

Lab Network Improves
PBMC Sample Quality to Support
Clinical Trials

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The collection and analysis of peripheral blood mononuclear cells (PBMCs) represents a significant technical challenge for clinical trials. PBMCs are a population of human blood cells that include T-lymphocytes, the “killer cells” of the immune system. Because these T-cells recognize and react to virus-infected and transformed tumor cells, they are critical tools for measuring the level of immune protection in patient blood samples. Functional PBMC activity assays are essential to accurately evaluate the performance of vaccines, as well as antiviral and anticancer agents in clinical trials.

These functional assays require high-quality PBMC samples. For large, international trials across hundreds of clinical sites, variation in cell harvesting protocols, the time from blood draw to sample freeze, and secure shipping practices are some of the potential obstacles to sample integrity and assay success. Based on research conducted in the course of a global clinical program to develop an HIV vaccine, optimal conditions for PBMC processing have been identified and a laboratory network was developed to ensure quality PBMC processing across multicenter trials. This network is now available through PPD to provide an industry platform that supports PBMC processing in global clinical research.

PBMC Processing Challenges

PBMC integrity declines rapidly over time after following the collection of blood samples. To perform successful functional assays, PBMCs must be isolated and frozen quickly to preserve cell integrity. Studies conducted by Kierstead, Dubey, Meyer and coworkers (2007) clearly demonstrate that PBMCs must be isolated within 12 hours from the time blood is drawn to insure optimal cell integrity and functional activity.

In the context of clinical trial operations, this means that PBMC processing must be scheduled to coincide with the patient visit. Blood drawn at the clinical site must be sent immediately to a processing lab for PBMC isolation and freezing. To meet the 12 hour deadline, the proximity of clinical site to processing lab is critical. Frozen samples must then be shipped to a lab where the functional assay is performed. In transit, frozen samples must be maintained at the correct temperature.

According to the researchers' report, “It is critical to the success of a clinical development program to establish optimized procedures for the handling and processing of PBMCs that not only will ensure the functional integrity of the cells but also can be implemented reproducibly in a multicentric, global clinical trial setting.”¹ Their work, described below, resulted in the development of an optimized and reproducible isolation procedure that was implemented through a highly successful PBMC processing network.

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Optimal Processing Conditions

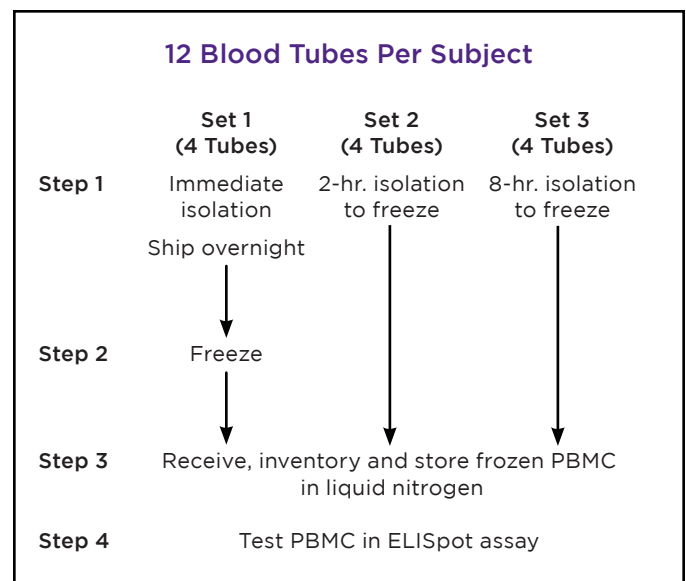
In support of a large multicenter clinical trial for an investigative HIV vaccine, Kierstead and colleagues were asked to develop an optimized assay to monitor the host immune response. Immune monitoring for HIV vaccines is especially difficult because the assays require PBMCs. In the early stages of the program, PBMC samples were processed at the site, collected from study patients and shipped, unfrozen, to a laboratory for freezing. However, this approach was not feasible for a large, multicenter trial, since distance and time to the laboratory would vary greatly among clinical sites and cell integrity could not be consistently maintained. The researchers designed a series of experiments to identify the optimal conditions for PBMC collection and processing. The goal was to develop a PBMC processing approach that would ensure consistent, high-quality assays globally across the clinical trial.

Experiments were conducted to compare three different processes for handling blood samples (see Figure 1). Twelve blood tubes were drawn from 11 healthy subjects and four tubes were used in each of the three different processing protocols. In Sample Set 1, PBMCs were isolated from blood samples within two hours of the blood being drawn from the patient, shipped overnight unfrozen, and then frozen at the lab on receipt. In Sample Set 2 and Set 3, PBMCs were isolated within 2 hours or 8 hours from blood draw, respectively. PBMCs in

both Set 2 and Set 3 were frozen immediately after isolation and shipped frozen. PBMCs obtained from each of the three processing protocols were thawed, cultured overnight, and comparatively tested for functional activity.

Results showed that for each subject, T-cell responses were significantly higher in the Set 2 and 3 samples (frozen immediately after blood draw), compared to the Set 1 samples frozen the following day. Kierstead and coworkers established that when the time from blood collection to PBMC freezing was reduced to 12 hours or less, on average the T-cell response improved more than three fold.

Figure 1. Flow Chart: Three PBMC Handling Processes



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Processing Network Becomes an Industry-wide Platform

Using these parameters for optimal PBMC processing, the research team developed a laboratory network to support the global clinical programs. They established processing facilities near major population centers, a key to the network's success. The network began in 2004 and within a year and a half, evolved to include 15 LabCorp laboratories in the United States and one in Canada. Since that time the number of participating laboratories has grown to include more than 15 additional private, hospital and academic labs.

To ensure consistent, high-quality PBMC samples and assays across multiple locations, the network relies on rigorous quality controls, staff training, monitoring and qualification. For example, couriers are trained to provide special handling for sensitive samples as they are transported to network processing labs. PBMCs are isolated and frozen within 12 hours. Documents from the processing lab are carefully examined to confirm strict adherence to the optimized procedure. Frozen samples are stored at the processing facility for no more than two weeks at minus 70 degrees, then shipped to the lab for storage in vapor phase liquid nitrogen prior to assay, with careful attention to maintaining proper temperature in transit.

PPD Extends Network Services

In 2009, PPD made the network's services available to the entire biopharma industry, providing comprehensive PBMC services from blood draw to delivery of assay results. Assays can be performed at PPD's Vaccine and Biologics Laboratory in Wayne, PA and results are transmitted directly to the client using secure data transfer technology.

Expanding the network's efficient and effective PBMC processing services has real value for clinical research. Biopharma's clinical development pipeline now includes more than 2,200 anticancer agents, 750 vaccines and 350 antivirals.² PBMC assays will play a vital role in delivering these and other new therapies to medical practice.

"There is a huge need for PBMC assays," says Research Fellow Lisa Kierstead, who is the scientific lead managing the PBMC network. "We've identified an optimal process, and we've been successful with it for six years now. Some clients tell us they can't process their samples within 24 hours—that kind of performance can have serious negative impact on the success of a clinical trial. The availability of this service will have real benefit for global research."

References

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