Best Practices for PBMC Processing from Leukapheresis Products & Large Volume Blood Draws

2017 ACTG Annual Network Meeting

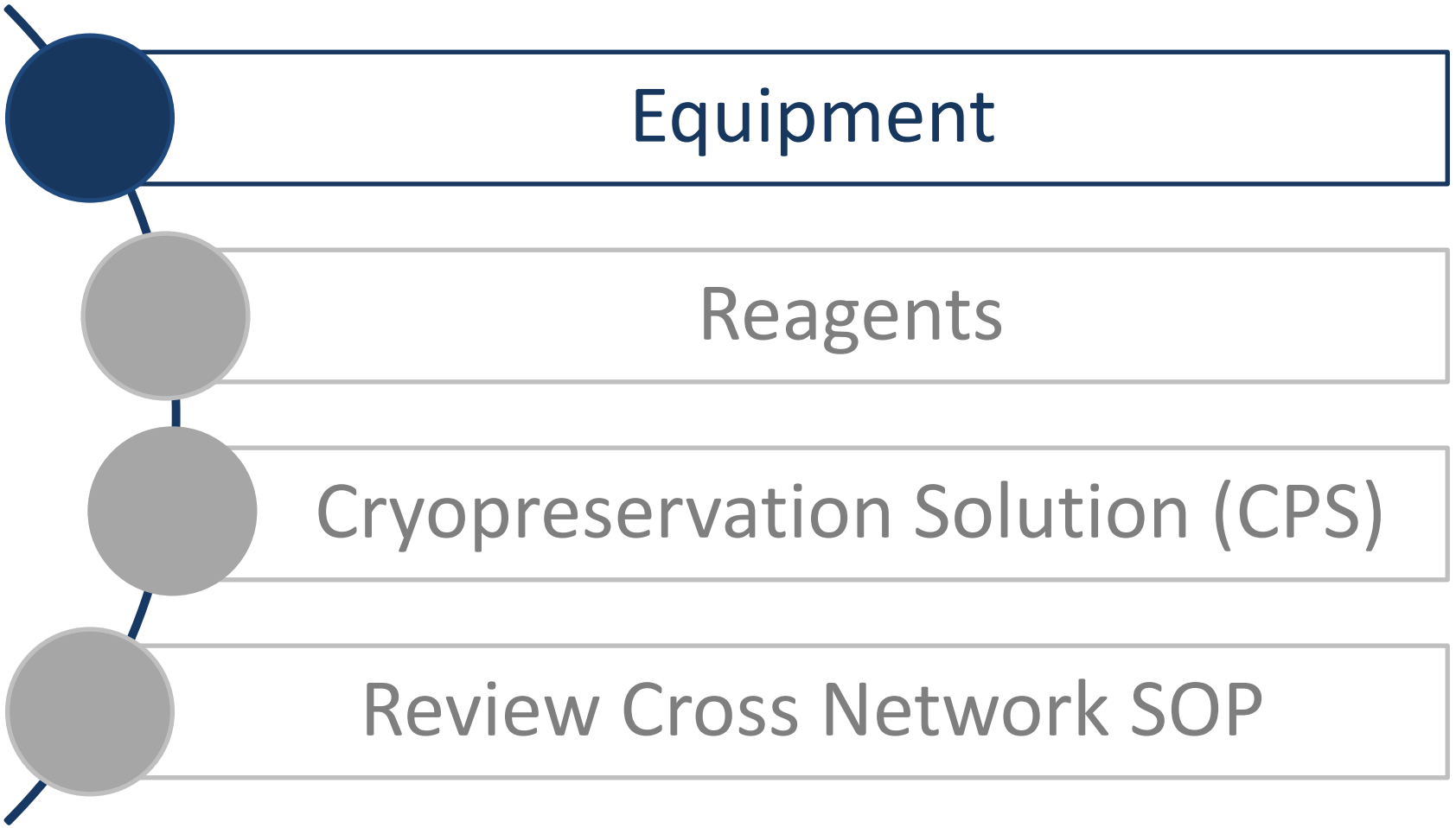
Presented by: Sarah Keinonen

June 25, 2017

IQA Immunology Quality Assessment Program An NIH, NIAID, DAIDS Program

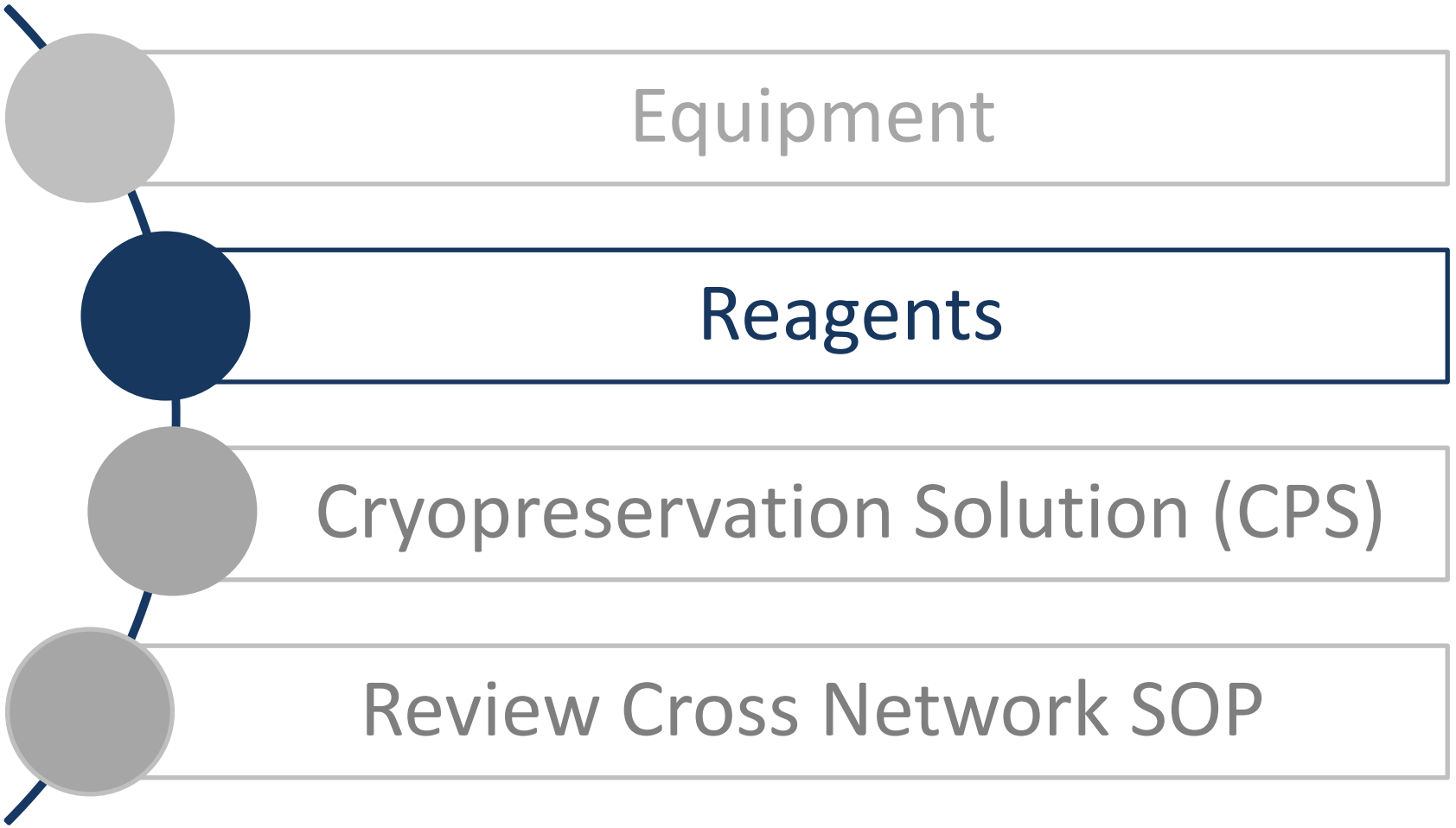
Duke Human Vaccine Institute

PBMC Processing Preparation



Equipment Preparation

PBMC Processing Preparation



PBMC Processing Reagents

Density Gradient Media (DGM)
(15-30°C)

Wash Diluent Reagent (WDR)
(15-30°C)

Fetal Bovine Serum (FBS)
(2-8°C)

Dimethyl sulfoxide (DMSO)
(15-30°C)

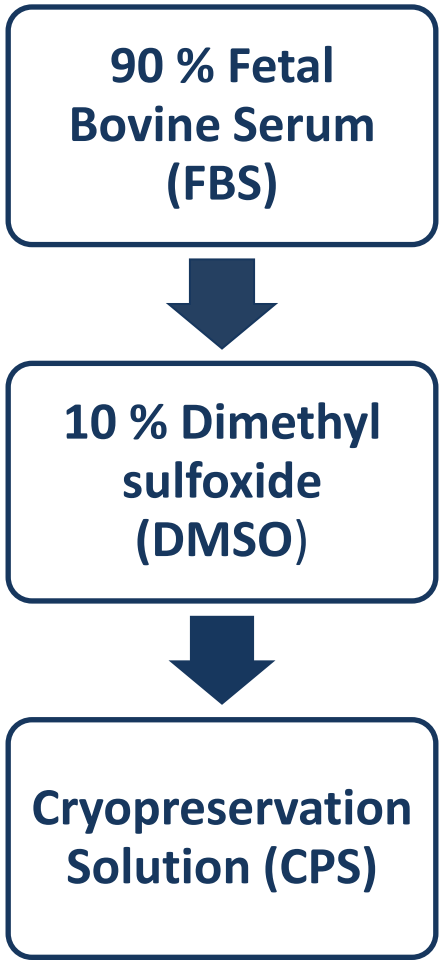
PBMC Processing Preparation

Cryopreservation Solution (CPS)

- Determine the volume of FBS (VFBS) needed using the expected number of PBMC for cryopreservation (Nc) and the final cell concentration (Cc) plus approximately 10%

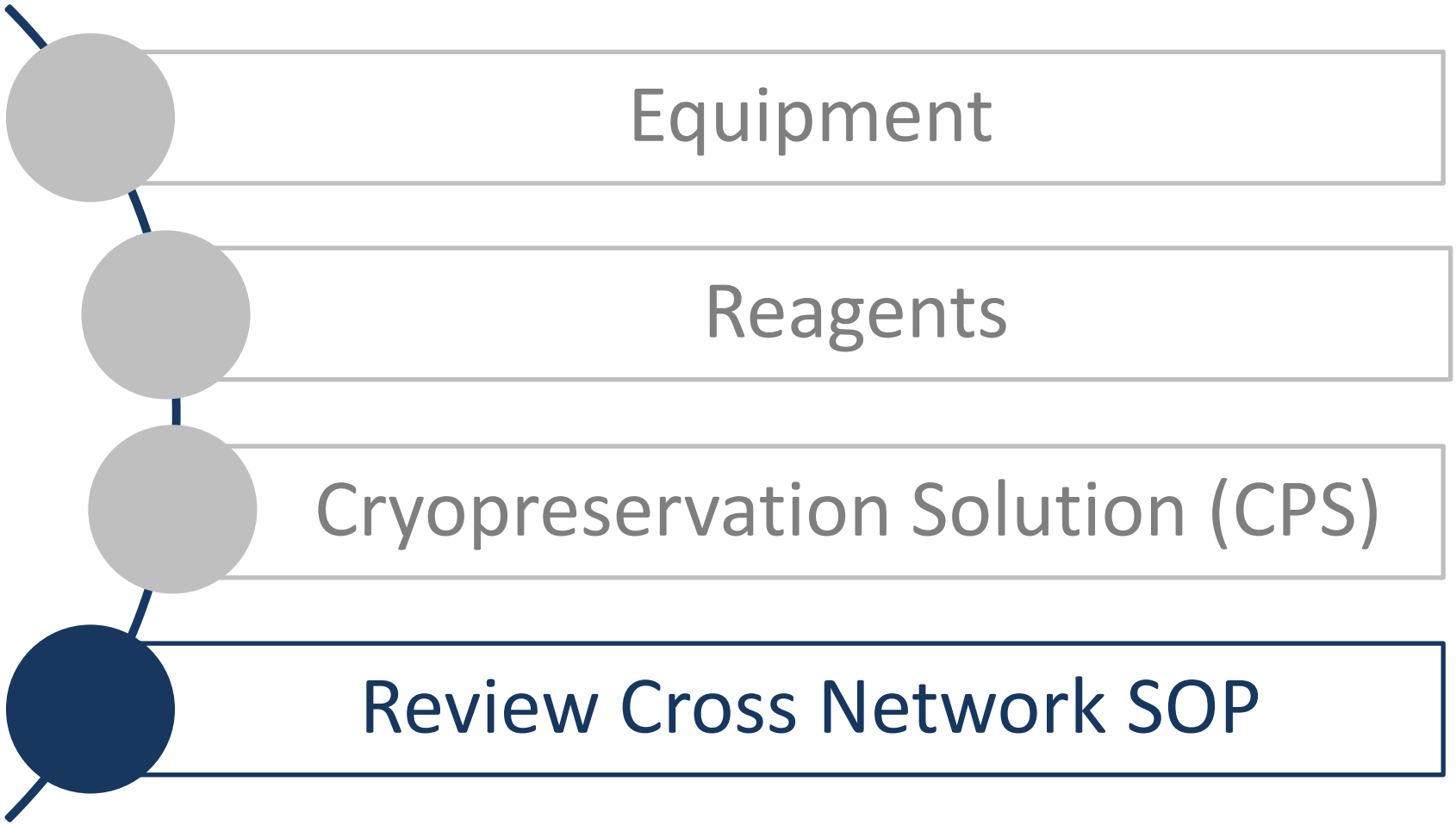
- *VFBS = Nc/Cc*

- *Example: If the protocol expects 1 x 109 cryopreserved PBMC at 10 x 106 cells/mL, VFBS = (1.1 x 109 cells)/(10 x 106 cells/mL) = 110mL.

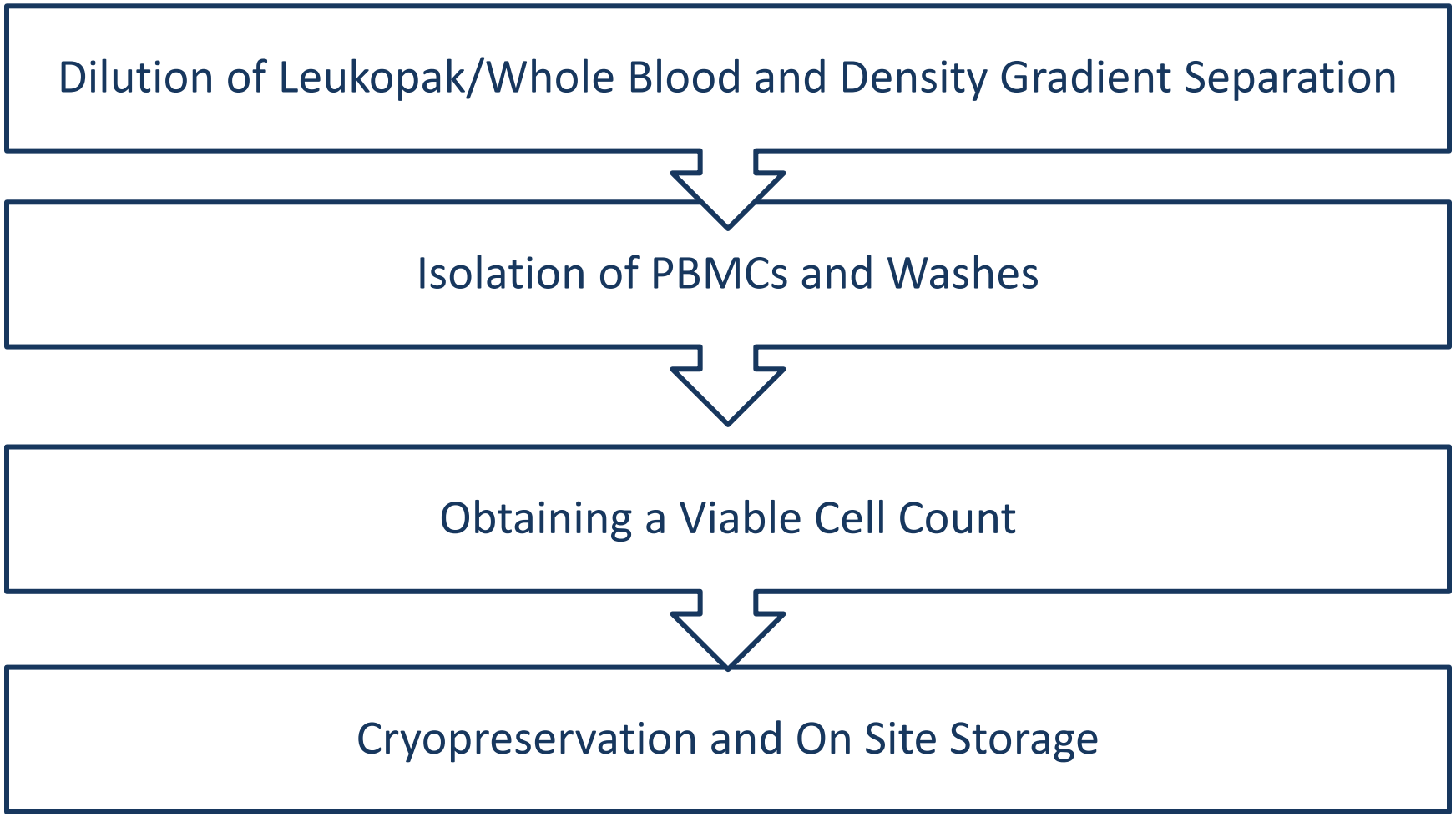


***Cross Network SOP-PBMC Isolation from Leukapheresis https://www.hanc.info/labs/labresources/procedures/ACTGIMPAACT%20Lab%20Manual/PBMC%20Isolation%20from%20Leukapheresis_v.1.0.pdfx**

PBMC Processing Preparation



Overview of Cross-Network* PBMC Isolation from Leukapheresis product and/or Whole Blood (≥ 150mL)

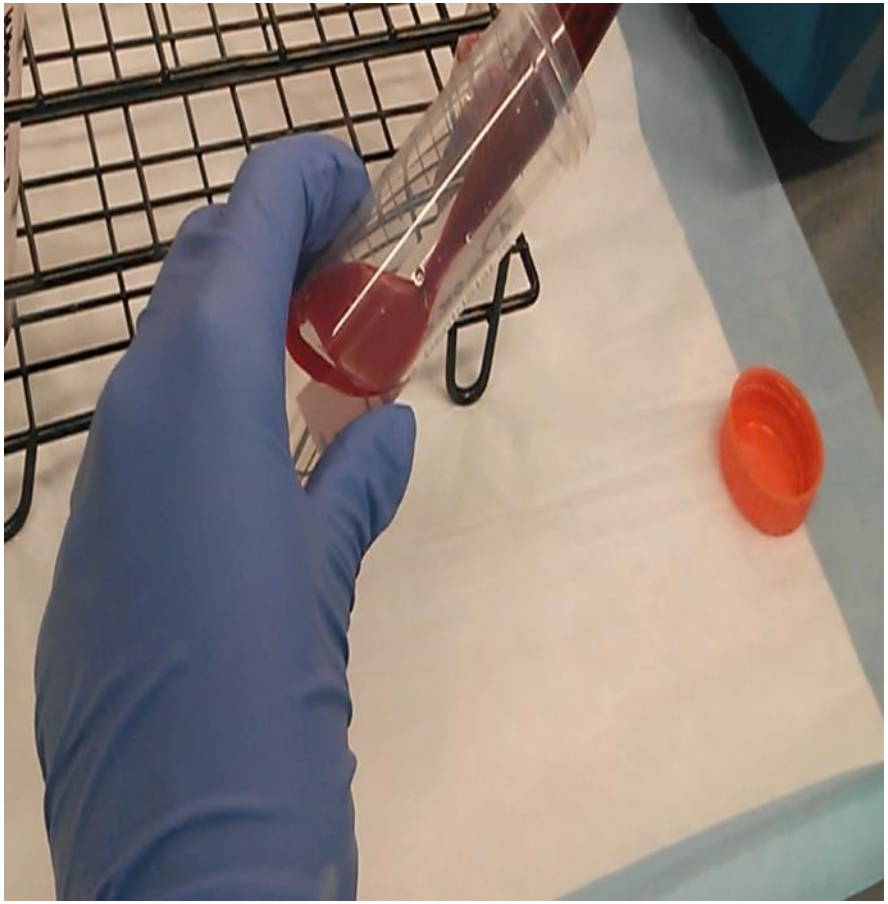


IQA Immunology Quality Assessment Program An NIH, NIAID, DAIDS Program

Duke Human Vaccine Institute

*https://www.hanc.info/labs/labresources/procedures/Pages/pbmcSop.aspx

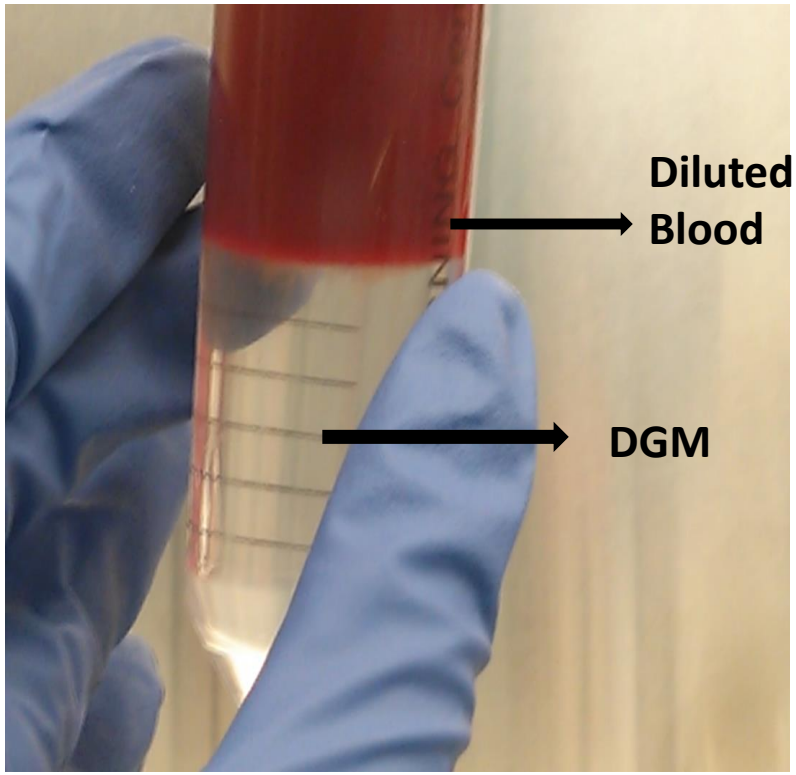
Dilution Of Leukopak/Whole Blood (≥ 150mL)



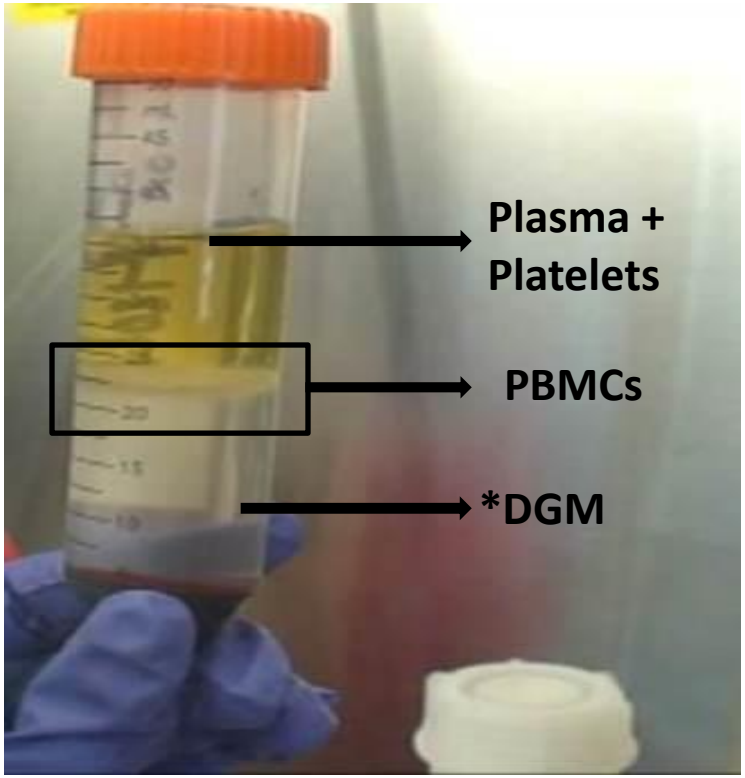
- Dilution with Wash Diluent Reagent (WDR)
  - *Leukopak :QS to 600 mL*
  - *Whole Blood: 1:1 ratio*
- Carefully Overlay Diluted Blood
- Centrifuge at 400 x g for 30 minutes at 15 to 30°C with the Brake OFF

Density Gradient Separation

Layers *Before* Density Gradient Separation Spin



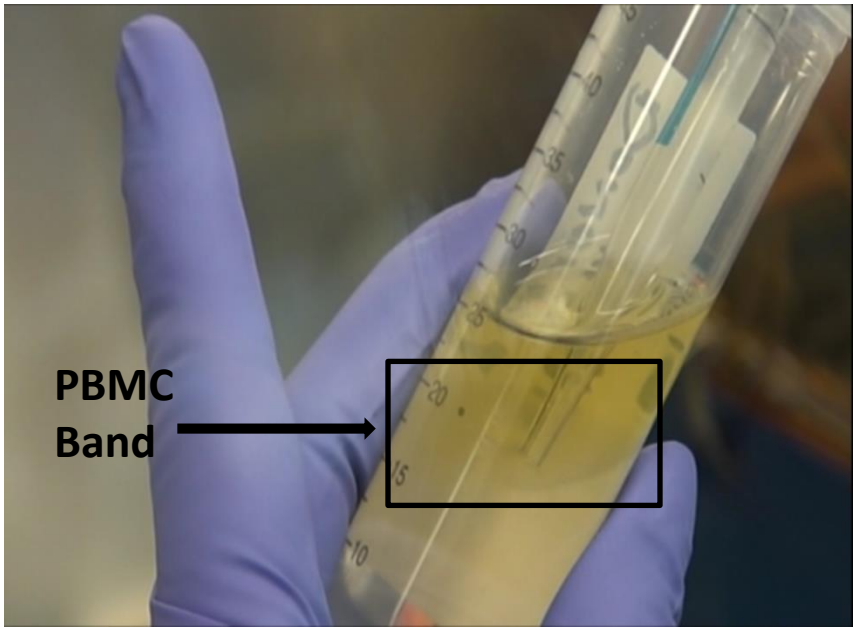
Layers *After* Density Gradient Separation Spin



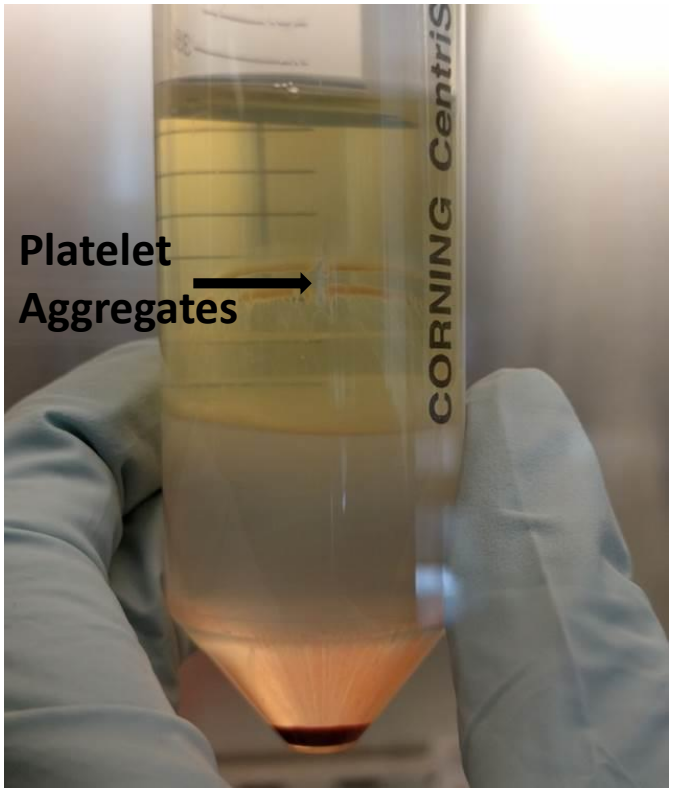
*DMG-Density Gradient Media

PBMC Isolation

Collect the PBMC band without removing excess amounts of Plasma and/ or Density Gradient Media limiting cell contamination



Avoid harvesting the platelet aggregates that form on the tube



PBMC Washes

Centrifuge twice @ 200 to 400 x g for 10 minutes at 15 to 30°C (brake optional)

- Quickly Decant
- Fully re-suspend the PBMC Pellet
- Consolidate PBMC pellets per Cross Network SOP

Obtain the Viable Cell Count

- If an automated cell counter that is not capable of distinguishing viable cells is used, viability must be determined with a manual cell count

Viable Cell Count Dilution

Leukapheresis Product

- Q.S. to 200mL with Wash Diluent Reagent

*Example:

-Remove 100 µL of cell suspension

-Add 160µL of WDR each to 2 tubes/wells and 50µL of trypan blue in a third tube/well.

-Transfer 40 µL of the cell suspension to the first tube/well with 160µL of WDR. Mix well.

-Transfer 40 µL of this cell suspension to the second tube/well with 160 µL of WDR. Mix well.

-Transfer 50 µL of this diluted suspension to the tube/well with 50µL of 0.4% trypan blue. Mix well.

*Cross Network SOP-PBMC Isolation from Leukapheresis
https://www.hanc.info/labs/labresources/procedures/ACTGIMPAACT%20Lab%20Manual/PBMC%20Isolation%20from%20Leukapheresis_v.1.0.pdfx

Large Volume Whole Blood

- Q.S to approximately 20% of the usable whole blood volume

*Example:

-Adjust the amount of cell suspension and Trypan blue as needed for desired dilution. A dilution range of 1:2 to 1:20 will cover most PBMC cell suspensions.

1:20- 20µL cell suspension + 380µL stain

1:10- 20µL cell suspension + 180µL stain

1:5- 20µL cell suspension +80µL stain

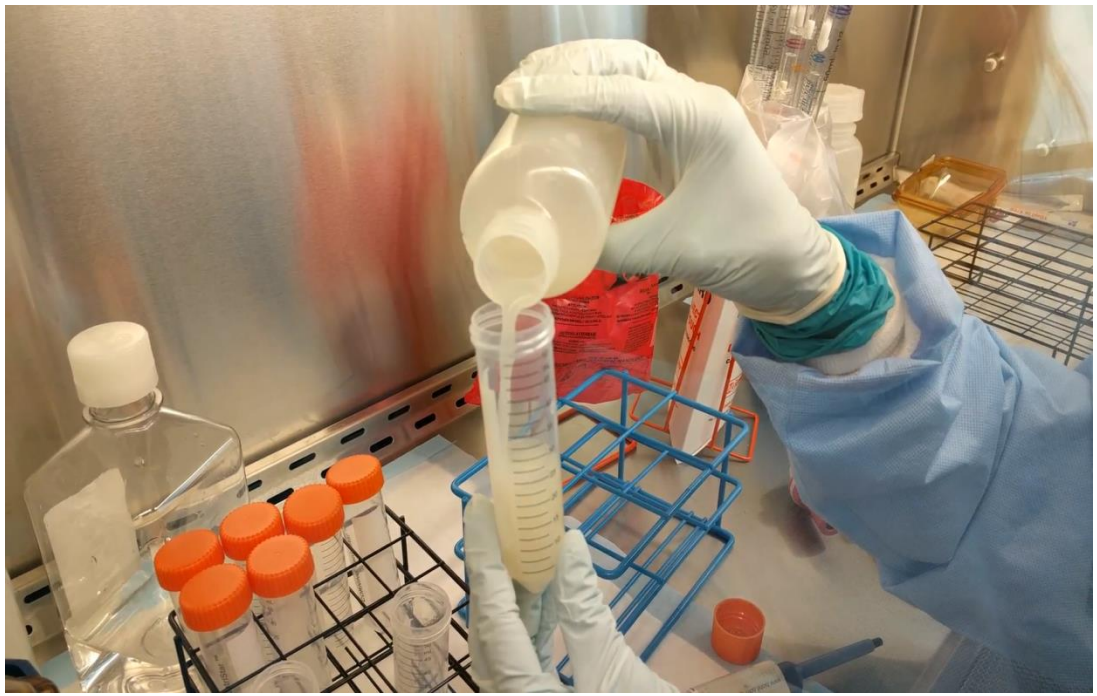
1:2- 20µL cell suspension + 20µL stain

*HANC-LAB-P0006_v1.0_2012-04-13
https://www.hanc.info/SearchCenter/Pages/results.aspx?k=ALL(hanc%20lab%20p0006)

Final Centrifugation

200 to 400 x g for 10 minutes at 15 to 30°C (brake optional)

- Prepare one conical centrifuge tube for each whole batch and each partial batch
- The appropriate number of aliquots per batch will depend on the capacity of the controlled-rate freezing vessel used



Aliquoting for PBMC Cryopreservation

- Work quickly once the Cryopreservation Solution (CPS) has been added to PBMC pellet
- Mix gently and thoroughly during the aliquoting process
- Pre-chilling and /or working on wet ice are allowed

Onsite Storage

- The cold-chain must be maintained during all transfer steps to avoid damage to the cells

- ACTG requires temporary onsite storage in a -70/-80°C freezer with shipment to testing laboratory or repository for long term storage
- Ship on dry ice within 4 weeks of cryopreservation
- Use a dry ice transfer pan during the packing steps

Acknowledgments

NIH

- Daniella Livnat

IVQAC Laboratory

- Tom Denny
- Raul Louzao
- Ambrosia Garcia
- John Wong
- Todd DeMarco
- Linda Walker
- Khalil Itani
- Meredith Carter
- William Tyson II
- Sylvia Hood
- Heidi Macht