Immunology Quality Assessment
Cryopreservation Proficiency Testing Program

Troubleshooting PBMC Processing

2016 IMPAACT/HPTN Annual Meeting

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IQA Cryopreservation Proficiency Test Program

- The optimization of peripheral blood mononuclear cell (PBMC) processing is essential to ensure the quality and function of the cells for ongoing studies in the development of vaccines and treatment strategies.
- The IQA Cryopreservation PT Program measures viability and viable recovery of PBMC samples processed at participating DAIDS-supported laboratories on a quarterly basis to ensure sample integrity.
- This enables the IQA to obtain insight regarding a number of difficulties that laboratories confront during the processing of PBMCs.

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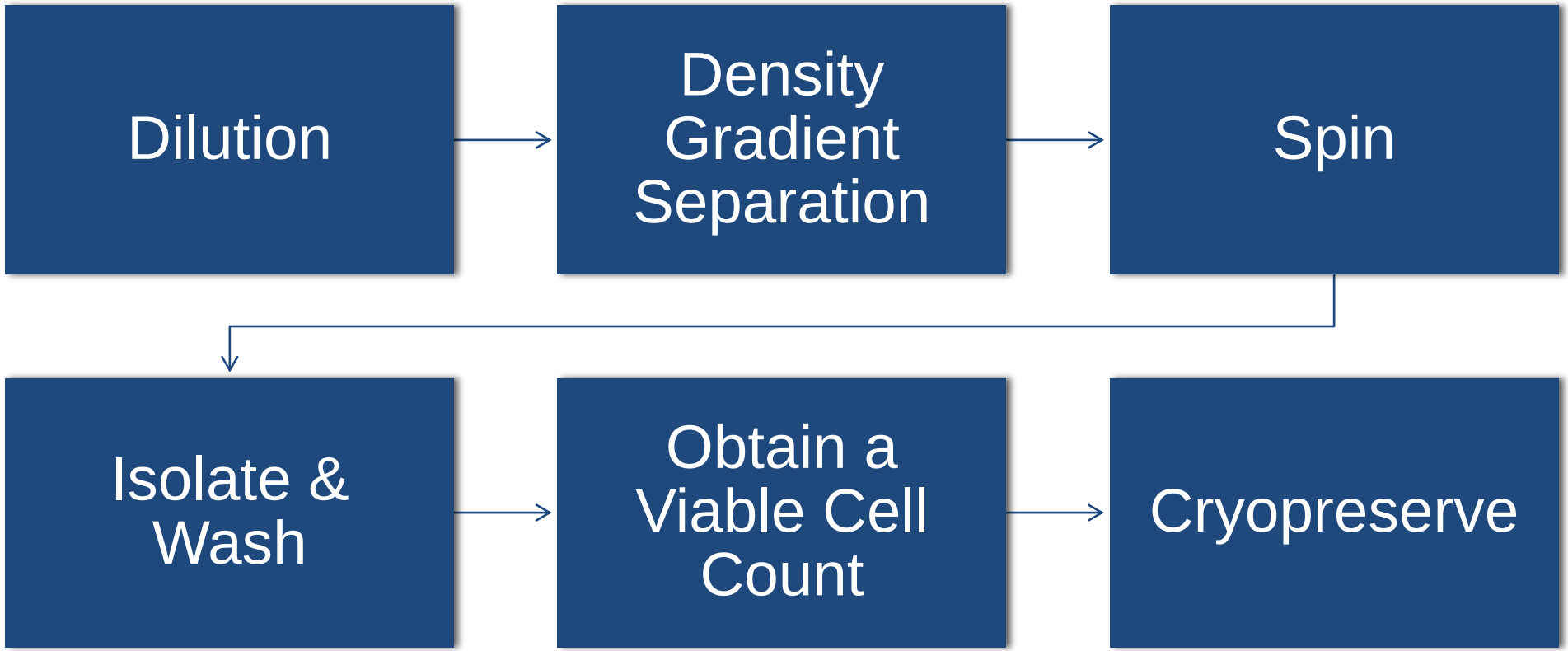
Difficulties Observed by the IQA

Out of Range Viable Recovery	Counting
	Cellular Contamination
	Calculations/Dilution
	Mixing/Aliquoting
Out of Range Viability	Processing time
	Use of Expired Reagents
	Lack of Cold Chain Method

Overview of PBMC Processing



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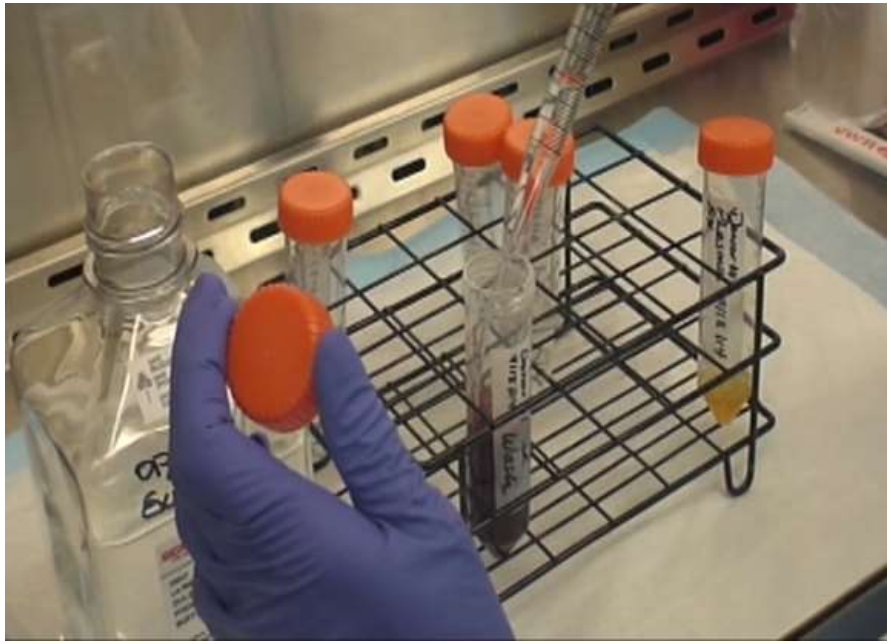
Whole Blood Dilution



- Suitable for the selected density gradient
- Higher yield of PBMCs
- Reduces the amount of the Red Blood Cell (RBC) contamination

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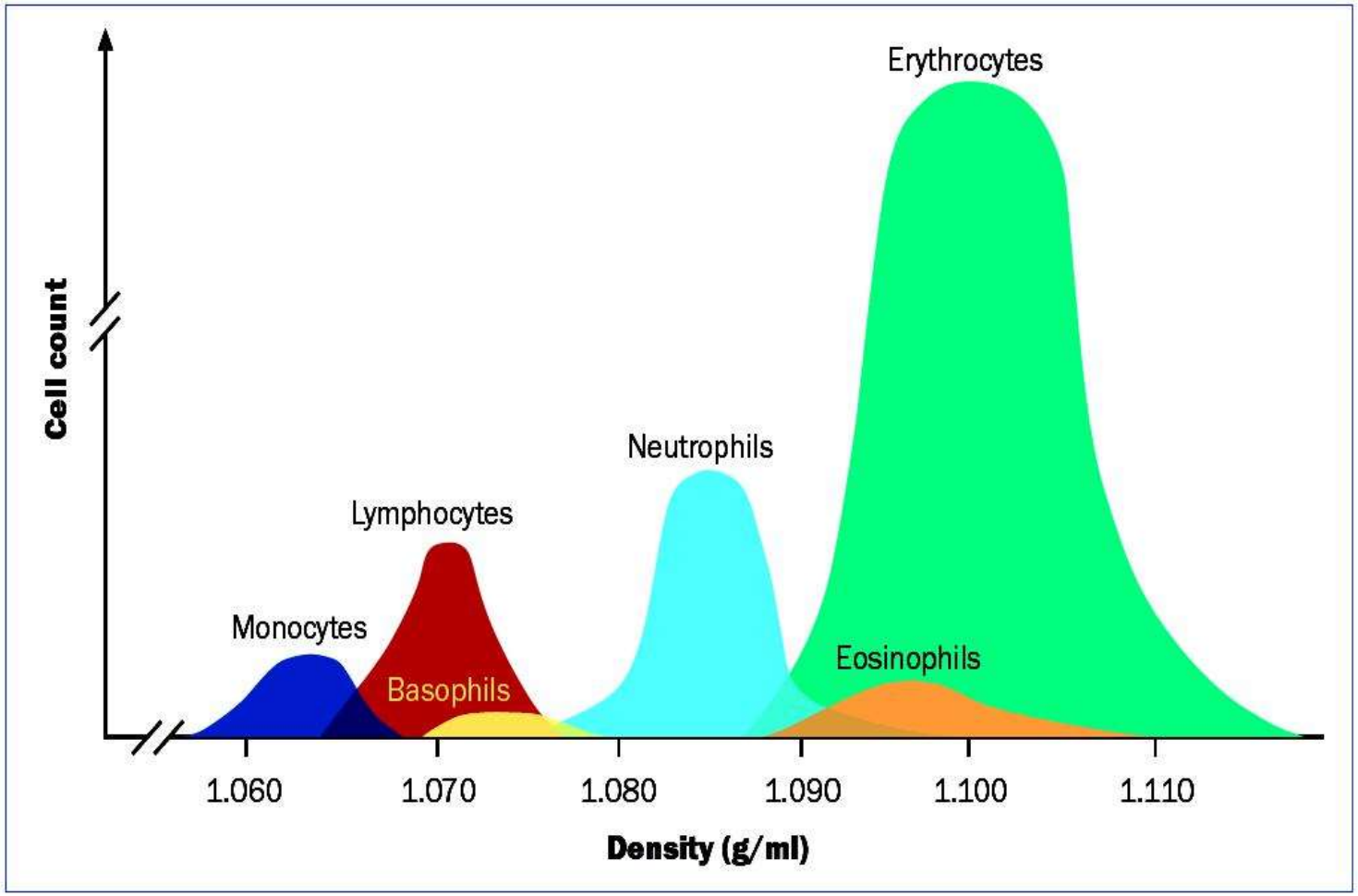
Density of PBMCs



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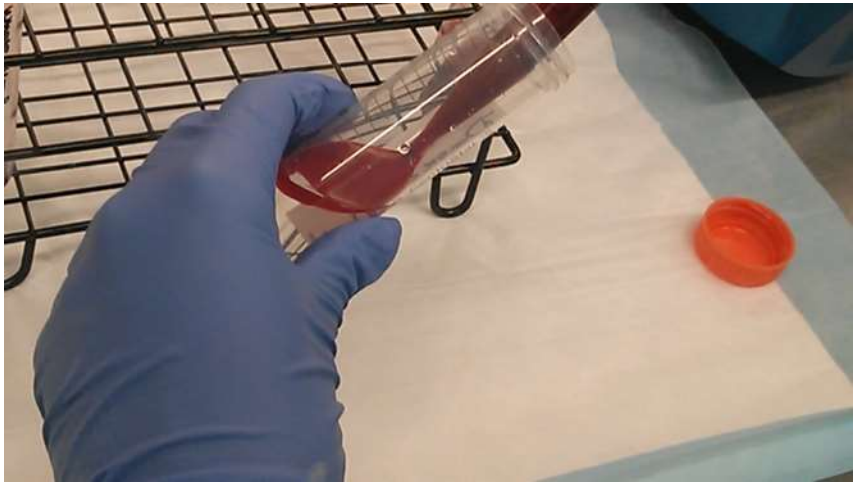
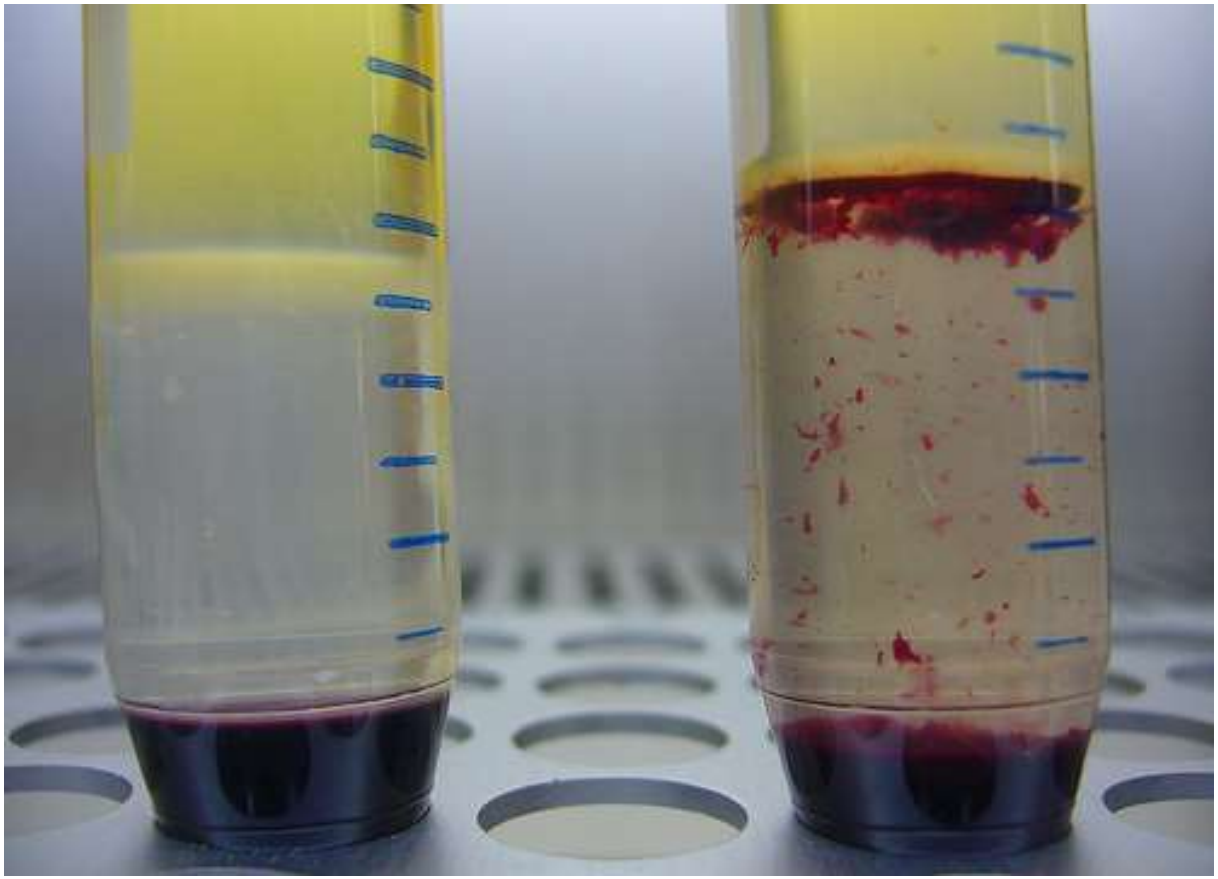
Density Gradient Cell Separation

- Whole Blood and Density Gradient Media (DGM) must be at 15–30 °C



- Carefully layer using proper technique

4°C Density Gradient Media



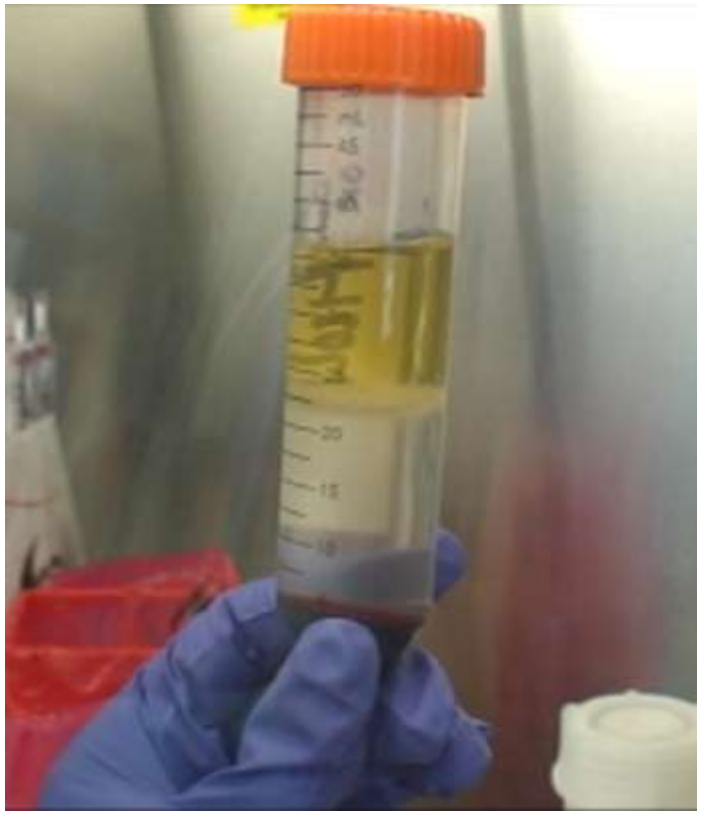
Centrifugation

Brake must be ***OFF*** and the rotor properly seated

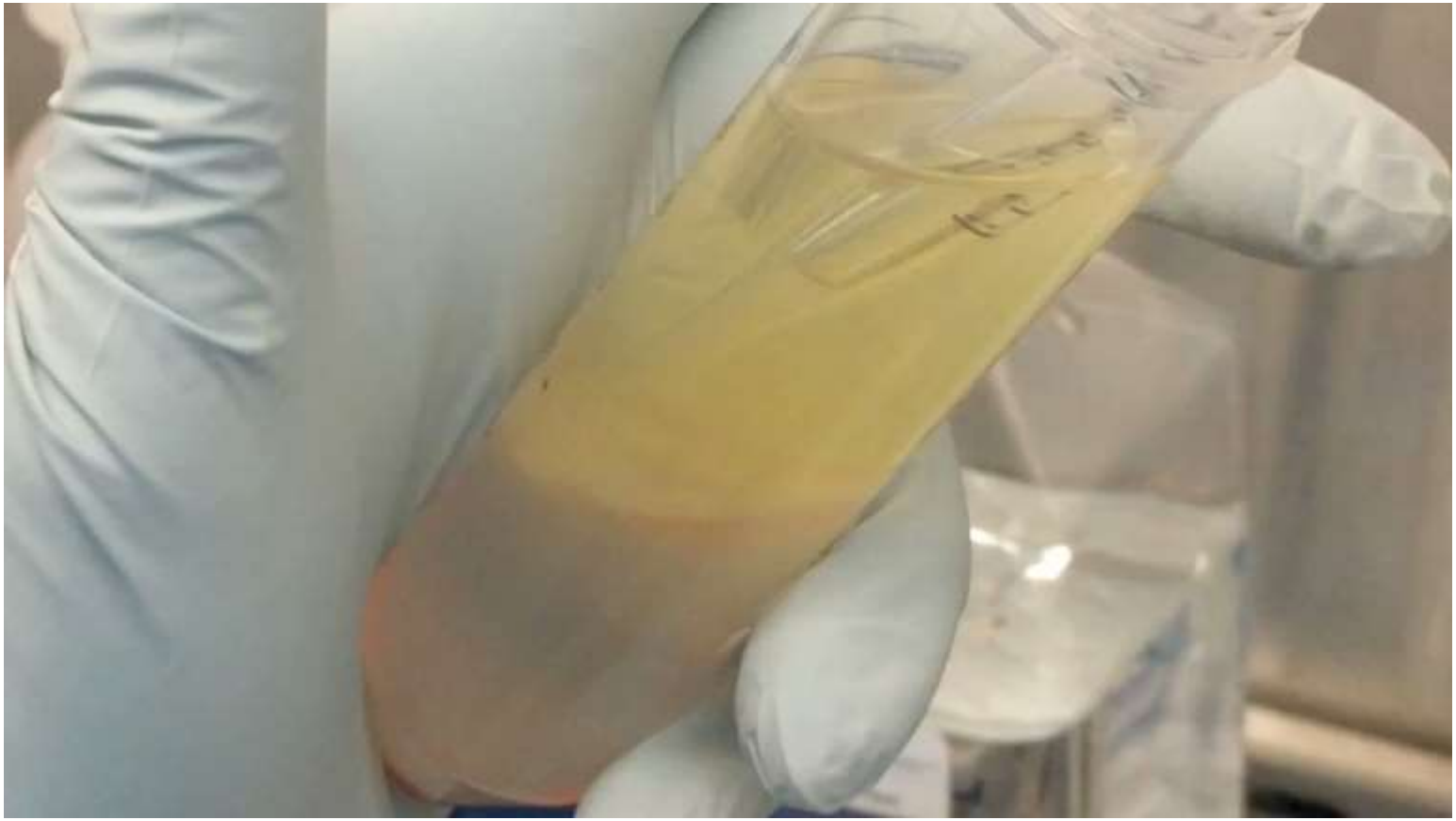
Use swinging buckets

Temperature must be at 15-30°C

Buffy Coat Isolation



- Once the centrifuge has completely stopped ***carefully*** remove the layered tube from the centrifuge as not to disrupt the layer.



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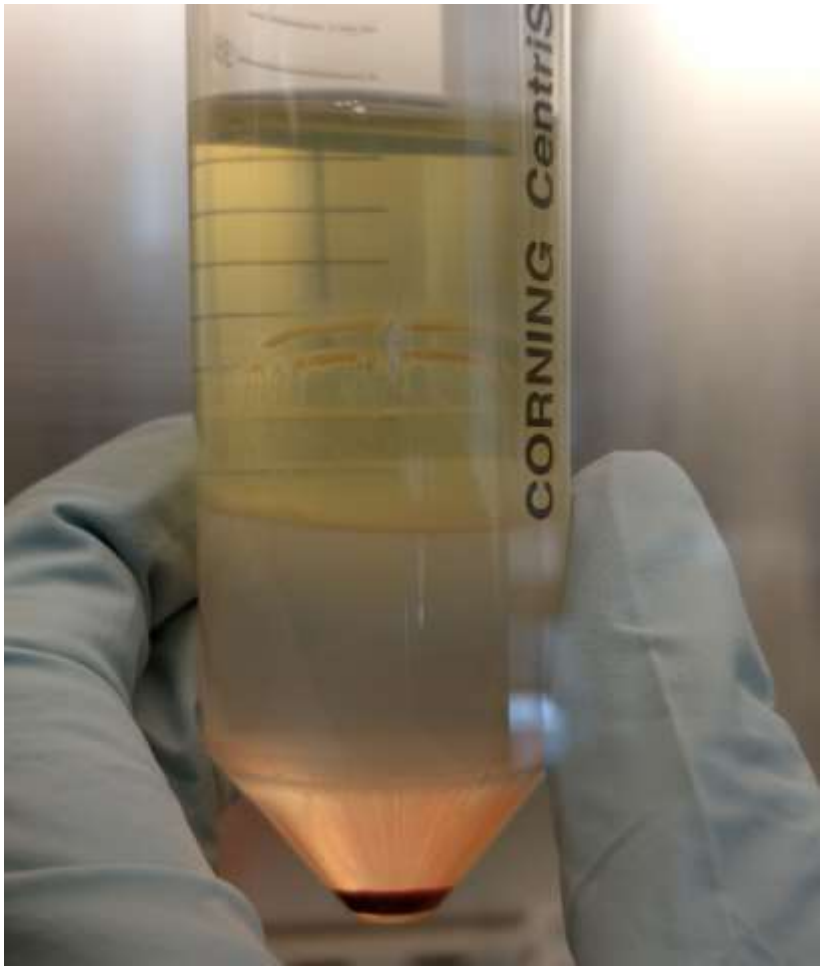
Isolation Recommendations

- Avoid harvesting the platelet aggregates
- Avoid removing excess amounts of Plasma and/ or DGM with the Buffy Coat



PBMC Washes

- Quickly Decant
- Fully re-suspend the PBMC Pellet
- Follow the number of required washes

Obtain the Viable Cell Count

- Accurate Volume
- Even distribution of PBMCs
- Dilute accordingly

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Manual Counting Sample Quality Control

Each quadrant should contain approximately 50-200 cells

Four quadrants should agree within ± 15 %

Allow the sample to settle for 30 seconds

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Cryopreservation Solution (CPS)

- Chill at 2-8°C for 30 minutes or in an ice bath for 15 minutes prior to use
- CPS can be stored at 2-8°C for up to 18 hours

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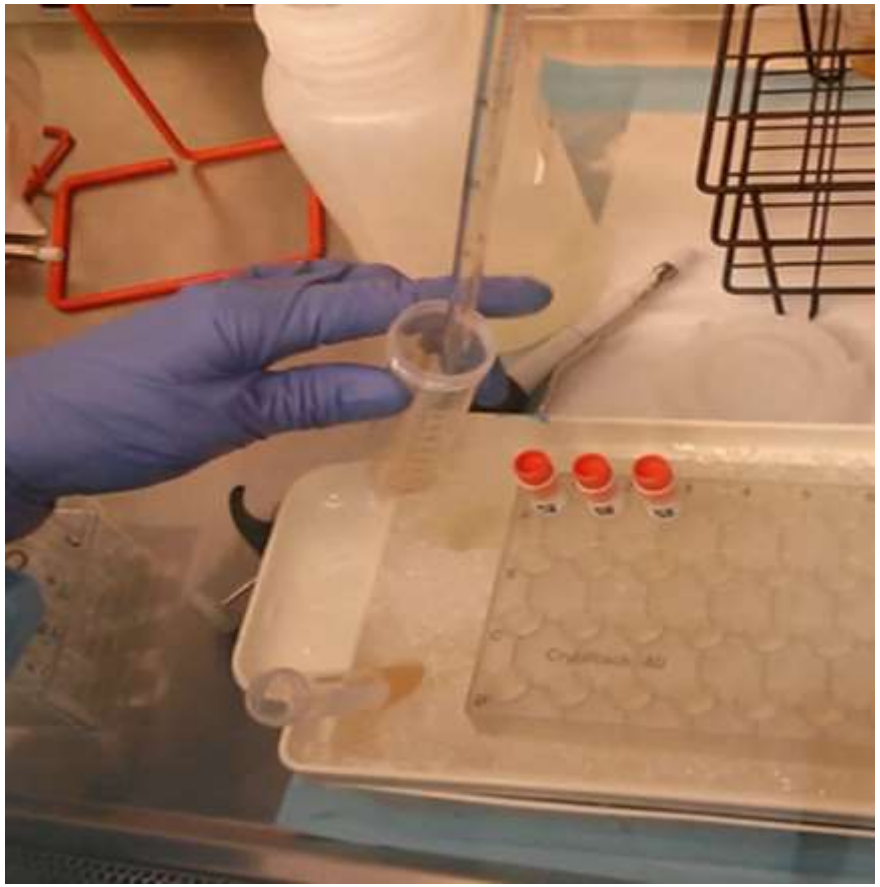
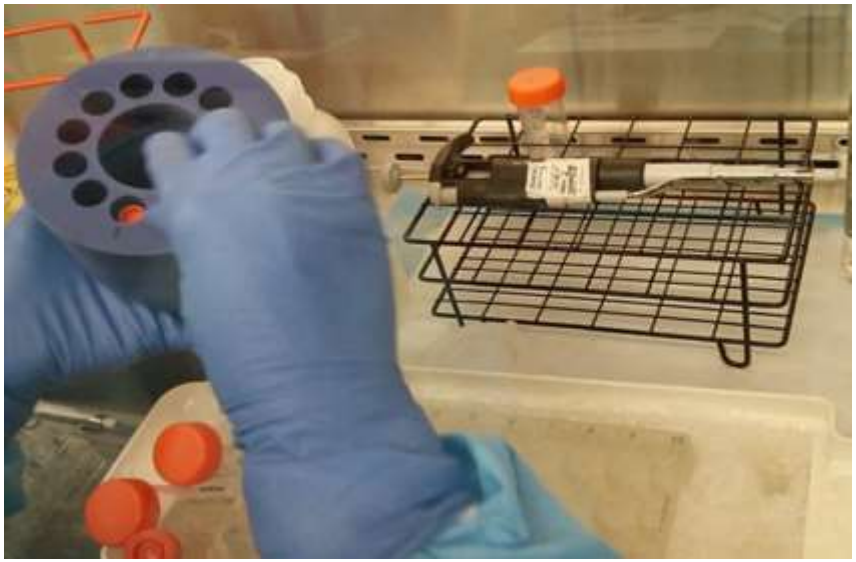
Addition of CPS



- Work quickly
- Mix gently and thoroughly during the aliquoting process
- Use an ice bath during the addition of CPS

Cryopreservation Process



- Maintain the cold chain method
- Follow all laboratory and network guidelines

Recommendations Prior to PBMC Processing

- Follow the Cross Network SOP
- Be Prepared
- Check all equipment
- Do NOT use expired reagents
- Prepare Cryopreservation Solution (CPS)

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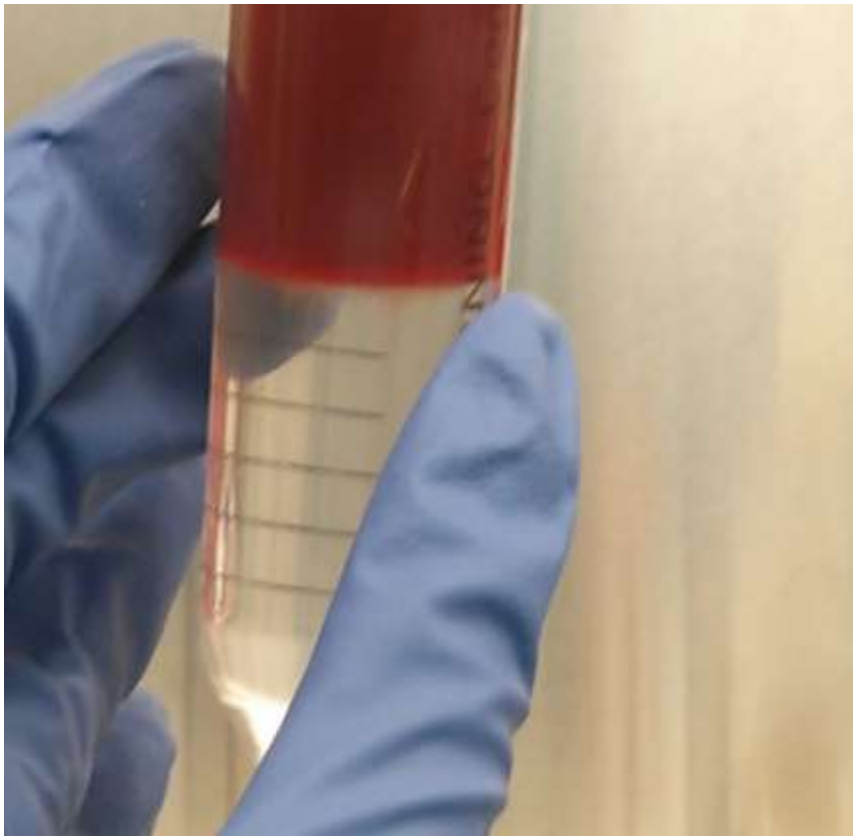
Recommendations During PBMC Processing



- Processing Time (8 hours from time of collection)
- Use accurate and precise pipetting techniques
- Maintain the proper temperature requirements
- Documentation

Acknowledgments

NIH

- Daniella Livnat

IVQAC Laboratory

- Tom Denny
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Questions?

Thank You!

References

- Cross Network PBMC Processing SOP (HANC-LAB-P0001v5.2, Effective date 2014-09-22)
- GE Healthcare/Isolation of mononuclear cells Methodology and applications(18-1152-69 AD 08/2010)
- Histopaque® Troubleshooting Guide, BioFiles Volume 6, Number 5 — Centrifugation
- PBMC Counting_HANC-LAB-P0006_v1.0_2012-04-13
- IVQAC SOP #007_Whole Blood Processing
- IVQAC CRYO #014_Cryopreservation of PBMCs Obtained from Whole Blood