaging and Transportation of Specimens (4)

Step 4:

- The serum specimens are transported to the testing laboratory on a weekly basis.
- Ensure that the specimens are delivered to the testing laboratory during working hours only (Ensure that its not a holiday before you leave).
- The samples should be accompanied by a duly completed and signed sample transportation sheet in duplicate.
- Once packed, the samples should reach the testing laboratory directly and there should be no deviation en route.
- The samples should remain in the fridge until the last moment and should not be taken home or elsewhere.

Packaging and Transportation of Specimens (5)

Step 5 :

- On reaching the HSS testing lab, the specimens along with the STS should be handed over to the testing lab in-charge or lab technician.
- Please wait while the samples are verified.
- Take back with you a signed copy of sample transport sheet and verification checklist.
- This should be handed over to the sentinel site in-charge on return and kept in a file for future reference.

Wrong Practices in Sample Transportation

Do not use rubber bands for packing



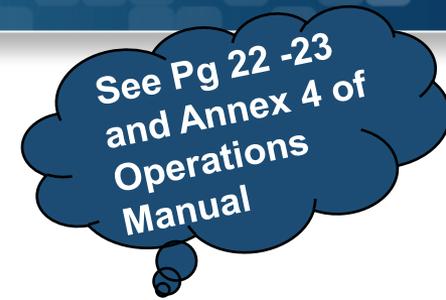
Do not use normal plastic Boxes for transporting Samples. Use Sample Transportation Box

Fig 35: Wrong practice of packing serum vials using rubber bands leads to chances of cross-contamination

Fig 36: Sample Transportation Box or Tiffin Box for School Kids? Use appropriate sample transportation box with numbered lid to avoid leakage of specimens during transport

Sample Transportation Sheet

General Instructions



- ▶ The responsibility of sending the blood specimens and the Sample Transportation Sheet (STS) is primarily that of the laboratory technician
- ▶ A properly filled STS, in duplicate, should accompany each set of blood specimens sent to the HSS testing lab. One more copy should be retained at sentinel site
- ▶ An acknowledgement of receipt from the HSS Testing Lab will be returned to the site, which should be stored at site for future reference

Instructions for Filling Sample Transportation Sheet

HIV SENTINEL SURVEILLANCE 2012-13

SAMPLE TRANSPORTATION SHEET

(To be sent in duplicate along with the samples)

1. Name and Complete Address of the Sentinel Site/Sub-site (Tick whichever is applicable) _____

District: _____ State: _____
2. A) Type of Site: B) Site Code:

--	--	--	--	--	--	--	--

 C) Sub-site No.
3. Period of Sample Collection: _____(dd/mm/yy) to _____(dd/mm/yy)

Instructions

- ▶ Clearly write the name and complete address of the sentinel site/sub-site, including district and state
- ▶ Mention the type of sentinel site i.e. ANC. Write the site code and sub-site number
- ▶ The period of sample collection i.e the period for which data forms are being sent, should be written in dd/mm/yy format

Instructions for Filling Sample Transportation Sheet

4. Total Number of Samples: _____
5. Total Number of Boxes: _____
6. Details of Sample Numbers:

S. No	Date of Collection	Sample No.	S. No	Date of Collection	Sample No.	S. No	Date of Collection	Sample No.	S. No	Date of Collection	Sample No.
1			26			51			76		
2			27			52			77		
3			28			53			78		
4			29			54			79		
5			30			55			80		
6			31			56			81		
7			32			57			82		
8			33			58			83		
9			34			59			84		
10			35			60			85		

Instructions

- ▶ Write the total number of serum samples and the number of boxes (containing the serum samples) being sent
- ▶ In the table, write the date of collection and sample number of each sample being sent
- ▶ If the space provided in the table is not sufficient, please attach another sheet

Instructions for Filling Sample Transportation Sheet

Samples Sent by: _____	_____	_____
(Name)	(Signature)	(Tel/ Mobile No.)
Date of Sending Samples: _____		
Samples Received by: _____	_____	
(Name)	(Signature)	
Date of Receipt of Samples: _____		

Instructions

- ▶ The sender should write legibly his / her name and telephone number and sign at the designated place before sending the of blood specimen
- ▶ Also write the date of dispatch of the of blood specimen
- ▶ The name, signature of the person receiving the of blood specimen and date of receiving the of blood specimen at the HSS Testing Lab will be written by the recipient and one of the two sheets will be returned to sentinel site
- ▶ The signed copy of STS received from the HSS Testing Lab should be securely stored at site for any future reference

Incorrectly filled Sample Transportation Sheet (STS) An Example

Period of sample collection is missing

National AIDS Control Organisation
Department of AIDS Control
Ministry of Health and Family Welfare, Government of India

Erroneous Site Code

Total number of samples not matching with the sample numbers

HIV SENTINEL SURVEILLANCE 2010
SAMPLE TRANSPORTATION SHEET

(To be sent in duplicate along with the samples)

1. Name and Complete Address of the Sentinel Site: General Hospital, Naharlagun
District: Papumpare State: Arunachal Pradesh
2. A) Type of Site: ANC B) Site Code: 12393031
3. Period of Sample Collection: ? (dd/mm/yy) to ? (dd/mm/yy)
4. Total Number of Samples: 1
5. Total Number of Boxes: 1
6. Details of Sample Numbers:

Period of sample collection is missing

4. Total Number of Samples: 1

5. Total Number of Boxes: 1

6. Details of Sample Numbers:

Two sample numbers are the same- not possible

S. No	Date of Collection	Sample No.	S. No	Date of Collection	Sample No.	S. No	Date of Collection	Sample No.	S. No	Date of Collection	Sample No.
1	15-10-10	001	26			51			76		
2	15-10-10	002	27			52					
3	15-10-10	003	28			53					
4	26-10-10	004	29			54			79		
5	26-10-10	005	30			55			80		
6	1-11-10	006	31			56			81		
7	1-11-10	007	32			57			82		
8	2-11-10	008	33			58			83		
9	2-11-10	009	34			59			84		
10	8-11-10	010	35			60			85		
11	9-11-10	011	36			61			86		
12	15-11-10	012	37			62			87		
13	15-11-10	013	38			63			88		
14	15-11-10	014	39			64			89		
15	16-11-10	015	40			65			90		
16	21-11-10	016	41			66			91		
17	21-11-10	017	42			67			92		
18	26-11-10	018	43			68			93		
19	27-11-10	017	44			69			94		
20	28-11-10	019	45			70			95		
21			46			71			96		
22			47			72			97		
23			48			73			98		
24			49			74			99		
25			50			75			100		

Date of sending samples is missing

Date of receiving samples is missing

If space provided above is not sufficient, please attach another sheet.

Samples Sent by: Md. Rapi
(Name)

[Signature]
(Signature)

94-360-59017
(Tel/ Mobile No.)

Date of Sending Samples:

Samples Received by: _____
(Name)

[Signature]
(Signature)

Date of Receipt of Samples:

Biowaste Management

General Principles

- ▶ Follow universal safety precautions during sample collection, storage, testing, transportation and disposal of bio-hazardous waste disposal
- ▶ Laboratory technician responsible for implementing safe bio waste management procedures under supervision of sentinel site-in charge
- ▶ Colour-coded bags to be used for disposal of waste materials and contaminated sharps
- ▶ Any spillage of potentially dangerous material should be properly cleaned and decontaminated following standard procedures

Disposal of waste material and contaminated sharps

- ▶ Used needles and syringes should be disposed off by using a needle cutter. After crushing hub of needles, put in a puncture-proof container containing freshly prepared 1% hypochlorite solution. At the end of the day, contents should be put in a bio-waste bag (blue colour) 
- ▶ Alcohol swabs, gloves, gauze pieces should be discarded into a biohazard bio-waste bag (yellow colour) 
- ▶ General waste such as wrapper of gloves, paper, should be discarded in bio-waste bags (black colour) 
- ▶ All bags should be finally disposed as per standard procedures at the site

Sometimes there are state-specific variations in the color specification of waste bags for different types of waste. Please comply with regulations of your state.

Preparation of 1% Sodium Hypochlorite Solution

Parts of Stock Solution: Parts of water

4% stock solution

5% stock solution

10% stock solution

15% stock solution

1:4*

1:5*

1:10*

1:15*

Management of Needle Stick Injury

- ▶ Needle stick, puncture wounds, cuts, open skin contaminated by spills or splashes should be washed thoroughly with soap and water
- ▶ Report injury to the laboratory in-charge or site in-charge as the case
- ▶ Assess individual for Post Exposure Prophylaxis (PEP). PEP, preferably should be started within 2 hours and no later than 72 hours of the accidental exposure
- ▶ Appropriate medical evaluation, treatment and counseling should be provided
- ▶ For details on PEP, please refer to NACO Guidelines for Post Exposure Prophylaxis on www.nacoonline.org

DISCUSSION

END OF SESSION 5