INTRODUCTION

Numerous agents threaten DNA in mammalian tissues and blood in vivo, including nucleases and reactive oxygen species. These threats become particularly acute in specimens collected for research and diagnostics when cell death removes genetically encoded mechanisms for preventing and repairing such DNA damage. Types of DNA damage that accrue in mammalian tissue specimens include: nucleolytic attack, oxidative damage to nucleobases and the sugar-phosphate backbone and hydrolysis (especially, depurination).

In this report, we describe a novel liquid stabilization technology for room temperature preservation of genomic DNA in human whole blood specimens. This technology extends Biomatrica’s platform technology that incorporates biochemical principles of anhydrobiosis to liquid solutions that protect biological macromolecules. DNAgard Blood encompasses mechanisms of physical protection of DNA with chemical means of inhibiting and denaturing nucleases. In addition, DNAgard Blood reduces the production of damaging reactive oxygen species and includes chemicals predicted to scavenge existing hydroxyl radicals. DNAgard Blood also provides a chemical environment for the blood specimen that is unfavorable to hydrolysis reactions such as depurination.

Here we compare the integrity and yield of genomic DNA recovered from blood samples stored in DNAgard Blood with conventional methods of blood storage and shipment.

DNAgard® Technology

DNAgard is designed for the immediate stabilization of DNA from mammalian whole blood with the convenience of room temperature shipping, processing and storage. The liquid storage reagent rapidly permeates cell membranes to stabilize and protect genomic DNA. The use of DNAgard Blood allows a streamlined workflow from blood collection in the field to sample processing in the laboratory.

RESULTS

No degradation after 5 months at room temperature

Figure 2. Genomic DNA in human whole blood is stable at room temperature for over five months in liquid DNAgard Blood. Human whole blood was immediately mixed with DNAgard Blood solution (DGB) or a competitor’s product (Comp.) and stored at room temperature. Control samples were stored without DGB: unprotected at room temperature (NP) or at -20 C. After 165 days, total DNA was recovered from the stored samples. DNA yield and integrity was analyzed on a 0.8% agarose gel (M = 1 kb ladder).

Multi-donor analysis indicates high DNA yields after room temperature storage

Figure 3. Genomic DNA yield from blood stored in DNAgard Blood is equivalent to frozen samples. DNA was extracted from triplicate blood samples from three donors that had been stored for 1 month at room temperature in DGB solution, in a competitor’s formulation (Comp.) or in the absence of stabilization solution (NP). DNA yield was quantified by RT-PCR (Fig. A) and normalized to recovery from blood that had been stored frozen (-20 C; Fig. B).

SUMMARY

• DNAgard Blood stabilizes genomic DNA in whole blood specimens for over 5 months at room temperature (Figure 2)
• DNAgard Blood is compatible with a number of DNA extraction methods
• DNA extracted from DNAgard Blood-stored samples is compatible with a number of downstream applications including:
  - RT-PCR (Figure 3)
  - Absorbance spectroscopy (Figure 3)
  - Long-range PCR
• DNA yield from blood stored at room temperature in DNAgard Blood is equivalent to frozen specimens (Figure 3)
• DNA integrity and quality from blood stored in DNAgard Blood is preserved during extreme temperature fluctuations (Figure 5)