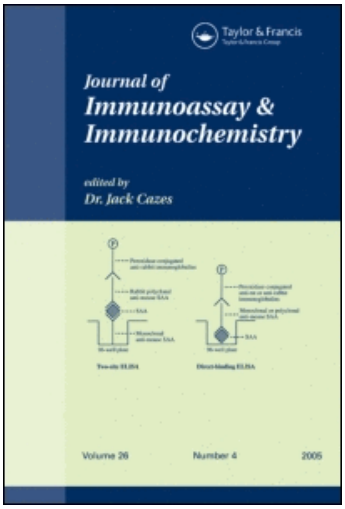

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A SIMPLE IMMUNOSORBENT ASSAY FOR DETECTION OF HUMAN BLOOD

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ABSTRACT

A simple, rapid and highly sensitive assay based on Enzyme Linked Immunosorbent Technique (ELISA) using human Haptoglobin antibodies for identification of human blood is described. The important feature of the present assay is absence of need for enzyme antibody conjugates. Human blood stains as old as 3 years show positive results by this method. This test is suitable for identification of human blood in forensic and epidemological studies. (Key words: ELISA, Haptoglobin).

INTRODUCTION

Blood stain is the most ubiquitous physical exhibit present at the scene of crime. Identification of the nature and origin of such a stain is essential for forensic use. A number of tests based upon the perioxidase activity of haemoglobin, it's crystalline nature, or its intrinsic optical properties (1) are in use for identification of blood stains. Blood stains are identified by the Ouchterlony method,[2] by crossover electrophoresis or by

immunoelectrophoresis (3). However, these methods are time consuming and require high technical skill.

Recently solid phase enzyme-linked immunosorbent assays (ELISA) have been developed for identification of blood (4-5), ABO grouping of stains (6), seminal identification (7), α-fetoprotein identification (8), and demonstration of methamphetamine (9) in blood serum.

In the present work a simple ELISA is described for the identification of blood stains, suitable for use in a police crime scene kit.

MATERIALS AND METHODS

Blood stains of various ages were collected from crime exhibits received for examination in this laboratory. Fresh stains on sterile cheese cloth were also prepared from blood donated by laboratory staff. The fresh stains were allowed to dry at room temperature and were examined after storage for one day, one, two, or three weeks, one, two or six months and one year. A few stains as old as three years were also used.

Blood of Cow, Goat, Fowl, Rabbit, Dog and Monkey were collecctd and stains prepared as described elsewhere (10) Human Saliva, Semen, Milk, Sweat, Tears and Vaginal fluid stains were also prepared by sample collected with the help of local Medical Colleges.

Reagents

Sheep anti-human haptoglobin antibody (Paesel, West Germany) was diluted 30 fold with phosphate buffer, 70m mol./L, pH 5.0, with saline 150m mol /L and Bovine serum albumin, 10g/L (Sigma Co. USA).

Ortho-phenylenediamine H_2O_2 and other routine reagents were purchased from Sigma Co. USA; B.D.H. E.Merck and Glaxo Co. India.

Principle of method

The haptoglobin-haemoglobin complex in blood has an intense perioxidase activity. The enzyme activity is retained for several years. A polystyrene ELISA plate coated with sheep anti-human haptoglobin antibodies was selected as solid phase. Haptoglobin-haemoglobin complex in blood stains binds to the anti-haptoglobin antibodies and can then be identified by its perioxidase activity. Thus a simple and sensitive ELISA results with the target ligand acting as its own marker molecule.

Procedure

The wells of polystyrene microtiter plates (Dynatech Alexandria) were coated with 200 µL. of 30 fold diluted sheep anti-human haptoglobin in carbonate buffer 0.50m mol /L (pH 9.6). Wells were incubated overnight at 4°C and were washed with phosphate buffered saline 0.70m mol /L, with 0.08% Tween 20 (PBS-T). The plates were allowed

to drain and blotted dry. Bovine serum Albumin 150 µL (10 g/L) was added to each well to block non-specific binding of antigen. Microtitre plates were incubated for 90 minutes and were washed twice with PBS-T before storing at 4°C. Coated microtitre plates were used upto 90 days after preparation.

Blood stains 1 x 1 mm in area or 50 µL of blood diluted 1000 times or more in PBS Tween was added to wells and incubated at room temperature for 60 minutes. The wells were drained and washed thrice with PBS-T. Finally a substrate-chrogen solution containing 100 µL of H_2O_2 3% v/v and 1.50 µL of 0.1% O-phenylenediamine (OPD) in 0.07 mol PBS pH 5.0 reagent was added to each well. The intensity of blue colour developed was noted visually after 10 minutes.

Human blood stains stored for various periods and 1 week old blood stains exposed to 20,30,40,50 and 60°C for one week were tested by this method. One week old blood stains of Cow, Dog, Rabbit, Goat, Fowl and Monkey were also tested.

The human saliva, semen tears, milk, vaginal fluid and sweat stains, all one week old, were also tested to check the specificity of this method.

RESULTS

It was observed that complete coating of wells with sheep anti-human haptoglobin antibody required 2

TABLE I

SPECIFICITY OF IMMUNOSORBENT ASSAY TO SPECIES OF BLOOD STAINS AND BODY FLUIDS

(One week old stains were used in the study)

Nature of stain	Origin	No.of stains examined	Intensity of Reaction
BLOOD STAINS	Human	20	+ + + + +
	Dog	10	- - - - -
	Cow	10	- - - - -
	Rabbit	10	- - - - -
	Fowl	10	- - - - -
	Monkey	5	+ - - - -
	Goat	5	- - - - -
HUMAN FLUID STAINS	Saliva	5	+ + - - -
	Semen	5	+ - - - -
	milk	5	+ - - - -
	sweat	5	+ - - - -
	tears	5	+ - - - -
	vaginal fluid	2	+ - - - -

+++++ Most strong positive reaction
++++- strong positive reaction
+++-- Moderate positive reaction
++--- Mild positive reaction
+---- Poor reaction
----- Negative reaction

TABLE II

SENSITIVITY OF IMMUNOSORBENT ASSAY TO BLOOD STAINS OF DIFFERENT AGES

AGE OF STAIN	NUMBER OF STAINS EXAMINED	POSITIVE RESULTS (expressed in %)	
		Immunosorbent Method	Ouchterlony & Cross over electrophoresis methods
Fresh	10	100	100
One week	10	100	90
Two weeks	10	100	80
Three weeks	10	100	60
One month	10	100	60
Two months	10	100	50
Three months	10	100	40
One year	5	80	20
Three years	5	60	20

hours incubation. Wells incubated with antibody for 24 hours were found to retain antibodies for a longer period and such precoated plates could be used for a period of three months. The binding of haptoglobin haemoglobin complex to immobilized antibody was complete within one hour. The method was found to be sensitive even to hemoglobin-haptoglobin complex at 1 g/L. Blood of Dog, Cow, Goat, Fowl and Rabbit did not show any binding and

TABLE III

SENSITIVITY OF IMMUNOSORBENT ASSAY METHOD TO BLOOD STAINS EXPOSED TO VARIOUS TEMPERATURES. (stains used in expt.4 weeks old)

S.No.	Temperature C°	Number of stains	POSITIVE RESULT (expressed in %)	
			Immunosorbent method	Ouchterlony & Cross over electrophoresis method
1	20	10	100	90
2	30	10	100	90
3	40	10	100	80
4	50	10	90	50
5	60	10	80	20

monkey gave only a trace reaction (Table I). Similarly, the stains of other human body fluids also gave uniformly weak reactions. The perioxidase reaction was decreased slightly in blood stains over 3 years old and stains exposed to temperature above 40° C. The present ELISA test was more sensitive than conventional methods (Table II & III).

DISCUSSION

Enzyme Linked Immunosorbent Assays (ELISAs) are widely used for detection and quantification of biological analytes (11-14).

The present method is a sensitive, simple and rapid means of species identification of blood stains, which compares favourably with conventional methods (15).

By conventional methods, it has been previously shown that positive identification of old and heat exposed stains is significantly reduced (10). In the present study, species of origin of stains upto three years age could be positively ascertained.

A commercially available polyvalent antiserum was found suitable for performing the present ELISA method. In keeping with findings of Hammack et al (11), we have found that antibody coated plates could be stored for 3 months without loss of antigen binding capacity.

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