DETECTION OF HUMAN BLOOD BY CLOTH-BASED ENZYME IMMUNOASSAY OF IMMUNOGLOBULIN G

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ABSTRACT

A specific and sensitive assay for the detection of human blood was developed using polyester cloth coated with goat anti-human IgG antibody to capture human IgG, an abundant and stable protein in blood. The captured IgG was detected by the reaction between goat anti-human IgG antibody-peroxidase conjugate and a chromogenic peroxidase substrate. Because the assay is simple and rapid, and permits simultaneous analysis of multiple samples, it has the potential to be used as a forensic test for human blood.

INTRODUCTION

The currently used forensic tests for blood stains are the hemastix (Miles Canada Inc.) and the hemochromagen tests (Takayama, 1912). The former is based on the catalysis of the reaction between cumene hydroperoxide and tetramethylbenzidine by the peroxidase activity of haemoglobin to produce a blue/green colour and the latter (Miles Canada Inc.) on the reaction between hematin and pyridine to produce orange-pink crystals (Takayama, 1912). However, these methods can generate false positive and false negative results, and cannot distinguish between human and animal blood.

Human immunoglobulin G (IgG), one of the most abundant protein components in blood, is found at a concentration of approximately 1700mg/mL in normal plasma (Sober, 1970). Due to the relative stability and abundance of IgG in human blood, we postulated that its enzyme immunoassay (EIA) could provide a specific forensic test for human blood.

Cloth-based EIA (CEIA) provides a simple, rapid and specific assay of antigens on antibody-coated hydrophobic cloth (Blais and Yamazaki, 1989). CEIA permits simultaneous processing of multiple samples on a sheet of cloth since incubation of the cloth in EIA reagents will equalize reaction times and eliminate multiple pipettings. The use of a chromogenic peroxidase substrate yields colour stains clearly distinct from the white, cloth background. The present communication demonstrates that a CEIA using
anti-human IgG antibody-coated cloth provides a specific and sensitive EIA for the
detection of human IgG, and hence human blood.

MATERIALS AND METHODS

Materials

The following reagents were obtained from Sigma Chemical Company: goat
anti-human IgG antiserum (I-1011), human IgG (I-4506), goat anti-human IgG-horseradish
peroxidase conjugate (A-8667), human serum (S-2257), dog serum (S-1757) and cat serum
(S-4634). The human IgG powder was resuspended in 150 mM NaCl to yield 10 mg/mL,
while the dog and cat sera were reconstituted in doubly-distilled water.

The blocker (non-fat dry milk) (170-6404) was obtained from BioRad. The
polyester cloth (Sontara 8100) was obtained from DuPont. The rat blood was obtained
from Dr. McIntyre of the Carleton University Psychology Department and the human blood
was donated by Sebastian Kowalski. The insoluble tetramethylbenzidine (TMB) substrate
for peroxidase was a powder formulation prepared by RICOH KyoSan Inc. of Japan.

Preparation of goat anti-human IgG-coated cloths

The polyester cloth was cut into 4 cm x 5 cm sheets, washed with 70% ethanol,
tollowed by 0.01 M sodium phosphate-0.85% NaCl, pH 7.3 (PBS), and blotted. Each sheet
was incubated overnight in 2.5 mL of goat anti-human IgG antiserum (diluted 1000X in
PBS to a final concentration of 5.3 μg/mL) in petri dishes at room temperature. After the
incubation period, each sheet was washed 5 times with 3 mL of PBS containing 0.05%
Tween 20 (PBST) to remove unbound antibodies, and blotted before being soaked in 3 mL
of 1% blocker in PBS for 1 hour, at room temperature. The cloth sheets were then washed
5 times with PBST, blotted, and stored in sealed petri dishes at 4°C until use.

Enzyme immunoassay

Five μL aliquots of appropriately diluted samples were spotted (in duplicate) onto
a cloth sheet. After the application of the last sample, the sheet was incubated for 10 min.
at room temperature, washed 5 times with PBST and blotted. The cloth sheet was then
incubated in 5 mL of goat anti-human IgG-peroxidase conjugate (diluted 2000X in PBS)
at room temperature for 10 min., washed 5 times with PBST, and blotted. The sheet was
soaked in 2.5 mL of TMB solution (32 mg/mL). Following the appearance of blue spots,
the cloth sheets were washed 5 times with PBST and blotted dry. A photocopy of the cloth
sheet was obtained and then scanned into the computer.

RESULTS AND DISCUSSION

Specificity of CEIA

Polyester cloth coated with goat anti-human IgG antiserum was used to capture IgG
in human serum, and the captured IgG was detected by its reaction with anti-human IgG
antibody-peroxidase conjugate and TMB. Undiluted human, dog and cat sera were assayed
in duplicates. Figure 1A shows that the human serum produced two large and intensely
dark spots (due to peroxidase activity), which were absent at the positions where dog and
cat sera were spotted. An assay of fresh human and rat blood samples yielded similari
results; human blood produced large blue spots while rat blood did not (Figure 1B). Since