

Dried blood spots for detection of autologous blood doping with targeted LC-MS/MS



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Introduction

- Dried blood spots (DBS) provide a cost-effective transport and archival method for monitoring of changes in the red blood cell (RBC) proteome due to disease or ex vivo storage, such as cryopreservation used for autologous blood doping (ABD).
- PTD has developed proprietary peptides for detection of ABD.
- We assessed the detectability of selected peptides with DBS as archival method.

Methods

- Whole blood (WB), RBCs and RBC cytosol (RBCC) were spotted onto Whatman 903 cards.
- Cards were dried, protein extracted (A) and Hb-depleted (B), using HemoVoid™, according to BSG's protocol (Table 1).
- Hb-depleted eluates were reduced, alkylated and digested with trypsin overnight, cleaned by solid-phase extraction and dried, before being analysed with multiple reaction monitoring (Figure 1).

Results

- Different spots yielded different protein mass (Table 1). The highest peptide areas were gained from RBC DBS, followed by WB and RBCC (Figure 1). Hb-depleted yield from RBCC was non-detectable.
- RBC DBS identified most targeted peptides: seven out of eleven.

Table 1. Protein yields as analysed by Bradford Assay

| Step | DBS Type | Yield (%) |
|----------------------------------|----------|-----------|
| (A): Protein extraction from DBS | WB | 42.5 |
| | RBC | 58.2 |
| | RBCC | 31.0 |
| (B): Hb-depletion | WB | 21.5 |
| | RBC | 5.2 |
| | RBCC | - |

Figure 2. Peak area sum of 3 selected peptides, important for detection of ABD

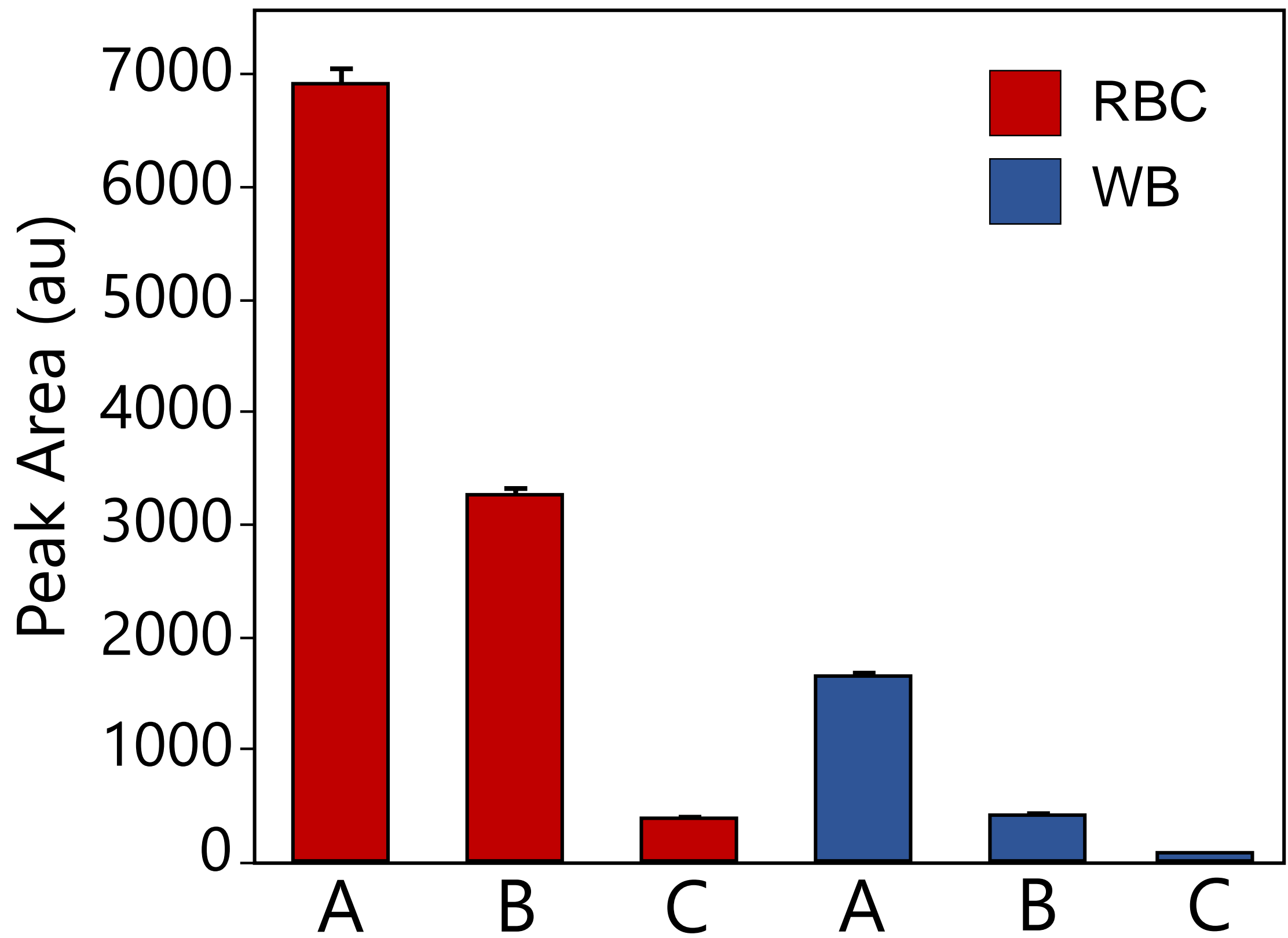
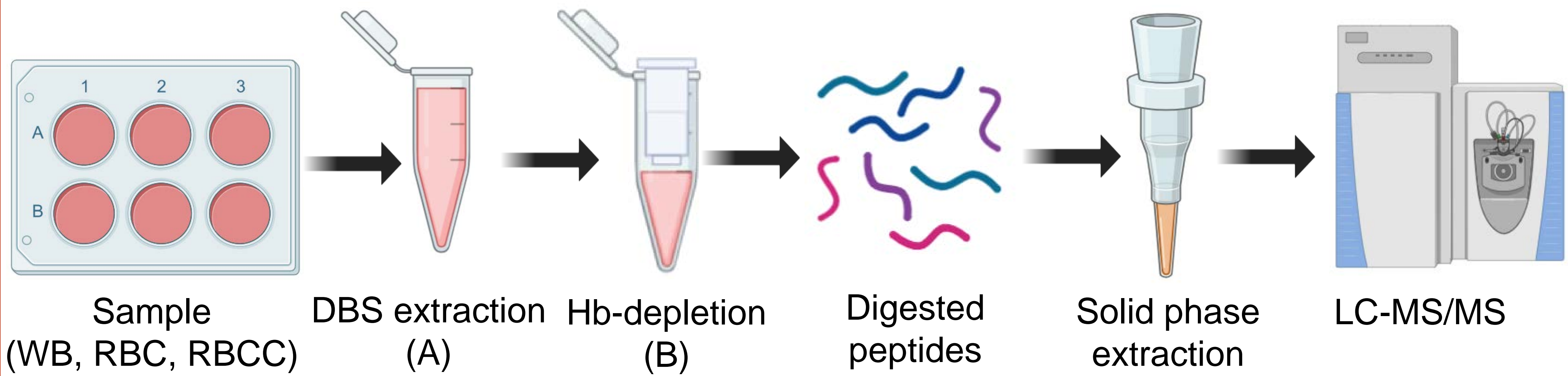


Figure 1. From DBS to peptide eluates for quantification in multi reaction monitoring



Conclusions

- WB DBS yields the highest protein mass, dominated by high abundance proteins, not of interest for detection of ABD.
- Detection of ABD using DBS necessitates removal of noise from high abundance proteins, especially Hb.
- Spotting RBCs, not WB, is preferred.
- Future increased yield, with enhanced signal, may results in more quantifiable peptides.

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Icons in Figure 1 was made using BioRender.