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| **STANDARD OPERATING PROCEDURE for Nigeria PreP Study** |
| **Study Site:**  | **SOPs Number** :LP-304 |
| **Title****ENUMERATION OF CD4+ T LYMPHOCYTE**  |
| **Version Number**:  | **Version Date:**  | **Effective date**:  |
| **Approval name Signature Date**  |

**Annual Review**

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| **Review date**  | **Revision Date**  | **Signature** |
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**Document History**

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| **Version number**  | **Reason for change**  | **Date**  |
| 1.0 | Initial release  | 28th March 2015 |
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**Distribution List**

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1. **Introduction**

Infection with HIV leads to the development of AIDS, which is characterized by the loss of CD4 T cells that are required for proper functioning of a person’s immune system. Assays that detect CD4 T cells in whole blood specimens are relied on to determine the appropriate time to initiate anti-retroviral therapy (ART) in HIV+ people. CD4 counts are also used as a tool to monitor disease progression and the effectiveness of ART.

The environment in which CD4 testing is performed must be conducive to efficient operations that do not compromise the safety of the staff or the quality of the testing process.

CD4 T-cell count is important in deciding when to commence ART for HIV infected patients, it is also used for monitoring progress of therapy.The Cyflow technique is used for all samples.

1. **Objectives**

This standard operating procedure (SOP) describes the procedure for CD4 estimation in the study.

1. **Responsibility**

All trained study laboratory personnel are responsible for this procedure.

1. **Abbreviations**
* CD----Cluster of differentiation
* QC – Quality Control.
* mAb – Monoclonal Antibody
* CCBG – Count check bead green
1. **Materials:**

**Reagent:**

Partec CD4 Easy Count Kit.

* No Lyse Buffer
* CD4mAb PE

Partec CD4 Easy Count Kit- dry

* 820ul No Lyse CD4 Buffer
* Lyophilized CD4mAb PE

Partec CD4 % easy count kit.

* Buffer 1
* Buffer 2
* CD4 mAb PE
* CD45 mAb PE- Dy647.

**Reagent preparation:**

Preparation of sheat fluid :

* 1g Sodium axide + 5 drops of liquid detergent + 5 litres Eva water

Reagent stability and storage**:**

* CD4 mAb PE kit is stored at 2 - 8◦C in the dark, the CD4 easy count kit is stable until expiration date on the kit label. Do not freeze or expose to elevated temperature.

**Sheath fluid preparation**

 **1.** Weigh one gram (1g) of sodium azide into sheath fluid container.

 2. Add five (5) drops of Tween 20.

3. Dissolve with one litre (1L) of Eva bottled water, make it up to five litre (5L) wih Eva bottled water.

 4. Label the container with date of preparation, date of expiration and name of personnel that prepared it.

**Note: this preparation is stable for one week.**

**Supplies:**

* Partec test tubes
* Micropipettes
* Pipette tips
* EDTA- Anticoagulated bottles

**Equipment:**

* Partec flow cytometry instrument (Cyflow Counter and Cyflow(R)Counter).

**Limitations:** Clotted sample is not used to run CD4+ T-Cell analysis, because the cells are trapped. Lysed sample, i.e. broken up cells and centrifuged sample are not used either.

**Sample Retention:** 1 week, but results are reliable only if run within 24hrs.

**Biosafety/precautions:**

1. Reagents contain 0.09% sodium azide that under acidic conditions yields hydrazoic acid, an explosive compound. Sodium azide may react with lead and copper plumbing to form potentially explosive metal azides. When disposing of sodium azide, flush plumbing with a large volume of water to prevent build-up. Very toxic if swallowed. After contact with skin, wash immediately with plenty of water.
2. Always observe good medical laboratory practice while handling control material.
3. Do not look into Laser beam. Use Laser protection glasses or block laser beam

**MAINTENANCE FOR CYFLOW COUNTER 2**

Daily Start-up Cleaning procedure

1. Make sure the sheath bottle is filled with sheath fluid and screw the cap tightly closed.
2. Tilt the sheath bottle in order to release air bubbles trapped under the yellow inline filter unit.
3. The waste bottle should be empty and tightly closed.
4. Switch on the main power at the back of the instrument, then push the green button on the left of the {Partec logo located at the front.
5. Run 1600µl of Partec cleaning solution.
6. Run 1600µl of sheath fluid in order to remove residual cleaning solution.

Background Check

* Acceptable background count cell after daily start-up procedures and after every clean is ≤5 cell/µl

Daily Shut-down Cleaning procedure:

1. Dispense 1600μl of Decontamination solution into the tube, and plug onto the machine to run.
2. Dispense 1600μl of cleaning solution into the tube and plug onto the machine to run.
3. Dispense 1600μl of sheath fluid into the tube and plug it to the machine.
4. Press **cancel** after 1 minute.
5. Leave the tube with sheath fluid attached to the sample port.
6. Allow the machine to stabilize for 10mins
7. Switch off the machine.

## PROTECT THE SAMPLE PORT

* Leave the final sample tube with the remaining clean Shealth Fluid connected to the sample tube-this avoids drying and crystallizing of any remaining material.

**Weekly cleaning and decontamination procedure:**

1. Run 1600μl of decontamination solusion for 15 seconds
2. Pinch the sheath fluid tube for 15 seconds and press the STOP button.
3. Release the pinched tube and let the Decontamination solution incubate for 15 minutes.
4. Press START to run the remaining Decontamination solution.
5. Run 1600μl of cleaning solution.
6. Run 1600μl of sheath fluid. After 2 minutes of measurement, press the STOP button and keep the sample tube with sheath fluid.
7. Add 10-20ml of hypochlorite solution to the waste bottle and let it incubate for 15 minutes before discarding the waste.
8. Switch off the instrument.

**CALIBRATION:**

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| **Calibrator** | **Stability** | **Frequency** | **Preparation** |
| Count check beads green | 2-8◦C  | **Once a week** | Ready to use |

**MATERIAL: Partec Count Check Beads green**

**Testing:**

* Bring Count Check Beads green out of the refrigerator to attain
* room temperature.
* Shake the Count Check Beads green bottle for about 2 minutes and pipette 850ul of Count Check Beads green into a sample tube.
* Plug into sample port and press the start button. Wait until measurement and the subsequent cleaning procedure have stopped automatically.
* Data are stored and printed on demand.

**Evaluating Calibration Results**

* The result of the Count Check Beads green measurement is indicated in the ‘’Result’’ area.
* Compare with the LOT specific concentration indicated on Label of the count Check Beads green bottle.
* Check if result is within ±10% range of the LOT specific concentration stated on the Count Check Beads green bottle.
* If the result falls within the ± 10% range, the Cyflow Counter is now ready for further analysis.
* If not, shake bottle vigorously for 2 minutes and repeat measurement.
* Perform an Emergency Cleaning of the Flow cuvettee.

**External Calibration Results:**

* The CCBG measurements are plotted on the LJ chart afterrun. At the end of the month, the chart is printed and pasted on the wall. See SOP for plotting LJ chart for details**.**
* **Monitor the coefficient of variation displayed on the LJ chart and ensure it does not exceed 10%.**
* **Monitor the gain setting to ensure it falls between 0 and 999.**

**Routine Quality Control (Alternative approach)**

* **Select one sample from each of the following range, prepare and store at 2 - 8º C:**
	+ **Low (180 – 220 cells/µL)**
	+ **Normal ( 315 – 385 cells/µL)**
	+ **High (450 - 550 cells/µL)**
* **Assay the samples next day and compare with previous day value.**
* **Control is said to have passed if the compared values are within ± 10% of the previous day values.**

**External Quality Control**

* Any staff on the bench analyses the EQA sample (according to the procedure used in analysing patients sample) and sends it.
* The personnel writes down the date and time the result was sent in the EQA register at the bleeding bench.
* The remaining EQA panel is stored in the refrigerator in Haematology Lab for proficiency testing.

**PROCEDURE:**

**Operating procedure for the cyflow machine**

* Switch on the light from the socket and stabilizer
* Switch on the UPS and allow it to stabilize until the “on line” light is stable
* Switch on the Cyflow machine and allow it to stabilize for 10 minutes.
* Perform daily cleaning.

**CD4 Abolute Count**

**Sample Preparation:**

* The CD4 antibody, dilution buffer and the count check beads must be at room temperature before use.
* ­Mix the sample on the Dynal mixer for 5 mins.
* Add 20ul whole blood to a partec test tube.
* Add 20ul of CD4 m Ab PE. Mix gently and incubate for 15 minutes at room temperature protected from light.
* Add 800ul of no lyse buffer and **mix** gently
* Plug the sample tube to the machine (tube must not touch the electrode)
* “Prerun” appears on the machine 1st, then “run” 2nd, “Count” 3rd and finally “ready” after counting the cells – (the sound on the machine must stop before printing the result).

**PERFORMING A MEASUREMENT**

* Load the respective script for a CD4 absolute or a CD4% measurement:

Setting → load script → CD4 script or CD4% script

* Attach the sample tube with the sample in it to the sample port.
* Click START to start the measurement. The instrument performs certain processes which are shown in the lower part of the screen.
* PRE RUN – creates a temporary, fast sample flow
* MEASURE – the particles are measured and counted
* LEVEL COUNTING – a certain volume gets measured and counted
* FLUSH – an automatic internal cleaning cycle gets performed
* During measurement, the position of the CD4 T-cell peak in the histogram or the CD45-SSc Dot plot and the CD4-SSC Dot plot by changing GAIN values only during the MEASURE phase.
* During level counting, do not change speed, Gain L-L or other software parameters.
* Wait until the measurement and a subsequent cleaning procedure have stopped automatically. During the process, FLUSH is indicated at the right side.
* If necessary, adjust the region to mark the cell or particle of interest.
* Save and /or print the windows result area.

**CD4 RESULTS INTERPRETATION**

Examples of a direct counting results using the CD4 easy count kit print out from a Partec Cyflow Counter as depicted in the histogram, the CD4 T cells (prominent peak on the right) can be clearly separated from the CD4 monocytes (left peak of weaker fluorescence intensity). The absolute concentration of CD4 T cells is displayed as number of cells/ul whole blood.

**Reference Range**

* Less or equal to 6 years : 750 – 999 cells/ul
* Adults and adolescents: 350 – 1571 cells/ul

**DOCUMENTATION:**

* The personnel RUNNING the sample is in charge of proper documentation of the number of samples received for CD4 tests on the bench forms.
* Make sure the tests is done within 6hrs of sample collection.
* The results are entered into the register according to how they are entered into the bench form and the focal person does the technical verification. Actors enter their names into the register for purpose of traceability.
* The instument used for each sample is also indicated on the bench form.

**REFERENCES:**

1. The Cyflow operating manual(Rev,009 Date 2009-07-07).
2. The Cyflow(R)Counter Operating Manual(Rev.006 Date 2008-07-29).
3. Partec CD4 Easy Count Kit-dry insert (Rev.005 Date 2009-07-23).

**This SOP has been read and understood by:**

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