Cell Pellet Training

Laboratory Break-Out Session

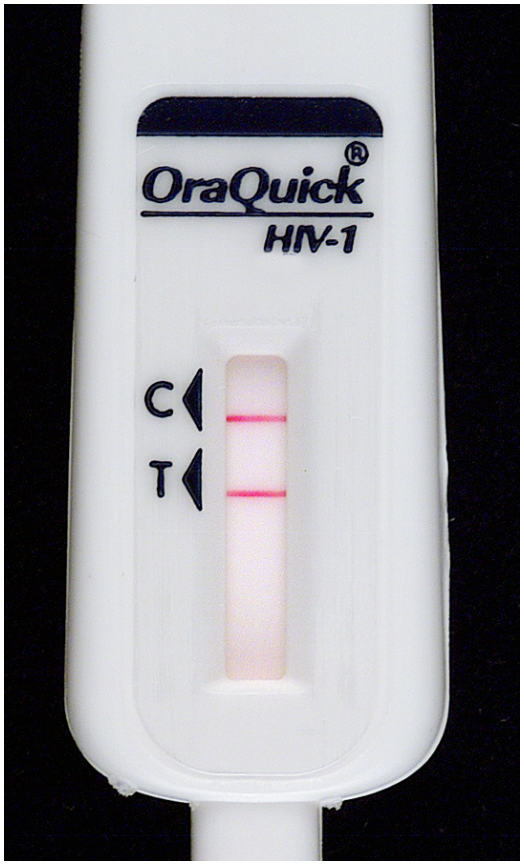
MTN Regional Meeting

Cape Town, October 2010

DNA PCR for Infant Diagnosis

Rapid tests and Western blots cannot be used to test infants for HIV

- DNA PCR used for HIV testing in infants under 18 months of age
- Antibody based tests look for the body's reaction to the virus
- Infants still have their mother's antibodies
- All HIV-exposed infants will be "positive" on antibody tests.



RNA vs DNA PCR

RNA PCR ("Viral Load")

- Quantitative
  - Copies/ml result
- Detects HIV-1 RNA from plasma virus
- Viral load can fluctuate from undetectable to high numbers

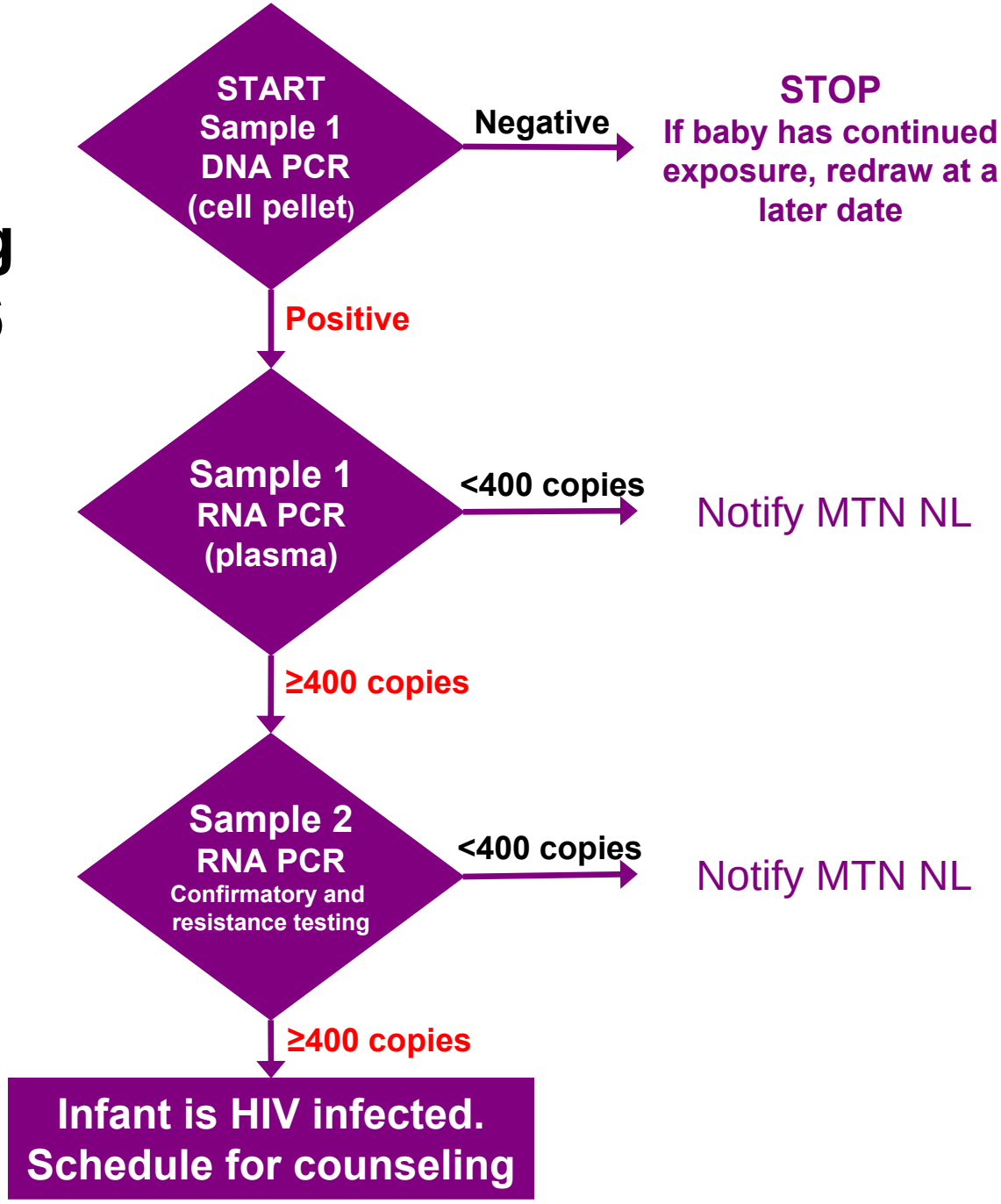
DNA PCR

- Qualitative
  - pos/neg result
- Detects HIV-1 DNA in peripheral blood cells
- Infected cells will always give a positive result, even when viral load is undetectable

When should I do DNA PCR?

- **MTN-003 or MTN-009**
  - When NL requests it for ambiguous HIV status
  - Not part of the algorithm
  - Not a routine test in these protocols; Special cases only
- **MTN-015**
  - Not expected; only if NL requests it for participant safety
- **MTN-016**
  - For infant HIV diagnosis – part of algorithm

Algorithm for Infant HIV Testing in MTN-016



Assays Available

- Roche
  - AMPLICOR HIV-1 DNA Test, v1.5 (MWP or COBAS®)*
  - COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test*,#
- Abbott *m*2000 HIV-1 DNA Qualitative#
- In-house

*For cell pellet
#For DBS and plasma

Sample Collection

- Blood should be collected in either Vaccutainer Blood Collection Tubes, using EDTA (lavender-top) or ACD (yellow top) or equivalent tubes as the anticoagulant
- Heel stick (infants) or venipuncture can be used
- Samples anticoagulated with heparin are unsuitable for this test

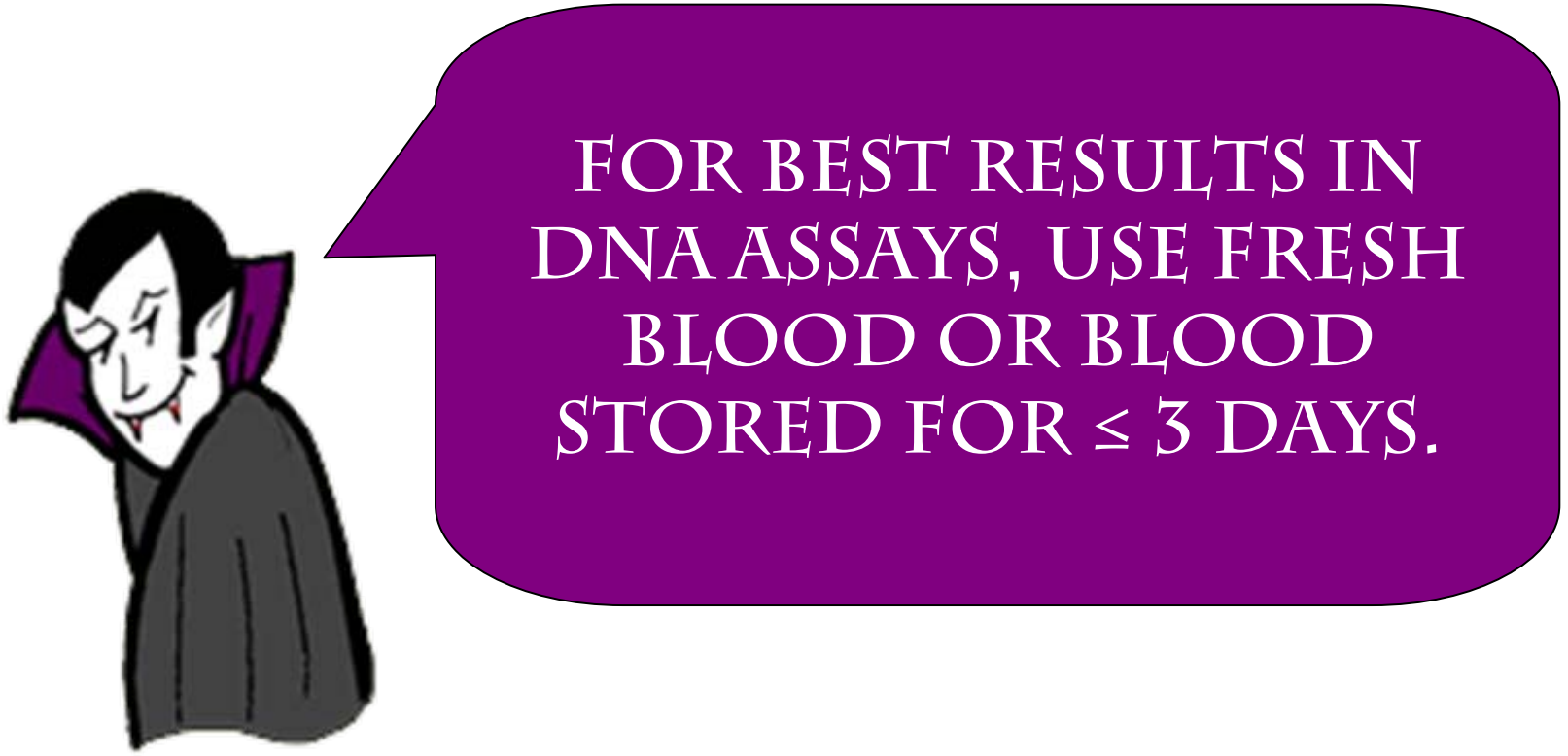
Blood volumes required

Participant	Total Blood Volume (ml)
Infant	EDTA tube 1.5 – 2 ml
Adult	Typical for the visit

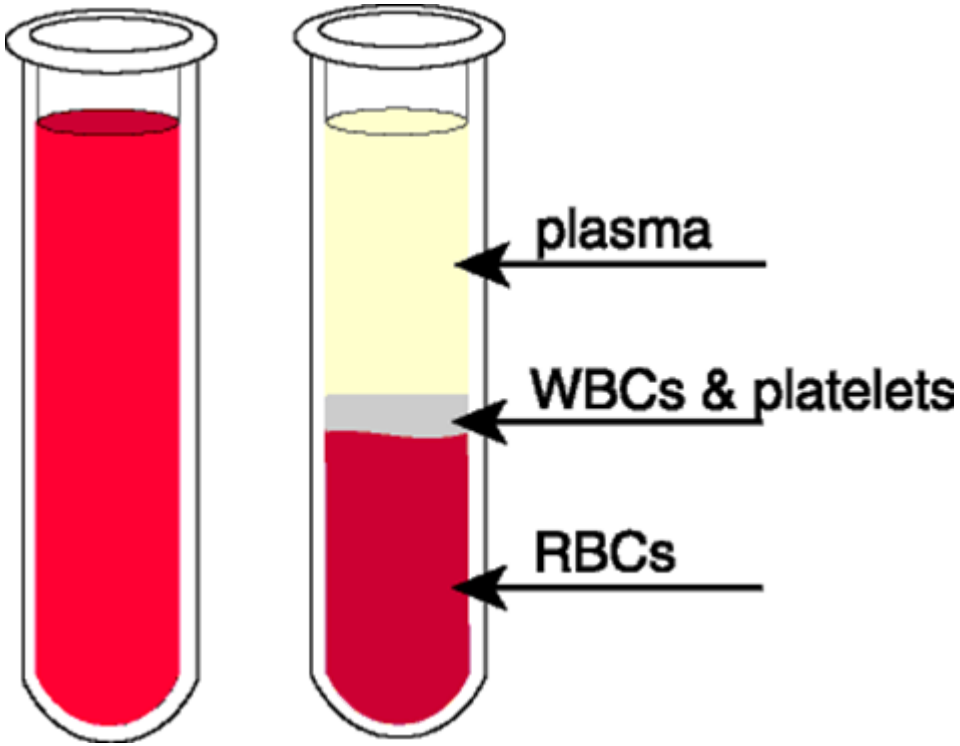
E.g, EDTA microtainer tube for infants

Sample Storage

- Whole blood should be stored at 2-25°C; Do not freeze.

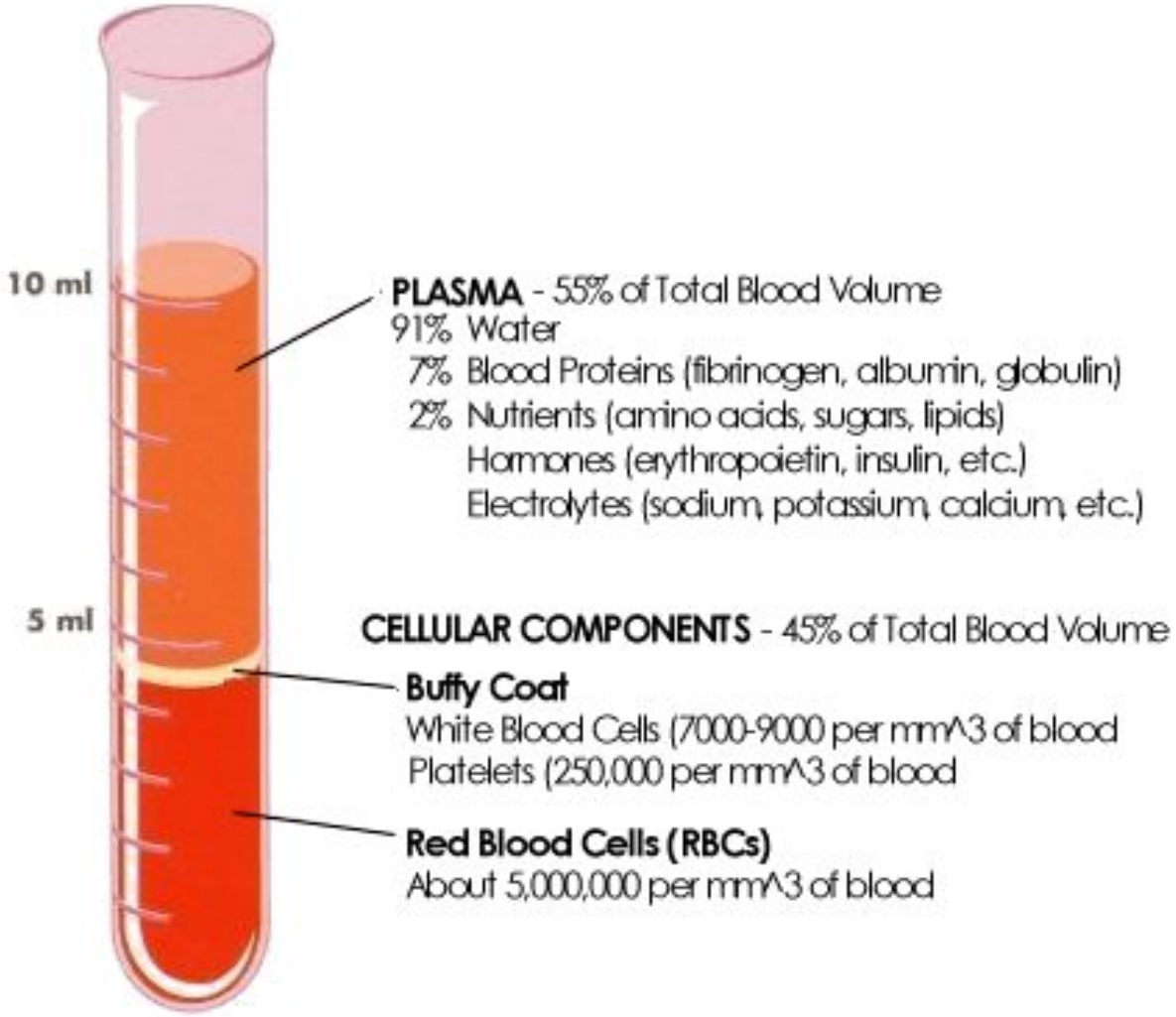


Overall Steps



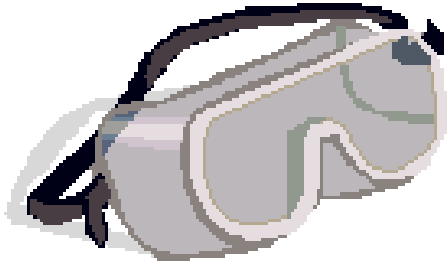
- **PROTOCOL A:** Plasma processing
- **PROTOCOL B:** Cell pellet processing

Sidebar: What is blood made of?

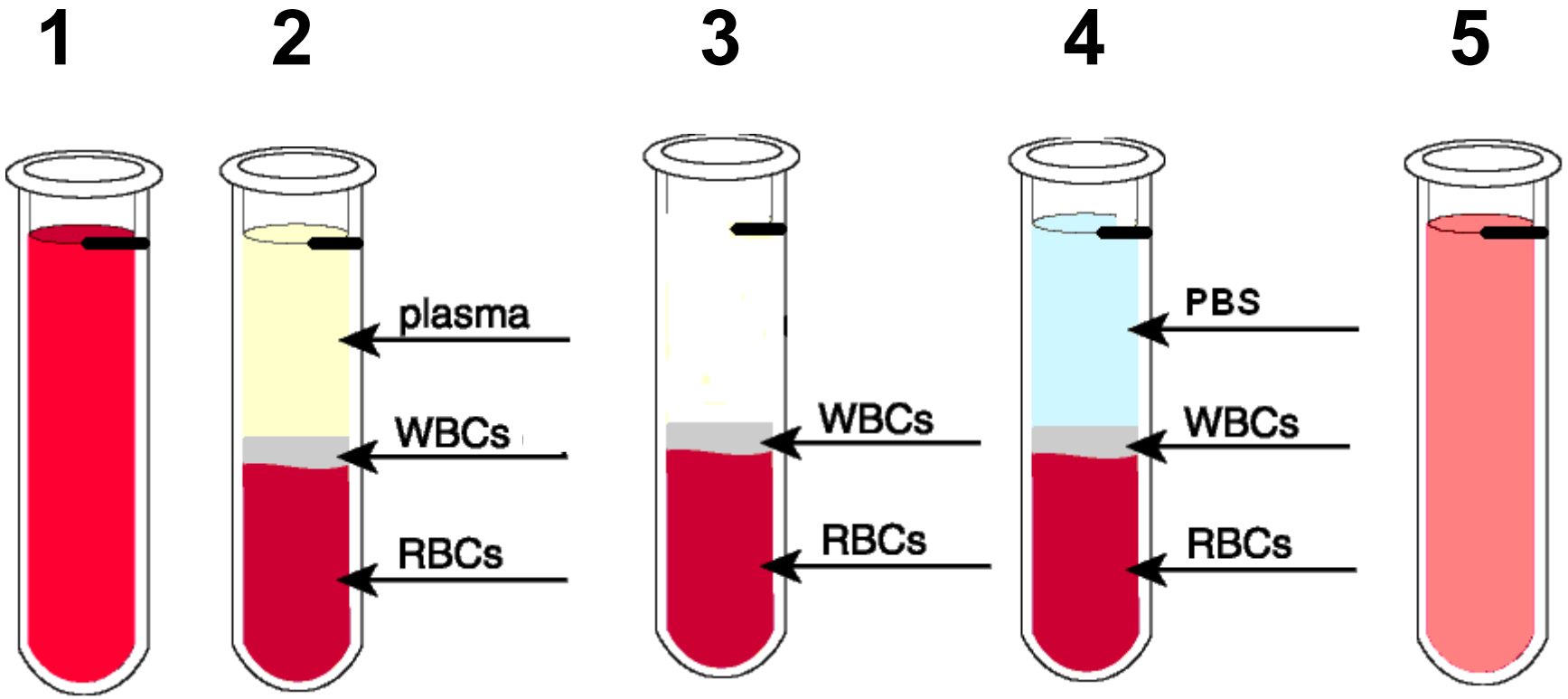


Reminder: Use Universal Precaution

Work in a biosafety cabinet



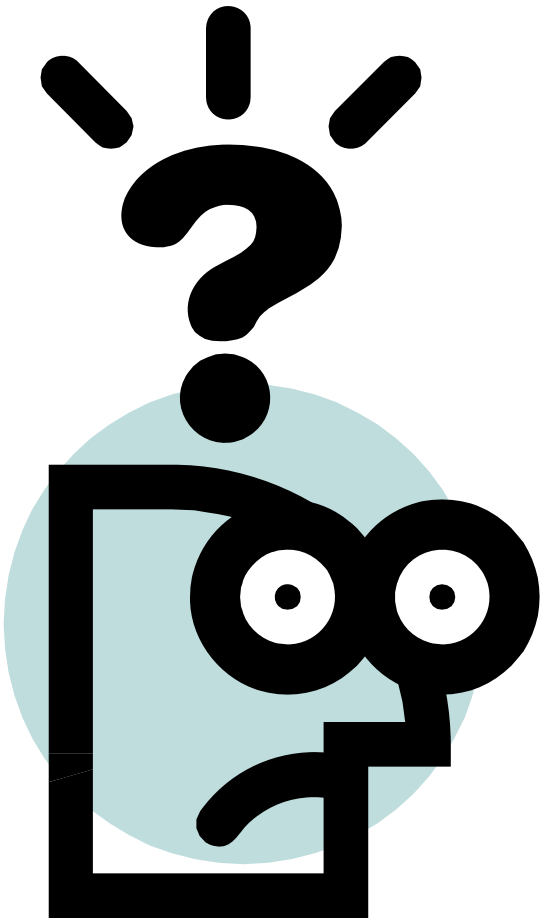
Protocol A: Plasma Processing



Protocol A: Plasma Processing

1. Mark level of whole blood with indelible ink on the outside of tube
2. Spin whole blood at 800-1600 x g for 20 min at room temp to separate plasma
3. Store a min of 0.5 ml/cryovial at -70°C
4. Bring specimen back up to original marked volume using PBS
5. Invert tube 10-15 times to mix

Protocol B: Cell Pellet Processing



What is happening?

- Red blood cells lysed
- White blood cells pelleted
- Supernatant removed
- Pellet frozen

Materials Needed

Transfer pipette

Safety glasses

Biosafety cabinet

Gauze pads

2 ml screw-cap tube

gloves

microcentrifuge

Aerosol filter tips

Pipette

Reagent Needed: Cell Lysis Buffer

- Can use:
  - BLD-WS: Specimen Wash Solution From Roche Amplicor HIV-1 DNA Test v1.5 Kit
  - RBC Lysis Buffer: Available separately; Roche Cat 1 814 389 001
- How does it work?
  - Lysis buffer contains Ammonium chloride (NH_4Cl) or sodium azide which effectively lyses non-nucleated cells (red blood cells), but not cells with nuclei (white blood cells)

Procedure

1. For each sample that will be processed, add **1.0 ml cell lysis buffer*** to a 2-mL screw-cap tubes

*Buffer BLD WS in Roche kit

More than 1 pellet can be made from each specimen if volume is adequate (500 µl whole blood per pellet.)

Procedure

3. Invert tube of whole blood 10-15 times to mix thoroughly

4. Remove cap from tube with a gauze pad to avoid aerosol contamination

5. Pipette 500 μl of whole blood into the tube containing lysis buffer using a pipette with an aerosol barrier tip

Procedure

6. Incubate for 5 min at room temperature
   - Invert tubes 10-15 times to mix thoroughly
   - Incubate 5 additional min at room temperature
7. Microcentrifuge the tubes for 3 min at max speed

Procedure

8. Using a fine-tip transfer pipette, aspirate the supernatant, being careful to avoid disturbing the pellet
9. Add 1.0 ml lysis buffer to each tube, recap and vortex to re-suspend the pellet
10. Microcentrifuge the tubes for 3 min at max speed

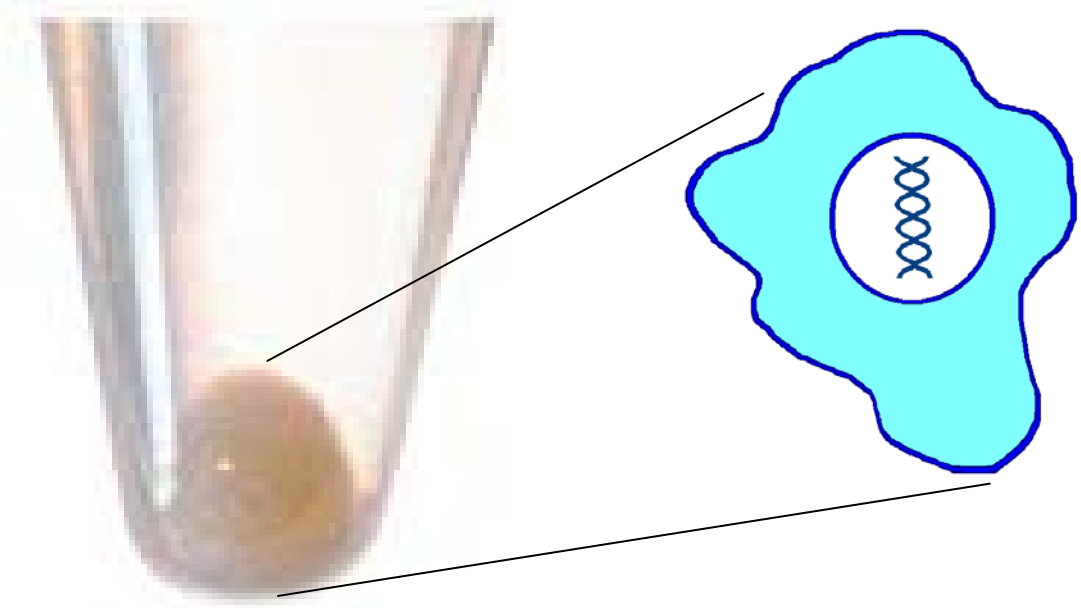
Procedure

11. Aspirate the supernatant being careful to avoid disturbing the pellet

12. The dry pellet may be extracted immediately or stored at -70°C until extraction.

Next step: Use pellet for DNA PCR assay.

What is in the cell pellet?



White blood cells are pelleted from whole blood, and DNA from the cell is extracted to check for the presence of HIV using a DNA PCR kit.

DNA PCR Proficiency Testing

- If you will be doing HIV-1 DNA Testing:
  - You must sign up for proficiency testing, similar to viral load
  - VQA will send you whole blood every other month
  - You must process the whole blood into cell pellets, and test using your DNA PCR assay
  - Requires pre-qualification
  - Contact Urvi or Ted (NL) or Cheryl Jennings (VQA) for further info

Final Note: LDMS

Test	Primary	Additive	Derivative	Sub Add/Derv	Primary Volume	Aliquot Volume	Units
*Blood for HIV DNA and RNA PCR testing	BLD	EDT	PL1/2	N/A	1 ml	Minimum of 0.5	ML
		EDT	CEL	PER	1 ml	5×10^6	CEL
		EDT	DBS	N/A	1 ml	100	UL

Don’t forget to log your specimens into LDMS

QUESTIONS ?