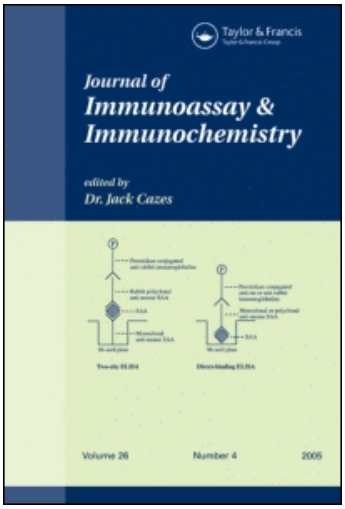

Journal of Immunoassay and Immunochemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Dot Blot Immunoassay for Detection of Human Semen

D. V. Rao[a]; V. K. Kash yap[a]
[a] Central Forensic Science Laboratory Bureau of Police Research & Development (M.H.A.) Government of India Ramanthapur, HYDERABAD, INDIA

To cite this Article Rao, D. V. and yap, V. K. Kash(1992) 'Dot Blot Immunoassay for Detection of Human Semen', Journal of Immunoassay and Immunochemistry, 13: 4, 537 — 544
To link to this Article: DOI: 10.1080/15321819208019834
URL: http://dx.doi.org/10.1080/15321819208019834

JOURNAL OF IMMUNOASSAY, 13(4), 537-544 (1992)

DOT BLOT IMMUNOASSAY FOR DETECTION OF HUMAN SEMEN

D V Rao & V K Kashyap

Central Forensic Science Laboratory
Bureau of Police Research & Development
(M.H.A.) Government of India
Ramanthapur, HYDERABAD - 500 013.
I N D I A.

A B S T R A C T

A sensitive and specific dot blot immunoassay based on prostate specific antigen (PSA) antibodies and a radiolabelled Protein A detection system was developed for detection of trace amounts of human semen in stains. The method was found highly sensitive and detected semen stain extracts diluted 10,000 times. Semen stains up to five years old were successfully detected by the method. The assay is found to be highly suitable for semen detection in forensic analysis.

(**KEYWORDS** : Dot blot immunoassay, Semen, Nitrocellulose, P30 antibodies).

INTRODUCTION

In cases of alleged sexual assaults, demonstrating the presence of semen on the genital area or the apparel of victims is important. Microscopic examination and biochemical tests based on the presence of acid phosphatase, choline or spermine (1-5) as routinely practiced for the detection of semen have limitations (6-7).

Immunological methods have provided better methods for the detection of biological substances including semen components (8-10) and many enzyme immunoassays for the detection of traces of semen its stains have been reported (11-14). Though enzyme immunoassays are simple and specific, sensitivities may be impaired by matrix effects (15). In

the present study, we have developed an ultrasensitive dot blot immunoassay using prostatic specific antigen (PSA) antibodies and I^{125} labelled Protein A for the visualization of immune complexes formed on the nitrocellulose membrane. The specificity of PSA antibodies and the sensitivity of the radiolabel makes the assay highly suitable for forensic investigation.

MATERIALS AND METHODS

Polyclonal antibodies against human PSA raised in rabbit were purchased from Dakopatts, Denmark. Protein A was obtained from Sigma Chemicals Co. USA and Nitrocellulose membrane was supplied by Advanced Microdevices Private Limited, Ambala, India. I^{125} was obtained from Bhabha Atomic Research Centre, Bombay, India. All other chemicals were of analytical grade and procured locally.

Semen and other body fluids : Semen from normal vasectomized persons and patients suffering from pathogenic and malignant diseases of the male genital system, vaginal smears, saliva and urine were collected from volunteers. Bull, buffalo, goat, dog, horse, monkey and pig semen were obtained from the Government Veterinary Hospital, Hyderabad. From all the body fluids, stains were prepared by pouring 0.05ml of sample over sterile cotton cloth. Human semen samples used in the study were from individuals aged from 15 to 70 years. The stains of various body fluids and semen were prepared from a minimum of five individuals.

Stain Extraction : The stains were extracted in 200 ul of phosphate buffered saline, pH 7.2, 150 mmol/L (PBS) as explained elsewhere (16). 5.0ul of the extract was used in the study.

Radioiodination of Protein A : Protein A was radioiodinated according to the method of Franker and Speck (17). Briefly, 0.5uci of radioactive

NaI was allowed to react with 500ug of Protein A at 40°C in a test tube coated with Iodogen. The reaction was allowed to take place for 15 minutes with occasional shaking. The labelled Protein A was separated from the free label using a 10ml column packed with Sephadex G-50.

Dot Blot Immunoassay : Stain extracts were spotted on nitrocellulose membrane and air dried. The non-specific binding sites were blocked with 2% bovine serum albumin (BSA) in PBS at 37°C for one hour. The membrane was washed three times with PBS containing 0.005% Tween-20 (PBST) and incubated with antibodies to PSA diluted 1:1000 times in PBS at 37°C for 4 hours. (This was the maximum dilution at which the antibodies were able to bind to the membrane bound PSA antigen (satisfactorily). The membrane was washed in three changes of PBST and incubated with 10 ul of I^{125} Protein A showing 10uci activity in PBS for one hour at 37°C. Then the membrane was washed with several changes of PBST for one hour at room temperature and air dried. The dried membrane was placed in a polythene bag and kept for autoradiography. The autoradiography was done at -20°C for 24 hours with ORWO X-Ray film and Kiran intensifying screens.

Specificity Study : Normal semen and semen from vasectomized persons, vaginal smears of human adult females who had abstained from sex for 15 days prior to collection, blood, saliva, urine, or normal humans and semen of bull, buffalo, goat, dog, horse, monkey and pig were included in the study. The extract of 20 stains of each type were tested.

Sensitivity Study : Normal human semen extract was diluted 1:10, 1:100, 1:1000 and 1:10000 in PBS. 5.0ul sample of each dilution was spotted on nitrocellulose membrane and the assay was performed.

Effect of Disease : Semen samples were also collected from patients suffering from pathogenic and malignant diseases of the male genital

system to find out the suitability of the assay in detection of semen stains derived from such persons. Semen samples from persons suffering from prostate cancer, non-malignant tumours, gonorrhea and syphillis were tested in the study and 10 samples for each case were included.

Effect of storage : Normal human semen samples prepared on cotton cloth were stored at room temperature ($25^{o}C$-$40^{o}C$) for one week, one month, six months, one year and five years and tested to ascertain applicability of the assay in detection of old stains. For each duration, extracts from 50 different stains were tested in the study. Semen stains of size $2.0cm^2$ were cut and extracted in PBS as explained above. 5.0ul of the extract was assayed from each.

RESULTS

All the human semen stains, irrespective of their fertility status, i.e. normal or vasectomized, were successfully detected by the assay. Other human body fluids and semen derived from non-human sources gave negative results.

Semen extracts diluted up to 10,000 times could be clearly demonstrated by the assay.

Semen from 40 patients with urogenital disease, 10 each of prostatic cancer, prostatic benign hyperplasia, gonorrhea and syphillis, all gave positive results. Positive results were obtained on every one of the 50 samples of human semen stains stored up to 5 years.

DISCUSSION

The assays for detection of semen have not lost their relevance in spite of the advent of DNA Profiling (18) which can provide infallible information about the identity of the source of semen in an alleged rape

case, because the characterization of semen is as essential as individualization.

Biochemical analysis of semen components has resulted in the discovery of a 32kd protein (PSA) which is highly specific to human semen (19). Because of the synthesis of PSA in the prostate, its presence in semen is independent of spermatozoa. PSA has been found to be a highly suitable marker of human semen in forensic analysis. Antibodies with high specificity to PSA have been characterized and several enzyme immunoassays for detection of PSA in semen and its stains have been reported (20). However, they often fail to detect minute quantities of semen in dried old stains. The present assay successfully detected semen stain extracts diluted 10,000 times.

As proteins strongly bind to nitrocellulose through hydrophobic interactions, in dot blot assays even nanogram quantities of PSA are sufficient for the test which is more sensitive than micro ELISA (21). The antibodies used in the assay are of high avidity, and the titration study has revealed that a 1:1000 dilution gives satisfactory results. This dilution has been used throughout the study.

The detection of PSA in vaginal washings of victims of rape after 48 hours is not possible, whereas prostatic acid phosphatase (PAP) can be detected at this time (23), but the specificity and stability of PAP in urinogenital swabs of victims and in semen stains is poor compared to PSA.

Protein A binds to a wide variety of immunoglobulins of different species and its affinity to rabbit IgG is high. This property of Protein A has led to the use of radiolabelled protein A for the detection of immune complexes, instead of enzyme labelled second antibody.

Further, the radiolabel is uninfluenced by the sample matrix which can affect the enzyme labels in ELISA. The other merit of the assay is its successful detection of semen stains as old as 5 years with remarkable sensitivity. The assay has better sensitivity than the commercially available PSA immunoassay kits.

Another advantage of the present assay is the availability of original results for long periods, unlike enzyme immunoassays (23). In the dot blot format, a large number of samples can be simultaneously processed. Antigen loaded membranes after blocking can be stored for periods of up to five years without any loss of the sample for further analysis.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. T R Baggi, Director, CFSL, Hyderabad, for providing facilities for carrying out the work. D V Rao thanks BPR&D for providing financial assistance. The authors also acknowledge the skillful secretarial assistance rendered by Mr. R Madhusudhan.

REFERENCES

1. Singh HP, Paramar SS and Gupta PK. Some biochemical studies on the seminal stains of normospermic, azoospermic and vasectomized persons. Ind J Forens Sci 1989; 3(2):83-5.

2. Gaensslen RE. Identification of semen and vaginal secretions. In sourcebook in Forensic Serology and Immunology and Biochemistry, United States Department of Justice, US Government Printing Office, Washington DC, 1983:149-81.

3. Sensabaugh G. The quantitative acid phosphatase test. A statistical analysis of endogenous post coital acid phosphatase levels in the vagina. J Forensic Sci 1979; 24:346-65.

4. Stubbins NA and Newall PJ. An evaluation of Gamma-glutamyl-peptidase(GGT) and P^{30} determination for the identification of semen on post coital swabs. J Forensic Sci 1985; 30(3):604-14.

5. Fair SR, Clark RB and Russev N. A correlation of seminal polyamine levels and semen analysis in the human. Fertil Steril 1972; 23:38-42.

6. King SJ, Werret DJ and Harrison DT. The evaluation of an ELISA for semen specific 19-OH Prostaglandin F1/F2 using Ex-casework swabs and stains. Forensic Sci Int 1989; 42:103-23.

7. Matsuzawa S, Itoh, Y, Migauchi C, Hara M, Inoue T and Tsude R. Immunologic determination of seminal secretions by a latex microtitre technique. J Forensic Sci 1982; 27(4):848-54.

8. Kashyap, VK. A simple immunosorbent assay for the detection of human blood. J Immunoassay 1989; 10(4):315-24.

9. Tamaki Y, Fakuda M, Kishida T and Takahasi N. Solid phase micro ELISA for Methamphetamine. Jap J Leg Med 1989; 37:417-20.

10. Menge AC, Shoultz GK, Keisey DE, Rutherfold P and Lee CYG. Characterization of monoclonal antibodies against human sperm antigens by immunoassays including sperm function assays and epitope evaluation. Am J Reprod Immunol 1987; 17:77-85.

11. Kyurchiev, SD, Kehayov RI, Tsankov Y and Dimitrova D. Application of an immunological method to detect trace amounts of dried human semen. Forensic Sci Int 1989; 43:27-35.

12. Herr JC and Woodward MP. An enzyme linked immunosorbent assay (ELISA) for human semen identification based on a biotinylated monoclonal antibody to a seminal vesicle specific antigen. J Forensic Sci 1987; 32:346-56.

13. Shigeta M, Watanabe T, Marayama S, Kayoma K and Isojima S. Sperm immobilizing monoclonal antibody to human seminal plasma antigens. Clin Exp Immunol 1980; 42:458-62.

14. Yan YC, Wang LF and Koise SS. Characterisation of sperm agglutinating monoclonal antibody and purification of human sperm antigens. Int J Fertil 1986; 31:77-85.

15. Boorman JL. Radiolabelling of Proteins. In Thorpe PJ Ed. Immunochemistry in practice. Blackwell Scientific Publishers. London 1986; 186-89.

16. Rao DV and Kashyap VK. An evaluation hepatitis B surface antigen as a forensic marker. Adli Tip Derg 1989; 6(1-20):19-24.

17. Fraker PJ and Speck JC. Protein and cell membrane iodination with a sparingly soluble chloramide. Biochem Biophys Res Commun 1978; 80:849.

18. Gill P, Jeffreys AJ and Werrel J. Forensic application of DNA Fingerprinting. Nature 1983; 318:577-79.

19. Sensabaugh GF. Isolation and characterisation of a semen specific protein for human seminal plasma. A potential marker for semen identification. J Forensic Sci 1978; 23(1):1066-15.

20. Herr JC, Summers TA, McGee RS, Sutherland WM, Sigman M, and Evans RJ. Characterisation of a monoclonal antibody to a conserved epitope on human seminal vesicle specific peptides. A novel probe/marker system for semen identification. Biol Reprod 1986; 35:733-85.

21. Lombardis and Espotito F. A new method for identification of mosquito bloodmeals by the immunobinding of peroxidase anti-peroxidase complexes on nitrocellulose. J Immunol Methods, 1986; 86:1-5.

22. Wilson EF. Sperm's morphological survival after 16 days in the vagina of a dead body. J Forensic Sci 1974; 19:561-64.

23. Cecks JM, Breidenthal S and Terasaki PT. Direct blood group typing of forensic samples using a simple monoclonal antibody assay. Forensic Sci Int 1987; 34:205-16.