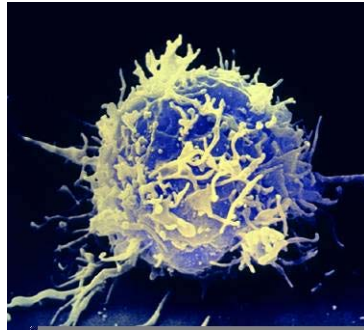


GOOD LABORATORY PRACTICE MANUAL CD4 ENUMERATION

THE CD4 CELL

- A molecule with a cluster of differentiation (CD) has a characteristic cell surface protein.
- These cell surface proteins are often associated with the cell's function.
- Cells with different functions will express different surface proteins and have unique CD designations.
- Over 300 CD molecules have been described.



CD4+ CELLS

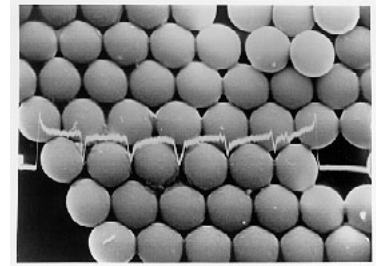
- CD4 is a protein found on the surface of different cell types, including a sub-class of lymphocyte called T-helper cells and monocytes.
- CD4 binds to the gp120 molecule which is expressed on the envelope of the HIV molecule.
- CD4+ (helper) T-lymphocytes are critical to normal immune responses
- The CD4 antigen is the receptor for initial HIV binding
- HIV infection causes a progressive decline in CD4 cell number that correlates with the degree of immunodeficiency



- Knowledge of CD4 cell number is crucial for patient care

- Lymphocytes are indistinguishable by (ordinary)light microscopy
- Can be distinguished by antigenic differences
- Employs inert latex beads coated with murine monoclonal specific for the CD4 surface antigen.

- CD4 coated latex spheres also react with monocytes but are blocked by MY4 CytoSpheres
- Beads attach to CD4+ cell surface antigen forming rosettes
- Rosettes are easily identified visually and counted
- Number of positive cells per unit volume quantified in Hemocytometer



KIT COMPONENTS

- Reagent A:MY4 Cyto-Spheres Monocyte Blocking reagent- (0.55-0.65 microns) for blocking reaction to monocytes
- Reagent B:CD4 Cyto-Spheres (1.8-2.4 microns)
- Reagent C:Lysing reagent(2%acetic acid in distilled water + crystal violet).

Reagent Storage

- Keep an inventory of all kits – lot numbers and expiry dates
- Rotate stock
- Store Cyto-Sphere kits at 2-8°C
- Avoid freezing and temperatures exceeding 37°C
- Always check for evidence of deterioration:
 - Leakage
 - change in colour
 - solidification, clumping, layering

If any of the above occur:

Document! **Do not use kit**

Reagent Preparation

- Allow reagents to reach room temperature 15 - 30 min at 20-25°C
- Normal appearance of reagents:
 - **Reagent A:** white cloudy suspension
 - **Reagent B:** white cloudy suspension
 - **Reagent C:** clear blue liquid

SPECIMEN PREPARATION

- Specimen collection – K₃EDTA (1.0-1.5 mg ml⁻¹ whole blood)
- Do not collect specimens in acid citrate dextrose (ADC)
- Treat all blood specimens as potentially infectious

BASIS FOR SPECIMEN REJECTION

- Haemolysis

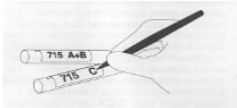


- Clotted blood
- Partial draw
- Temperature Extremes
- Improper specimen labeling
- Specimen integrity

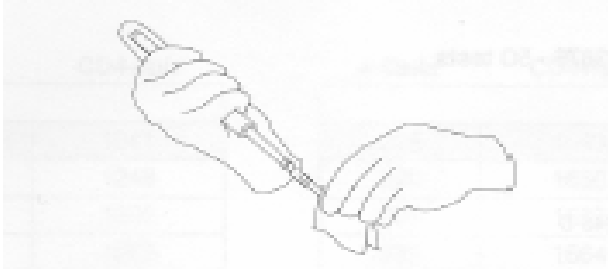
Start assay within 6 hours of sample collection.

Blood should remain at 20-25⁰C .**DO NOT REFRIGERATE**

TEST PROCEDURE – PREPARE TUBES



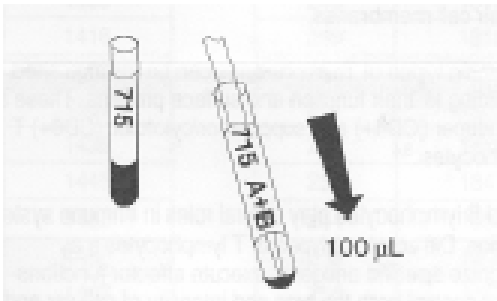
1. Label 1 tube “A+B” and 1 tube “C”. Label both tubes with specimen ID.



2. Note: Wipe outside surface of pipette tip with tissue before dispensing.

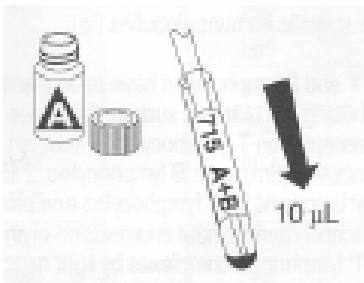


3. Place 100 µl of reagent C into tube labeled “C”



4. Place 100µl whole blood into tube labeled “A+B”

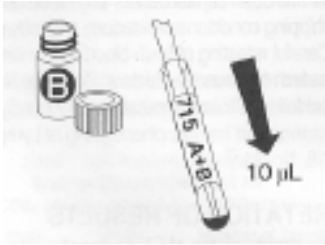
TEST PROCEDURE – MIX REAGENTS



Add 10µl Reagent A to tube labeled “A+B”



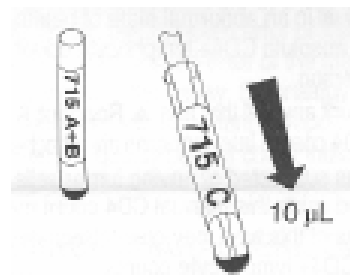
Shake for 2 minutes



Add 10 µl Reagent B to tube labeled “A+B”



Shake for 2 minutes

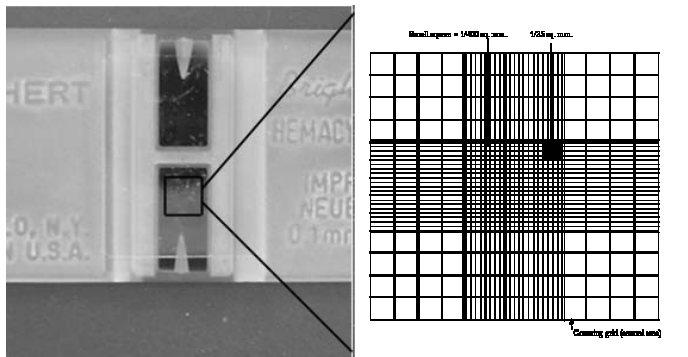


Add 10µl blood-latex spheres from the tube labeled “A+B” to the tube labeled “C”



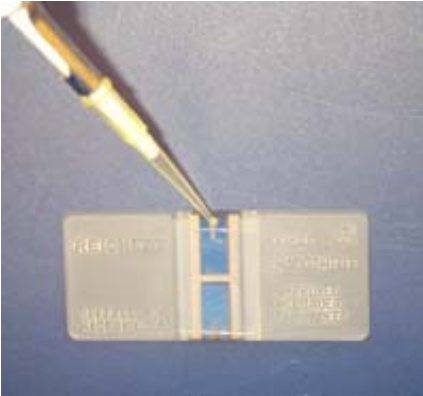
Shake for 15 seconds

HEMOCYTOMETER

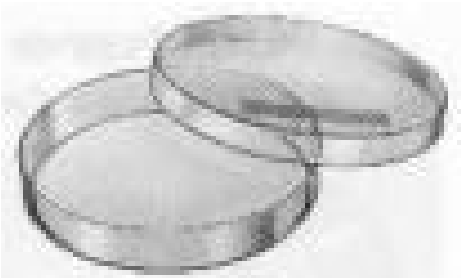
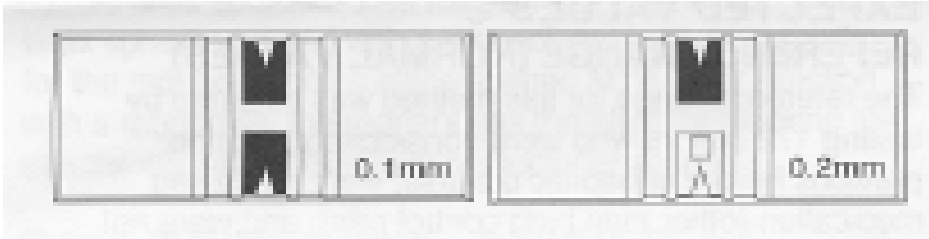


COUNT CELLS IN ALL NINE MAJOR SQUARES

TEST PROCEDURE LOAD CHAMBER

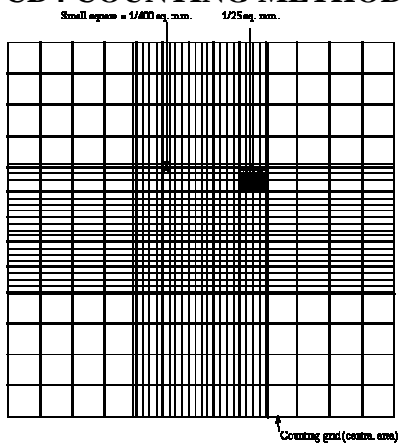


Load chamber (both sides of 0.1mm chamber)

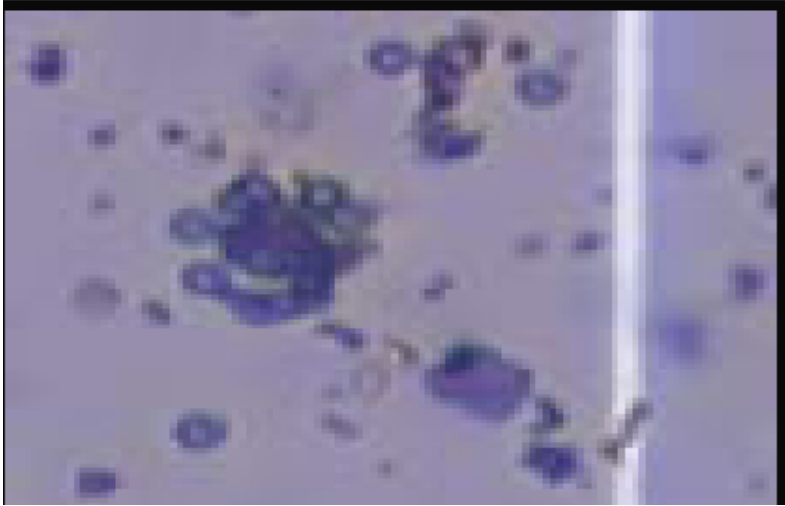


Allow cells to settle in moisture chamber for 2 min
Sample stable for 15 min

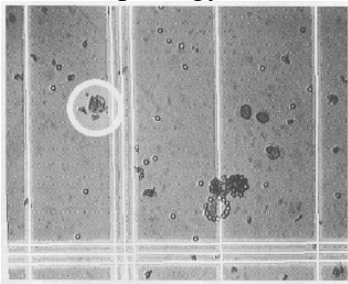
CD4 COUNTING METHOD



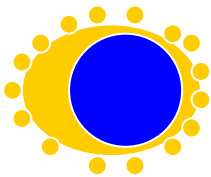
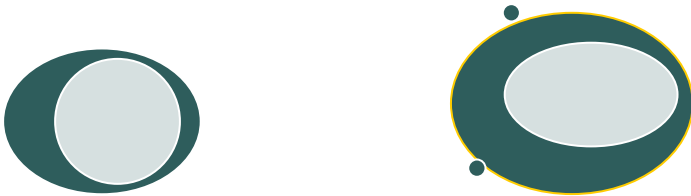
CELL MORPHOLOGY



Cell Morphology 2

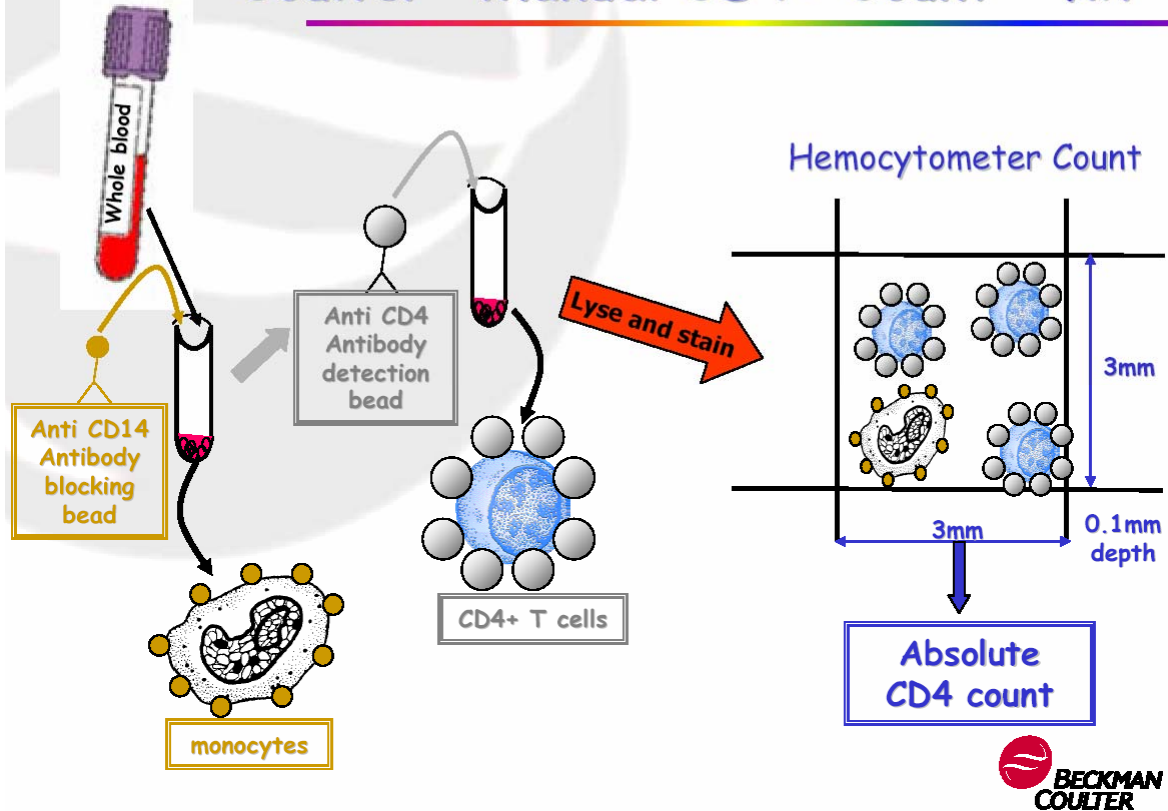


COULTER MANUAL CD4 COUNT



SUMMARY OF PROCEDURE

Coulter® Manual CD4+ Count™ Kit



ABSOLUTE COUNTS

Count both sides of the 0.1 μm chamber and multiply the numbers of CD4+ cells by 7.3

This will give an absolute count:

The number of CD4 + lymphocytes μl^{-1}

LIMITATIONS OF ASSAY

Reportable range is 30-2000 CD4+ cells μl^{-1}

If >2000: Dilute with native plasma (or an appropriate ratio of reagent C) and retest – REPORT THAT THIS HAS BEEN DONE and record calculations!!!!!!

If <30: Should be reported as “LESS THAN 30 CD4+ cells μl^{-1} ”

LIMITATIONS OF ASSAY

- To ensure maximum viability, analyze cells promptly.
- Reagents should not be diluted.
- Use only as packaged

QUALITY CONTROL USING CD-CHEX PLUS

- QC should be carried out with every run – a “run” usually means the 8-hour working day AND whenever you change operator or use a new kit with a different lot number
- QC Results should be meticulously recorded
- Process CD-Chex as you would for any other sample
- Use either the low OR normal control each time

Normal	178 cells in chamber (1303 CD4+ cells μ l-1)
Low	27 cells in chamber (194 CD4+ cells μ l-1)

PROCEDURAL PRECAUTION

- Samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
- Never pipette with mouth.
- Reagent A and Reagent B contain sodium azide. Sodium azide under acid conditions yields hydrazoic acids, an extremely toxic compound. Flush with running water while discarding.
- Reagent C contains 2% acetic acid and 0.025% crystal violet. Crystal violet may be a potential mutagen. Therefore wear appropriate laboratory safety equipment.
- Do not use individual kit reagents beyond the expiry date on the kit label.
- Do not mix reagent of one kit with reagents from any other kit.
- Incubation times and temperatures other than those specified may give erroneous results.
- Avoid microbial contamination of reagents or incorrect results may occur.
- Harmful if any of the reagent is swallowed.
- After reagent contact with skin, wash immediately with plenty of water.
- Reagent vials should be repeatedly inverted until the latex spheres are smoothly suspended.
- Reagents must be @ RT before use
- Indicate acceptance or rejection of the test run and remedial actions taken should the run be rejected.

LABORATORY SAFETY

- Use universal precautions with all specimens
- Establish the following safety practices
 - Wear laboratory coats and gloves when processing and analyzing specimens, including counting of cells on the microscope.
 - Never pipette by mouth. Use safety pipetting devices.
 - After working with specimens, remove gloves and wash hands with soap and water.

SPECIMEN COLLECTION

- Select appropriate anticoagulant
 - Use tripotassium ethylenediamine tetra-acetate (K3EDTA, 1.5 + 0.15 mg/mL blood) and perform the test within the time frame allowed
 - Reject a specimen that cannot be processed within this time frame
- Collect blood specimens by venipuncture into evacuated tubes containing an appropriate anticoagulant, completely expending the vacuum in the tubes.
- Draw pediatric specimens in pediatric tubes to avoid under drawing.
- Mix the blood well with the anticoagulant to prevent clotting.
- When hematology and CD4 immunophenotyping will be performed in the same laboratory on the same specimen, use one tube containing K3EDTA.
- Label all specimens with a unique patient identifier, date, and time of collection.
- Assure that patient information and test results are accorded confidentiality.
- Provide on the submission form pertinent medications and disease conditions that may affect the immunophenotyping test
- Follow up specimens should be taken at about the same time of the day with the previous specimen (to avoid nocturnal variation)

SPECIMEN TRANSPORT

- The effect of cool temperatures (4oC) on immunophenotyping results is not clear
- Maintain and transport specimens at room temperature (18–22o C)
- Avoid extremes in temperature so that specimens do not freeze or become too hot. Temperatures above 37o C may cause cellular destruction and affect both the hematology as well as CD4 measurements.
- In hot weather, it may be necessary to pack the specimen in an insulated container and place this container inside another containing an ice pack and absorbent material. This method helps retain the specimen at ambient temperature.

SPECIMEN INTEGRITY

- Inspect the tube and its contents immediately upon arrival.
- Take corrective actions if the following occur:
 - If the specimen is hot or cold to the touch but not obviously hemolyzed or frozen, process it but note the temperature condition on the worksheet and report form.
 - Do not rapidly warm or chill specimens to bring them to room temperature because this may adversely affect the immunophenotyping results.
 - If blood is hemolyzed or frozen, reject the specimen and request another.
 - If clots are visible, reject the specimen and request another.
 - If the specimen is >6 hours old (from the time of draw), reject it and request another.

QUALITY ASSURANCE

Assure the overall quality of the laboratory's CD4+ T-cell testing by monitoring and evaluating the effectiveness of the laboratory policies and procedures for the pre-analytic, analytic, and post-analytic testing phases. The practices and processes to be monitored and evaluated include:

- Methods for collecting, handling, transporting, identifying, processing, and storing specimens.
- Information provided on test request and results report forms.
- Process for reviewing and reporting of results.
- Employee training and education, which should consist of:
 - Basic training on CD4 manual count and additional training in hands-on workshops for supervisors. through workshops and other programs personnel through attendance at meetings and workshops.
 - Adherence to IHV-N regulations for training and education.
- Assurance of satisfactory performance. Laboratories must successfully participate in a performance evaluation program.
- Satisfactory participation in EQA proficiency testing as organized by IHVN