BRIEF COMMUNICATION

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Interferences with Semen Detection by an Immunoassay for a Seminal Vesicle-Specific Antigen

REFERENCE: Chen JT, Hortin GL. Interferences with semen detection by an immunoassay for a seminal vesicle-specific antigen. J Forensic Sci 2000;45(1):234–235.

ABSTRACT: A commercial enzyme-linked immunosorbent assay (ELISA), the SEMA® assay, for a seminal vesicle-specific antigen (SVSA) provides highly sensitive detection of semen. Here we show marked interference of proteins such as albumin, serum proteins, or mucin with the assay. This would substantially decrease the sensitivity for detecting semen mixed with other biological fluids such as blood or vaginal secretions.

KEYWORDS: forensic science, semen, identification, seminal vesicles, immunoassay seminal vesicle specific antigen

Forensic identification of traces of semen depended for many years on detecting acid phosphatase activity or microscopic identification of sperm (1,2). However, sperm identification is a relatively insensitive method for semen detection and fails to detect semen from vasectomized or azoospermic males. Acid phosphatase is found in other tissues and secretions albeit at lower concentrations than in semen. To improve sensitivity and specificity of semen detection, assays for two other semen proteins have been investigated—prostate-specific antigen (PSA), also known as P-30 (3,4), and SVSA (5,6). There has been interest in these markers both as better forensic markers and as markers of semen exposure in studies of condom effectiveness (7,8). An ELISA for SVSA has appeared very promising for improved semen detection; SVSA is expressed only in seminal vesicles and this gland's secretions, and the assay yields detect semen diluted more than one million-fold (5,6). However, there has been evidence that components in vaginal fluid may reduce assay sensitivity (5). In the present study we examined potential interference with detection of SVSA.

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Materials and Methods

The SEMA® assay kit for SVSA was purchased from Humagen Fertility Diagnostics, Inc. (Charlottesville, VA) and was performed as described in the package insert, except additional washes were performed before the final color reaction, and color in each well was measured in a spectrophotometer at 415 nm after diluting the contents with 1 mL water. A buffer with 300 mmol/L NaCl, 25 mmol/L tris(hydroxymethyl)aminomethane, and 0.5 mmol/L EDTA at pH 7.65 was used for dilutions and as a negative control. Semen was from previous studies (8) and stored frozen. Serum for competition studies was pooled from several women. Bovine serum albumin and bovine submaxillary mucin were from Sigma Chemical Co. (St. Louis, MO).

Results and Discussion

The ELISA for SVSA yielded a positive response at semen dilutions (in saline) as high as 1:10,000,000. Dilutions of 1:10,000, 1:100,000, and 1:1,000,000 yielded absorbances of 1.4, 0.6, and 0.05, respectively. However, adding other proteins to semen samples markedly decreased assay response (Fig. 1). When increasing amounts of serum up to 50% specimen volume were added to a constant amount of semen, the assay response (as equivalent semen dilution) fell progressively up to 10,000-fold (top panel of Fig. 1). Similar effects were observed with bovine albumin and with bovine submaxillary mucin (middle and bottom panels of Fig. 1). Thus, biological fluids such as blood, saliva, or vaginal fluid containing albumin, mucins, and other proteins should inhibit the ELISA. This interference may explain much lower sensitivity of this ELISA versus an assay for prostate-specific antigen in detecting semen in vaginal swab samples (8). The ELISA is likely to work well for analysis of semen spots or other unmixed specimens, but should have much reduced sensitivity for semen detection in specimens containing blood or mucus. Interference by proteins probably relates to the ELISA's direct antigen capture step in which the antigen must adsorb directly to the surface of a plastic well before detection. Modification of the assay into a sandwich-type format with a capture antibody may overcome the interference observed here and provide a highly sensitive and specific assay for semen detection.

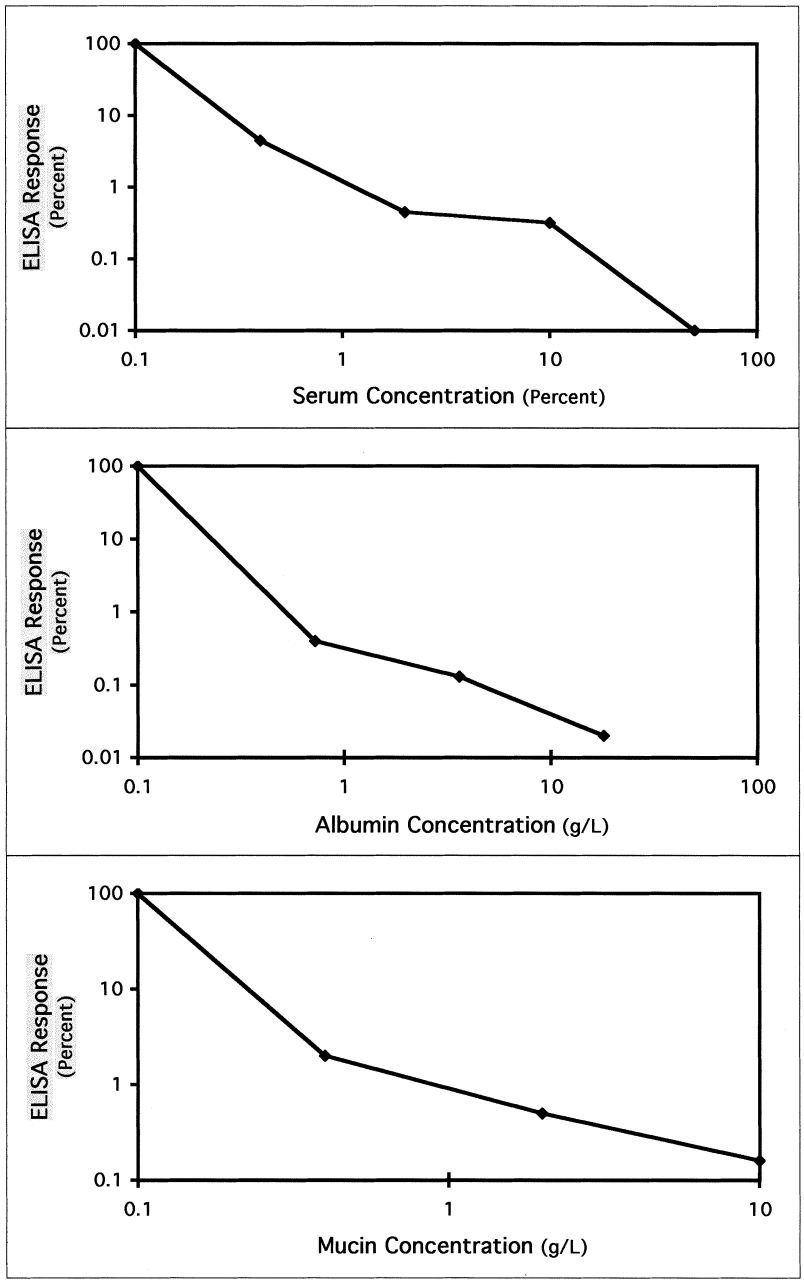

FIG. 1—*Inhibition of ELISA responses to a constant dilution of semen (1:1000) with the addition of varying amounts of serum (*top*), bovine albumin (*middle*), or mucin (*bottom*). Results are expressed as the semen dilution (as a percentage) yielding an equivalent absorbance.*

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