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Document Title: Spermatozoa Capture During the Differential Extraction Process for STR Typing of Sexual Assault Evidence

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Phase 1 involved the evaluation of three different anti-sperm antibodies obtained through ATCC. The first was the MHS-10 antibody developed by John C. Herr and R.M. Wright, University of Virginia Health System, and covered by U.S. Pat. 5,602,005. It is an IgG monoclonal antibody which reacts with an intra-acrosomal antigen (designated SP-10. It does show some cross-reactivity with analogous sperm antigen from other primates. It reacts with adluminal spermatids and mature sperm. The MHS-10 antibody does not react with other primate tissue other than sperm, and we have demonstrated that after attachment to magnetic beads it has the ability to capture 90-95% of spermatozoa. The second anti-sperm antibody is NUH-2. It was developed by E. Nudelman et al., The Biomembrane Institute; Hyogo Medical College, and covered by U.S. Pat. 5,227,160. It is an IgM monoclonal antibody which reacts with a ganglioside containing disialyl I structures, and inactivates human sperm. We have demonstrated that after attachment to magnetic beads it can capture approximately 80% of spermatozoa, primarily binding to the tail region. The third anti-sperm antibody that we evaluated is HS-21. It was developed by D.P. Wolf et al., and described in Biol. Reprod. 32; 1157-1162, 1985. It is an IgG monoclonal antibody directed against mammalian sperm, and has been shown to react with an antigen on the acrosomal cap of mammalian sperm. We have demonstrated that after to attachment to magnetic beads it can capture approximately 60% of spermatozoa, primarily binding to the acrosomal cap. Cell lines producing each of the three antibodies were grown, and milligram quantities of each antibody were purified.

Phase 2 involved the evaluation of commercially available magnetic beads and the development of an attachment chemistry that would maximize the binding of the antibodies to sperm. Three different types of magnetic beads were evaluated including the Dynal M450-epoxy bead, the Dynal M450-tosyl activated bead, and the Bangs Laboratories amino functionalized bead. We selected the Dynal M450-epoxy magnetic bead due to its highest antibody loading potential. The Dynal M450-epoxy magnetic beads were functionalized by the addition of a maleimide group. The maleimide group is then used to attach the antibody to the bead by the addition of a sulfhydryl group. The sulfhydryl groups were produce by reduction of the antibodies with 2-amino-ethanethiol. Each of the three antibodies was successfully attached to the Dynal M450-epoxy magnetic beads. The binding efficiencies of each of the anti-sperm/magnetic bead reagents were then evaluated. To simulate sexual assault evidence we obtained cultured vaginal epithelial cells. The genetic profiles for the vaginal epithelial cell line and all anonymous sperm donors were determined for the 13 core STR loci. To demonstrate the specificity and sensitivity of the initial antibody coated magnetic beads, the ability to capture of spermatozoa was tested. Mixtures consisting of varying numbers of vaginal epithelial cells and sperm were prepared and placed into 1.5 ml centrifuge tubes. The ratio of the antibody-coated magnetic beads to cells was also varied. Both the bound and unbound portions were stained and visualized by light microscopy. The number of recovered cells (both sperm and vaginal cells were counted. The MHS-10 antibody coated magnetic bead proved to be the most efficient, binding between 90-95% of the input sperm. None of the three antibodies bound the epithelial cells. Following the magnetic capture of the sperm, a rigorous washing is desirable to ensure the separation of

the sperm from any other cells or cellular debris present in the evidentiary sample. After 30 seconds of vortexing or sonication, greater than 95% of the sperm were released from the magnetic beads. This experiment demonstrated the necessity to modify the antibody/magnetic bead reagent to enhance the retention of the sperm.

Phase 3 we chemically modified the antibody/magnetic bead reagent to allow the formation of a more permanent covalent bond to enhance the retention of the sperm. The formation of covalent binding between the spermatozoa and the antibody through photochemical activation will enhance the retention of the spermatozoa to the magnetic beads. This enhanced binding would be advantageous during the rigorous wash cycles performed during sperm/epithelial cell separation. We also postulated that the covalent attachment of the sperm to the magnetic bead should provide a mechanism to retain cellular components of the spermatozoa following the final lysis of the sperm head. This would allow the direct amplification of the lysate without the need for any additional cleanup. We have evaluated several different photoaffinity labels for the covalent attachment of the sperm to the antibody/magnetic bead reagent. We concentrated on photoaffinity labels which utilize the arylazide group for the light activated attachment step. When light activated, arylazides lose diatomic nitrogen producing the reactive nitrene intermediate. Nitrenes are known to form covalent bonds to neighboring molecules by addition to unsaturated linkages or insertion into single covalent bonds (C-H or C-C). Thus, attachment of the arylazide group to one molecule allows it to be covalently coupled to a second molecule. Active esters of photoaffinity labels such as the arylazides are commercially available and protocols for their use in decorating proteins are well developed. NHS activated esters, for example, will react with primary amino groups of proteins (*e.g.* lysine residues) producing stable amide bonds. Photoactivation of the decorated protein, after binding to a receptor, will produce a covalent adduct between the photoaffinity label and the receptor. Several photoaffinity labels with differing reactivities and geometries were tested for sperm binding. The photoaffinity labels can be selectively attached to either the N-terminus of the protein, or to lysine residues throughout the protein. Our initial experiments involved the attachment of the photoaffinity label ANB-NOS (N-5-azido-2-nitrobenzoyl-oxysuccinimide). At the concentrations we attempted, the binding efficiency of the MHS-10 antibody/magnetic bead was dramatically reduced to only a few percent. However, those sperm that were attached, could not be dislodged by either vortexing or sonication. Modifications to the antibodies which alter the recognition site will have an adverse affect on the initial sperm capture. We subsequently tried the attachment of a photoaffinity label with a longer spacer group, SFAD (sulfosuccinimidyl[perfluoroazido benzamido]-ethyl 1,3 dithioprppionate). After the initial sperm binding and photoactivation, approximately 30% of input sperm appear to be covalently attached to the photoaffinity labeled MHS-10 antibody/magnetic bead reagent. Control experiments have shown that using the same photoaffinity labeled MHS-10 antibody/magnetic bead reagent without the photoactivation step, the bound sperm were released after vortexing or sonication. **The photoaffinity labeling event was found to strengthen the attachment of the sperm to the magnetic bead such that it was no longer disturbed by vortexing or sonication.** Other photoaffinity labels and attachment chemistries will continue to be evaluated in an attempt to maximize the binding and recovery of sperm

MiraiBio Inc. entered into discussions with the University of Virginia and Dr. John Herr's lab in an attempt to develop some collaborative efforts. Dr. Herr has purified the SP10 protein which is the antigen in sperm recognized by the MHS-10 antibody. In November of 2001, Dr Herr's lab provided MiraiBio, Inc. with a sample of the SP10 protein. The binding of the photoaffinity labeled MHS-10 antibody bead complex to the SP10 protein following photoactivation is monitored by gel mobility shifts on denaturing PAGE gels. Initial gel shift experiments have not provided any significant data. We remain confident that the availability of the SP10 protein will be extremely valuable in optimizing the photoaffinity labeling of the MHS-10 antibody. In addition Dr. Herr has other sperm specific antibodies that may be as good if not better than the MHS-10 antibody we are currently evaluating. It may be possible to photoaffinity label a cocktail of several sperm specific antibodies to maximize the recovery of sperm from evidentiary samples.

Phase 4 demonstrated that DNA isolated from sperm captured following binding with the SFAD labeled MHS-10 antibody/magnetic bead and photoactivation could be amplified with the CODIS core STR loci. Varying numbers of sperm (5,000; 1,000; 500; and 100) were mixed with 10^4 vaginal epithelial cells in 0.5mL PBS containing 0.1% BSA. The samples were incubated at 4°C for 1 hour with inversion (circa 60 rpm). The samples were submerged in ice-water and were photolysed for 5 minutes with a mercury vapor lamp (300 watts) with gentle agitation. After photolysis, the beads were captured with a stationary magnet (Dynal MPC-S), the supernatant was removed, and the bead pellet was washed (with vortexing) 3 times with PBS containing 0.1% PBS. The magnetic beads with covalently bound sperm were resuspended in TE⁻⁴ containing 1% DTT. The magnetic beads containing bound sperm protein were captured with the Dynal magnetic. The supernatant containing the sperm DNA was concentrated by Microcon filtration. Complete profiles were obtained from mixtures with 5×10^3 sperm cells. Only partial profiles (D3S1358, vWA, amelogenin, D8S1179, D21S11, D5S818, D13S317, and THO1) were obtained from mixtures containing 500 sperm. No profiles were obtained from mixtures containing 100 sperm. The presence of DNA from the epithelial cells was not detected in any of the mixtures following magnetic bead capture. These preliminary results indicated that the capture, photactivation and wash procedure would yield clean type able DNA. Further experiments in which the Microcon concentration/filtration is omitted have yet to be performed.

The one year funding provided by the National Institutes of Justice, Office of Justice Programs has been successfully used to demonstrate the proof of concept for the development of a sperm specific antibody/magnetic bead reagent. The necessity for the further modification of this antibody/magnetic bead reagent with a photoaffinity label has also been demonstrated. The optimization of the photoaffinity labeling has yet to be completed. We believe that the photoactivation and the formation of a covalent linkage between any sperm specific antibody/magnetic bead reagent is necessary for the ultimate goal of automation. Future funding will be required to further optimize the photoaffinity labeling process and the incorporation of the reagent into an automatable process for the recovery of sperm from sexual assault evidence.

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Office of Justice Programs

CATEGORICAL ASSISTANCE PROGRESS REPORT

The information provided will be used by the grantor agency to monitor grantee cash flow to ensure proper use of Federal funds. No further monies or other benefits may be paid out under this program unless this report is completed and filed as required by existing law and regulations (Uniform Administrative Requirements for Grants and Cooperative Agreements — 28 CFR, Part 66, Common Rule, and OMB Circular A-110).

1. GRANTEE University of North Texas Health Science Center 3500 Camp Bowie Blvd Fort Worth, Texas 76107-2699		2. AGENCY GRANT NUMBER 2000-IJ-CX-K0009	3. REPORT NO. 2
4. IMPLEMENTING SUBGRANTEE		5. REPORTING PERIOD (Dates) FROM: July 1, 2001 TO: Dec 31, 2001	
6. SHORT TITLE OF PROJECT Spermatozo Capture During the Differential Extraction Process for STR Typing of Sexual Assault Evidence		7. GRANT AMOUNT \$272,870	8. TYPE OF REPORT <input type="checkbox"/> REGULAR <input type="checkbox"/> SPECIAL REQUEST <input checked="" type="checkbox"/> FINAL REPORT
9. NAME AND TITLE OF PROJECT DIRECTOR Arthur J. Eisenberg, Ph.D. Assoc Professor, & Director DNA Identity Laboratory		10. SIGNATURE OF PROJECT DIRECTOR <i>Arthur J. Eisenberg</i>	11. DATE OF REPORT 2/6/02

12. COMMENCE REPORT HERE (Continued on plain paper)

The funding provided by the National Institute of Justice was used to develop a cellular sorting method (antibody/magnetic bead reagent capture system) for the recovery of sperm from sexual assault evidence. The design of the antibody/magnetic bead capture system was such that:

1. It utilized differentiating features (extracellular antigenic sites unique to sperm) so that the separation of cell types would be complete.
2. It would provide a more efficient means of sperm recovery, increasing the yield of DNA from the sperm fraction.
3. The DNA isolation process would be compatible with the amplification of the CODIS core STR loci.

The project was funded for one year and was given a no cost extension to December 31, 2001. The long term goal would be to develop an efficient, reliable and cost effective system for the automation of the recovery of sperm from sexual assault evidence.

The goals for the initial year of funding project were divided into four phases:

1. Evaluate a panel of antibodies directed against sperm for their ability to bind sperm heads.
2. Attach the anti-sperm antibodies to magnetic beads in such a way as to maximize their binding efficiency.
3. Chemically modify the antibodies, so that when bound to sperm they can be photoactivated, resulting in a covalent linkage.
4. Develop conditions for lysing the sperm heads and releasing the DNA, which will allow the direct amplification of the CODIS core STR loci.

Continued on next page

13. CERTIFICATION BY GRANTEE (Official signature)

Arthur J. Eisenberg

14. DATE

2/6/02