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NACO currently has 100 (existing & upcoming) operational ART centres catering to the needs of HIV infected individuals. A number of these ART centre are currently equipped with CD4 machines and there is a plan to equip some more centres with CD4 machines. However, in order to maximize the capacity utilization of the CD4 machines at the various existing and upcoming centres, NACO proposes to develop linkages between the various centres (for CD4 testing) by devising an effective sample transport network.

The purpose of the current document is to provide a guideline to the various ART centres for collection, handling and transportation of specimens for CD4 testing to the CD4 testing laboratories. The guidelines cover the following:

- Standard operating procedures for collection and handling of samples for CD4 testing
- Recommendations for transport of samples including instructions for sample packaging.
- Rejection criteria for samples.
- Devising a unique patient identification number .
- Instructions for the laboratories receiving the samples for CD4 testing.
- Chain of custody from sample collection to reporting of results.
- Data Management
- Release of results
- The CD4 specimen and report log in registers
- CD4 test requisition and report form

Ideally, when monitoring a patient, blood specimen should be collected from the same person at similar times of the day. Suggested timing for collection is between 9-11 AM. Fasting conditions are not required.

- K₂/K₃ EDTA vacuum based collection tubes (3-5ml capacity)
- Collection needles compatible with the vacutainers.
- Tourniquets, spirit swabs
- Needle destroyer
- Plastic or thermocol box for packaging and shipping samples. A bread box or a vaccine carrier box can also be used for this purpose.
- Cotton gauze as absorbent material to be placed inside the sample packaging box.
- A tube rack which fits the vacutainer tubes.
- Cool gel packs



Procedure for sample collection and handling:

1. Approximately 3 to 5 ml of peripheral blood should be collected in K₃ or K₂ EDTA vacutainer tube.
2. The tube should be labeled with patient's identification, date and time of collection as well as the name of the collecting personnel. The tube labels should be double checked for accuracy with the sample request forms before sending it to the testing lab
3. The blood should be mixed properly by inverting the tube 6-8 times immediately after collection. Formation of small clots may affect accuracy of the count and ability to run the instrument.

Sample transportation

- For CD4 machines using the CD3/CD4/CD45 combination (BD FACS Calibur), anticoagulated blood samples shipped at ambient temperature (20-25⁰C) must be stained within 48 hours of draw and then analyzed within 6 hours of staining¹. Similarly, for CD3/CD4/CD8 and CD3/CD4 reagent combination used on BD FACScout, whole blood (anticoagulated blood) samples shipped at ambient temperature (20-25⁰C) are stable for 48 hours after draw².
- The specimens should not be refrigerated or frozen. The samples must be transported and stored at ambient temperature (20-25⁰C). If the external temperature is high, the samples should be shipped with a cool (not frozen) gel pack.
- Extremes in temperature should be avoided, so that specimens do not freeze or get heated above 32⁰C.

Rejection criteria

The sample should be rejected if

- Sample is hemolysed
- Sample is frozen
- Sample is clotted
- Sample tube is cracked / broken
- Sample has not been drawn in a K₂ / K₃ EDTA vacutainer
- Sample not shipped under ambient conditions (20-25⁰C)
- Quantity not sufficient (at least 3-5 ml of sample must be available).

Recommended instructions for transportation of blood samples for CD4 testing

The shipment of infectious agents is regulated by the Transportation of Dangerous Goods Act and the International Air Transport Association (IATA) dangerous goods regulations. HIV infected specimens are classified as infectious class 6.2 substances under the United Nations (UN) no. 2814. The packaging instructions are given below:

¹BD Tritest CD3 FITC/CD4PE/CD45PerCp Technical Data Sheet

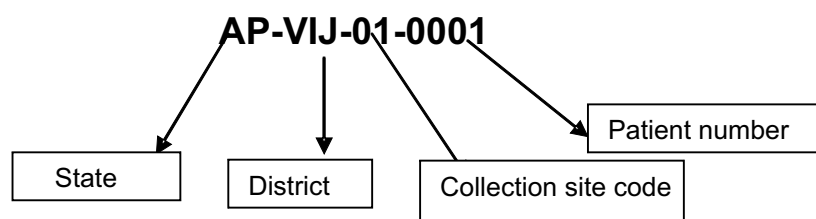
² BD Biosciences – Immunocytometry systems; Technical specifications BD FACScout system, 2005.

- The specimen should be carefully packaged to protect it from breakage and insulated from extreme temperature
- Label for “**CD4 count**”. The collection site should make use of a unique identification number as sample identity. Names of the patients should be avoided to prevent confusion on account of duplication of names as well as to maintain confidentiality.
- Secure the vacutainer cap carefully and seal it further with sticking tape (placed so that it covers the lower part of the cap and some part of the tube stem).
- During packaging, the tubes containing specimens should be placed in a tube rack and packed inside a cool box (plastic or thermocol) with cool gel packs (so that the temperature during transport is maintained at 20-25°C) placed below and on the sides of the tube rack. Place some cotton or other packaging material between the tubes to ensure that they do not move or rattle while in transit. Cool box required for transportation could be a plastic bread box or a vaccine carrier. Seal/secure the lid of the cool box .
- This cool box should then be placed in a secure transport bag for purposes of shipping to the testing facility. The request slips should be placed in a plastic zip lock bag and fastened securely to the outside of the cool box with a rubber band and sticking tape.
- A biohazard label should be pasted on the visible outer surface of the package containing the samples. The package must be marked with arrows indicating the 'up' and 'down'side of the package
- Samples should be transported to the receiving laboratory by commercial courier or be hand delivered by a trained delivery person.
- The collection site must have prior knowledge of the designated testing days of the CD4 laboratory to which the samples are being sent.
- No transport should be done during weekends and holidays or non-testing days of the CD4 testing laboratory unless prior arrangement has been made with the receiving laboratory.

Note: Overnight carriers with an established record of consistent delivery should be used to ensure arrival of specimen within the specified time. The infectious nature of the sample must be declared to the courier company. Alternatively in case of non-availability of commercial couriers or for shorter distances, lab staff may be used to hand deliver the samples.

As mentioned earlier, all patient samples must carry a unique patient identification number. This unique patient identifier is required to avoid any confusion on account of similarity in patient names and also for the purpose of maintaining confidentiality .

An alphanumeric code could be deployed to serve as the unique patient identifier. Following is an example; AP (State of Andhra Pradesh), VIJ (District - Vijaywada), 01 (collection centre code) and 0001 (patient number)

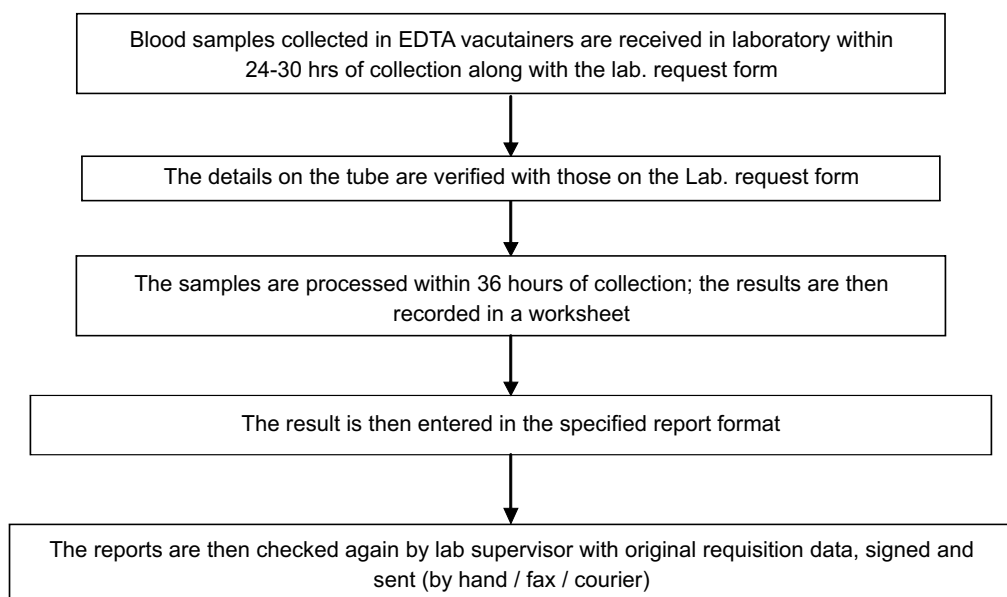


Each ART site transporting the samples to the nodal CD4 testing site should be assigned the unique site code as generated above.

Instructions for receipt of the sample in the laboratory (nodal CD4 testing laboratory) for purpose of testing

- Gloves should be worn before opening the courier package.
- The package should be checked for any visible leakage before opening.
- After opening the package, the details on the specimen request form should be checked and confirmed against the label on the sample tube. The same should be entered in the appropriate lab register.
- A unique lab number must be assigned to every sample. The lab register should also record the time of draw of the sample and the time at which the sample was received in the lab for testing.
- In case of any doubts regarding the identity of the sample, the testing lab personnel should immediately get in touch with collection site to check / confirm the identity of the sample. No testing should be done without confirming the identity of the sample (in case of doubt).
- Similar procedure should be followed to confirm the identity of the sample in case the package is received without a test request form.
- The sample must be rejected if:
 - Sample is hemolysed
 - Sample is frozen
 - Sample is clotted
 - Sample tube is cracked / broken
 - Sample has not been drawn in a K₂ / K₃ EDTA vacutainer
 - Sample is not shipped under ambient conditions (20-25⁰C)
 - Quantity is not sufficient (at least 3-5 ml of sample must be available).
- After retrieving the sample from the package and noting all details, the package must be discarded as per standard waste disposal guidelines.

Chain of custody from sample collection to reporting of the result



Important Instructions

- Samples must be collected in K₂/K₃ EDTA vacutainer tubes only. Anticoagulants such as heparin and sodium citrate should not be used.
- Written policies and instructions on sample collection and transport should be available to all lab personnel.
- The courier company should have clear instructions on the timing for pickup of samples from collection sites and delivery of samples at testing sites.
- Collection site should label tube correctly and double check tube labels and test forms for accuracy before sending to the nodal CD4 testing laboratory
- Extremes in temperatures should be avoided during shipping of samples:
 - Temperatures >32⁰C may cause cellular destruction and affect both hematology and flow cytometry measurements.
 - Under such conditions, ship samples with refrigerated (not frozen) cool gel Packs.
- Samples should not be stored in the refrigerators before processing. They must be maintained at ambient temperatures (20-25⁰C). Follow manufacturer's instructions / SOPs.
- If a canister is being used for sample transport the specimen tube should always be packed in a leak proof container (e.g., a tube or plastic zip lock bag) and then in a bigger insulated container with absorbent material to contain an accidental spill.
- The test requisition forms should always be kept separate from the specimen container to prevent smudging in case of leaks from breakage or spills.
- Samples should be transported to the CD4 laboratory as soon as possible
- Samples should be transported by commercial courier or clinic vehicle. Transport is possible by many methods including air, car, bike, motorcycle and hand-carriage.

Data Management

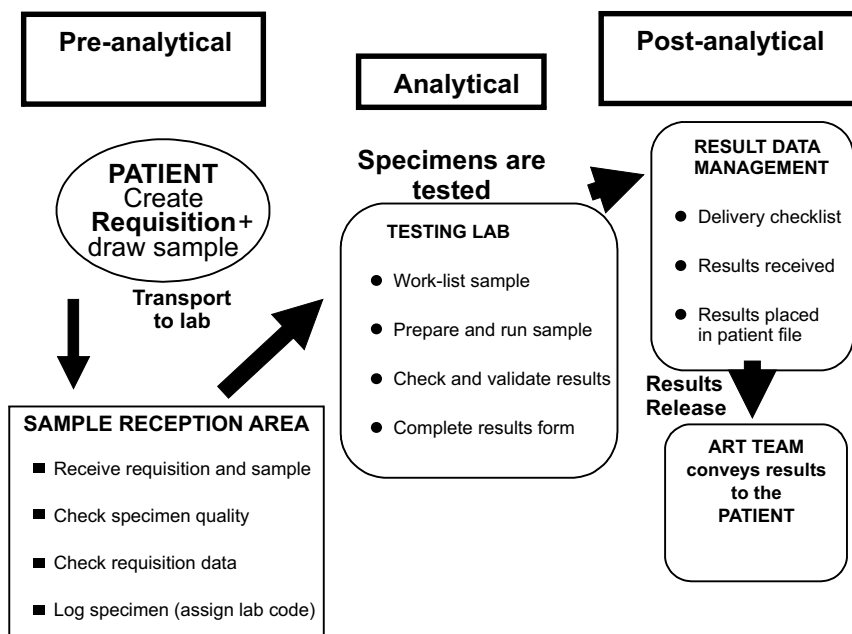
Strict procedures for data management during the preanalytical, analytical and post-analytical phases of testing are required to ensure the reliable production and delivery of accurate test results. Log-Books should be used to record receipt of samples (Patient ID, date and time, test requested will be recorded) and the production and release of results (date of analysis, the result and the date of release to clinic site). The SOPs will ensure:

- Reliable and rapid delivery of results to referral ART sites.
- That testing laboratories have reliable systems for receiving and processing result data
- Uniform basic data handling, storage, and reporting standards
- That the testing laboratory maintains records of result data for defined periods to allow repeat reporting of lost test results as well as aggregation for monitoring and evaluation or research purposes
- Preparation and dispatch of periodic reports to NACO, easily and quickly

See annexure for sample lab registers for specimen log in (Appendix 2) and entry of test results (Appendix 3).

A copy of the test request form (Appendix 4) must be maintained by the collection site as well in case the same gets lost during transit to the testing laboratory.

Fig. 1 Flow of Data associated with CD4 testing



Release of results

Testing will not be complete until the result has been transmitted back to the collection site and is placed in the patient file ready for the next patient visit. The laboratory must ensure the results are transferred from the lab to the clinic site. This may be done through any of the following:

- Manual collection of results from laboratory by site representative
- Delivery of results via courier system
- Faxing results or other electronic or telecommunications transfer

In addition, a logbook or copies of the test results should be archived within the laboratory for a set period of time to provide back-up data in case of queries or lost results. Systematic archiving will be strictly adhered to in each facility. Manual or electronic records may be maintained. In case of electronic records, suitable back ups must be maintained .

APPENDIX 3

CD4 CELL COUNT TEST REQUEST AND RESULT FORM												
SECTION 1: FOR REFERRING ART CLINIC USE ONLY												
1	Patient ID:	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	Sex: M F
	Name/Other ID:	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	Year of Birth:	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	Age:	<input type="text"/>
2	Authorizing clinician name:						3 Date and Time Blood Drawn (dd/mm/yy):					
	<input type="text"/>						<input type="text"/> / <input type="text"/> / <input type="text"/> <input type="text"/> : <input type="text"/> (hh:mm)					
	Signature:						4 Clinic Code:					
	<input type="text"/>						<input type="text"/>					
5	Clinic Name:										Tube additive: EDTA	
	<input type="text"/>						<input type="text"/>				<input type="text"/>	
SECTION 2: LABORATORY USE ONLY												
6	Sample lab number:						9 Was a result produced for this sample?					
	<input type="text"/>						<input type="checkbox"/> Y <input type="checkbox"/> N If no, state reason: _____ Other comments _____ _____					
7	Date sample received in lab (dd/mm/yy):											
	<input type="text"/> / <input type="text"/> / <input type="text"/>											
8	Date test conducted (dd/mm/yy)											
	<input type="text"/> / <input type="text"/> / <input type="text"/>											
10	RESULTS:						11 Technician initials and signature:					
	Absolute CD4 count (cells/ μ l)						<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> _____					
	CD4 % of Lymphocytes:						12 Verified by: _____					
	<input type="text"/> <input type="text"/> . <input type="text"/>											

APPENDIX 4

Sample transport check lists for the blood collection site and blood testing site for enumeration of CD4 cells

The blood collection site and the blood testing site should fix the days for transporting the samples, so that patients are called on the designated days for sample collection. There can be one or more days fixed for the purpose commensurate with the patient load and the testing capacity of the testing site. At the same time the testing site should be ready to receive and test the blood samples for CD4 cells on the same day. The number of samples to be collected should also be decided in consultation with the testing site / lab. All the materials required and the systems should be in place as per the guidelines for success of sample transport exercise.

Blood collection site (transporting the samples to the testing site)

- Call the patients on the day fixed for collection of blood for sample transport.
- Enter the patient details on the appropriate test requisition form (TRF) in duplicate-one to be retained at the collection site and other to be sent with the sample) and in the blood collection register.
- Each patient blood sample is given a unique code (as per the example shown in the guidelines) which is entered in the register as well as all the the relevant forms (test requisition & report forms & the register wherein sample details are entered).
- The blood sample is collected in the appropriate labeled container (as per the guidelines)
- The collection vial is closed tightly. In case the outside of vial is soiled it is wiped with sodium hypochlorite (5%). The vial is then packaged as given in the guidelines.
- All the samples properly labeled and packaged are placed in a container in such a way that the samples stay upright. The container can be thermocol or plastic box. The temperature during transport of sample should be maintained around 20-25⁰C.
- The samples along with the requisition forms should be handed over to the technician/relevant person for taking by hand to the testing site immediately (can be given to the courier/sent by air as the case may be).
- The samples should reach the testing site on the same day preferably but not later than 36 hours so that these can be received and tested within 48 hours.
- The test requisition forms should be placed in a zip lock bag and attached to the outside the container in which the samples have been packed. This is done to avoid smudging of the test request form from any spillage.

CD4 testing laboratory (testing site)

- The technician at the CD4 testing site receives the samples along with the requisition forms.
- Gloves must be worn before opening the sample collection box.
- The samples should be rejected if:
 - Sample is hemolysed
 - Sample is frozen
 - Sample is clotted
 - Sample tube is cracked / broken
 - Sample has not been drawn in a K₂ / K₃ EDTA vacutainer
 - Sample not shipped under ambient conditions (20-25⁰C)
 - Quantity not sufficient (at least 3-5 ml of sample must be available).
- In case the identity of the sample is not clear from the test request form, the test site should confirm the identity with collection site before testing the sample.
- After receipt of the samples in the lab, the samples should be maintained at an ambient temperature (20-25⁰C) till they are processed (processing time should be confirmed from the product insert/-25⁰C) till they are processed guidelines. Under no condition should the samples be placed in the refrigerator
- The sample meeting the required criteria are accepted and processed for CD4 testing as per the guidelines.
- The valid results are entered in the register and the reporting formats (see guideline for formats).
- The results are verified by the officer in-charge of the testing site and duly signed.
- The results are handed over to the person who brought the samples.
- The results are taken by hand / courier to the blood collection site where the results are entered in the register and the reports are sent to the MO ART center to be handed over to the patient during the next visit which is usually scheduled within a week for collecting the report
- The patient is managed as appropriate by the MO, ART center

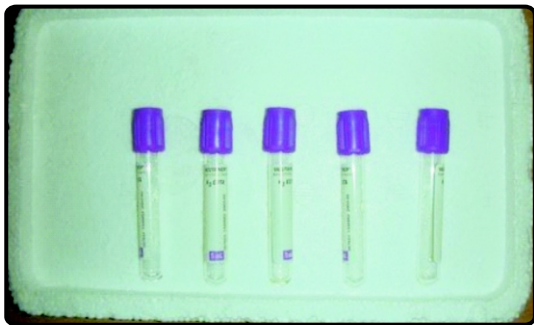
APPENDIX 5
Materials to be used for sample transport



1. Disposable Gloves



2. Sample collection box



3. K₂ EDTA Vacutainers



4. Gel packs and the rack for vacutainers



5. Gel pack as kept inside the sample collection box



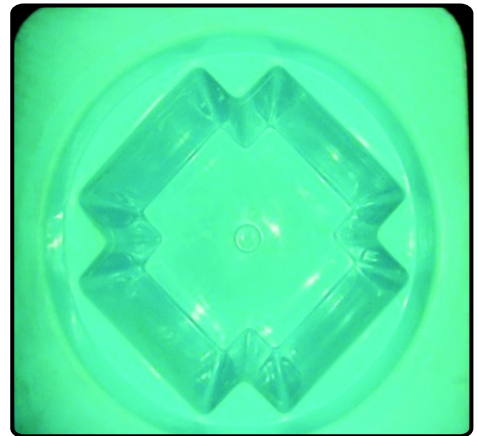
6. Vacutainer rack as placed over the gel pack during packaging



7. This canister can be used in case of fewer samples



8. A vaccine carrier cool box can also be used for shipping samples



9. The canister with samples can be placed inside the vaccine box



10. Zip lock bag for keeping the test requisition form