Evaluation of Sperm Specific Lactate Dehydrogenase Isoenzyme C4 (LDH C4). Application to Semen Detection in Stains

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INTRODUCTION

Lactate dehydrogenase is present in most tissues as a series of 5 tetrameric isoenzymes, but in semen 6 isoenzymes can be found. The sixth is LDH C4 or X which is specific for semen and has never been observed in any male and female tissues other than testes and spermatozoa (Wheat and Goldberg 1983).
LDH C4 activity accounts for about 80 % of the total LDH activity in spermatozoa, and is present largely in the mitochondria but partly also in the cytosol. In previous experiments (Pawlowski et al. 1988) we observed that LDH C4 is relatively stable in liquid semen and mixtures of semen with vaginal washings incubated in vitro.
The main aim of the work was the analysis of LDH C 4 in semen, semen stains and mixtures using IEF and to establish a reliable and sensitive method of semen detection, which could be used as an alternative to micros-copical searching for spermatozoa.

MATERIALS AND METHODS

Samples analysed: Fresh semen, blood, saliva, vaginal swabs. Semen stains and mixed stains were stored at room temperature for up to 8 months.

Electrophoretical methods: LDH isozymes were separated on 1% agarose gel (Shaler 1981), and on ultra-thin PAGIEF, pH 3-10 and 6-8 with 0.4 M beta alanin as a separator. LDH C was determined semiquanitatively using rocket immunoelectrophoresis, with subsequent SDS electrotransfer and avidin-biotin detection system, and using a dot blot method.
Total LDH activity was detected using lactate staining.LDH C was detected specifically using alpha hydroxyhexanoate or antibody against LDH C (LeVan et al.1991).

PCR: Presence of sperm and other male cells in vaginal swabs was analysed with X and Y chromosome specific primers flanking a segment of the amelogenin gene (Nakahori 1991).

RESULTS AND DISCUSSION

Conventional agarose gel electrophoresis (Fig. 1) and PAGE of LDH isoenzymes present in semen or testicular tissue homogenates show one additional band in comparison with blood and other human fluids or tissue extracts (Goldberg 1963).

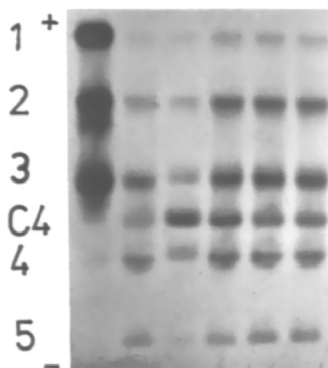

Fig. 1: Agarose gel electrophoresis of LDH isoenzymes.
From left to right: Blood, seminal fluid, lysed spermatozoa and three semen samples. Staining for total LDH activity

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Figure 2 presents IEF pattern of LDH pattern of LDH in semen, blood and mixtures analysed in broad pH 3-10. Multiple bands of LDH are present both in blood and semen samples with some additional bands in semen. Under these conditions it is possible to detect LDH C bands up to dilutions where semen consist of 1/50 part of the mixture.
Semen specific bands are more easily recognized in the narrow pH range from 6 to 8. The use of 0.4 M beta alanine as a separator improved separation pattern.
Figure 3 shows LDH C4 pattern obtained after immunodetection with anti LDH C. Multiple bands with different activities are present showing microheterogeneity of LDH C, indicating that LDH C subunits are able to form heterotetramers with other LDH subunits similar to the A and B homo- and heterotetramers present in fluids other than semen.
No variants were observed in 120 samples, of fresh semen lysed using mechanical or chemical methods. Pseudopolymorphism of LDH C, could be observed after 2-3 freeze-thaw cycles. Microheterogeneity of LDH A and B in blood and some tissue cytosols was found in 1988 using IEF (Romero-Saravia et al. 1988). No papers presenting microheterogeneity of LDH C have yet been presented.

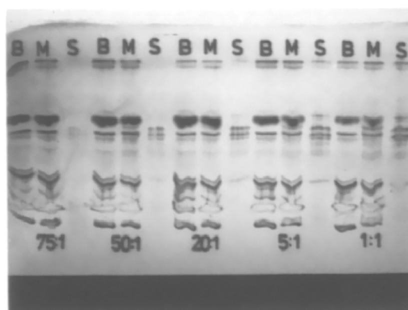

Fig. 2: IEF pattern of blood and semen LDH isozymes separated on pH 3-10 polyacrylamide gel. Semen sample /S/ and blood /B/ were mixed /M/ in different ratios /S:B 1:1 - 1:75/ and subjected to IEF. LDH activity was detected using lactate staining

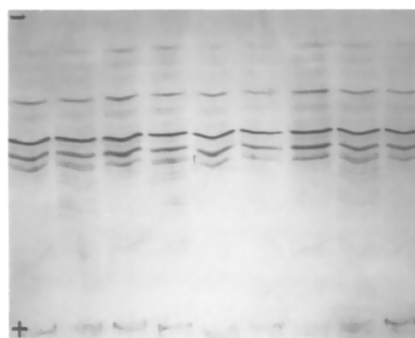

Fig. 3: IEF pattern of LDH C isozymes from human semen. Semen samples were separated on 200 μm thick PA gel at pH gradient 6-8 with beta alanin as a separator. After blotting LDH C bands were detected using specific antibody against human LDH C and biotin system

FORENSIC APPLICATION OF LDH C

The presence of semen in stains or swabs can only be documented with certainty by the microscopic detection of spermatozoa or by presence of other semen specific components such as LDH C4, p 30 etc. Because LDH C is a semen specific component it could be used as a semen specific indicator in mixtures of semen with other body fluids.

Dot blot analysis. LDH C and other LDH isoenzymes are tetramers, which in stains are usually present in a denatured form. During the extraction process special conditions are needed, to restore the tertiary and quaternary structure of the enzyme.
The best results were achieved after at least 12 hours extraction at 4°C in a buffer containing DTT, Triton X-100, BSA and potassium chloride at pH 8.0.
The stability of LDH C in semen stains as a function of storage time and sperm count is shown in Fig. 4. After 8 months about 50 % stains with normal sperm counts still gave positive reactions. It was also possible to detect LDH C in semen samples with very low sperm concentrations. Even some semen stains with no detectable sperm gave weak positive reactions.

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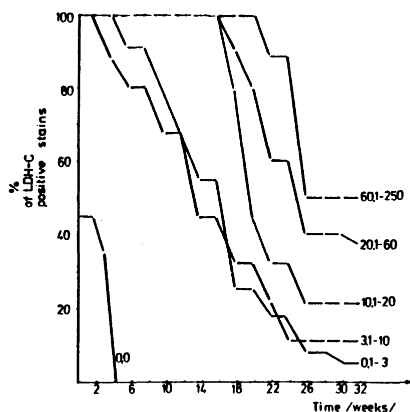

Fig. 4: Stability of LDH C in semen stains.Semen stains with different sperm counts ranging from 0-250 mill/ml were stored at room temperature for 8 months. LDH C was analysed using dot blot method and avidin-biotin detection system

We also compared different methods of LDH C detection (agarose gel electrophoresis, IEF, rocket immunoelectrophoresis and dot blot) with microscopical searching for sperm. Results from some stains showed that dot blot and rocket immuno-electrophoresis gave better results than microscopical searching for spermatozoa. LDH C analysis was also applied to the semen detection in vaginal swabs.The results of microscopic searching for spermatozoa and LDH C analysis using dot blot method were similar, but PCR amplification of a Y chromosome specific sequence was superior to both these methods.

SUMMARY

IEF analysis of semen specific LDH C reveals the microheterogenic nature of this enzyme but polymorphism of LDH C was not observed.

LDH C could be used as a semen indicator in stains. Sometimes LDH C analysis gave better results than classical sperm searching method.

The LDH C is detectable in stains over relatively long time periods but depends on sperm concentration.

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