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Spermine and Choline Identification by Thin-Layer Chromatography

Various methods are available for the detection of prostatic acid phosphatase [*1*, *2*], spermine, choline [*3*], and spermatozoa. Aspermatic seminal stains are increasing in number due to vasectomies. In these cases location of spermatozoa is not possible. Difficulties are also frequently encountered in the location of spermatozoa even when present in dried seminal stains. This paper sets forth a practical, rapid, inexpensive, and sensitive thin-layer chromatographic method for the detection of spermine and choline. No interferences have been noted from blood, vaginal secretions, saliva, douche powders, urine, or the azo dye (Fast Blue B) for the detection of prostatic acid phosphatase.

Spermine occurs in numerous human organs and fluids [*4*]. Choline is also present in various biological materials but, similarly to spermine, in much lower concentrations than in seminal fluid. Spermine and choline are found in combination and in high concentrations only in seminal fluid [*5*].

Experimental Materials and Reagents

Glass plates with a 250-μm layer of Silica Gel G or GF (Analtech, Inc., Newark, Del.) are used with no further preparation. The plates are 10 by 20 cm or 20 by 20 cm depending on the number of samples to be analyzed.

The standard materials, spermine and choline chloride, were purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis., and Eastman Kodak Co., Rochester, N. Y., respectively. The aqueous standards contained 0.15 mg spermine/ml and 1.0 mg choline chloride/ml. A semen control was prepared by diluting 1 ml of frozen seminal fluid to 5 ml with 1 *N* hydrochloric acid (HCl). Frozen control seminal fluid was obtained from a local pathology laboratory. All standards were stored in the refrigerator and no changes have been noted over a one-year period. Fast Blue B and alpha-naphthyl phosphate calcium salt were purchased from Dajac Laboratories, Philadelphia, Pa.

Cloth stained with control seminal fluid was air dried and stored in the laboratory for a period of 8 months.

Potassium iodoplatinate was used to detect the spermine. The reagent consisted of 2 ml of a 10 percent platinum chloride solution, 50 ml of a 4 percent potassium iodide solution, and enough water to dilute to a volume of 200 ml. Concentrated (18 *M*) sulfuric acid was used for the detection of choline.

The developing solvent was 1 *N* HCl, which could be reused several times.

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Procedure

Approximately 1 cm^2 of dried, stained fabric is used for the sample in both unknown and control. The swatch is finely shredded and placed in a 10- by 75-mm culture tube. Approximately 5 drops of physiological (0.9 percent) saline are added to the tube and thoroughly mixed with the fabric. A vortex mixer may be used to aid in this mixing but is not necessary. The samples are allowed to incubate at room temperature for approximately 30 min. Leaving them longer (two to three days) does no harm.

The samples are spotted on the plates using (1) 10 μl of the spermine standard, (2) 10 μl of the choline standard, (3) 10 μl of the seminal fluid standard, and (4) 30 μl of the saline wash from each of the questioned, stained fabrics. The samples are spotted using 10 μl disposable pipets while applying cool air to the plate from a hair drier.

After spotting and drying the plates are introduced into a tank containing 1 *N* HCl as a mobile phase and are allowed to develop to approximately 10 cm. The plate is removed from the tank and the solvent front is carefully marked and then dried for 15 min, using hot air from a flameless air gun (at approximately 300°C).

The plate, while still hot, is sprayed with the potassium iodoplatinate reagent to develop the spermine as a blue spot, R_F approximately 0.85. The plate is then oversprayed with concentrated sulfuric acid to develop the choline, R_F approximately 0.58. Choline is a second blue spot and the spermine spot darkens in color with overspraying.

Discussion

Thin-layer chromatography offers a rapid, practical, sensitive, and inexpensive method for the detection of seminal fluid by the separation and detection of spermine and choline. The entire procedure takes less than 1 h.[2] The minimum detectable amount of spermine is 0.1 μg and of choline is 3.0 μg.

The pure choline chloride standard has a slightly higher R_F value (0.65) than choline in the seminal fluid. The tentative explanation for this difference is the fact that choline is present in seminal fluid not only as free choline, but also as glycerylphosphorylcholine, and phosphorylcholine [6]. The concentration ranges of these three compounds are 258–380 mg/100 ml, 50–100 mg/100 ml, and 70–2000 mg/100 ml, respectively [6]. Numerous other body salts are also present [6]. Attempts to remedy the R_F value difference by hydrolysis have been unsuccessful. For this reason the control seminal fluid is always used as the choline standard.

Spermine is present in seminal fluid at a concentration of 20–250 mg/100 ml [6]. No appreciable R_F difference is noted between the spermine standard and seminal spermine.

Reversing the order of detection reagents and spraying leads to a negative test result.

The acid phosphatase test was performed on the control seminal stain periodically over an 8-month period with consistent positive results. Detection of spermine and choline was also possible on the dried stain over this 8-month period with some deterioration of the choline noted. It was possible to detect spermine and choline in a swatch which had also been previously used for the acid phosphatase test. This one swatch can be used for both purposes, allowing good results in cases where very minute samples are available.

Summary

A thin-layer chromatographic procedure for separating and identifying spermine and choline in seminal stains is described. The identification of spermine and choline, along

[2] The procedure has been used in over 30 actual cases and has been used twice in testimony in criminal court cases in the State of Iowa.

with the detection of prostatic acid phosphatase, is used to identify a seminal stain which is aspermatic. Silica Gel G or GF plates are developed in 1 *N* HCl with a saline extract of the stains, using aqueous standards. Potassium iodoplatinate is used for the detection of spermine, and concentrated sulfuric acid is oversprayed for choline.

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