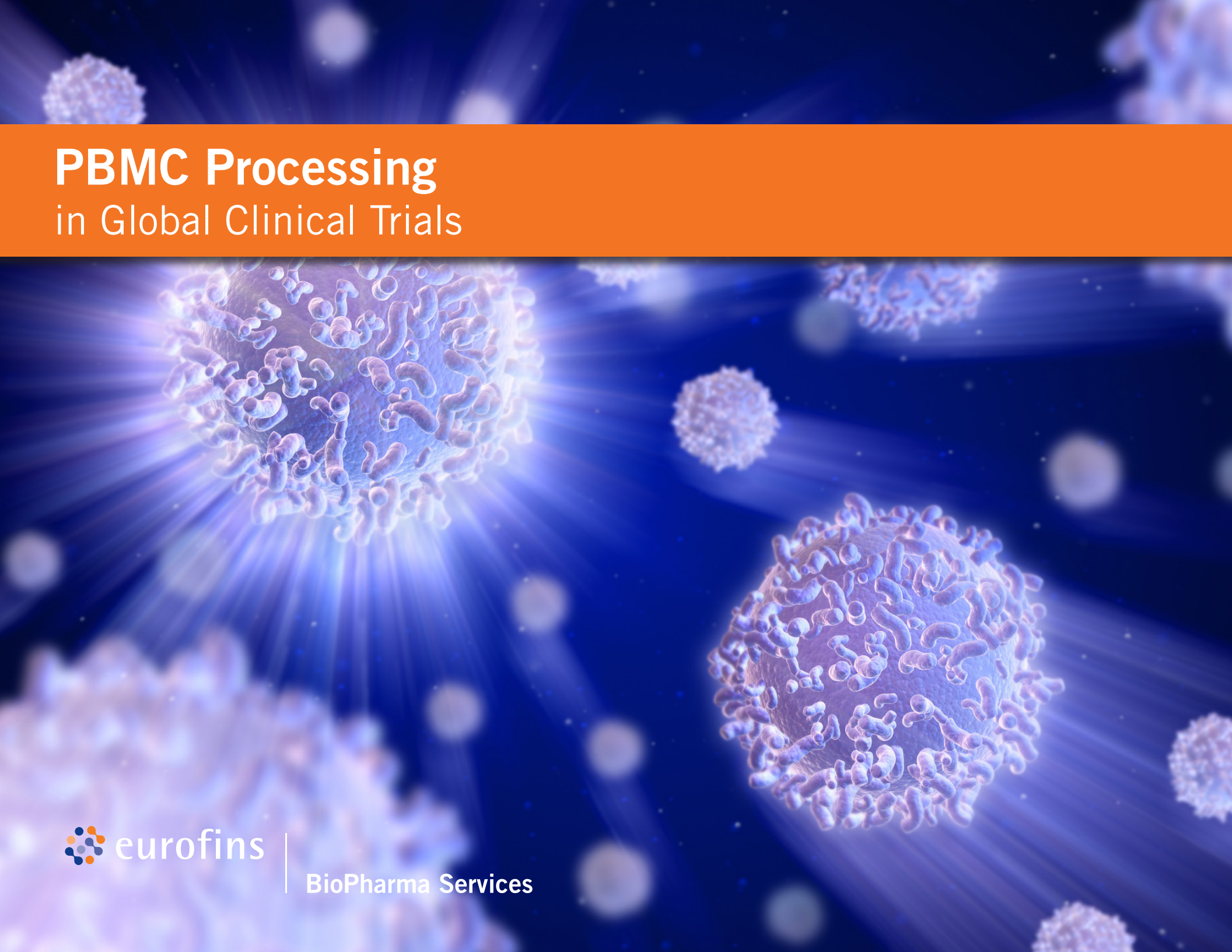
PBMC Processing
in Global Clinical Trials



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Integrated Lab Performance and BioPharma Solutions From the Organization You Trust

From compound discovery and clinical research through manufacture and release of commercial product and post approval/marketing, Eurofins BioPharma Services provides seamless, end-to-end solutions to help clients progress through the drug development cycle through a single, experienced provider. Our integrated solutions deliver the most comprehensive range of state-of-the-art analytical technologies with an expansive geographic reach in order to support our clients' specialized testing needs and stringent quality and safety requirements around the world.

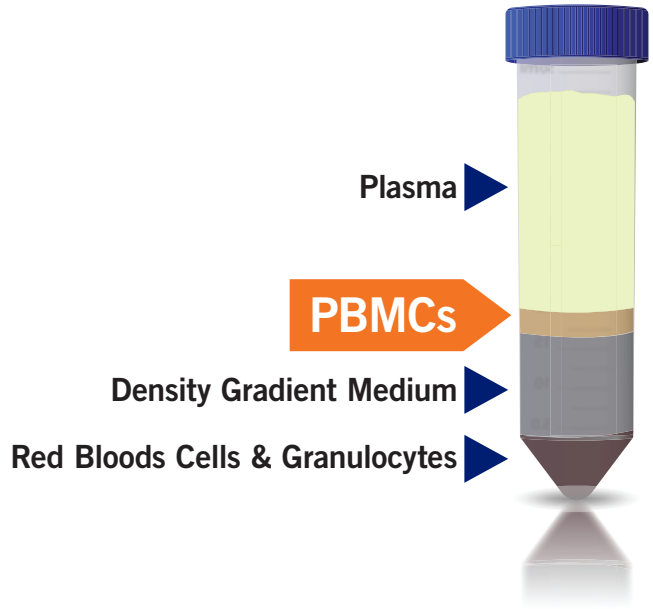
WHAT IS A PBMC?

A peripheral blood mononuclear cell (PBMC) is any blood cell having a round nucleus. These could include lymphocytes, monocytes, or macrophages. These blood cells are a critical component in the immune system to fight infection and adapt to intruders. They can be extracted from a person's blood using "ficoll," which is a hydrophilic polysaccharide that separates layers of blood, followed by gradient centrifugation, which separates the blood into a top layer of plasma, followed by a lower layer of PBMCs, a fraction of polymorphonuclear cells (such as neutrophils and eosinophils) and ending with a bottom layer of erythrocytes. These polymorphonuclear cells can be further isolated by lysing the red blood cells. PBMCs are widely used in research and toxicology applications, as well as in clinical research.

The method for mononuclear cell isolation was first developed by Boyum in 1968. PBMCs are isolated by a process known as density gradient centrifugation, as different components of blood have different densities and should be separated accordingly. This medium is denser than lymphocytes, monocytes, and platelets (meaning these will remain above it), but less dense than granulocytes and enthrocytes, which will fall below it.

PBMCs are used in cell-based analytical assays, which may lead to many operational challenges with regard to transport methods, isolation, speed, quality of isolation, freezing, and harmonization to keeping as many cells alive as possible.

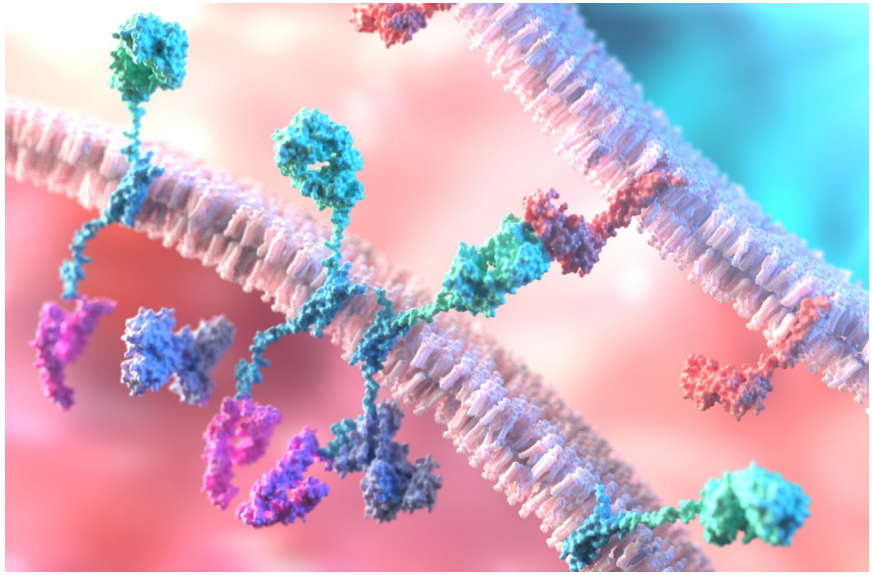
Because these assays demand rigorous handling techniques to maintain their integrity within clinical trials, these operational challenges pose a threat to the trial as a whole. After PBMCs are isolated they must be cryopreserved immediately as well as have a specified timeline determined to maintain viability and time of recovery.



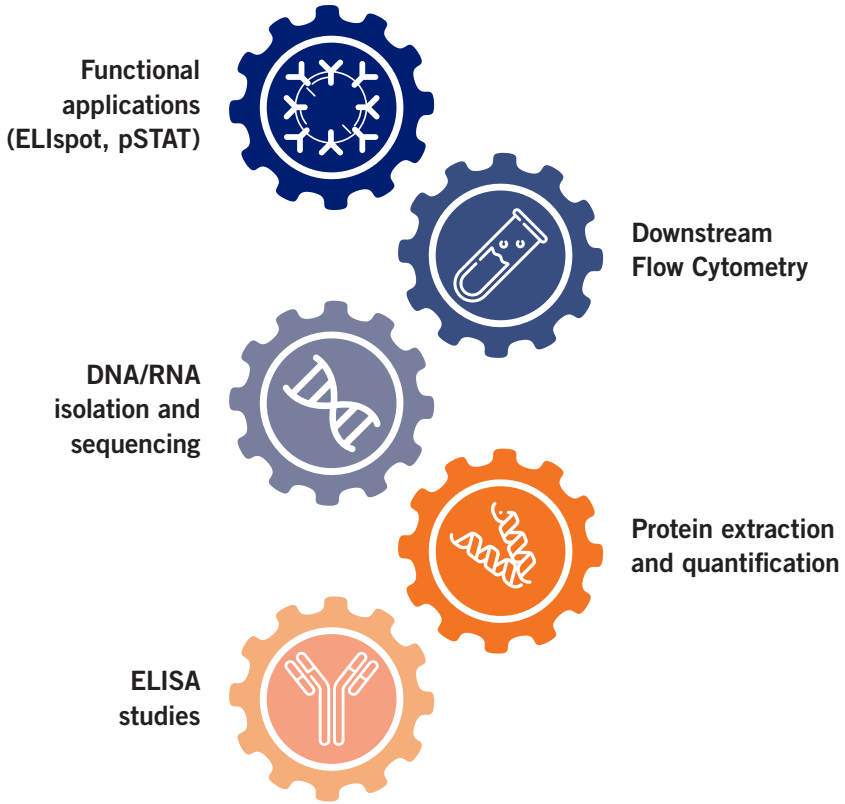
How Are PBMCs Deployed?

Many scientists conducting research in the fields of Immunology (including Autoimmune Disorders), Infectious Disease, Hematological Malignancies, Vaccine Development, Transplant Immunology, and High-Throughput Screening are familiar in the realm of PBMCs.

Immunologists use a form of gene therapy known as CAR T-Cell Therapy. This immunotherapy method uses a part of the immune system, specially altered T-cells, to fight cancer. This involves using a sample of a patient's T-cells collected from their blood, then modifying them to produce special structures known as chimeric antigen receptors (CARs) on their surface.



Analytical Methodology Downstream of PBMC Isolation



IMPORTANT CONSIDERATIONS

There are several important factors to understand when considering PBMCs in your clinical trial. Here, we will overview the process of analyzing PBMCs in a scientific clinical trial.

First, a scientist will draw a blood sample from a clinical trial subject in a clinic.

Second, there are three options for processing the sample:

1. On-site processing takes place in the clinic where the blood was drawn.
2. The sample is shipped to a sample processing laboratory close to the site.
3. The sample is shipped to a central laboratory.

Depending on Sponsor specific PBMC requirements and processing protocols, the best fit-for-purpose scenario is selected.

Third, the PBMCs are isolated from the sample blood. Eurofins supports standard PBMC processing protocols include Ficoll Pacque Method, CPT Mononuclear Cell Preparation Tube and Accuspin PBMC Isolation Tube, and has supported 15 Sponsor specific protocols as well.

Fourth, the PBMCs are put through an immediate deep Nitrogen freeze, to enable ease of shipping the sample to another laboratory location for downstream analysis.

Lastly, the sample is thawed and downstream analysis is initiated.

After the process is completed, it is important to examine the number of PBMCs (Yield) before freezing, to the number of PBMCs after freezing. It is also important to examine the Viability of the PBMCs before and after the freezing process. If all the cells are dead after freezing, they are not viable to use in the trial.

Training, harmonization, and quality control are crucial to maintaining the integrity of the manual process of PBMC processing in clinical trials.

EUROFINS SOLUTIONS

Three Different Levels of PBMC Processing Support

Eurofins differentiates itself from the BioPharma Industry by offering three distinct solutions for Rapid TAT PBMC Stabilization. These include:

Utilization of Eurofins Core PBMC network and supplementary Eurofins global network of laboratory-based locations. The existing Eurofins core laboratory locations have historically existed for provision of 24-hour TAT processing from any clinical trial site on a global level.

Utilization of a “Train the Trainer” model. In this model, Eurofins would assume responsibility for training personnel on-site on Sample Processing Laboratories (SPLs).

Utilization of Travelling Laboratory Technician services. These services would include CAP-Certified Eurofins Laboratory staff travelling to laboratory sites and performing standardized and harmonized processing of specimens on-site.

EUROFINS PBMC NETWORK – ANY CORNER OF THE WORLD

The Eurofins PBMC Network currently comprises 34 active global laboratory locations. The Eurofins supplementary network also includes 125 potential wholly-owned biopharma laboratory locations within the Eurofins organization which could be brought in based upon business needs or requirements.

Eurofins regularly identifies and contracts with additional laboratories to meet the needs of specific projects. There is also a dedicated referral laboratory liaison tasked with identifying high-quality partner laboratories, as well as establishing contractual and technical relationships. Eurofins would deploy at least two certified technicians to each laboratory site in order to best manage the PBMC workload for a Sponsor Protocol.

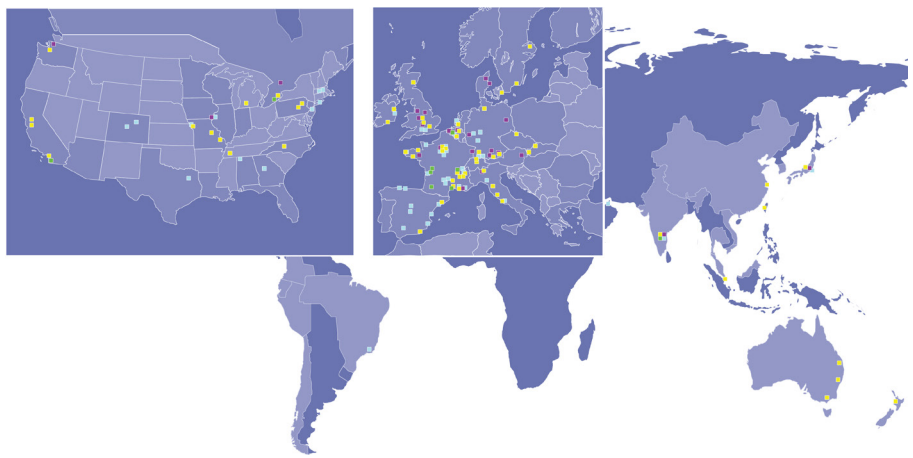
Eurofins Standard PBMC processing protocols include:

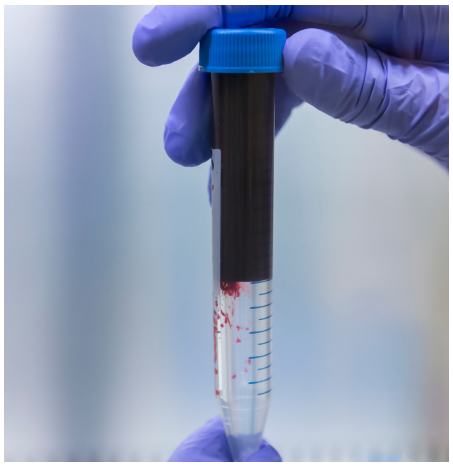
- Ficoll-Paque™ Method
- Vacutainer® CPT Mononuclear Cell Preparation Tube
- Accuspin™ PBMC Isolation Tube

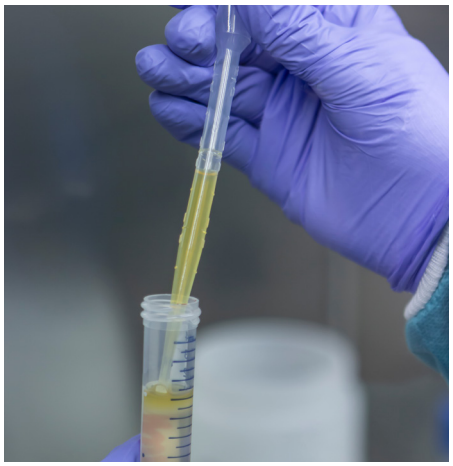
In addition to these, Eurofins has also supported 15 other customized Sponsor-defined processing protocols.

With a 24-hour TAT requirement, all PBMC shipments are expedited. At the onset of any project Eurofins verifies the exact time points at which PBMC isolation is required, and we request notification by the site so that we can ensure adequate staffing ahead of time.

With less than 24-hours TAT requirement, Eurofins either utilizes the “Train the Trainer” Model or deploys a Travelling Technician to the laboratory site. The latter of these services is currently provided for Testicular Toxicity protocols, which have stability TAT criteria of only 45 minutes.







FIVE STEPS OF QUALITY CONTROL

1. Initial Laboratory Assessment

Eurofins deploys due diligence when selecting and monitoring Sample Processing Laboratories. Management of the performance of an SPL and tasks to be performed by all involved parties are defined in the study documentation, as well as approved by the project Sponsor and all participating laboratories.

The initial assessment criteria are detailed in the Subcontractor Assessment Form. The laboratory should be accredited to the relevant local laboratory accreditation standards which include: CAP, GCP, GLP, ISO17025/15189.

The laboratory should also demonstrate expertise in analytical services and exhibit suitable quality characteristics. Some of these characteristics would include method validation, internal quality control, external QA schemes, trained staff, maintained equipment, etc. The laboratory should also show evidence of provision of other service requirements. Some of these requirements would include turnaround time, reporting facilities project management, IT systems meeting requirements, etc. Lastly, the laboratory must be willing and able to supply analytical services and sample processing as defined by Eurofins Central Laboratory, along with display evidence of long-term financial stability.

2. PBMC Training and Certification

Typically, it would take three to six months to qualify a new potential laboratory site, which would be trained and ready for participation in Eurofins' PBMC laboratory network. Eurofins Central Laboratory deploys a Scientific Affairs group to support the global laboratory operations.

The Scientific Affairs Group gives scientific advice on assays, analytical platforms, stability and feasibility, specimen collection, transport and storage, method development, transfer and validation. The Scientific Affairs Group is also responsible for approving, establishing and managing analytical subcontractor arrangements as well as the work performed.

3. Internal Quality Control

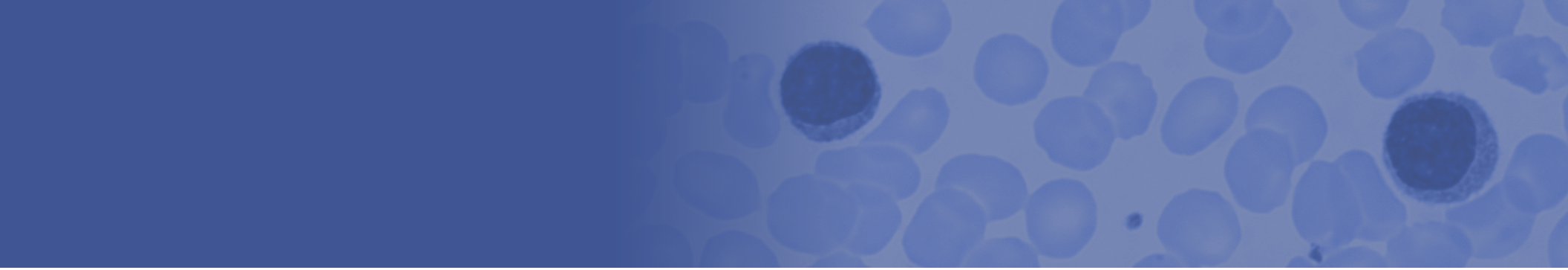
Indicators for internal quality control may include, based on your protocol:

- Fresh Cell Yield:
  Number of harvested PBMC per milliliter of fresh whole blood.
- Total Time to Cryopreservation:
  Time from specimen collection to initiation of cryopreservation.
- Actual Processing Time:
  Time from initiation of blood processing to cryopreservation.
- Percentage of Viable Fresh Cells

The processing time represents the time elapsed from the initiation of processing (i.e., opening the blood tubes in the biosafety cabinet to initiate processing steps) to the initiation of cryopreservation using rate-controlled freezing. Processing times are required to be less than 4 hours. Processing times in excess of 4 hours may suggest an inexperienced processing technician, workflow interruptions, or equipment problems within the SPL.

The Total Time to Freeze represents the time elapsed from collection of the blood specimens to the initiation of cryopreservation. Total times are required to be less than 8 hours. Total times in excess of 8 hours suggest possible specimen transport delays from the clinic to the processing laboratory, scheduling problems between the clinic and the processing laboratory, or excessive workload for the level of staffing within the laboratory.

Internal quality indicators can be helpful in monitoring performance to the specific Sponsor protocol and identifying operational or technical challenges in the SPLs. Technician Comparison Reports are useful for management and staff to visualize differences in quality between the technicians at a single site and allow further training.



4. External Quality Control (EQC)

All Eurofins PBMC network sites and qualified contracted SPLs will be trained to follow the Sponsor specific PBMC isolation protocol. Internal Eurofins sites are trained following global training procedures. SPL training is overseen by Eurofins by deploying the “Train the Trainer” model.

Once initial training has been performed, ongoing monitoring is performed through proficiency testing, oversight from Scientific Affairs, and QA Auditing within the entire active PBMC isolation network deployed to support the Sponsor protocol.

In general, samples are selected covering all active laboratory sites and each technician’s monthly EQC results help to monitor performance in and between laboratories, identify outliers in IQC, issues with freezing/thawing, shipping deviations, and monitoring of new technicians. Each EQC evaluation includes multiple samples. Internal PBMC controls are included at the beginning and end of each evaluation batch.

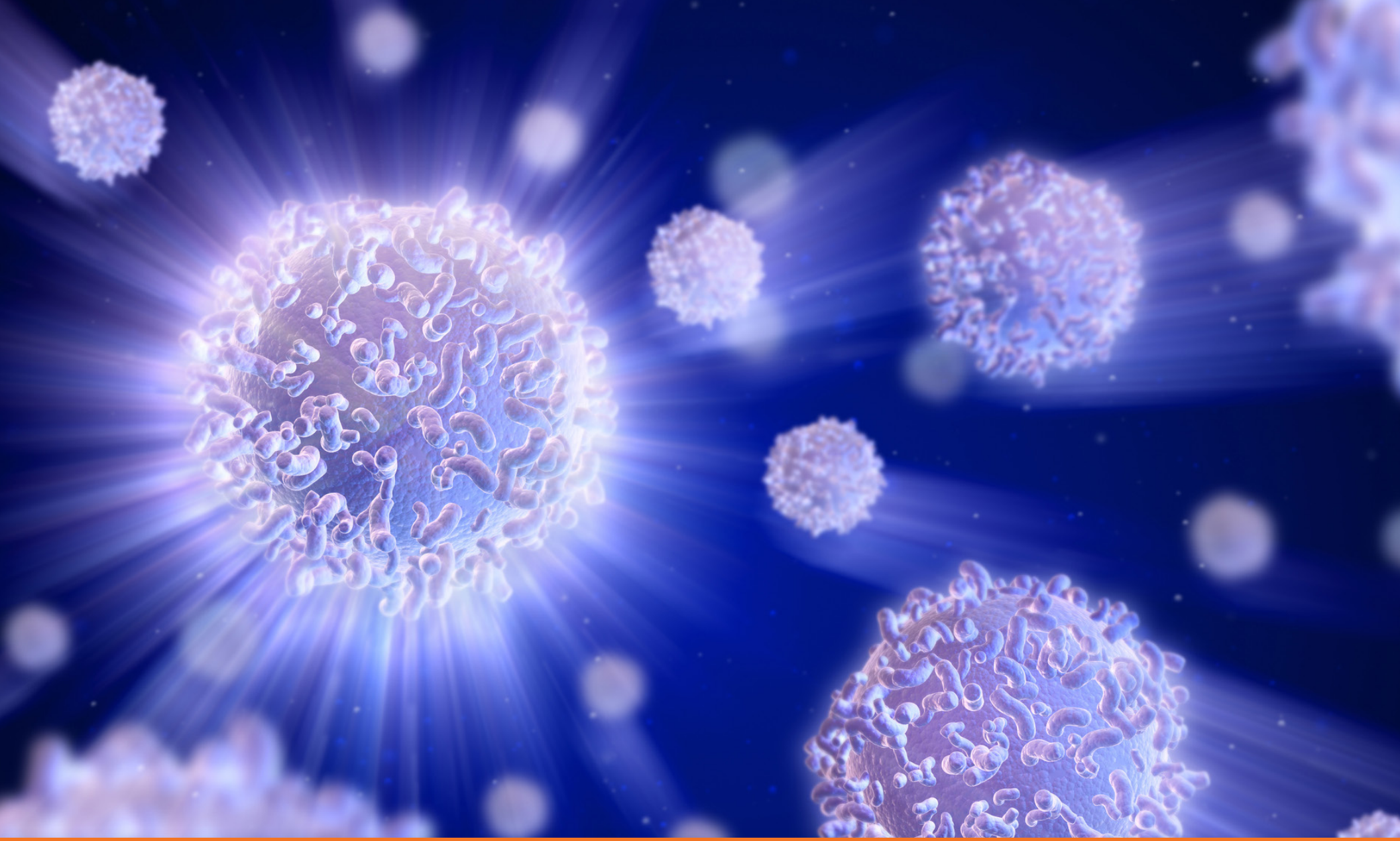
5. Assay Specimen Quality Control

Assay Quality Control (AQC) provides an assessment of the quality of the PBMCs that were used in the clinical trial downstream assays. Viability and recovery data is captured when cryopreserved PBMCs are thawed and counted in preparation of the assays.

Performance reports can be provided by protocol or by SPL. AQC results provide information on the quality of specimens used in the cellular assays as well as feedback on the effectiveness of the EQC program. Comparisons can be made between vials obtained from the same venipuncture that were thawed separately for EQC and AQC.

Final Conclusion

It is imperative to maintain a high-level of quality at all stages of PBMC clinical laboratory testing. This is important beginning at the point of specimen collection all the way through to processing, shipping, handling, storage, and assaying of the specimen.



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