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Acid Phosphatase Test for Seminal Stains--The Importance of Proper Control Tests, The

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THE ACID PHOSPHATASE TEST FOR SEMINAL STAINS

The Importance of Proper Control Tests

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The acid phosphatase test is now widely used to identify stains of seminal origin. (1, 2, 3, 4) Tests for acid phosphatase which use a phenolic ester as a substrate are non-specific; experimental interferences were studied and a simple method of detection of interferences is reported.

The test methods consist of reacting the suspected stain or an extract of it with a solution of suitably buffered phenolic phosphate ester. If acid phosphatase is present, the phenolic phosphate ester is hydrolyzed to phosphate ion and phenol. The phenol formed, if any, is then reacted with a third substance to form a colored product. The presence of this colored product indicates a positive test for the phosphatase. If the quantity of colored product is sufficient within the time and concentration limits stated in the test, a conclusion that the stain is of seminal origin is justified. The difficulty with this process is that extraneous phenols will react with the chromogenic color forming substance to produce colored substances which may interfere or produce a false positive reaction. With some very reactive chromogenic substances, such as the diazonium ions, other classes of chemicals, such as aromatic amines and their salts, imidazoles, pyrroles, indoles, ammonium ion, histidine, hydrazine, hydroxylamine, phenylalanine, tryptophane, tyrosine, arginine, also react to form colored products under suitable conditions.

Hence, with the use of this type of test the presence of a phenol, cresol, aromatic amine, or its salt in the material tested may cause an interfering or misleading reaction. This occurrence is of more than academic interest because many commercial vaginal douches, mouth and foot washes, gargles, suppositories, hemorrhoid medicants, anti-perspirants, contraceptive preparations, deodorizing formulas, sheepdips, poultryhouse sprays, and antiseptics contain amounts of phenolic substances sufficient to give interference or false positive reactions. This type of false results can be reliably detected by the use of proper controls.

The control procedure is usually not detailed by the authors of the various references and texts, and in at least one published report is not considered at all. Inasmuch as the omission of, or improper use of controls may lead to a gross error in results of the test, it was proposed to test a number of theoretical interferences and outline test methods in such a manner so as to preclude as much interference as possible.

PROCEDURE

The method of Seligman and Manheimer as described by Walker (4) was first used. The substances tested were placed on cloth and the reagent added directly to the cloth. No control tests were used.

TABLE I

	Seligman-Manheimer Reagent W/O Controls	Modified Seligman-Manheimer Reagents W/Controls
Known seminal stain	++ Red-brown color	++ Red-brown color
1. Aniline sulfate EKC	+ Yellow-brown	Reacted with chromogen
2. Acetyl salicylic acid USP	NR	NR
3. Thymol USP	+ Red-brown	Reacted with chromogen
4. 6-Chlorothymol EKC	+ Red-brown	Reacted with chromogen
5. Phenol USP	++ Red	Reacted with chromogen
6. Salicylic acid USP	NR	NR
7. p-Ethylphenol EKC	++ Red	Reacted with chromogen
8. o-Toluidine EKC	++ Yellow-brown	Reacted with chromogen
9. b-Naphthylamine EKC	++ Purple	Reacted with chromogen
10. p-Aminobenzoic acid USP	NR	NR
11. Menthol USP	NR	NR
12. Eucalyptus oil USP	NR	NR
13. Lysol USP	++ Red-brown	Reacted with chromogen
14. Diethyl stilbestrol USP	NR	NR
15. Sulfanilamide USP	NR	NR
16. p-Amino salicylic acid USP	+ Yellow-brown	Reacted with chromogen
17. Hydroquinone EKC	NR	NR
18. Metol EKC	+ Yellow	Reacted with chromogen
19. Di-n-propyl phenol EKC	+ Yellow	Reacted with chromogen
20. Creosote USP	++ Red-brown	Reacted with chromogen
21. Hydroxylamine hydrochloride EKC	++ Brown	Reacted with chromogen
22. Ammonium chloride	NR	NR
23. Indole (Delta)	NR	NR
24. Hydrazine sulfate	+ Yellow	Reacted with chromogen

NR No reaction + Slight color reaction ++ Intense color reaction

In order to facilitate proper control tests, essentially the same method and reagents were used, except the buffer solution was divided into two equal parts and the substrate (sodium alpha naphthyl phosphate) and one-half of the aerosol was added to one portion and the chromogen (anthroquinone-1 diazonium hydrochloride) and the remainder of the aerosol was added to the other. A portion of each of the substrate solution and chromogen solution was reserved and an equal volume of buffer added to each to equalize control and test solution concentrations. The test solution proper consisted of a mixture of equal volumes of each of the undiluted substrate and chromogen solutions.

Three control tests were made for each evidence test:

1. A test using buffered substrate (diluted) on the stain
2. A test using buffered chromogen (diluted) on the stain

One small drop of each of these two solutions was added directly to the stain at separate points

3. A test using the mixed test solution

One small drop of the mixed reagent was added to the cloth adjacent to but not touching the stain.

After 2 minutes the control tests were examined. If any of the control tests develop color, the acid phosphatase test may give unreliable or misleading results. If the control tests produced no color, a small drop of the mixed reagent was added to the

TABLE II

Name and Manufacturer	Composition According to Label	Tests on 2 mgs Powdered Medicinal Substance a.	b.	Tests on Solution of Medical Substance* a.	b.	Tests on Stain Found by Evap. of Solutions of Col. IV a.	b.
Antiva Suppositories, A. O. Schmidt Co., San Francisco, Calif.	Boric acid, alum, thymol, berberine hydrochloride, monochlorothymol, aromatics, cocoa butter	NR	NR	Not Soluble		Not Soluble	
Bo-Car-Al, Hygienic Powder, Sharpe & Dohme, Philadelphia, Pa.	Boric acid, potassium alum, phenol, oil of of eucalyptus, menthol	++ Red-brown	RWC	NR	NR	Not tested	
Creolin, Merck & Co., Rahway, N. J.	Coal tar, neutral oils, phenols, soap	++ Red-brown	RWC	++ Red-brown	RWC	+ Red brown	RWC
Lysol, Lehn & Fink Products, Corp., Bloomfield, N. J.	Soap, o-hydroxydiphenyl alcohol, cresylic acid, propylene glycol, glycerol	++ Red-brown	RWC	++ Red-brown	RWC	+ Red-brown	RWC
Massengill Powder, S. E. Massengill Co., Bristol, Tenn.	Boric acid, ammonium alum, berberine salt, carbolic acid, menthol isomers, thymol, eucalyptol, methyl salicylate	+ Orange	RWC	NR	NR	Not Tested	
Pazo Suppositories, Grove Laboratories, St. Louis, Mo.	Bismuth subgallate, resorcin, camphorated phenol, zinc oxide, benzocaine, camphor, boric acid, cocoa butter	NR	NR	Not Soluble		Not Soluble	
Pepsodent Antiseptic, Lever Bros. Co., Chicago, Ill.	Chlorthymol, boric, malic, citric acids, alcohol	NR	NR	NR	NR	Not Tested	
Stomaseptine, Stom Aseptine Corp., New York	Sodium perborate, sodium bicarbonate, sodium chloride, borax, menthol, thymol, eucalyptol, methyl salicylate, aromatics	+ Red-brown	RWC	NR	NR	NR	NR
Takara Douche Powder, Takara Laborities, Hollywood, Calif.	Alum, oil of peppermint, boric acid, carbolic acid	NR	NR	NR	NR	Not tested	
Trichotine, Fesler Company, Stambord, Conn.	Sodium lauryl sulfate, sodium perborate, sodium borate, thymol, eucalyptol, menthol, methyl salicylate	+ Red-brown	RWC	NR	NR	NR	NR
Tyrees Antiseptic Powder, J. S. Tyree, Washington, D. C.	Zinc sulfate, carbolic acid, boric acid, salicylic acid, menthol, eucalyptol	+ Brown	RWC	NR	NR	Not tested	

* Prepared per instructions on label to prepare douche. a. Without controls. b. With controls. RWC. Reacted with Chromogen.

stain, and the results noted after 2 minutes at room temperature. If a red coloration was formed, sufficient acid phosphatase was present to justify a conclusion that the stain was of seminal origin.

Results

Results on 2 mgs. each of a number of pure chemicals are shown in Table I. Ten substances responded to the test with production of red, red-brown, or yellow-brown color.

Results on eleven commercially available proprietary drugs as received, in solution recommended by manufacturer for use as vaginal douche, and on the stain caused by evaporation of such a solution are shown in Table II. Six of these preparations responded to the test as pure substances; two responded in dilutions recommended as a douche, and these two also gave positive tests 24 hours after the douche had been applied and allowed to dry. Several of the douche solutions responded with faint reactions when diluted as recommended as a vaginal douche; if the active material concentrations were higher, as when these substances were prepared for use as a general antiseptic, germicide, or with accidental increase in active material, additional false positive tests could be expected.

Discussion

Many chemical substances and some medicinal substances may interfere or may cause serious error with this type acid phosphatase test unless proper controls are used. Especially remarkable is lysol and creolin, both recommended by manufacturer for vaginal douches, which gives a marked positive test in solution diluted 1:200 with water. In all interference cases tested, proper control tests revealed the fact that an interfering substance was present. The same principles are applicable to other test methods for detection of enzymes which use as a test indicator the presence or absence of a colored substance formed from a hydrolytic product of an added substrate and a very reactive, non-specific chromogen.

References

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