Flowcytometry - A Novel Approach to Isolate Sperm and Vaginal Cells from Postcoital Swabs

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The preferential lysis is a well established method for isolating sperm DNA from mixed stains. However, this process is tedious and lacks potential for automation. Moreover, chances for successful typing are limited when only few spermatozoa and numerous vaginal cells are recovered from a swab. To overcome these problems a protocol involving fluorescence activated cell sorting (FACS) of vaginal cells and sperms on a flowcytometer was developed. To that end different quantities of sperm were added to vaginal swabs which were afterwards cut into two identical portions. One portion of each swab was extracted using the preferential lysis protocol. The other portion was prepared for flowcytometry: The cells were extracted from the swabs, fixed with paraformaldehyde and saponin and labeled with monoclonal pancytokeratin antibodies followed by staining with FITC marked MHCI, Anti-CD45 antibodies and FITC marked secondary pancytokeratin antibodies. Finally the DNA was stained by incubation with RNAse and propidiumjodide. Using a flowcytometer with a piezosorter (FACSort, Beckton Dickinson) sperms and vaginal cells were sorted using the different quantity of the DNA, the different scattering of light, and differences in the expression of MHCI antigen, CD45 antigen and pancytokeratin as parameters. From both fractions DNA was extracted with a standardized organic extraction protocol. Finally both flowcytrometrically and conventionally isolated sperm DNA was amplified for three STRs in a multiplex assay.

All swabs which had been successfully typed with DNA isolated by preferential lysis were also positive using the DNA isolated after flowcytometry. Therefore, due to its high potential for automatation and its sensitivity, the flowcytometrical isolation of sperms seems to be an interesting alternative to conventional preferential lysis, especially for laboratories with high sample throughput.