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A MORE SENSITIVE MODIFICATION OF THE ZINC TEST FOR SEMINAL TRACES SUITABLE FOR STABLE TEST PAPER STRIPS

P. HOOFT, H. VAN DE VOORDE and P. VAN DIJCK

Katholieke Universiteit Leuven, Laboratory of Forensic Medicine, Kapucijnenvoer 35 niv. 03, B-3000 Leuven, Belgium

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Summary

A more sensitive modification of the zinc test for semen has been developed, which can be used either as a solution or incorporated into test papers. The latter retain their sensitivity for at least 3 months.

Key words: Semen; Presumptive test; Zinc; Test papers

Introduction

In 1983 Suzuki et al. suggested that the high concentration of zinc in seminal plasma could be a valuable basis for the primary qualitative detection of seminal stains [3]. For their experiments they used the following 1-(2-pyridylazo)-2-naphthol (PAN) reagent: 10 mg of PAN were emulsified in 2 ml of Triton X-100, to which 98 ml of a 0.5 M Tris solution (6.057 g tris(hydroxymethyl)aminomethane in 100 ml distilled water) was added.

The experiments of Suzuki, however, did not contain any comparison to the classical acid phosphatase tests, which can be seen as a golden standard in most Western countries. Such experiments have been carried out by the present authors and showed that the zinc test had an inherent lower sensitivity than the classical acid phosphatase test, but is less fragile and more specific for semen [1]. In addition, a study on vaginal swabs taken after different time periods of sexual abstention showed a very great consistency between a positive zinc test and the microscopical finding of spermatozoa [2].

When used as a spot test reagent the zinc test is not suitable for the investigation of dark colored textiles and it may involve the spoiling of the evidence material being investigated. As some stable test paper variants of the acid

Correspondence to: P. Hooft, Katholieke Universiteit Leuven, Laboratory of Forensic Medicine, Kapucijnenvoer 35 niv. 03, B-3000 Leuven, Belgium.

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phosphatase test are largely available, this might (inter alia) be a reason why the zinc spot test did not receive much further attention. In view of the earlier reported positive evaluation of the zinc test, however, it seemed worthwhile to the authors to develop stable test paper strips for the zinc test. As the PAN reagent proposed by Suzuki has the disadvantage of being rather hydrophobic due to the Triton X-100 component, a number of other detergents and solvents was studied. Indeed, since the naphthol dye used in the zinc spot test is insoluble in water, a detergent is needed anyhow.

Modifications and Results

In an initial step, several detergents and solvents were tried as replacements for Triton X-100. The results are summarized in Table 1. Although the naphthol dye could be emulsified or dissolved in some of the detergents and solvents, most of them showed a precipitation on the addition of the buffer solution. None of the alternatives tested was any better than the Triton X-100.

Next, the minimal volume of Triton X-100 needed to keep the dye in solution was assessed without modification to the other components. The volume could

TABLE 1

OBSERVATIONS WITH DIFFERENT SOLVENTS AND DETERGENTS USED FOR EMULSIFICATION OF 1-(2-PYRIDYLAZO)-2-NAPHTHOL IN THE ZINC TEST REAGENT

Detergent/solvent	*Observation after addition 0.5 M Tris buffer*
Diethyleneglycol (Merck)	Precipitation
Dimethylsulfoxide (Merck)	Precipitation
Propyleneglycol (Merck)	Precipitation
Methanol (Merck)	Precipitation
Ethanol (Merck)	Precipitation
Acetone (Merck)	Precipitation
Tween 80 (BBL)	Precipitation
Chremophor EL (BASF)	Solution, positive test pale red, low sensitivity
Chremophor RH 40 (BASF)	Precipitation
PE/F 127 (ICI) 10 g%	Partial solution, intermediate precipitation
BRIJ 78 (ICI) 10 g%	Partial solution, intermediate precipitation
20 g%	Solution, weak color reactions, decrease in sensitivity, late precipitation
BRIJ 98 (ICI) 10 g%	Partial solution, intermediate precipitation
BRIJ 700 (ICI) 10 g%	Partial solution, intermediate precipitation
Triton X-15 (Rohm & Haas)	Insoluble
Triton X-45 (Rohm & Haas)	Cloudy solution
Triton X-102 (Rohm & Haas)	Clear solution, comparable to Triton X-100
Triton X-114 (Rohm & Haas)	Cloudy solution
Triton X-305 (Rohm & Haas)	Partial solution, intermediate precipitation
Triton X-405 (Rohm & Haas)	Partial solution, intermediate precipitation
Triton N-42 (Rohm & Haas)	Insoluble
Triton N-60 (Rohm & Haas)	Cloudy solution
Triton N-111 (Rohm & Haas)	Clear solution, comparable to Triton X-100

be reduced to 0.7 ml, at the cost, however, of the occurrence of a dye precipitation within 3 days of storing and a decrease in sensitivity of the test (mean minimal detection titer for semen 1:16 compared to 1:64 with the original reagent).

As a result of the experiments where mixing of several detergents and solvents was investigated the following modified reagent was finally retained: 10 mg of 1-(2-pyridylazo)-2-naphthol (UCB, Belgium) was emulsified in 0.7 ml Triton X-100 (Aldrich Chemie, Belgium). This emulsion was left standing at room temperature for 30 min, then 1.3 ml dimethylsulfoxide analytical grade (Merck, Germany) were added with stirring. Finally, 98 ml 0.5 M Tris buffer (6.057 g tris(hydroxymethyl)aminomethane (Merck, Germany) in 100 ml distilled water) were added. This reagent composition showed a higher sensitivity than the original reagent (mean minimal detection titer for semen 1:128). This might be the result of the enhanced color difference between positive and negative tests. The original reagent changes from yellow to rose-red, whereas the modified reagent changes from yellow to the darker purple-red. In addition, the modified reagent is as stable as the original one and can be stored in the dark for more than 3 months without loss of sensitivity.

Finally, experiments were carried out with the modified PAN reagent to develop a test paper variant of the test. Several zinc-free carrier papers (Whatman filter paper, chromatography paper, drawing paper) were tried with similar results. They were saturated with the modified PAN reagent and left to dry in an incubator at 37 °C. Dried stains of a twofold dilution series of semen were used to study the sensitivity. The stains were moistened with one drop of physiologic saline and the paperstrip was pressed onto them. The color change of the positive test was the same as the one observed in test tubes. The mean minimal detection titer was the same as for the spot test variant (1:128). As could be expected the color reaction fades with lower semen concentrations. When stored dry in an airtight opaque container the paperstrips were stable for over 3 months.

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