B146 A Capillary Electrophoresis Immunoassay for Forensic Identification of Human Blood

Katie M. Horsman, MS, University of Virginia, Department of Chemistry, Mccormick Road, Charlottesville, VA 22901; Michael G. Roper, PhD, Florida State University, Department of Chemistry and Biochemistry, Tallahassee, FL 32306; and Alexander S. Greene, and James P. Landers, PhD, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22901*

The goal of this presentation is to introduce a multicolor capillary electrophoretic immunoassay for identification and species testing of suspected blood stains.

This presentation will impact the forensic community and/or humanity by demonstrating how the immunoassay represents an alternative to the Ouchterlony double immunodiffusion method for identification and species testing of bloodstains. This method significantly reduces sample volume, analysis time, and improves assay sensitivity compared to the conventional method.

For admittance into court proceedings, forensic scientists routinely identify a suspected bloodstain as blood of human origin before proceeding to DNA analysis. Conventionally, this is performed via double immunodiffusion (also known as the *Ouchterlony method*) in which both the antibody to adult human hemoglobin (or, antisera for only species testing) and antigen (suspected human blood) diffuse in an agarose gel. Agglutination occurs at an equivalence zone resulting in a precipitated band representing the antigen-antibody complex.[1] This technique is often multiplexed such that the antigen is placed in a single central well and anti-sera of various species are placed in surrounding wells to confirm the species of origin. The advantage of this technique, when using anti-human hemoglobin compared to historical methods, is the combination of blood identification with determination of species of origin in a single test.[2] However, the disadvantages of this technique are many, including: assessment of precipitin bands is subjective, analysis times are lengthy, and interpretation of cross-reactivity is difficult. Consequently, a capillary electrophoresis immunoassay (CE-IA) has been developed that incorporates the positive attributes of the immunodiffusion assay but circumvents the drawbacks of the conventional technique.

The work presented here focuses on improving species-specific identification of human blood by replacing the double immunodiffusion assay with a capillary electrophoresis-based immunoassay. This non- competitive immunoassay utilizes multiple antibodies to hemoglobin to assess species of origin. Fluorescent tags are used to identify each species uniquely, from which a decrease in free, labeled antibody indicates complexation with the analyte. This assay can be performed in less than 10 minutes and has limits of detection of 1 nM, which significantly improves both the speed and sensitivity compared to the *Ouchterlony method*.

The approach exploited here utilized a non-competitive CE-IA for detection of human hemoglobin (Hb) via laser-induced fluorescence detection. In this assay, a fluorescently-tagged antibody against human Hb (Ab*) is detected as a single peak, while addition of antigen (adult human Hb) reduces the peak area of the Ab* peak. An internal standard, fluorescein, is included with the sample to account for any variability in injection volume. The presence of hemoglobin is determined and the amount quantified by comparing the Ab* peak area before and after addition of Hb. Cross-reactivity of Ab* was assessed by examining the change in peak area after incubation with mouse, rat, pig, and dog blood. Two Ab* were tested for minimal cross-reactivity to hemoglobin from other species.

As in the *Ouchterlony method*, the developed immunoassay tests reactivity of the sample to hemoglobin from other species. In this manner, a multi-color CE-IA was developed where antibodies against each species are tagged with spectrally-resolvable dyes. The cross- reactivity of a suspected blood-stain can then be assessed for species-of- origin determination.

The multi-color capillary electrophoresis-based immunoassay presented improves the speed and sensitivity of the identification of suspected bloodstains in comparison to the conventional methods. While outside of the scope of this work, it is anticipated that this method will be easily translated to microdevice technology for potential rapid crime scene testing in the near future.

References:

1 Saferstein, R. *Forensic science handbook*; Prentice-Hall: Englewood Cliffs, N.J., 1982.

2 Gaensslen, R. E.; National Institute of Justice (U.S.) *Sourcebook in forensic serology, immunology, and biochemistry*; U.S. Dept. of Justice National Institute of Justice : For sale by the Supt. of Docs. U.S. G.P.O.: Washington, D.C., 1983.

Immunoassay, Capillary Electrophoresis, Ouchterlony Method

 ** Presenting Author*