PROCESSING PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) FOR CLINICAL STUDIES: POINTS TO CONSIDER FOR QUALITY RESULTS

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INTRODUCTION

With the importance of flow cytometry and functional assays (e.g., enzyme-linked immune absorbent spot; ELISpot) in evaluating the potential of immunotherapeutic candidates[1,2], providing high quality peripheral blood mononuclear cells (PBMC) is a critical first step in the process. This paper discusses pre-analytical variables that affect the performance of PBMC in downstream analyses, as well as other considerations that can be managed cooperatively with the Sponsor to ensure reliable outcomes using this sample type in clinical research.

The literature provides abundant examples where pre-analytical variables affect ideal cell recovery and viability, or meaningful performance in subsequent fit for purpose testing[3,4]. This pre-analytical phase is broad and includes specimen handling issues during the time of collection, through transit to the receiving facility, as well as subsequent sample handling and processing, or storage prior to downstream analysis. Similarly, the typical life cycle of PBMC for clinical studies begins with a whole blood sample collected at the trial site using a vacutainer with anticoagulant. This is then shipped to a laboratory facility for processing and purification before immediate analysis. Often the purified PBMC are cryopreserved and stored cryogenically for long periods before they are retrieved and shipped to another laboratory for thaw and acclimation prior to additional testing.

Below is a framework of principles or points to consider (PTC), which Medpace laboratories uses for ensuring the best results from PBMC processed for clinical studies.

PTC 1: UNDERSTANDING REQUIREMENTS BEFORE STUDY START

Consideration: What is the use or intent of the PBMC sample and what is required by the downstream testing? Will PBMC be used for receptor/biomarker analysis, functional assays, molecular assays, regenerative medicine, or only for future use biobanking?

Though the expectation might be that PBMC destined for downstream analytics must demonstrate 100% recovery and 100% viability, the truth of the matter is that acceptance criteria are actually established by the validation of the method used at the particular testing laboratory. As an example, depending on the specifics of the particular ELISpot, acceptance criteria for minimum viability for PBMC at thaw can range from 60-80% with similar ranges for recovery after overnight rest prior to start of the functional assay. PBMC destined for genetic testing may require less cell count and cell viability.

Medpace manages those scientific discussions to ensure all teams are aware of the requirements and limitations, and expectations for results. It is important to understand the conditions for acceptance criteria by the downstream analytics prior to setting up the trial.

PTC 2: SITE CAPABILITIES AND SPECIMEN COLLECTION

Consideration: The collection site is the starting point where procedures must happen correctly. Understanding the qualifications and capabilities of the collection site as well as their hours of operation can facilitate patient processing and prompt courier pick up. With studies that rely on a shorter time from collection to process for PBMC functionality, the sites may consider scheduling patient visits later in the day to shorten transit time to the processing lab. Be aware of any limitations the site may have, which could influence time for immediate processing (i.e., centrifugation) as this pre-analytical variable can affect cell counts, viability, and performance in downstream assays[5,6].

Preference for collection tubes must be based on all downstream analytics anticipated. Certain collection tubes contain preservatives, fixatives, or anticoagulants, which can interfere with molecular assays or can affect viability of PBMC especially after cryopreservation[7]. When short turnaround time for processing is required, consider using collection tubes with gel barriers specifically designed for separation of mononuclear cells. These tubes can reduce variability with processing and often work best for the overall turnaround time at the final PBMC processing lab.

Medpace provides detailed lab manuals to the sites, which include collection kits and instructions for processing and shipping. Where appropriate, the collection site will also receive training especially with any study-specific processes. This ensures consistency with sample collection and processing at the site level.

PTC 3: LOGISTICS FROM SITE TO PROCESSING LAB

Consideration: Minimizing the time in transit of samples from the collection site to the processing lab will improve turnaround time to processing. Delays in transit during inclement weather or seasonal weather extremes may also create suboptimal temperature conditions, which can influence PBMC processing and ultimately their utility in a clinical study[8].

Labs that evaluate the function of PBMC have developed requirements for testing (e.g., % viability, cell number and recovery, etc.) as fit for purpose, presumably based upon their particular method validation. There is an expectation that the testing lab has determined the acceptable limit from time of sample draw to time of processing. However, an optimum time of 6 hours from collection to processing PBMC for ELISpot may not be practical or maintained with collection sites too distant from the central processing lab. It should be noted that depending on the particular assay, an acceptable maximum time from collection to processing PBMC could vary up to 48 hours[9].

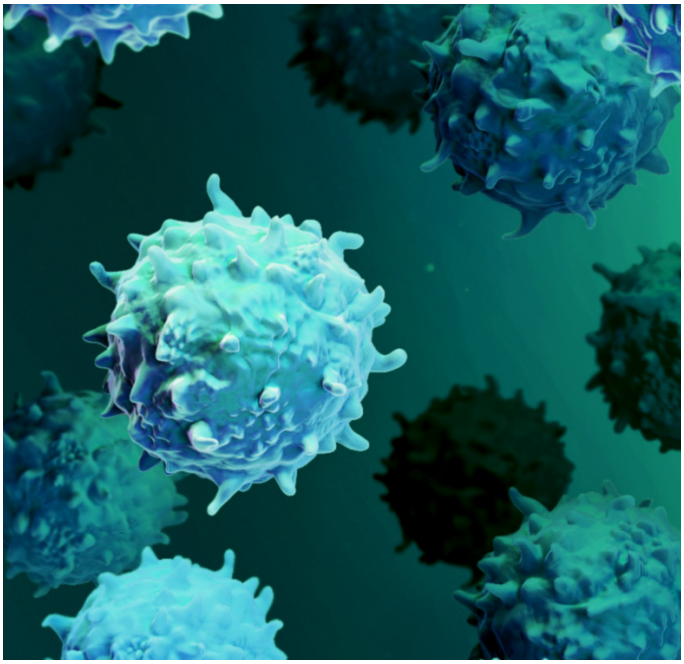
Medpace manages relationships with couriers that will ensure 'white glove' urgency and provide solutions for expediting the sample from the site to the lab. Our operations also use color-coded shippers to cull out critical shipments for quicker processing. Additionally, Medpace has practical experience managing the extremes in temperatures during shipment of blood samples using warming gel packs preconditioned prior to shipment and using dataloggers to record temperature excursions during transit.

PTC 4: PBMC PROCESSING LAB

Consideration: The selection of the processing lab is a final, but critical requisite. The lab should have a practical use with a sample management system capability of recording critical parameters including time of sample receipt, sample disposition, condition of blood sample and shipper received, time of processing, time of freeze as well as cell count and viability. Registering samples with a robust, automated process will provide the basis for quantifying preanalytical variables as potential sources influencing future assay results.

The sample processing lab staff must be skilled in performing standard PBMC processing methods as well as managing sponsor-specific instructions when modifications are required or validating new sample processing methods as fit for purpose.

Medpace guarantees PBMC processing within 2 hours of sample receipt in the lab. Medpace uses validated methods for PBMC processing, which show optimal results with cell count, viability and performance in ELISpot as early as 6 hours and up to 24 hours from time of collection to time of processing. Our ongoing long-term stability evaluation with cryopreserved PBMC utilizes flow cytometry and ELISpot to confirm functionality.



SUMMARY

Medpace PBMC processing procedure has been effectively vetted through external qualification and accreditation as well as real-time results feedback from our clinical sponsors. Any successes Medpace has had are attributed to the relationships established early on with sponsors and their scientific decision makers. Even after the clinical study starts, Medpace teams work closely with sponsors to ensure the quality of PBMC suits the downstream analysis.

FULL-SERVICE CLINICAL DEVELOPMENT

Medpace is a scientifically-driven, global, full-service clinical contract research organization (CRO) providing Phase I-IV clinical development services to the biotechnology, pharmaceutical and medical device industries. Medpace's mission is to accelerate the global development of safe and effective medical therapeutics through its high-science and disciplined operating approach that leverages local regulatory and deep therapeutic expertise across all major areas including oncology, cardiology, metabolic disease, endocrinology, central nervous system and anti-viral and anti-infective.

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