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Research paper

Assessment of sample collection and storage methods for multicenter immunologic research in children

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ABSTRACT

Multicenter studies involving both large and small centers separated by significant distances pose unique challenges to biological sample collection. The objective of this study was to evaluate protocols for determining inflammatory biomarkers that are cost and manpower efficient for handling blood destined for a sample repository. Tempus® (Applied Biosystems) and Paxgene® (Qiagen) blood collection systems were evaluated for RNA isolation. P100® tubes (BD), containing propriety stabilizers for preservation of plasma proteins, were evaluated for protein content and compared with plasma collected in conventional tubes. Blood for plasma separation was spiked with recombinant TNF-α and IL-2 prior to being processed and stored under various conditions. The Tempus® RNA system produced a significantly greater yield of RNA at comparable quality when stored at 4 °C and shipped at ambient temperature than any other condition tested. The Tempus® system was 20% less expensive and required approximately 40% less processing time thereby reducing costs. The P100® system preserved recombinant TNF-α in blood shipped at ambient temperature significantly better than conventionally collected plasma that was shipped on dry ice. There was no significant difference in IL-2 levels between the two collection methods and shipping temperatures. The Tempus® RNA blood collection tubes and the P100® protein stabilization system provide the opportunity for reliable collection and ambient temperature transport of samples in multicenter studies. This cost-effective, standardized protocol for a large multicenter trial ensures the integrity of biological samples and maximizes study participation by both large and small centers.

1. Introduction

To effectively study rare childhood diseases, such as juvenile idiopathic arthritis (JIA), multicenter collaborative research is required. Establishing a centralized tissue repository for storing biological samples is a strategy that can help ensure access to samples for current and future studies and can limit the burden to the child by minimizing the number and volume of samples required. In Canada, as in other regions, the vast geographical area and widely dispersed population must be considered when designing reliable, standardized, practical and straightforward protocols for multicenter sample collection, handling and storage.

The desirability of a common sample repository warrants a systematic approach to sample collection to ensure reliable and reproducible assessment of parameters that may be pathologically important. Peripheral blood is often collected as the biological sample of choice to investigate levels of inflammatory mediators such as cytokines and other potential biomarkers. Cytokines modulate immune function, provide clues to understanding the immune response, and their relative amounts may be useful to monitor and characterize disease course and

Abbreviations: JIA, Juvenile idiopathic arthritis.

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activity. Cytokines in biological fluids can be quantified at both the transcriptional and translational stages, as reflected by the mRNA and protein levels, respectively. However, mRNA and protein have a short half-life *in vivo* and are also subject to rapid degradation *in vitro* following sample collection if appropriate storage and handling procedures are not adopted. Our experience is in accord with studies showing that sample collection, processing and storage are critical for achieving accurate and reproducible results of cytokine protein levels in serum or plasma samples (Riches et al., 1992; Thavasu et al., 1992; Aziz et al., 1999).

In recent years, there has been a substantial increase in interest in the quantitative analysis of cytokine mRNA profiles. This is based on the potential use of cytokine mRNA expression and its profile as a unique and sensitive marker for *in vivo* immune cell activation in a variety of clinical settings (Whiteside, 1994). However, it is unknown to what extent differences in blood collection and preparation techniques may cause *ex vivo* alteration of cytokine mRNA levels and is therefore necessary to identify optimal conditions for collection, storage and analysis of patient blood samples (Hartel et al., 2001).

Additionally, conventional methods for RNA stabilization require a substantial investment in manpower and equipment which may not be available at centers with only one clinical investigator collecting patient information and biological samples. In particular, small centers face the challenge of having limited access to research laboratory support and may be unable to carry out conventional RNA isolation immediately following blood collection. Ideally, sampling techniques that improve RNA stability would allow blood collection to take place at any time of the day as well as permit processing to take place at a later more convenient time.

There are commercially available sample collection systems designed for these specific downstream applications. Qiagen and BD Biosciences (Mississauga, ON, Canada) have blood collection kits that are marketed to stabilize blood collected in a clinical research setting. These companies have recognized that simply refrigerating the sample until transferring to the processing laboratory for long-term storage would have advantages for the creation of a tissue repository within a multicenter study. A system that preserves the sample integrity while keeping processing and shipping costs low will revolutionize the traditional approach to sample collection and storage. If successful, these changes should improve data accuracy and precision, and ultimately improve research outcomes.

In the present study, the effect of room temperature cross-country shipping of blood samples was assessed using commercially available blood collection kits designed for this purpose. Analyses were performed on the integrity of TNF-α and IL-2 cytokine levels as well as RNA transcripts. Data generated from this study helped determine the appropriate methods for collection and storage of patient blood products to aid in preservation of the samples for future assays in an 12 center Canada-wide childhood arthritis study.

2. Materials and methods

2.1. Sample collection for protein analyses

Blood from 20 healthy adult volunteers was collected into either 4 ml Vacutainers® (Beckton Dickinson (BD) Diagnostics; Oakville, ON, Canada) containing lithium heparin (final concentration 15.2 U/ml) or P100® tubes (BD) which contain a proprietary blend of protease inhibitors. Within 15 min of collection, blood was spiked with a degradable cytokine (Riches et al., 1992), human recombinant TNF-α (eBioscience, San Diego, CA, USA), and a relatively stable cytokine (Panicker et al., 2007), IL-2 (eBioscience) at levels high enough to ensure detection (1000 pg/ml and 500 pg/ml, respectively). The heparinized blood collection tubes were immediately processed conventionally: plasma was separated by centrifugation for 10 min at 1300 ×*g* and aliquots of 250 μl were either frozen immediately at −80 °C or stored at 4 °C for 4 days (Table 1). The 4 day time-line replicates the maximum shipping delays for samples collected prior to a long holiday weekend. The frozen aliquots were shipped on dry ice overnight and stored immediately at −80 °C at the receiving laboratory. The remainder of the blood was immediately stored at 4 °C for 4 days. On the fourth day P100® tubes and the refrigerated plasma aliquots were shipped overnight at ambient temperature to the processing laboratory (Table 1).

2.2. Cytokine assays

Plasma cytokine concentrations were determined using ELISA-based assays. TNF-α levels were measured in all

Table 1
Sample collection, processing and shipping regimens

Collection tube	# of samples	Processing at the collection site	Storage prior to shipping	Shipping	Processing and storage at the receiving lab
Lithium heparin	10	Spiked [a], centrifuged, aliquoted	−80 °C for 4 days	Dry ice, overnight	Stored at −80 °C
Lithium heparin	10	Spiked [a], centrifuged, aliquoted	4 °C for 4 days	Ambient temperature, overnight	Stored at −80 °C
P100®	20	Spiked [a]	4 °C for 4 days	Ambient temperature, overnight	Centrifuged and aliquoted. Stored at −80 °C
Paxgene®	10	–	−80 °C for 4 days	Dry ice, overnight	Stored at −80 °C [b]
Paxgene®	10	–	4 °C for 4 days	Ambient temperature, overnight	Stored at ambient temperature [b]
Tempus®	10	–	−80 °C for 4 days	Dry ice, overnight	Stored at −80 °C [b]
Tempus®	10	–	4 °C for 4 days	Ambient temperature, overnight	Stored at ambient temperature [b]

[a] Samples were spiked with human recombinant TNF-α and IL-2 at 1000 pg/ml and 500 pg/ml, respectively to ensure detectable cytokine levels.
[b] Processing was carried out according to the manufacturer's instructions on the same day the samples were received after shipping.