## Validation of novel extraction reagents to protein analysis with dried blood spots.

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- **Abstract:** Scientific methods of analysis are used to reconstruct crime scenes. However, a lack of sufficient blood for analysis is common at crime scenes. To apply scientific analysis methods to crime scenes, it is important to extract components from the very small amounts of blood samples without damage. We previously developed a novel extraction reagent for analyzing bloodstains at crime scenes. The developed extraction reagent contains Tris-EDTA (TE), which preserves DNA and phosphate-buffered saline (PBS), which preserves proteins. The developed extraction reagent exhibited superior performance with respect to DNA extraction. Blood was collected from 23 healthy adult men and women in vacutainers that did not contain coagulants or anticoagulants. These samples were used to generate DBSs under different temperature and humidity conditions. Proteins were extracted at five time points (day 1, 7, 14, 21, and 30) using the developed extraction reagent, previously used agents TE and PBS, as well as the conventional agent double distilled water (D.D.W). The protein extraction performances of these extraction agents were compared and analyzed using western blotting. We also quantitatively confirmed the amount of protein extracted using commercialized GAPDH. We cross-validated the protein extraction capability of the extraction reagent developed using two verification substances from the blood cells of the crime scene and confirmed that the protein extraction performance was excellent.
- **Keywords:** Dried blood spot, protein, extraction reagent, GAPDH, western blot, crime scene