John C. Herr,[1] Ph.D. and Michael P. Woodward,[2] Ph.D.

An Enzyme-Linked Immunosorbent Assay (ELISA) for Human Semen Identification Based on a Biotinylated Monoclonal Antibody to a Seminal Vesicle-Specific Antigen

REFERENCE: Herr, J. C. and Woodward, M. P., "**An Enzyme-Linked Immunosorbent Assay (ELISA) for Human Semen Identification Based on a Biotinylated Monoclonal Antibody to a Seminal Vesicle-Specific Antigen,**" *Journal of Forensic Sciences,* JFSCA, Vol. 32, No. 2, March 1987, pp. 346-356.

ABSTRACT: Monoclonal antibody mouse antihuman semen-5 (MHS-5) (immunoglobulin G_1 [IgG_1]) was biotinylated using *N*-biotinyl-*w*-aminocaproic acid-*N*-hydroxysuccinimide ester. This monoclonal antibody-biotin conjugate recognized low molecular weight peptide bands between 10.5 and 20 kilodaltons on immunoblots of liquefied semen. Immunodominant peptides had molecular weights of 10.5, 11.5, and 13.5 kilodaltons. An enzyme-linked immunosorbent assay (ELISA) developed with the biotinylated-MAb and streptavidin peroxidase demonstrated sensitivity curves with lower limits of 10 ng of seminal fluid protein per microtiter well using 50 ng per well of monoclonal antibody-biotin conjugate. Cross-reactivity studies on a panel of human biological fluids and tissues demonstrated no cross-reactivity or false positives using the monoclonal antibody-biotin conjugate. The sensitivity of the monoclonal antibody-biotin ELISA was compared to ELISA based upon a polyclonal secondary antibody-peroxidase conjugate. These findings indicate that this ELISA assay, based on a biotinylated monoclonal antibody to a seminal vesicle-specific antigen, may be useful for semen identification.

KEYWORDS: criminalistics, immunoassay, semen, ELISA

Microscopic observation of sperm cells in sexual assault evidence has long served to verify the presence of semen, and thus scientifically corroborate an alleged rape. Because some men lack spermatozoa in the ejaculate as a result of aspermia, azoospermia, or vasectomy, and because elution and recovery of sperm cells is often hampered by adherence to material evidence, tests for seminal fluid markers, such as prostatic acid phosphatase, prostate specific antigen (P30) [*1,2*], gamma glutamyl transpeptidase (GGT), choline, spermine, and lactate dehydrogenase (LDH) have been reported [*1-3*]. Choline, GGT, spermine, and LDH tests each have significant drawbacks [*1,3*], and are not in general use.

In current forensic science practice, the test for prostatic acid phosphatase is considered a

Supported by the Humagen Corporation and Grants FBI 115744 and NIH 16767 to J. C. Herr. Received for publication 10 Feb. 1986; revised manuscript received 1 May 1986; accepted for publication 2 May 1986.

[1]Assistant professor, The Department of Anatomy and Cell Biology, University of Virginia Medical Center, Charlottesville, VA.

[2]Staff scientist, Humagen, Inc., Charlottesville, VA.

Copyright © 1987 by ASTM International

presumptive, rather than a diagnostic, semen assay by most pathologists and forensic specialists. Acid phosphatase activity from endogenous vaginal sources or the many plant materials which contain the enzyme may give false positive results [*1*]. The enzyme also declines in activity upon room temperature storage. For these reasons, when small amounts of semen may be present, as in eluates of dried semen stains from vaginal swabs or undergarments, the acid phosphatase activity cannot be attributed exclusively to semen. Because of the relatively high levels of acid phosphatase in semen, it has been suggested that acid phosphatase determinations may be useful for estimating how much semen is present in evidence material [*4*].

Prostate specific antigen (P30), a 32-kilodalton protein of prostatic origin, has been utilized by the forensic science community as a semen marker since its introduction in 1978 [*5*]. Immunoassays based upon polyclonal antisera are generally employed [*6*]. Although the prostate contributes from 15 to 30% of the ejaculate volume, the seminal vesicles contribute the major fluid volume estimated from 50 to 80% [*7*]. A new monoclonal antibody probe mouse anti-human semen-5 (MHS-5), which recognizes a novel seminal fluid protein specific to the seminal vesicles has recently been identified [*8*].

The MHS-5 monoclonal antibody has been reported [*8*] to be directed to a conserved epitope present in all samples of semen tested from 421 human donors including vasectomized patients. By indirect immunofluorescence, this antigen localized to the surface of ejaculated sperm over postacrosomal, midpiece, and tail regions. The antigen has been detected in no human biological fluid other than semen, nor is it found in semen of common domestic animals and monkeys. However, it is present in orangutang, gorilla, and chimpanzee semen. The antigenic epitope recognized by the monoclonal antibody has been identified by Western blots of proteins obtained following 15 h of liquefaction of semen from vasectomized males. The epitope is located on low molecular weight polypeptides ranging from 8 to 17 kilodaltons with peptides of 10 to 13-kilodaltons being most immunoreactive. Fresh semen contains immunoreactive molecules over a wide molecular weight range. During 15 h of semen liquefaction, higher molecular weight immunoreactive peptides disappear and the 10 to 13-kilodaltons components persist [*8*]. ELISA assay of homogenates of human tissues and reproductive organs has indicated that the antigen originates in the human seminal vesicles. Immunocytochemical studies of the human reproductive tract have localized the antigen's site of secretion to the principal cells of the seminal vesicle epithelium [*9*]. Antibody binding to this seminal vesicle-specific antigen has been found to persist in mixtures of semen and vaginal secretion maintained at 37°C for 4 h, attesting to the stability of the antigenic epitope in the female tract. Because this protein antigen is present in semen from all tested donors, it has been proposed as a new forensic science marker for the management of sexual assault casework using assay systems based on the MHS-5 monoclonal antibody probe [*8*].

The superior properties of monoclonal immunoreagents, which include uniformity (constant class and isotype), constant affinity, and availability in virtually unlimited supply, suggest that forensic science assays based upon monoclonal antibody reagents offer opportunities for standardization of tests between forensic science laboratories using a reagent that remains constant for decades. These advantageous properties of monoclonal antibodies confer an added level of certainty to forensic science testimony not previously possible with polyclonal immunoreagents. In this report, a new ELISA assay is described which employs a biotinylated conjugate of the MHS-5 monoclonal antibody.

Methods

Semen Samples

Semen from 20 donors was diluted 1:1 in phosphate buffered saline and frozen within 4 h of donation in 1-mL aliquots. These aliquots were periodically thawed for analysis.

Ascites Production

An MHS-5 clone was thawed, subcloned, reassayed by ELISA [*8*] on human semen, and a rapidly growing subclone expanded for ascites production. Forty male Balb/c mice were primed with an intraperitoneal injection of 0.5 mL of pristane on Day 0. On Day 14, 5×10^6 cells were injected into each mouse. Ascites fluid was collected over a one-week period and pooled. Cells and other debris were removed by centrifugation. Approximately 180 mL of ascites fluid was obtained.

MHS-5 Purification

Immunoglobulins were precipitated from the pooled ascites by addition of an equal volume of saturated ammonium sulfate [50% $(NH_4)_2SO_4$]. The precipitate was collected by centrifugation (20 min, 10 240 $\times$ *g*), dissolved in 0.01*M* sodium phosphate buffer (pH 6.8), and reprecipitated. Following the second ammonium sulfate precipitation, the pellet was dissolved in a minimal volume of 0.01*M* $NaPO_4$ (pH 6.8), and centrifuged for 10 min at 10 600 $\times$ *g*. The resulting supernatant was applied to a Bio-gel P6-DG column (1.5 by 40 cm) (BioRad, Rockville Centre, NY). Fractions containing protein were pooled and applied to a hydroxylapatite column which had been equilibrated with 0.01*M* $NaPO_4$ (pH 6.8) [*10*]. Proteins were eluted with a linear 0.01 to 0.3*M* $NaPO_4$ gradient. Column fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and ELISA (as below). Tubes containing maximal MHS-5 activity were pooled and concentrated. An aliquot of this concentrate was analyzed by SDS-PAGE.

Biotinylation of MHS-5

Approximately 2 mg of MHS-5 were biotinylated with *N*-biotinyl-*w*-aminocaproic acid-*N*-hydroxysuccinimide ester (ENZOTIN, Enzobiochem, NY) at a protein to ENZOTIN ratio of 1:22 [*11*]. The stoichiometry of biotin to antibody in the conjugate was not determined.

SDS-PAGE

Column fractions and purified immunoglobulin were analyzed on 10 or 10 to 20% linear gradient polyacrylamide gels in the presence of 0.1% SDS. Immunoblot analysis of the MHS-5 antigen was done with 15% polyacrylamide gels. Low molecular weight standards (Sigma and LKB) were used for molecular weight estimations.

Immunoblots

Immunoblots of the MHS-5 epitope were performed using liquefied semen as source material. Following electrophoresis, proteins were transferred to nitrocellulose (100 mA, 1 to 3 h, room temperature) using 25m*M* Tris-192m*M* glycine containing 10% methanol [*12*]. Unoccupied sites on the nitrocellulose were blocked by incubating the blots in 1% bovine serum albumin (BSA)-50m*M* Tris-150m*M* sodium chloride (NaCl) for a minimum of 1 h. Subsequently the blots were washed twice in 0.05% Tween 20, 50m*M* Tris-NaCl (TTN) and then incubated with a soluble biotinylated MHS-5-streptavidin-horseradish peroxidase (HRP) complex for 1 h at room temperature. Following five rapid washes with TTN, bound MHS-5 was localized with 1m*M* diaminobenzidine-100m*M* Tris (pH 7.0)-0.01% hydrogen peroxide (H_2O_2).

ELISA

Immulon II plates were coated with seminal fluid, forensic science samples, or tissue homogenates for 1 h at room temperature. Unless specified, 100-μL sample volumes were applied per well. Protein concentration was determined by the method of Bradford [*13*] and samples were placed in the wells at various protein concentrations ranging from 5000 to 0.005 μg/mL. Plates were then emptied, filled with TTN plus 0.1% BSA, and incubated at room temperature for 10 min. Next, freshly prepared (biotinyl MHS-5-streptavidin-HRP) was added and the plates incubated for 30 min. Following five washes, the presence of bound MHS-5 was detected with 1m*M* 2,2-azino-di-(3-ethylbenzthiazoline sulfonic acid) (ABTS)-citrate-phosphate buffer-0.03% H_2O_2. Plates were read in an automated plate reader (Multiskan, Flow Laboratories, McLean, VA) after 30 min and then photographed.

Reproducibility

To calculate intra-assay reproducibility, 88 wells were coated with a standard seminal fluid pool (0.7 μg/well) and a uniform sample of monoclonal antibody-streptavidin-HRP mixture was added. Eight wells coated with human serum (7 μg/well) were used as a negative control. Reproducibility was defined as 100% minus the coefficient of variation, following published methods [*14*].

Results

MHS-5 antibody was purified using ammonium sulfate precipitation and hydroxylapatite chromatography. Our protocol yielded approximately 2 g of antibody from 180 mL of ascites fluid. SDS-PAGE of the purified immunoglobulin shows single heavy and light chain bands (Fig. 1). MHS-5 light chain migrates more slowly than most mouse light chains (data not shown). This behavior makes it possible to detect easily contaminating immunoglobulins. Based on the dilution series shown in Fig. 1 and other electrophoretic analyses, we estimate that our preparation is at least 95% pure immunoglobulin.

Previous evidence [*8*] has indicated that the epitope recognized by MHS-5 occurs on many proteins in nonliquefied seminal fluid, but that liquefication considerably reduces this complexity to peptides with molecular weights from 11 to 13 kilodaltons predominating after 15 h. Our immunoblots of liquified semen exhibit a complex pattern of bands between 10 500 and 20 000 daltons which is dominated by bands with apparent molecular weights of 10 500, 11 500, and 13 500 daltons (Fig. 2). Antigen dilution experiments indicate that the lower molecular weight doublet is the major antigenic species, because at low concentrations of antigen, the doublet remains immunoreactive while other bands disappear. This pattern is unaffected by incubation of seminal fluid for 24 h at room temperature (Fig. 2).

The initial ELISA assay described for the seminal vesicle specific antigen [*8*] involved several assay steps. It relied upon polyclonal anti-immunoglobulin conjugates for detection of bound MHS-5. Such secondary reagents may give rise to variable backgrounds in assays as a result of cross-reactivities. We sought to streamline the assay procedure and to eliminate the reliance on polyclonal anti-immunoglobulin conjugates. In our initial experiments, we observed that the monoclonal antibody and the goat anti-mouse immunoglobulin-peroxidase conjugate could be mixed together and applied simultaneously for 30 min with no apparent loss of assay sensitivity. This combination of primary monoclonal antibody and secondary anti-monoclonal into a single-step reagent reduced assay time. We next investigated the feasibility of using a biotinyl MHS-5-streptavidin-HRP complex as a single-step reagent. We chose streptavidin-HRP because of its low nonspecific binding to eukaryotic tissues. Figure 3 demonstrates the steps involved in the assay procedure. Both the primary

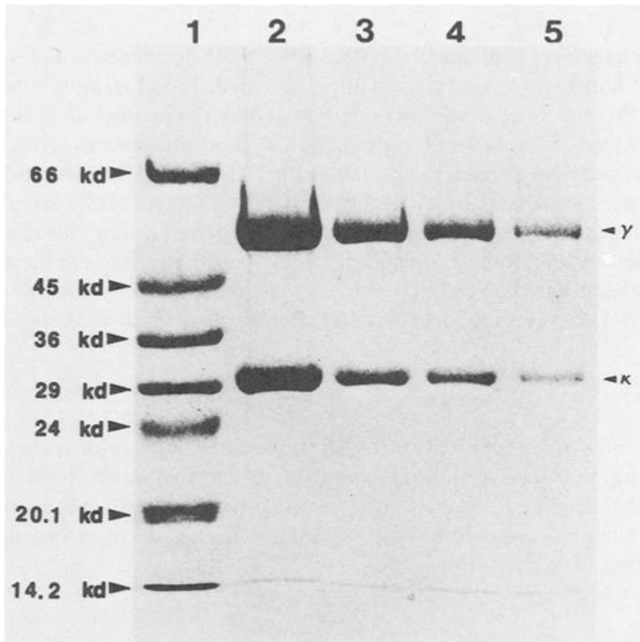

FIG. 1—*A 10% polyacrylamide gel stained with Coomassie blue showing the purified MHS-5 monoclonal antibody. Of purified MHS-5 antibody, 10 μg were applied in Lane 2; 5 μg, Lane 3; 2.5 μg, Lane 4; and 1.25 μg, Lane 5. Before electrophoresis, 5% b-mercaptoethanol-1% SDS was added to the samples which were heated at 95°C for 2 min. A single band is observed for both light* (κ) *and heavy* (γ) *chains of the monoclonal antibody (IgG_1). Molecular weight standards were bovine serum albumin (66 kilodaltons), ovalbumin (45 kilodaltons), glyceraldehyde 3-phosphate dehydrogenase (36 kilodaltons), carbonic anhydrase (29 kilodaltons), trypsinogen (24 kilodaltons), trypsin inhibitor (20.1 kilodaltons), and α-lactalbumin (14.2 kilodaltons) (Lane 1). The α-lactalbumin runs at the dye front on these 10% gels.*

biotinylated monoclonal antibody and the secondary streptavidin-peroxidase are mixed together as a single-step reagent before addition to the sample.

The (biotinyl MHS-5-streptavidin HRP) complex detects as little as 10 ng of seminal fluid protein per well (Fig. 4). This is a maximal estimate of antigen since we have not determined what percentage of the 10 ng of seminal fluid protein added to the assay well is actually bound to the plastic plate. The optimal concentration of MHS-5 in this assay appears to be between 0.25 to 0.5 μg/mL (Fig. 5) when 0.25 μg of streptavidin-HRP is utilized. This optimum value is in good agreement with the optimum observed by Herr et al. [*8*]. The decrease in signal above 0.5 μg/mL (prozone-like effect) is likely due to subsaturating levels of streptavidin-HRP in the system. Negative control wells coated with 7 μg/mL of human serum consistently give optical densities less than 0.05. It has been our experience that an optical density greater than 0.100 exceeds the mean negative control optical density plus three standard deviations.

To determine the reproducibility of the ELISA, 88 samples of a constant antigen pool were tested with a constant concentration of the monoclonal antibody-streptavidin-HRP complex. At an antigen concentration of 0.7 μg/mL and a monoclonal antibody-streptavidin-HRP complex ratio of 0.5 μg/mL:0.25 μg/mL, the intra-assay reproducibility was 87.5% (Table 1).

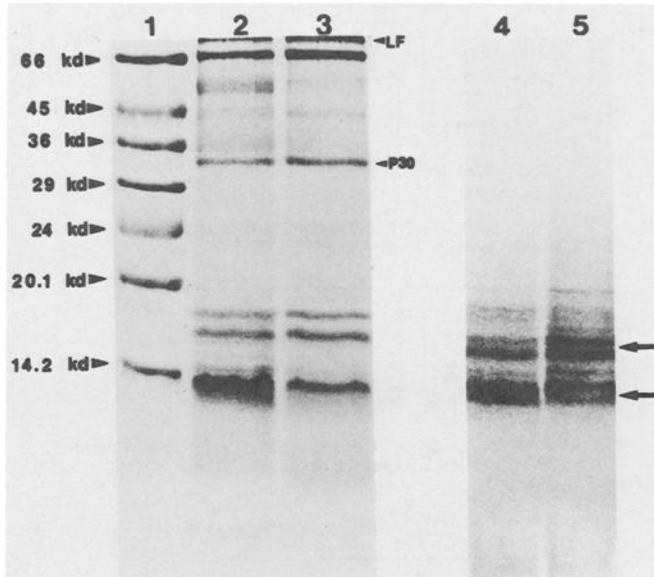

FIG. 2—*Immunoblot of liquefied seminal fluid using biotinyl MHS-5-streptavidin-HRP complex. Seminal fluid samples were allowed to liquify for 24 h at room temperature after which they were reduced in 1% SDS-5%* b-*mercaptoethanol by heating at 95°C for 2 min. Approximately 90 µg of seminal fluid proteins per well were separated on a 15% polyacrylamide gel and either stained with Coomaissie blue* (a) *or transferred to nitrocellulose and visualized with biotinyl-MHS-5-streptavidin-HRP* (b). *Lane 1 shows low molecular weight standards (66, 45, 36, 29, 24, 20.1, and 14.2 kilodaltons). Lanes 2 and 3 are Coomassie-stained seminal fluid proteins following 24 h of liquefaction. Seminal fluid following 24 h of liquification (Lanes 4 and 5, respectively) was immunoblotted. The blot was probed with 0.4-µg/mL biotinyl MHS-5-0.25-µg/mL streptavidin-HRP and bound. MHS-5-streptavidin-HRP was detected with 1m*M *diaminobenzidine. The major immunoreactive peptides are indicated at the arrowheads. (*LF = *lactoferrin;* P30 = *prostate-specific antigen.)*

A direct comparison of the biotinyl MHS-5-streptavidin HRP complex to the previously reported MHS-5-goat anti-mouse immunoglobulin Gs (IgGs)-HRP complex [*8*] is shown in Fig. 6. The titration curves are virtually identical.

For this assay to have applicability in forensic science analysis, it should have minimal nonspecific interactions with human tissues or fluids. Table 2 lists the various human tissues and fluids tested. A range of antigen concentration was used in these cross-reactivity tests to insure that we were not artifactually blocking binding. No nonspecific reactivity was observed with any tissue tested even after several hours of color development.

Discussion

The ELISA assay described in this report is rapid, inexpensive, and shows specificity for human seminal fluid and lack of cross-reactivity with other biological fluids. Because the MHS-5 monoclonal antibody has been shown to react with peptide antigens secreted by the principal cells of the seminal vesicle epithelium [*9*], it is a useful probe for detection of vasectomized or azoospermic seminal fluids.

Use of the biotinylated monoclonal antibody streptavidin-HRP complex on Western blot analysis of seminal fluid peptides after 24 h of semen liquefaction demonstrated major im-

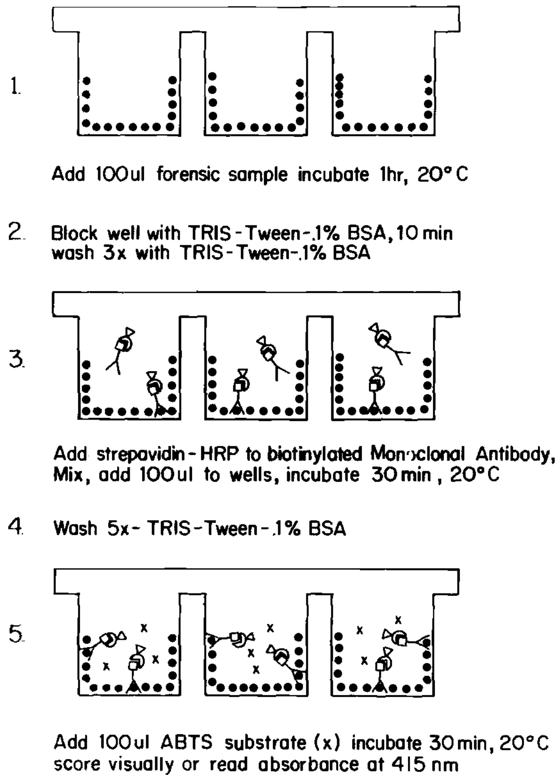

FIG. 3—*Summary diagram of the ELISA protocol based on monoclonal antibody MHS-5 and streptavidin-HRP.*

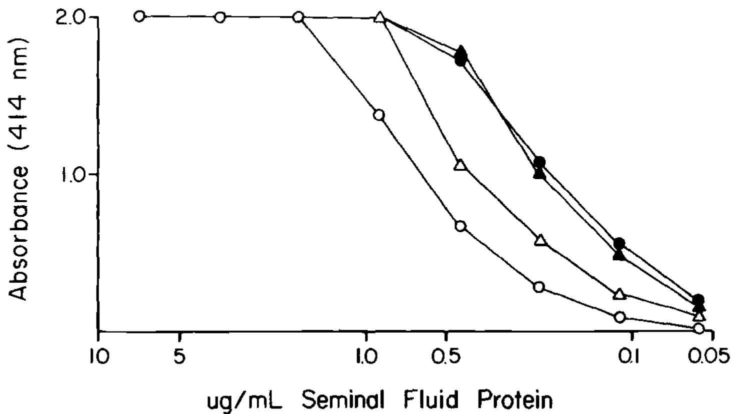

FIG. 4—*Sensitivity of the biotinylated MHS-5-streptavidin-HRP based ELISA. The binding of the biotinylated MHS-5 streptavidin-HRP complex to adsorbed seminal fluid proteins was titrated. An Immulon II plate was coated with two-fold dilutions of seminal fluid proteins (starting concentration 7 µg/mL) and then probed with four concentrations of the biotinylated antibody. In this experiment, the concentration of biotinylated antibody was varied between 2 and 0.03 µg/mL and the streptavidin-HRP concentration held constant at 0.25 µg/mL. Four concentrations of the biotinylated antibody are graphed: ○ = 2 µg/mL, △ = 1 µg/mL, ● = 0.5 µg/mL, and ▲ = 0.25 µg/mL. Wells lacking antigen served as reagent blanks. These values were subtracted from the experimental points. These control wells had optical densities less than 0.05.*

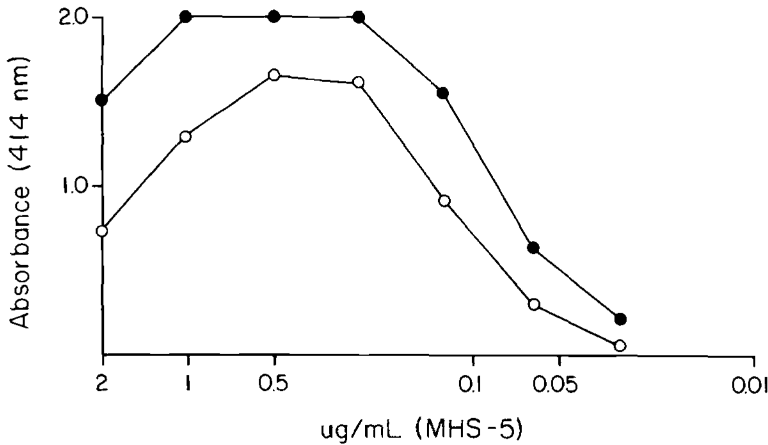

FIG. 5—*Determination of optimal MHS-5 concentration in the assay system. Two-fold dilutions of biotinyl MHS-5 (starting concentration 2 μg/mL) were assayed on an Immulon II plate coated with 100 μL of seminal fluid over a wide concentration range (2 to 0.01 μg/mL). Values for 0.88 and 0.44 μg/mL of seminal fluid are plotted.* ● *= 0.88 μg/mL and* ○ *= 0.44 μg/mL.*

TABLE 1—*Intra-assay reproducibility.*

	Absorbance (Optical Density) 414 nm	
Blank	0.017 ± 0.010	$n = 8$
Sample	0.546 ± 0.068	$n = 88$
% Reproducibility	87.5	

munoreactive bands in the 10- to 13-kilodalton range. These findings are comparable to the results previously reported using a secondary antibody-HRP conjugate [*8*], and verify retention of specificity of the antibody following the biotinylation procedure. Herr et al. [*8*] reported the dominant immunoreactive peptides to be 10, 11.9, and 13.7 kilodaltons. The calculations made in this study, which give 10.5, 11.5, and 13.5 kilodaltons, are within the expected range of variability in molecular weight determinations from SDS-PAGE.

In its current format, the assay can be performed in approximately 2 h. We have observed that the plate-coating step with semen samples or forensic science evidence can be reduced to 30 min without substantial loss of sensitivity. Our preliminary experiments with extracted forensic science specimens and laboratory-generated semen stains indicate that positive signals generally develop within 5 to 10 min with optical densities of 2 (the maximum reading on the spectrophotometer) being reached in 15 min. The assay we have developed can detect the equivalent of 1 mL of seminal fluid protein in 200 L. Studies are currently underway to see if such sensitivity is necessary for routine forensic science determinations.

Our immunoblot evidence indicates that following semen liquification the MHS-5 epitope is stable for at least 24 h at room temperature in a liquid state. The MHS-5 epitope has been shown to be detectable in mixtures of vaginal secretion and semen after 4 h of coincubation [*8*]. We are currently investigating the effect of vaginal secretions, blood, and saliva on the temporal stability of the antigenic epitope over several days of coincubation.

The intra-assay reproducibility of this monoclonal antibody-based ELISA assay for semen identification is 87.5%. This result is in accord with reproducibility values reported by

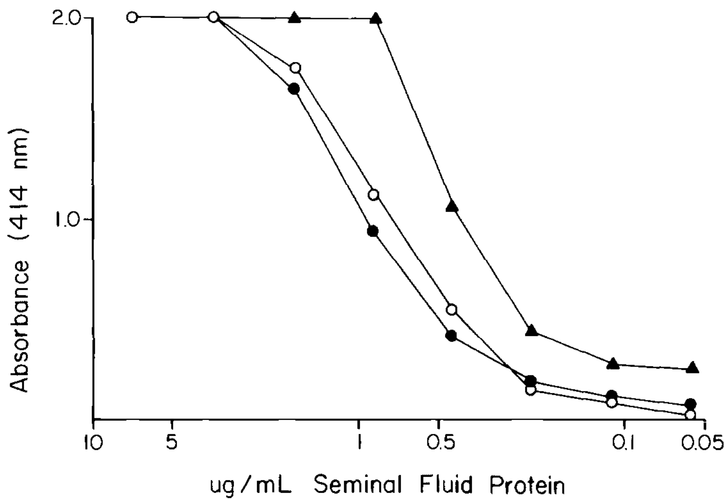

FIG. 6—*Comparison of biotinyl MHS-5-streptavidin-HRP with MHS-5-goat anti-mouse immunoglobulin-HRP in assay for seminal vesicle specific antigen. An Immulon II plate was coated with 100 μL of seminal fluid over a wide concentration range. Soluble complexes of either biotinylated MHS-5-streptavidin-HRP (0.4 to 0.25 μg/mL and 0.4 to 0.125 μg/mL) or MHS-5-goat anti-mouse IgGs (0.4 μg/mL—1:1000 dilution) were employed. The streptavidin-HRP based assay was as sensitive or more sensitive than the system employing conventional HRP-conjugated secondary antibody.* ○ = *0.25-μg/mL dilution streptavidin-HRP,* ▲ = *0.125-μg/mL dilution streptavidin-HRP, and* ● = *1:1000 dilution goat anti-mouse IgGs-HRP.*

TABLE 2—*Fluids and tissues tested for MHS-5 binding.*

Fluids and Tissues	Range Tested, μg/well	Result
Swine serum	400-0.13	−
Orangutang serum	450-0.14	−
Gorilla serum	450-0.14	−
Chimp serum	450-0.14	−
Human serum	350-0.11	−
Human liver	50-0.016	−
Human pancreas	250-0.08	−
Human kidney	500-0.16	−
Human milk	200-0.065	−
Human vaginal secretion[a]		
(2D)	1600-0.5	−
(3D)	2000-0.65	−
(5D)	2500-0.8	−
Seminal fluid	0.7-0.01	+++

[a]2D, 3D, and 5D refer to days elapsed since last coital contact.

other investigators using polyclonal antisera in ELISA determinations for anti-sperm antibodies [*14*].

For any assay to be useful in the identification of semen it should show no cross-reactivity with other human or animal tissues. The MHS-5 probe has been tested on a large panel of biological fluids and no cross-reactivities observed [*8*]. We have found that commercial polyclonal anti-immunoglobulin conjugates can be one source of false positive results in ELISA

assays. Thus far, we have observed no cross-reactivity with other human tissues or fluids nor with the sera of several animal species tested using the streptavidin-HRP complex.

The method we employ involves the addition of streptavidin-HRP to the monoclonal antibody in a mixing step before addition to the unknown sample. The streptavidin-HRP is added to the antibody at concentrations below a 1:1 ratio. The dissociation constant for the avidin-biotin complex is of the order of $10^{-15}M$ [*15*]. Because of this high dissociation constant and the use of subsaturating amounts of streptavidin-HRP/monoclonal antibody-biotin, we assume that little free streptavidin-HRP is available in the system to give false positive results on endogenous biotin in biological samples.

Monoclonal immunoreagents offer advantages of constant affinity and class, uniformity, and availability in virtually unlimited supply. Hybridomas may be frozen in liquid nitrogen for decades and identical antibody recovered. Thus, by using monoclonal immunoreagents, standards of uniformity and reproducibility are now possible that were previously difficult or impossible to achieve with polyclonal antisera. Forensic science tests employing monoclonal antibodies may gain wider acceptance as more monoclonal antibodies which have been thoroughly evaluated in forensic science casework become available.

References

[*1*] Gaensslen, R. E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry,* United States Department of Justice, U.S. Government Printing Office, Washington, DC, 1983, p. 149.

[*2*] Poyntz, F. M. and Martin, P. O., "Comparison of P30 and Acid Phosphatase Levels in Post-Coital Vaginal Swabs from Donor and Casework Studies," *Forensic Science International,* Vol. 24, No. 1, Jan. 1984, pp. 17-25.

[*3*] Stubbins, N. A. and Newall, P. J., "An Evaluation of Gamma-glutamyl Transpeptidase (GGT) and P30 Determinations for the Identification of Semen on Postcoital Vaginal Swabs," *Journal of Forensic Sciences,* Vol. 30, No. 3, July 1985, pp. 604-614.

[*4*] Sensabaugh, G. F., "The Acid Phosphatase Test," in *Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence,* U.S. Government Printing Office, Washington, DC, (LC 84-601146), 1983, pp. 65-81.

[*5*] Sensabaugh, G. F., "Isolation and Characterization of a Semen Specific Protein from Human Seminal Plasma: A Potential Marker for Semen Identification," *Journal of Forensic Sciences,* Vol. 23, No. 1, Jan. 1978, pp. 106-115.

[*6*] Graves, H., Sensabaugh, G. F., and Black, E. T., "Post Coital Detection of a Male-Specific Semen Protein: Application to the Detection of Rape," *New England Journal of Medicine,* Vol. 312, No. 6, Feb. 1985, pp. 338-343.

[*7*] Mann, T. and Lutwak-Mann, C., *Male Reproductive Function and Semen,* Springer-Verlag, Berlin, 1981, p. 183.

[*8*] Herr, J. C., Summers, T. A., McGee, R. S., Sutherland, W. M., Sigman, M., and Evans, R. J., "Characterization of a Monoclonal Antibody to a Conserved Epitope on Human Seminal Vesicle Specific Peptides: A Novel Probe/Marker System for Semen Identification," *Biology of Reproduction,* Vol. 35, Oct. 1986, pp. 773-784.

[*9*] Evans, R. J. and Herr, J. C., "Immunohistochemical Localization of the MHS-5 Antigen in Principal Cells of Human Seminal Vesicle Epithelium," *Anatomical Record,* Vol. 214, April 1986, pp. 372-377.

[*10*] Stanker, L. H., Vanderlaau, M., and Juarez-Salinas, H., "One-Step Purification of Mouse Monoclonal Antibodies from Ascites Fluid by Hydroxylapatite Chromatography," *Journal of Immunological Methods,* Vol. 76, No. 1, 1985, pp. 157-170.

[*11*] Hofmann, K., Titus, G., Montibeller, J. A., and Finn, F. M., "Avidin Binding of Carboxyl-Substituted Biotin and Analogues," *Biochemistry,* Vol. 21, No. 5, March 1982, pp. 978-984.

[*12*] Towbin, H., Staehelin, T., and Gordon, J., "Electrophoretic Transfer of Proteins from Polyacrylamide Gels to Nitrocellulose Sheets: Procedure and Some Applications," *Proceedings of the National Academy of Sciences,* Vol. 76, No. 9, Sept. 1979, pp. 4350-4354.

[*13*] Bradford, M. M., "A Rapid and Sensitive Method for Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein Dye Binding," *Analytical Biochemistry,* Vol. 72, No. 1, May 1976, pp. 248-254.

[*14*] Ackerman, S., Worthan, J., and Swanson, R., "An Indirect Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection and Quantitation of Antisperm Antibodies," *American Journal of Reproductive Immunology,* Vol. 1, No. 4, Aug. 1981, pp. 199-205.

[15] Hsu, S., Raine, L., and Fanger, H., "Use of Avidin-Biotin-Peroxidase Complex (ABC) in Immunoperoxidase Techniques," *Journal of Histochemistry and Cytochemistry*, Vol. 29, No. 4, April 1981, pp. 577-580.

Address requests for reprints or additional information to
Dr. John C. Herr
Anatomy and Cell Biology
Box 439
Medical Center
University of Virginia
Charlottesville, VA 22908