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GRANT NUMBER DAMD17-95-1-5054

TITLE: Risk Factors for Chorioamnion Infection and Adverse
Pregnancy Outcome Among Military Women

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REPORT DATE: October 1996

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

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DTIC QUALITY INSPECTED 2

19971210 101

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE October 1996	3. REPORT TYPE AND DATES COVERED Annual (15 Sep 95 - 14 Sep 96)	
4. TITLE AND SUBTITLE Risk Factors for Chorioamnion Infection and Adverse Pregnancy Outcome Among Military Women			5. FUNDING NUMBERS DAMD17-95-1-5054	
6. AUTHOR(S) Gail H. Cassell, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Alabama at Birmingham Birmingham, Alabama 35294-0111			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Commander U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, MD 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Distribution authorized to U.S. Government agencies only (proprietary information, 14 Oct 96). Other requests for this documents shall be referred to Commander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012.			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200) Cervicovaginal ureaplasma infection alone is not predictive of preterm birth. Only a subpopulation of women infected in the lower genital tract are at risk for chorioamnion invasion and premature birth. The major goal of the proposed study is to identify microbiologic factors that predispose to and/or predict chorioamnion invasion and premature birth. This study will determine if the presence of bacterial vaginosis is a risk factor for ureaplasma invasion of the chorioamnion. 243 women have been enrolled to date. Vaginal cultures from 145 of these women have been assessed for <i>Ureaplasma urealyticum</i> colonization and Bacterial Vaginosis (BV). 90/145 or 62% are positive for <i>U. urealyticum</i> . 14/68 or 21% of the gram stains for BV are positive. To date, no women enrolled in the study have gone into labor so there are no results to report from placental and amniotic cultures or baby assessment.				
14. SUBJECT TERMS Defense Women's Health Research Program			15. NUMBER OF PAGES 90	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Limited	

FOREWORD

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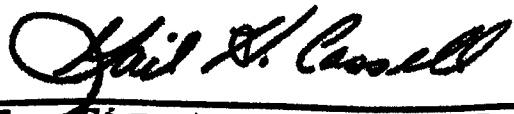
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"Risk Factors for Chorioamnion Infection and Adverse Pregnancy Outcome Among Active-duty Military Women and Dependent Women."

INTRODUCTION:

Women are playing increasing roles in active duty military service and suffering unexplained adverse outcomes of pregnancy. In the Navy, up to 20% of enlisted women become pregnant each year and nearly 15% of these pregnancies suffer adverse effects. This percentage is in excess of that experienced in most other U.S. female populations and despite a number of preliminary investigations, the difference does not appear to be related to environmental exposures.

Some of the traditional factors associated with adverse outcomes of pregnancy such as limited access to prenatal care or poor nutritional status are not operative among naval personnel, nonetheless at least 10% of Navy live births are premature (<37 weeks gestation) or low birth weight. In addition, among pregnant, enlisted women, spontaneous abortions occur in 9.9%, 2.7% of pregnancies are ectopic and 1.5% result in fetal death (0.7% early, 0.8% late). There is evidence that rates of spontaneous abortion among shipboard women may be higher than other military women and that shipboard women may frequently practice unprotected sexual intercourse (both while in port and at sea) and subsequently may acquire sexually transmitted pathogens. Bacterial infections of the lower genital tract may in part explain poor pregnancy outcomes.

Preterm birth complicates 8-10% of all pregnancies in the United States and is the leading cause of infant morbidity and mortality in the United States. We have shown that *Ureaplasma urealyticum* is the single most common microorganism isolated from the chorioamnion of women in spontaneous labor with intact membranes and in whom there are no chances for cervicovaginal contamination of the placenta (i.e. they delivered by cesarean section). Furthermore, ureaplasma infection of the chorioamnion in the absence of other bacteria was associated with birth <37 weeks even after multifactorial analysis to adjust for labor and other obstetric and demographic factors that could confound the association. Infection was inversely related to gestational age and birth weight. Other related studies indicate that ureaplasma infection is a significant cause of respiratory disease, meningitis and death in very low birth weight infants.

Cervicovaginal ureaplasma infection alone is not predictive of preterm birth. It is clear that only a subpopulation infected in the lower genital tract are at risk for chorioamnion invasion and premature birth. In order to logically design therapeutic intervention trials, one must first be able to identify those at risk.

The major goal of the proposed study is to determine whether chorioamnion infection, in particular infection by *U. urealyticum*, is associated with adverse pregnancy outcome, specifically premature birth. The study population will include both active-duty military personnel, and dependents of active-duty military personnel.

The design of the study will also allow us to identify factors that may predict chorioamnion invasion and premature birth. Specifically we will determine if the presence of bacterial vaginosis (BV) is a risk factor for ureaplasma invasion of the chorioamnion. Potential confounders of these data will be controlled through multivariate analyses. They include the presence of other sexually transmitted pathogens, in particular *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, Group B streptococci, *Trichomonas vaginalis*, *Mycoplasma hominis*, and *Mycoplasma genitalium*.

In a 3-year enrollment period, approximately 11,000 women will be screened for *U. urealyticum* cervicovaginal colonization and BV. Of these women, about 1,272 will be followed at delivery and be re-cultured for BV and *U. urealyticum*. The population of 1,272 women will consist of: 672 cesarean sections (CS) with intact membranes; 300 CS with ruptured membranes and 300 vaginal deliveries. In addition to cultures for vaginal *U. urealyticum* and BV, the 1,272 women

will also undergo culture of placental and amniotic fluid for aerobes, anaerobes, and ureaplasma/mycoplasma (placental tissue within one hour of delivery). Babies being born to these mothers will be assessed for gestational age, birth weight, and health status. Certain obstetric conditions will be examined and results recorded on the questionnaire for all 1,272 study participants: whether or not PROM occurred (membrane rupture before the beginning of contractions regardless of gestational age); whether or not there was prolonged membrane rupture or labor (membrane rupture or labor for > 24 hr). Discharge diagnostic data from the delivery admissions of the other 9,828 (11,000 - 1,272 = 9,828) study participants will also be examined using an existing computerized hospital data base.

Upon first visit for prenatal care at Naval Medical Center, San Diego (NMCS D), both active-duty and dependent pregnant women, will be invited to participate in this study. After informed consent is granted, and in conjunction with other clinical specimen collection, study volunteers will be asked to permit collection of a vaginal swab and a 10cc blood specimen. A study nurse will complete the first four pages of the Mother's and Infant's Questionnaire for the volunteer, through an interview procedure and medical record review.

At delivery, the estimated 1,272 volunteers will undergo placental tissue cultures, amniotic fluid analyses and maternal sera analyses. It is anticipated that study serum will be collected along with other admission specimens. A 5- to 10-cc specimen of cord blood will also be taken after the umbilical cord is cut. This cord blood sample poses no inconvenience, danger or discomfort to the mother or infant and is routinely collected for clinical study. The infants' nasal secretions (at external nares) will be swabbed and cultured at birth. Other infant specimens (CSF and lower respiratory secretions) will only be collected if clinically indicated as determined by the patient's attending physicians.

The studies outlined in the present proposal will allow us to determine risk factors for chorioamnion infection and association with adverse pregnancy outcome (including prematurity and spontaneous abortion). These studies will also provide a comprehensive analysis of the incidence of sexually transmitted pathogens in pregnant women in the Navy. These data not only will facilitate the future design of rational treatment strategies but they will also allow comparison of different populations of women in military service and potential environmental and sociological effects on pregnancy outcome. Considering the fact that we have recently shown *U. urealyticum* chorioamnion infection to be a significant risk factor for postpartum endometritis, the studies in the present proposal should also provide information useful for reducing hospital costs and morbidity associated with this delivery complication.

To accomplish this study, a study nurse coordinator, a clinical microbiologist and an after hours on-call technician have been hired specifically to screen and enroll patients, process specimens, examine cultures and identify all microorganisms, and interpret data. These UAB employees live in the San Diego area and are enrolling the women (active duty and dependent wives) being seen at the OB/GYN clinic of the Naval Medical Center, San Diego as their patient population.

BODY:

Experimental methods to be used in this study are identical to those detailed in the original proposal and are also included in Appendix 1.

The original start date of this study was September 15, 1995. Due to a number of unanticipated obstacles, enrollment was not begun until June 10, 1996. The reasons for delay are as follows:

1. The Naval Medical Center San Diego(NMCS D) scientific review committee reviews all research projects prior to review by their IRB. Even though this project had been approved and

awarded by the DOD, the hospital wanted to add additional patient populations. This resulted in only slight changes in the protocol but the proposal had to be presented to them in a different format. After three attempts, we finally were awarded their approval to conduct the study in their facility (Revised protocol enclosed Appendix 2). Our original proposal was to culture the placenta and amniotic fluid from only women that delivered by Cesarean section with membranes intact to preclude cervical/vaginal contamination. The cultures were to be accessed for aerobes, anaerobes and ureaplasma/ mycoplasma. The infants born to these mothers would also be followed during their hospital stay. Clinical assessment: term, pre-term, oxygen requirement, sepsis, death, meningitis, pneumonia, good general health, etc. Alterations suggested by the committee to the original proposal are as follows:

- a. For comparison over the 3 year study period, 300 women delivering by cesarean section with ruptured membranes should be cultured and their infants followed.
- b. For comparison over the 3 year study period ,300 women delivering vaginally should be cultured and their infants followed.

The overall goal for the study has not changed.

2. NMCS D IRB absolutely refused for many months to include the father's signature line on the Consent Form. The DOD and HHS require this line but the Navy did not feel that legally it was required. Finally, these two agencies came to an agreement.

3. There was a misunderstanding in Grant's Administration about the type of legal document binding the University of Alabama at Birmingham and the Naval Health Research Center (NHRC) for the transfer of funds from this institution to the Navy in San Diego. This issue has been resolved.

The clinical microbiologist reported to NMCS D in December of 1995 to begin organizing the laboratory, (space, equipment, supplies, procedure manual). The nurse study coordinator began working on this project 1/8/96.

During the six months, prior to the first enrollment, the microbiologist trained in Dr. Sydney Finegold's laboratory for two weeks as a refresher course in the current technology available for the isolation and identification of anaerobes. He also attended a three day workshop on the maintenance of the anaerobe chamber that has been purchased for this study.

Both the microbiologist and the nurse study coordinator spent many days and weeks finalizing the consent form, data questionnaire, and protocols. Appendix 3, A written protocol for each of the participating divisions of the hospital i.e. Labor and Delivery, Neonatal Intensive Care Nursery, and OB/GYN clinic were developed. In-service training in each of these areas has also been performed by the study personnel. The results of these efforts are summarized in Appendix 4. Much time and effort has been spent in educating the hospital personnel about the proposed study. Their collaborative efforts are necessary for the success of this study. Time has been spent in each of these areas to insure that our protocols do not conflict, hinder, or inconvenience the health care providers. Although enrollment space in the clinic continues to be a problem, we are confident that we will be able to meet our goals established in the original protocol.

Since patient enrollment began on June 10, 1996, 243 consented patients have been enrolled and are summarized in the following table. This number is lower than our projected enrollment. This can be explained as follows. All pregnant women (active-duty and dependent) receiving military medical care, attend clinic at the NMCS D for their initial pre-natal visit. At this visit, they make an appointment for all follow-up visits. Many of these women are assigned to outlying clinics and do not return to NMCS D until delivery. Since at this initial visit, only lab work is performed (no physical exam by physician), we are losing potential enrollees because we are not able to obtain the cervical/vaginal swabs necessary to determine the presence of bacterial vaginosis and *Ureaplasma urealyticum* colonization. Among these lost enrollees are the active duty women

which we can not afford to lose since they represent the most important population to be studied. Because of budget reductions by the DOD in the original amount requested for personnel, we cannot provide personnel for the outlying clinics. Our original budget for this project was reduced and our study personnel was cut from a Study Nurse Coordinator and 1 additional nurse and two laboratory technicians to 1 Study Nurse Coordinator and 1 technician. Additional funds of \$63,403.00 (\$35,000 FTE + \$9030 FB + \$19,373 Indirect costs) would be required to hire an additional nurse for this project.

TABLE

WEEK	# Registered	# Seen by UAB	# Consented	% of Registering Women seen by UAB	% of Women seen by UAB who enrolled
1	79	25	17	30	65
2	91	28	17	30	62
3	101	40	30	40	75
4	70	20	15	28	75
5	106	17	9	12	40
6	109	17	10	13	59
7	102	30	21	29	70
8	110	28	21	25	75
9	83	34	25	41	74
10	63	15	10	24	67
11(1Day)	18	5	3	28	60
12	102	18	15	18	83
13	85	21	15	25	71
14	101	28	15	28	54
15	101	33	20	33	61
TOTALS	1321	359	243	27	68

A solution to this problem is to have the patients collect the vaginal cultures by self-swabbing. This procedure has been used at the STD clinic at UAB and at other institutions and provides adequate specimens for analysis. A pilot study to compare self-swabbing and physician obtained specimens has been approved by the NMCS D IRB and the Naval Health Research Center (NHRC) CPHS. If the results of the pilot study are favorable, we will alter the protocol to use this method of collection. Being able to collect these specimens at the initial pre-natal visit could triple our enrollment. However, to do this, we would need additional personnel in the NMCS D clinic to

assist with patient interviews, patient chart reviews, specimen processing and to aid laboratory personnel in transporting specimens from clinic to hospital laboratory. Interviewing the potential enrollees is time consuming and processing the specimens collected and transporting them to the laboratory has proven to be extremely difficult for our two employees.

Of the 243 patients enrolled, ureaplasma/mycoplasma cultures have been completed on 145 patients. The results are as follows:

<i>Ureaplasma urealyticum</i> only	89
Negative	52
Mycoplasma species only	1
<i>Ureaplasma urealyticum</i> and Mycoplasma species	1
Overgrown with Bacteria	1
Overgrown with Yeast	1

Bacterial vaginosis is being accessed by the Nugent Gram stain method. 14/68 or 21% are positive.

To date, no women enrolled in the study have delivered their babies. The notification system is in place and the on-call person has been hired and trained. Her start date was October 1, 1996. The first enrolled patients have a projected delivery date November 1, 1996. We anxiously await the next phase of the study which includes placental and amniotic fluid cultures, and baby assessment.

CONCLUSION

The demographic, maternal health related behavior, maternal reproductive and medical history and preliminary laboratory results on the first 100 patients are summarized in Appendix 5. Of the 145 women screened, the *U. urealyticum* cervicovaginal colonization rate is 62% and of the 68 BV slides read, 21% are positive. These results fall in line with what we previously predicted.

The population is well defined and now that all the logistics have been taken care of, we propose that we can complete the study as designed. We are currently looking for additional funds (University) to hire personnel to facilitate the changes and increasing enrollment.

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APPENDIX 1

UAB STUDY

UAB STUDY STAFF PROCEDURE FOR ENROLLMENT

Each possible study participant will be identified and approached during her pre-registration appointment at NMCSD in the OB/GYN clinic Bldg. 3, 1st floor. Every effort to keep disruption of the clinic at a minimum should be made. An attempt to keep contact brief with each possible study participant should be made. However, the UAB study staff must remain available to explain the study and answer questions appropriately to the woman.

1. Identify and approach each possible study participant.
2. Introduce yourself and explain that you are contacting her to ask for her participation in an "important" study being conducted at NMCSD. Explain the study and review important points of the consent form with her. Ask her if she would like to read the consent form completely at this time. If so, provide her the opportunity to do so. If not, ask her to read it at her convenience and to call study personnel if she should have any questions. Ask her if she would like to participate in the study.
3. If consent is received at this time: Give her a pen, pencil, data questionnaire, army questionnaire, and consent form. Ask her to fill out the appropriate information.
4. When forms completed, check consent form, army form, and data questionnaire to ensure all questions have been filled out properly.
5. Give copy of bill of rights, privacy act, and consent form to participant.
6. Give her a UAB STUDY lab slip to present to lab tech.
7. Place UAB STUDY sticker on the front cover of prenatal chart (upper right corner as you are facing the chart).
8. Thank her for her participation.
9. If questions or concerns are identified, discuss study with possible participant.
10. If consent is denied:
 - a. ask her to share her reason for not wanting to participate
 - b. offer further discussion about study and answer any questions that she may have
 - c. if consent is granted after b, see #3 above
 - d. if consent is still denied; take documents back
 - e. thank her for her time and consideration
 - f. document in denial book reason for denial

UAB STUDY
NURSE COORDINATOR/CLINICAL MICROBIOLOGIST
PROCEDURE FOR FIRST PRENATAL VISIT

Six Vaginal Swabs

1. After the Clinician has obtained the vaginal swabs, the Standby will place the blue top test tube with swabs in the central green rack in "check out," upon completion of the examination.
2. UAB staff will collect test tubes from "check-out" area of OB clinic after being paged for processing in the UAB Study laboratory.
3. The swabs will be recorded in the log book and used as follows:
 - a. Express swab #1 into Mycoplasma/Ureaplasma transport media (white top) and freeze at -70°C.
 - b. Express swab #2 into sucrose buffer (red top) and freeze at -70°C.
 - c. Express swab #3 into PBS/PCR buffer (green top) and freeze at -70°C.

Vials for swabs 1,2, & 3 will be shipped on dry ice to UAB weekly (on Monday or Tuesday).

- d. Express swab #4 into InPouch Tv and onto a slide with saline for wet prep and culture. After the wet preparation is performed, the InPouch will be placed in the 37°C incubator.
- e. Swab #5 will be touched to pH paper (3.0-7.5) and before discarding the swab, it will be expressed into PBS buffer (blue top) for mucosal antibody detection

The PBS buffer for mucosal antibodies will be frozen at -70°C and shipped on dry ice to UCSD.

4. Swab #6 will be used for a Gram Stain Smear to detect Bacterial Vaginosis and before discarding the swab, it will be expressed into KOH to perform a Whiff Test. The smear will be read by the Clinical Microbiologist.
5. The results of InPouch wet prep & culture (swab #4), pH(swab #5), and the Gram Stain & KOH will be recorded.

UAB STUDY
NURSE COORDINATOR/CLINICAL MICROBIOLOGIST
PROCEDURE FOR INITIAL VISIT

Mother's Blood Serum

1. The Red Top Vacutainer tube of blood will be taken from the OB/GYN clinic laboratory refrigerator to the UAB Chorioamnion Infection Study laboratory for processing.
2. The Red Top tube will be spun at 2000 rpm's for 10 to 15 minutes and logged into the record book while spinning.
3. The serum will be aliquoted into three 1.8 ml Nalgene Cryogenic Vials (approximately 0.75 ml) and frozen at -70°C.
4. The serum will be sent to NHRC on dry ice for storage.

THE UAB CHORIOAMNION INFECTION STUDY

UAB STUDY
NURSE COORDINATOR/CLINICAL MICROBIOLOGIST
PROCEDURE FOR *Trichomonas vaginalis* CULTURE

InPouch Tv culture, *Trichomonas vaginalis*

1. The InPouch Tv culture will be read for five consecutive days and turned out on the fifth day logged in the culture book and on the daily Wet Prep and InPouch worksheet.
2. Should any *Trichomonas vaginalis* be isolated, it will be taken to Dr. Corbeil's Laboratory at UCSD during log phase to be frozen in liquid nitrogen.

Wet Prep and InPouch Tv

Reviewed By:_____

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UAB STUDY
CLINICAL MICROBIOLOGIST
PROCEDURE FOR GRAM STAINING
BACTERIAL VAGINOSIS SMEARS

Microscopic examination:

1. Direct smear from cervical swab (Gram Stain)
2. One minute crystal violet
3. Rinse, tapping off water
4. One minute Iodine
5. Rinse, tapping off water
6. Fifteen seconds decolorization
7. Rinse, tapping off water
8. Two minutes Safranin
9. Rinse, tapping off water
10. Dry
11. Examine and record results

[illegible]

UAB STUDY

UAB STUDY STAFF PROCEDURE FOR LABOR AND DELIVERY

1. Proceed to L&D when notified of delivery.
2. Collect 4 vaginal swabs, placenta, amniotic fluid, cord blood, and nasal swab (if done in L&D) from L&D staff.
3. Ask delivering physician if he/she would like the placenta taken to pathology.
 - a. If so, get pathology "chit."
4. If infant is in transition nursery or NICU proceed to those areas to collect nasal swab.
5. Make sure infant's chart/paperwork has been flagged with UAB STUDY sticker as a reminder to staff, should infant require intubation and/or an L/P further specimens should be collected for the study.
6. Thank staff for their help in collecting specimens.
7. Take specimens to UAB lab.
8. Follow lab procedures for processing specimens.
9. When procedures on placenta are complete, walk over placentas that have "chits" to the pathology department; all others should be returned to L&D.

Placenta Protocol:
 Last edited 5-28-96
 (from Wilma's protocol)

Beeped: return call to determine when the placenta will be ready.
 Allow media to warm to room temperature.

(All plates should be prepared beforehand)

1. Label everything and check to make sure information is complete.
2. enter in placenta log book
3. Fill microbial lab sheet
4. Fill in results of chlamydia & other tests micro tests performed
5. Centrifuge Amniotic Fluid in 15ml tube
6. Assemble supplies:

MEDIA needed:

Swabs:	Tissue:	Amniotic Fluid:
	1 -sucrose phosphate buffer & petri dish	
1 -T.v. Pouch	1 -T.v. Pouch	1 -T.v. Pouch
1 -PCR tube	1 -PCR tube	1 -PCR tube
1 -Thio	1 -Thio	1 -Thio
Mycoplasma transport media	Mycoplasma transport media	No mycoplasma culture
1 -Campy	1 -Campy	1 -Campy
1 -choc, BAP	1 -choc, BAP	1 -choc, BAP
1 -BrucBAgr,PEA,LKV,BBE	1 -BrucBAgr,PEA,LKV,BBE	1 -BrucBAgr,PEA,LKV,BBE
1 -HBT or V Agar	1 -HBT	1 -HBT
Glass slide	Glass slide	Glass slide

Vaginal Deliveries: SABw/CHL, Mac, MTM, PEA

CS w/ Rupture membranes:

Aerobic workup will be done in the biologic safety cabinet.

Anaerobic workup will be done in the chamber.

Label media: name and number, date, **s** or **t** or **af**
 Sarstedt tube the same
 Mycoplasma & Ureaplasma tubes

Label campy plate 37°C.

Procedure for setting up placenta.

1. Put all media and equipment in the Biological Safety Hood.
3. Open drape aseptically, touching only the corners, with an absorbent pad underneath.
4. Drop instruments onto drape.
 - blade
 - 3-4 Dacron swabs
5. Gown up.
6. Put gloves on using sterile technique
7. Open bag. (hand is no longer sterile)
8. Place placenta on the sterile field and place suction canister of amniotic fluid in hood.
9. Throw bag away. Put on new pair of gloves.
10. Pull apart the chorion and the amnion..
11. Swab area between membranes and inoculate media. *Culture #1.*
12. Make 5 pairs of parallel slits on placenta around area separated.
14. Pour media into petri dish
15. Cut 5 small pieces of tissue from the slits. *Culture #2.*
16. Cut them in half and inoculate into anaerobic transport media, evacuate in the chamber (further processing in the chamber.)
17. Mince the tissue using the scissors.
18. Cut piece of placenta at edge & place in 50 ml tube to be frozen at -70°C.
19. Dispose of blades in sharps container.
20. *Measure length-weight-depth in cm. from total I have already cut pieces*
21. Rinse instruments in sink & put in disinfectant detergent and milk.
22. Record descriptions of the amniotic fluid.
 - a.f.:
 - cloudy-white
 - clear-yellow
 - bloody
 - meconium-brownish
- cord placement:
 - center
 - out of center
 - marginal (on side)

23. Take placenta to pathology.
24. Amniotic Fluid: Take off old needle and put on new sterile one.
25. Centrifuge Amniotic Fluid in ml tube at rpm for minutes.
26. Squirt a few drops into Thio
27. Squirt a few drops into T.v. Pouch
28. Put remaining in 15 ml in conical tube.
29. Take loop and inoculate plates.
30. Freeze 15 ml tube at -70°C.
31. Inoculate Mycoplasma/Ureaplasma media

SP4 and 10B: 0.5 ml added to 2ml of media → freeze -70°C

Aerobic Plates:

37° C 5% C incubator:

Campy atmosphere:

37° C incubator

Anaerobic Plates:

37° C incubator

Twins:

Same as single birth but in duplicate.

UAB STUDY

UAB CLINICAL MICROBIOLOGIST PROCEDURE FOR MYCOPLASMA/UREAPLASMA CULTURES

BLOOD CULTURES

Blood cultures are performed only for genital mycoplasmas.

1. Inoculate 0.5 mg blood which has been collected aseptically and free of anticoagulants into 4.5 ml of 10B broth. The addition of blood to the broth will cause a slight rise in pH and color change. A 0.1 ml sample inoculated into 0.9 ml of 10B broth is acceptable from infants.
2. Once this culture is received in the lab, perform tenfold dilutions out to the fifth dilution tube of 10B. Serially dilute a 0.1 ml sample in SP4 medium (tube 2 through 5). Plate dilutions of 10B on A8 agar and plate SP4 dilutions on SP4 agar.
3. Incubate broths at 37 degrees C aerobically and the plates are incubated at 37 degrees C in CO₂.
4. Broths and plates are held for 14 days before being reported out as negative.
5. Report a positive culture by phone to the ordering physician or attending nurse as soon as mycoplasma growth has been verified.

CEREBROSPINAL FLUID (CSF)

CSF is cultured for mycoplasma species as well as *U. urealyticum* in infants.

If specimen is from a patient regardless of age where *M. pneumoniae* is to be considered, an additional SP4 plate should be plated and incubated anaerobically. Both plates and SP4 broths should be held 2 months prior to reporting as negative.

If quantity is sufficient, spinal fluid should be concentrate. See procedure for amniotic fluid or urine. Inoculate 0.1 ml fluid into 0.9 ml 10B broth with Cefobid and SP4 broth with no antibiotics and perform tenfold dilutions out through the fifth dilution tube.

1. Inoculate 0.02 ml of each 10B dilution and the original specimen onto one A8 plate. Inoculate 0.02 ml of the undiluted specimen and of each SP4 dilution onto one SP4 plate.
2. Freeze an aliquot (0.3 ml) of the original specimen at -70 degrees C for future reference.
3. Incubate broths at 37 degrees C aerobically and the A8 plate and the SP4 plate at 37 degrees C in CO₂.
4. Hold the plates and broths 14 days. Report out as negative at the end of this time frame.
5. Report positive cultures by phone to the ordering physician or to the baby's nurse as soon as mycoplasmal growth is verified. Report preliminary results and final results.

ET ASPIRATES

ET aspirates for large colony mycoplasmas as well as *U. Urealyticum*. For baby ET aspirates

received, usually the aspirate has already been diluted one to ten in 10B media. This tube even though it is already diluted is considered the neat sample.

Inoculate 0.1 ml fluid into 0.9 ml 10B broth with Cefobid and SPAN (this is second dilution) and perform tenfold dilutions out through the fifth dilution tube. (Media with antibiotics is used because of the potential for specimen to be contaminated with oral bacteria) Remove an aliquot of the original specimen and incubate as the neat sample with the diluted broths.

Inoculate 0.2 ml of each dilution and the original specimen onto one SP4 and one A8 plate.

Freeze an aliquot of the original first dilution specimen at -70 degrees C for future reference.

Incubate broths at 37 degrees C aerobically and the SP4 plate and the A8 plate at 37 degrees C in CO₂.

Hold the plates and broths 14 days. Report out as negative at the end of this time frame.

Report positive cultures by phone to the ordering physician or to the baby's nurse as soon as mycoplasmal growth is verified. Report preliminary results and final results.

BRONCHOALVEOLAR LAVAGE, PLEURAL FLUID, PERITONEAL FLUID, SYNOVIAL FLUID, WOUND ASPIRATES

Body fluids are cultured for all mycoplasma species including *U. urealyticum*, *M. hominis*, *M. pneumoniae*, *M. fermentans*, and *M. genitalium*. Fluids from potential sterile sites should be cultured in SP4(no antibiotics). Bronchoalveolar lavage should be treated as a respiratory specimen and cultured in SPAN (SP4 with antibiotics). Omit 10B/A8 cultures for *U. urealyticum* from routine BAL cultures unless BAL is from AIDS patient.

1. If volume allows, all fluids should be concentrated prior to processing for culture. Concentrate 10 fold. Centrifuge fluids at 5000 RPM for 10 minutes. Remove supernatant and resuspend pellet in at least 1 ml of residual fluid.
2. Inoculate 0.1 ml fluid into 0.9 ml 10B broth with Cefobid and SP4 broth and perform serial ten-fold dilutions out through the fifth dilution tube. Inoculate 0.02 ml of the undiluted specimen and each 10B dilution onto an A8 plate. Inoculate 0.02 ml of the undiluted specimen and each SP4 dilution onto 2 SP4 plates.
3. Make one cytospin slide and stain by DAPI from any aspirate or fluid from a sterile site.
4. Freeze an aliquot of the original specimen at -70 degrees C for future reference.
5. incubate BROTHS AT 37 degrees C aerobically, 1 SP4 plate and the A8 plate in CO₂ at 37 degrees C and the second SP4 plate at 37 degrees C anaerobically.
6. Hold A8 plates for 14 days. Hold 10B broths for 14 days. Hold SP4 broths and plates for six weeks. Report out as negative at the end of each time frame.
7. Report all positive cultures by phone to the ordering physician or nursing units as soon as growth of a mycoplasmal organism is verified.

MISCELLANEOUS CULTURES

Specimen SWABS are acceptable for the following culture sites: CERVIX/VAGINAL, URETHRA, NASOPHARYNX, THROAT, WOUND

General principles:

Use only Dacron or polyester swabs with plastic or wire shafts. Do not use wood sticks or cotton tipped swabs. Calcium alginate swabs have been found to cause inhibition in PCR reactions; therefore, we discourage the use of these swabs. These materials may be inhibitory to mycoplasmal growth.

OTHER acceptable specimens when collected aseptically in sterile container:

URINARY CALCULI (STONES)

BONE CHIPS

TISSUES (other than respiratory)

PLACENTA

LUNG TISSUE for *M. pneumoniae* and *Ureaplasma urealyticum* (in infants)

NASOPHARYNGEAL CULTURES

Depending on the age of the patient, different culture protocols should be followed. Infant cultures where *U. urealyticum* is being sought, the swab should be inoculated into 10B transport broth at bedside and transported to lab for processing. Refer to ET Aspirate procedure for neonates. For study participants or older patients where *M. pneumoniae* is suspected: Inoculate NP swab into one ml vials of SPAN. This is considered to be the original sample. Refer to sputum culture procedure and proceed.

Use only Dacron or polyester swabs with wire shafts. Calcium alginate swabs have been shown to cause inhibition in PCR reactions; therefore, we discourage the use of these swabs.

To collect a good posterior nasopharyngeal culture, insert the swab at least 1 to 2 cm into the nostril and rotate swab 360° against the mucosa. Immerse it into the vial of SPAN media or inoculate the plate directly.

Diagnostic Mycoplasma Laboratory

General Protocol

Body Fluid Collection of Mycoplasma and Ureaplasma Culture

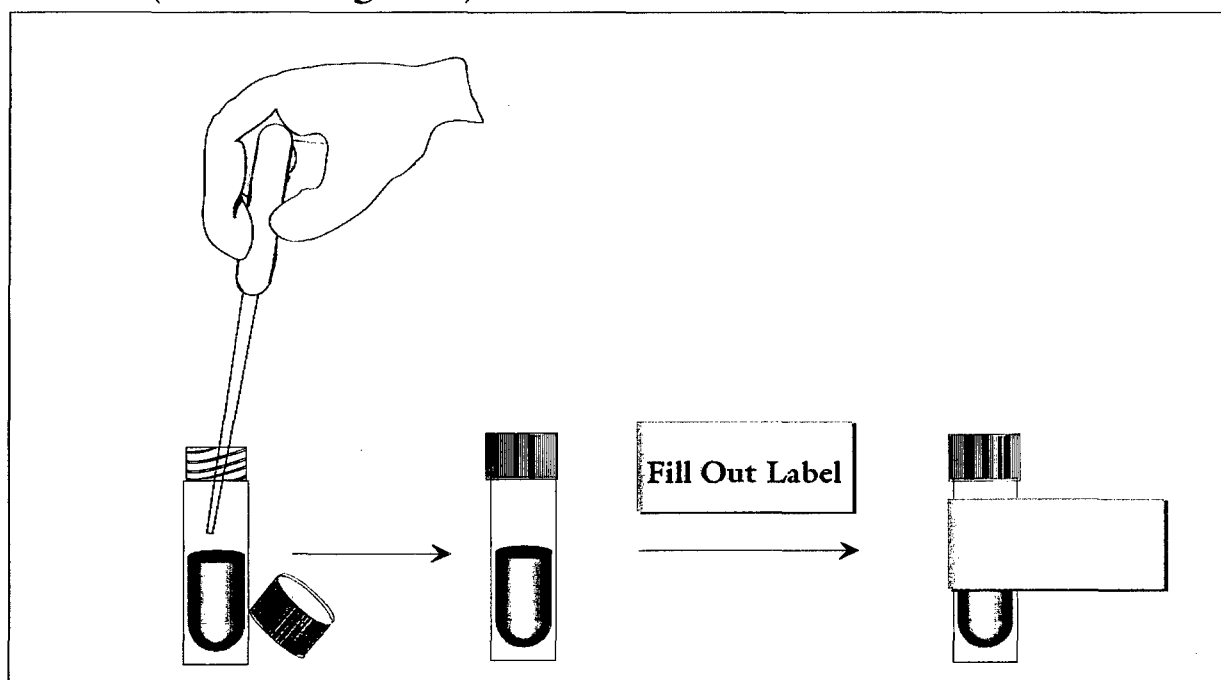
1. Collect Specimens. Specimens may include:

Sputum	Pleural Fluid
Endotracheal Aspirate	Bronchial Washing
Synovial Fluid	Cerebrospinal Fluid
Wound Aspirates	Peritoneal Fluid
Blood*	Semen
Amniotic Fluid	

2. Using a sterile transfer pipet, place 0.5 to 1.0 mL of the sample into 2mL of the transport medium
3. Fill out patient label with appropriate information and affix as pictured to the cryovial containing the respective specimen. Make sure the lid of each cryovial is tightened well.

Immediately freeze each cryovial upright in a -70°C freezer or place on dry-ice.

*Collect blood specimen in a 10mL Red Stoppered or SerumSeparator Tubes (no anti-coagulant).



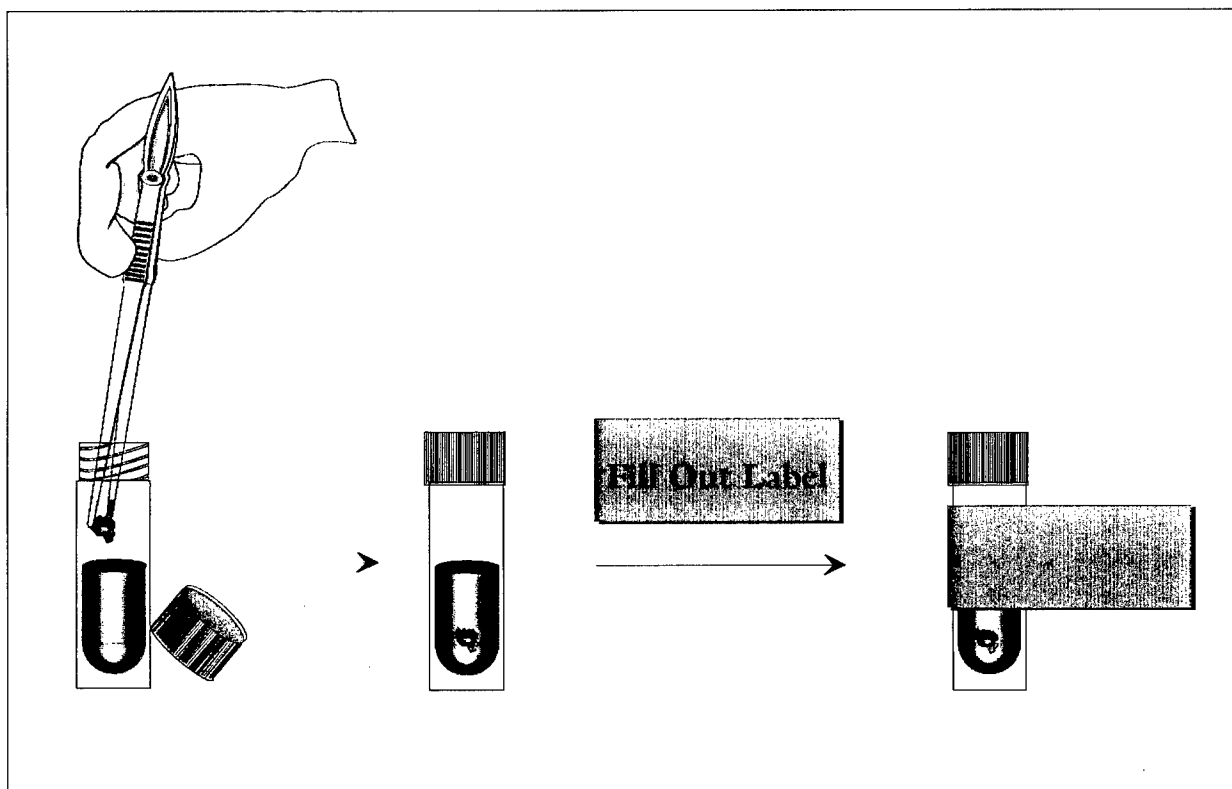
Diagnostic Mycoplasma Laboratory

General Protocol

Tissue Processing for Collection of Mycoplasma and Ureaplasma Culture

1. Collect tissue specimen. Specimens may include:
Lung Biopsy Lymph Node
Bone Chips Endometrial Biopsy
Placental Tissue
2. Place tissue into a cryovial containing the transport medium.
3. Fill out the patient label with appropriate information and affix to the cryovial, *before freezing*, containing the respective specimen (see figure). Make sure the lid of each cryovial is tightened well.

Immediately freeze each cryovial upright in a -70°C freezer or place on dry-ice.



Diagnostic Mycoplasma Laboratory

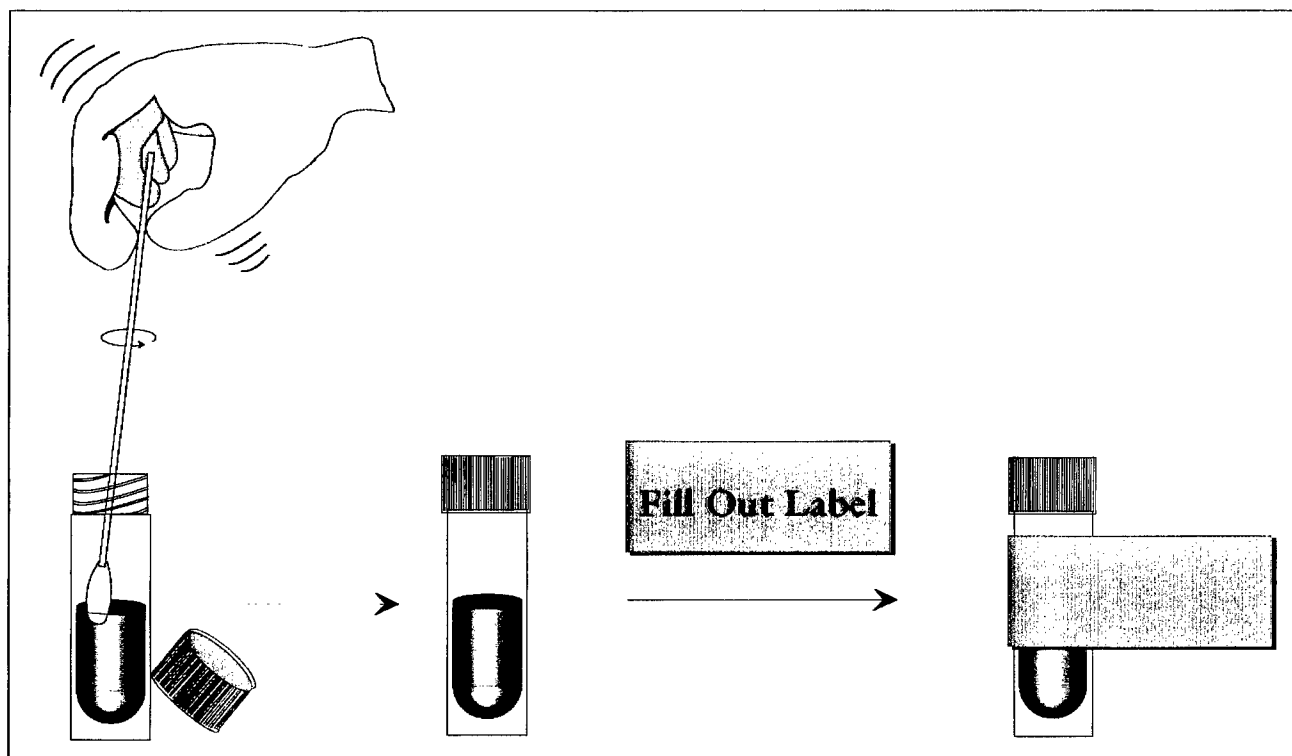
General Protocol

Swab Culture Collection of Mycoplasma and Ureaplasma Culture

1. Collect swab specimen. Use only calcium-alginate, dacron or polyester swabs with plastic or aluminium shafts. **Do not use cotton swabs!** Do not use swabs with wooden shafts! Specimens may include:

Throat	Nasaopharyngeal
Wound	Cervical/Vaginal
Urethral	
2. Place swab into the cryovial containing the transport medium.
3. Swirl. Express excess liquid from the swab by pressing the swab against the inside of the tube.
4. **Discard the swab!**
5. Fill out the patient label with the appropriate information and affix to the cryovial containing the respective specimen (see figure). Make sure the lid of each cryovial is tightened well.

Immediately freeze each cryovial upright in a -70°C freezer or place on dry-ice.



APPENDIX 2

Revised Protocol
5-10-95

Gray, 95-30284 , Risk Factors and Adverse Pregnancy Outcomes

V. SCIENTIFIC BACKGROUND AND OBJECTIVES

1. Background

Preterm birth (World Health Organization definition = birth of less than 37 weeks' gestation; Low birth weight = < 2,500 g) is the single most common cause of infant morbidity and mortality, affecting 8-10% of all births and contributing to more than 60% of all perinatal morbidity and mortality. A number of microbiologic factors have been suggested as etiologic risk factors or correlates with adverse pregnancy outcomes. A growing body of evidence supports a causal role of subclinical infection of the chorioamnion for adverse pregnancy outcome.

We have recently shown that *Ureaplasma urealyticum* (Uu) infection of the chorioamnion, in the presence of intact membranes and in the absence of other bacteria, is associated with preterm birth. Uu, a sexually transmitted bacteria, was found to be the single most common organism isolated from the chorioamnion and amniotic fluid, often in association with histologic chorioamnionitis, but in the absence of any clinical signs of infection (manuscript in process). Infection was found to be inversely related to gestational age and birth weight. Related studies indicate that Uu infection is a significant cause of pneumonia, meningitis, and death in very low-birth-weight infants (1). Uu has also been found to be a significant risk factor for postpartum endometritis (2).

We undertook a study to determine the role of Uu in chronic lung disease (CLD) of low-birth-weight infants. Endotracheal aspirates from 200 infants < 2,500 g (note the infants were not from the chorioamnion study previously described) with evidence of respiratory disease were cultured within 24 hr of birth for mycoplasmas, chlamydiae, viruses, and bacteria to evaluate the relationship of lower respiratory tract infection to development of CLD and/or death (1,3). One or more microbial agents were isolated from the tracheal aspirate of 37% of the infants. Uu was isolated from 17% and was the single most common microorganism isolated. Infants < 1,000 g who had Uu isolated from the endotracheal aspirate within 24 hr after birth were twice as likely to develop CLD and also twice as likely to die than were infants of similar birth weight but who were uninfected, or infants > 1,000 g. Very-low-birth-weight infants infected with ureaplasmas did not differ demographically from those uninfected, nor did they differ with respect to other potential risk factors for development of CLD.

That the endotracheal isolations represent true infection of the lower respiratory tract is supported by recovery of the organism in pure culture from 85% of the infants, and concomitant recovery of the organism from blood in 26% of infants. In fact, Uu was the single most common microorganism isolated from blood. That the tracheal isolates were not merely a reflection of contamination from the nasopharynx is supported by the discrepancy in isolation rates between the two sites. Further evidence that tracheal isolates represented true infection of the lower respiratory tract include:

initial isolation from tracheal aspirates in numbers exceeding 10^3 , and in some cases greater than 10^6 colony-forming units, and repeated isolations of the organisms from tracheal aspirates over a period of months. As part of another ongoing study (1,3), the cerebrospinal fluid (CSF) of infants enrolled in the CLD study was cultured and often found to be positive for Uu, again indicating the invasive nature of this organism in preterm infants. Fourteen percent of isolates were from infants born by CS with intact membranes indicating in utero infection.

Recent data indicate that enlisted women in the U.S. military forces are at greater risk for delivery of preterm birth, yet correlates of adverse outcomes of pregnancy among military personnel are unclear (4-9). Epidemiologic data suggests that groups of military women suffer from an increased incidence of spontaneous abortion, ectopic pregnancy, intrauterine growth restriction, pregnancy induced hypertension, transfer to a tertiary care facility for preterm complications, abdominal and operative vaginal deliveries, and perinatal mortality (4-7,10). The reason for these pregnancy complications and poor perinatal outcome are unknown. Several hypotheses have been or are being tested as explanations for these pregnancy complications and poor outcomes. Environmental risk factor studies have not demonstrated the aberrations are due to occupational exposure (11-13). In early studies, military women experiencing moderate to severe mental stress have evidence of increased risk but potential confounders in these studies, such as psychosocial makeup merit more comprehensive analyses (11,12). Little data has been collected regarding the sexual activities of Navy women; however, studies conducted among male Navy personnel indicate that despite education to avoid dangerous sexual contact, as many as 42% of men reported contact with a prostitute during a six month cruise (14). Uu infection (non-gonococcal urethritis) is often a result of such non-protected sexual contact and if untreated, Uu is often transmitted to future female sexual partners. Women with Uu cervicitis or lower genital tract colonization are frequently asymptomatic and readily serve as a reservoir for continued sexual transmission.

As stated earlier, numerous microorganisms have been implicated in preterm delivery and/or premature rupture of the membranes (PROM). These include: Uu, *C. trachomatis*, Group B streptococcus, *L. monocytogenes*, *N. gonorrhoeae*, and more recently a group of organisms associated with bacterial vaginosis (BV). BV occurs in 15-20% of pregnant women. Complaints of a watery discharge and a fishy odor occur in symptomatic women. However, about half of patients with this infection have no symptoms, or only very mild symptoms. Investigations have consistently shown an increased prevalence of *G. vaginalis*, selected anaerobic bacteria (most notably, *Bacteroides* and *Mobiluncus*), *M. hominis*, and a decreased prevalence of facultative lactobacilli (15-20). A thousandfold increase in the concentration of anaerobic microorganisms and a hundredfold increase in the concentration of *G. vaginalis* have been documented (21). Although Uu

is not independently associated with BV, the prevalence of vaginal colonization by *Uu* is increased about twofold, and the intravaginal concentration of these organisms is increased a hundredfold (21). The precise relationship between BV and *Uu* and premature birth is not known. Some have postulated that the increased intravaginal concentrations of BV organisms may result in increases in the synthesis of phospholipase A2 and production of prostaglandins, which may lead to preterm labor or PROM (15-19). Alternatively, others (19) have suggested that *Bacteroides* species in the lower genital tract could produce enough protease to weaken the fetal membrane strength, causing PROM and invasion by other organisms. In addition, it is possible that certain BV-associated microorganisms, like *Uu*, may be more likely to invade the intact fetal membranes simply because they are present in larger numbers (1,3). However, this latter possibility cannot be the total explanation for *Uu* association with prematurity since intravaginal concentrations of *Peptococcus* species are also increased in BV but are found infrequently in the chorioamnion and amniotic fluid.

The presence of BV is independently and significantly associated with preterm birth when cervical organisms, obstetrical and demographic factors are taken into consideration (15-19). However, these studies have not determined whether BV is associated with premature delivery independent of chorioamnion infection with organisms other than those associated with BV (i.e., *Uu*). In the study by Hillier et al. (22), multiple logistic regression was carried out to determine the strength of the relation between the recovery of any organism from the chorioamnion and BV. After adjusting for factors that were related to both BV and the recovery of organisms from the chorioamnion, patients who had an organism recovered from the chorioamnion culture had a high relative odds of BV (Odds Ratio [OR], 3.0; 95% Confidence Interval [CI], 1.1 to 6.6). As in our study, the most common organisms recovered in their study were *Uu*, *M. hominis*, *G. vaginalis*, *Peptostreptococci*, and *Bacteroides*. Our isolation rate of *Mobiluncus* was somewhat lower. This could have been due to the fact that most of their patients had PROM, or to differences in cultural methods. *Mobiluncus* is notoriously difficult to recover. Their laboratory is indeed one of the first to describe this organism.

Unfortunately, due to the small patient numbers ($n = 38$) it was not possible for Hillier and colleagues to determine the effect upon individual organisms nor to address the question of whether BV is associated with preterm birth independent of chorioamnion infection, or for that matter whether *Uu* chorioamnion infection can occur independent of BV. In our study, almost 50% of the *Uu* chorioamnion isolates were in pure culture. Although *M. hominis* and *G. vaginalis* were the next most common organisms isolated, they were not independently associated with preterm birth. Thus, the relationship between BV and prematurity with or without *Uu* infection remains unclear. A distinct possibility based upon current evidence is that both BV and *Uu* may be of etiologic significance independent of each

other; however, when present simultaneously, they may be additive. In this regard, it is of interest that in our study, the effect of Uu plus other bacteria appeared additive.

Some of the traditional factors associated with preterm birth are limited access to prenatal care and/or poor nutritional status. Military women and spouses (dependents) of active-duty military members, are screened or have access to screening for underlying medical problems, have mandatory and/or universal medical care, and job security. For these reasons they represent a unique population that is not affected by these traditional factors, thus providing a unique study population for which such confounding factors may be better controlled.

All of the above mentioned findings reveal the potential harm of a Uu infection of the chorioamnion for both the mother and infant, and show the need for a closer examination of the relationship between chorioamniotic infection and preterm birth, and BV infection and preterm birth. Our previous studies consisted of study populations with low social economic status (SES) backgrounds, varied standards of health care, and varied access to prenatal care. The current study will control for these potentially confounding factors by using a military and dependent population.

2. Objectives

a. Hypothesis to be tested

The major goal of the proposed study is to determine whether chorioamnion infection, in particular infection by Uu, is associated with adverse pregnancy outcome, specifically premature birth. The study population will include both active-duty military personnel, and dependents of active-duty military personnel.

b. Other objective

The design of the study will also allow us to identify factors that may predict chorioamnion invasion and premature birth. Specifically we will determine if the presence of BV is a risk factor for ureaplasma invasion of the chorioamnion. Potential confounders of these data will be controlled through multivariate analyses. They include the presence of other sexually transmitted pathogens, in particular *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, Group B streptococci, *Trichomonas vaginalis*, *Mycoplasma hominis*, and *Mycoplasma genitalium*.

VI. EXPERIMENTAL METHODS

1. Methods

In a 3-year enrollment period, approximately 11,000 women will be screened for Uu cervico-vaginal colonization and BV. Of these women, about 1,272 will be followed at delivery and be re-cultured for BV and Uu. The population of 1,272 women will consist of: 672 cesarean sections (CS) with intact membranes; 300 CS with ruptured membranes; 300 vaginal deliveries (see detailed description in Sample Size Determination). In addition to Uu and BV culturing, the 1,272 women

will also undergo placental tissue and amniotic fluid processing for aerobes, anaerobes, and ureaplasma/mycoplasma (placental tissue within one hour of delivery). Babies being born to these mothers will be assessed for gestational age, birth weight, and health status. Certain obstetric conditions will be examined and recorded on the questionnaire for all 1,272 study participants: whether or not PROM occurred (membrane rupture before the beginning of contractions regardless of gestational age); whether or not there was prolonged membrane rupture or labor (membrane rupture or labor for > 24 hr). Discharge diagnostic data from the delivery admissions of the other 9,828 (11,000 - 1,272 = 9,828) study participants will also be examined using an existing computerized hospital data base, but hospital charts will not be reviewed.

Upon first visit for prenatal care at Naval Medical Center, San Diego (NMCS), both active-duty and dependent pregnant women, will be invited to participate in this study. After informed consent is granted, and in conjunction with other clinical specimen collection, study volunteers will be asked to permit a vaginal swab and a 10cc blood specimen. A study nurse will complete the first four pages of the Mother's and Infant's Questionnaire (Appendix I) for the volunteer, through an interview procedure and medical record review.

At delivery, the estimated 1,272 volunteers will undergo placental tissue cultures, amniotic fluid studies and maternal sera studies (10cc blood specimen). It is anticipated that study serum will be collected along with other admission specimens. A 5- to 10-cc specimen of cord blood will also be taken after the umbilical cord is cut. This cord blood sample poses no inconvenience, danger or discomfort to the mother or infant and is routinely collected for clinical study. The infants' nasal secretions (at external nares) will be swabbed and cultured at birth. Other infant specimens (CSF and respiratory secretions) will only be collected if clinically indicated as determined by the patient's attending physicians.

The study procedure is summarized as follows:

These visits should require no more than 15 min to complete all procedures both upon enrollment and at delivery. Specific procedures for specimen collection are found in Appendix H. This study will be conducted at NMCS with the cooperation of the Departments of Obstetrics and Gynecology, Neonatology, and Clinical Microbiology Laboratory.

Initial Prenatal Visit (n = 11,000):

- Vaginal swab for Gram stain for BV evaluation
- Cervicovaginal culture (as per hospital routine)
- Serum (10cc of blood collected by venipuncture)
- A research nurse will complete a questionnaire (Appendix I) partially through personal interview, but largely from a medical record review. Much of the information on the questionnaire will be obtained from the patient's chart, and will be a secondary source of information.

At delivery (n = 1,272):

- Placenta from CS for microbiological analysis within one hour of delivery (Refer to Appendix H for a procedural description of placental tissue collection)
- Amniotic Fluid (collected at delivery for research purposes only from mothers with intact membranes)
- 10cc of Maternal blood (serum) taken during preadmission blood stored in hospital blood bank
- Vaginal swab for Gram stain for BV evaluation
- 5- to 10-cc cord blood
- Nasal swab for culture from infant

Study personnel will include a Study Nurse Coordinator, a clinical microbiologist, and an after hours on-call technician. The duties of each are as follows:

Study Nurse Coordinator (UAB staff):

Prenatal visit and Follow-up:

1. Enroll patients. This includes explanation of study to participants and acquiring signature on consent form.
2. Complete patient questionnaire page 1-4.
3. Obtain BV smear, cervicovaginal culture for ureaplasma/mycoplasma and sera.
4. Collect chlamydia and GC data from patient hospitalization laboratory records.

Delivery:

1. Obtain cord blood, BV smear, cervicovaginal culture for ureaplasma/mycoplasma, and mother's serum.
2. Obtain nasal culture on baby for UU colonization and submit to laboratory for processing.
3. Assist in processing of placenta and amniotic fluid for culture.
4. Complete data packets on women that meet study criteria.
5. Complete data packets on babies born to Study Mothers.

Clinical microbiologist (UAB staff):

1. Process placentas and amniotic fluids for aerobes, anaerobes, and ureaplasma/mycoplasma 0800-1700 Monday through Friday.
2. Read all cultures and identify all microorganisms.
3. Read all Uu cultures from prenatal screening.
4. Process and read all infant cultures.
5. Store placental tissue, amniotic fluid, mother's sera, and cord blood in -70°C freezer.
6. Assist in data collection and entry.

After hours on-call technician (UAB staff):

This person will be responsible for being "on-call" from 1700 Monday until 0700 Tuesday and likewise for Tuesday, Wednesday, and Thursday. On-call for the weekend begins at 1700 Friday and runs through 0700 Monday. Duties include placental and amniotic fluid

culture processing for aerobes, anaerobes, and ureaplasma/mycoplasma.

Cooperation from Labor and Delivery will be required for notification of study participants. All study personnel will wear pagers for easy accessibility.

For the purpose of this study, gestational age will be determined by CDR Everett Magann, M.D., NMCSD. The criteria used to make this determination will be last menstrual period (LMP), ultrasonography when available, infant's Dubowitz score, or his best estimate. Dr. Magann will be blinded with respect to questionnaire or study laboratory data.

The purpose for collection of vaginal secretions at the initial prenatal visit and at delivery is to assess whether the patient has BV. Serum collection at the initial prenatal visit and at delivery will be used to determine if lack of specific antibody (Uu) correlates or denotes a risk factor for chorioamnion infection and premature delivery. Cord blood analysis for specific antibody (Uu IgM) could be used to assess if the infant was exposed in utero.

If, at any time during hospitalization, the infant requires intubation for supplemental oxygen, respiratory secretions will be collected for research purposes. If the infant requires CSF to be tapped, CSF will be collected for culture for research purposes. These procedures will not require any alteration or increase the sampling volumes over what is normally obtained.

2. Sample Size Determination

Assumptions:

- NMCSD has approximately 3,600 deliveries each year; 1,000 of which are preterm deliveries.
- Approximately 800 of the 3,600 deliveries are via CS.
- From a NMCSD medical record review of 194 consecutive CS deliveries, the proportion of CS with intact membranes was found to be 35%.
- Conservative estimates of Uu chorioamnion infection is about 9.9% in a parturient population.
- $800 \times 35\% = 280$ CS/year with intact membranes. Assuming 80% participation rate, in three years we would have data from 672 women who delivered by CS with intact membranes.
- In three years we will have data from 300 women who delivered by CS with ruptured membranes by taking a probability sample from our study volunteers.
- In three years we will have data from 300 women who delivered vaginally by taking a probability sample from our study volunteers.
- The probability samples will be taken at the time of delivery such that each mother will have an equal chance of selection.

Then, considering our volunteers with intact membranes, assuming a power of 0.80, an alpha error of 0.05, and a prevalence of Uu

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infection of 10%, our necessary sample size for various relative risks is:

Baseline prevalence of preterm delivery in those without Uu	Relative Risk (Uu + / Uu -)	Total Sample
35.0%	1.6	520
35.0%	1.8	300
35.0%	2.0	190
35.0%	2.5	80

Conservative estimates of BV incidence in a parturient population is approximately 15% (as determined by our current study). Considering our volunteers with intact membranes, assuming a power of 0.80, an alpha error of 0.05, and a prevalence of BV infection of 15%, our necessary sample size for various relative risks is:

Baseline prevalence of preterm delivery in those without BV	Relative Risk (BV + / BV -)	Total Sample
35.0%	1.6	367
35.0%	1.8	213
35.0%	2.0	140
35.0%	2.5	60

We have chosen to focus largely on CS with intact membrane deliveries to reduce the potential bias of bacterial contamination (BV or Uu) in placenta cultures from lower tract bacterial colonization. We expect the CS intact membrane population to have less contamination risk. For the deliveries other than CS with intact membranes, we will endeavor to reduce this potential contamination bias by following the placenta culturing procedures outlined in Appendix H.

3. Statistical Procedure

Our chief outcome of interest is premature birth. We may also examine other outcomes such as chorioamnion infection and infant mortality. Potential risk factors associated with outcomes will be many and include demographic data (age, race, socioeconomic status), BV, history of sexually transmitted diseases (STD), active-duty service, and Uu culture.

Covariates will be examined by descriptive analyses, tables, and graphical methods and compared by chi-square tests, nonparametric tests, and analysis of variance. The primary analyses will be

performed using logistic regression methods. Additional analyses will be done using the Cox model to study the impact of risk by gestational age. Logistic analyses will be used to determine pertinent variables for model building and for the assessment of possible confounding variables. The isolation of subgroups will involve numerous repeated tests.

4. Justification for Exclusion of Specific Groups

No specific groups will be excluded. Necessarily, all subjects will be pregnant women.

5. Required Equipment and Supplies

The Diagnostic Mycoplasma Laboratory, Birmingham, Alabama, occupies 400 square feet and operates as a state-licensed clinical microbiology laboratory culturing for and identifying only mycoplasmas. The equipment available includes: 37°C incubator, CO₂ 37°C incubator, biological safety cabinet for culture processing, inverted microscope, Leitz epifluorescent microscope, stereomicroscope, 50-cubic-foot refrigerator, autoclave, pH meter, slow-speed refrigerated centrifuge, high-speed refrigerated centrifuge, horizontal flow hood for PCR specimen processing, Beckman UV Spectrophotometer, water bath, and electrophoresis and immunoblot equipment. Microbiological and PCR data will be recorded and analyzed in the Diagnostic Mycoplasma Laboratory using Statistical Analysis System (SAS) on a Dell 325 D computer. User-friendly software will enable immediate searching and sorting and will assure sensitive, flexible, and prompt patient database analyses as required for planning and evaluation of clinical research.

VII. ORGANIZATION OF RESEARCH EFFORT

1. Duties and Responsibilities

The Diagnostic Mycoplasma Laboratory will perform specimen processing for culture and PCR.

Patient enrollment and follow-up, data collection, and routine laboratory studies will be conducted at NMCSO. Laboratory personnel will perform cultures for aerobic and anaerobic bacteria. Cultures for H₂O₂-producing lactobacilli will be performed by individuals trained by University of Alabama at Birmingham (UAB) personnel.

The Naval Health Research Center (NHRC), San Diego, will coordinate data collection, data management, and report generation.

The principal investigator, Dr. Cassell (or her replacement), or the alternate principal investigator, Dr. Gray (or his replacement), will supervise all protocol work and delegate responsibilities and resources to co-investigators and contract personnel. All manuscripts, presentations, and interviews regarding this work first

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must be approved by the principal investigator, Dr. Cassell.

2. Chain of Command

The alternate principal investigator, Dr. Gray, will be responsible to his Division Head, CAPT Brodine, his Department Head, Dr. Garland, his Scientific Director, Dr. Nice, and Commanding Officer, CAPT Dean, as per the routine chain of command at NHRC. The co-investigators will be responsible to the principal investigator, Dr. Gail Cassell.

VIII. RISKS AND DISCOMFORTS TO RESEARCH VOLUNTEERS

1. Risks to the Volunteer and Means of Mitigation

The patient may feel some mild pain at the site where blood is taken. Sometimes a bruise may occur. Rarely, a small clot may develop or local swelling and bleeding from the puncture site may be seen. The cervical and vaginal cultures will be performed with a soft swab and will be no more uncomfortable than undergoing a Pap smear. The patient will not feel any discomfort from specimens obtained from the placenta, amniotic fluid, or umbilical cord. The infant will be cultured by placing a soft swab into his/her nostril for approximately 3 sec.

2. Special Risks to Pregnant or Potentially Pregnant Women Volunteers

No significant risks are anticipated for patients who participate in this study. Of more than 600 women and neonates who have been enrolled thus far in our previous studies, to our knowledge no complications have arisen as a consequence of participation.

3. Safety Precautions and Emergency Procedures

All procedures will be conducted in a hospital or in a clinic setting and will be performed by trained medical personnel available to respond to emergencies if any should arise.

4. Assessment of Sufficiency of Plans to Deal With Untoward Events or Injuries

Venipuncture drawing is a standard laboratory technique that, when performed by trained personnel, should cause no risk to the patient. Venipuncture may result in a slight pinch or bruise. Sterile techniques will be used. BV smears frequently can be self-collected by inserting a swab into the vagina, but for this study they will be collected by trained personnel. This procedure should cause no risk to the patient or emergency procedures. Less discomfort occurs in collecting this swab than by self-insertion of a tampon. In screening more than 1,000 women at UAB, no emergency procedures have been required.

5. Qualification of Medical Monitor and Medical Support Personnel

CAPT Brodine, MC, USN (at NHRC) will serve as the medical monitor for this project. Dr. Brodine is a board-certified internist and infectious diseases specialist with extensive clinical and operational research experience. All procedures will be performed by trained physicians and nurses. A nurse coordinator (to be named) will assist the study in the collection of patient information, processing of specimens for culture, and shipment of specimens to UAB.

IX. DESCRIPTION OF THE SYSTEM FOR MAINTENANCE OF RECORDS

1. Experimental Data

All study data will be managed by the alternate principal investigator and stored in a locked space (Dr. Gray's office). Computerized Privacy Act data will be similarly handled in a confidential, controlled access fashion as per Navy regulations.

2. Research Protocol, Consent Forms, and Related Documents for Protection of Human Research Volunteers

NHRC will hold consent forms and related documents in locked storage for 3 years after the completion of the study.

3. Individual Medical Records

Medical records will be summarized onto study data forms.

X. APPENDICES

APPENDIX 3

The UAB Study

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM CHORIOAMNION INFECTION STUDY

PURPOSE

You have the opportunity to participate in a very important research study being conducted at the Naval Medical Center, San Diego. The purpose of this study is to determine if a certain bacterial infection found in some women's vagina or placenta, is associated with premature delivery and infections of brain and lungs of newborn infants. This is an observational study only and will not change your medical care or put you or your baby at any risk.

GOAL

The Naval Medical Center, San Diego wishes to provide the best obstetrical care possible to military families. For this reason Naval Medical Center is collaborating with the University of Alabama at Birmingham and the Naval Health Research Center to investigate reports that infection causes premature labor. The results of this study may aid in the prevention of poor outcomes of pregnancy (premature birth, sickness and death of infants) among military active-duty and dependent women.

QUESTIONS YOU MAY HAVE

What will I have to do if I choose to participate?

Upon enrollment you will be asked to read and sign a consent form as well as to complete a questionnaire of approximately 30 questions.

What laboratory specimens will I need to provide?

You will need to donate an equivalent of approximately 1 teaspoon of additional blood while your routine prenatal blood work is being drawn.

At your first prenatal doctor's visit, during routine examination, your doctor will swab your vagina with soft swabs looking for evidence of known harmful bacterial. If you chose to participate in this study, your doctor will collect several additional soft swab samples. This will not be painful to you.

At delivery you will again be asked to permit clinical staff to perform several soft vaginal swabs during routine examination. Again, an extra teaspoon of blood will be drawn while routine admission laboratory tests are being drawn by the labor and delivery staff. After your baby is delivered, investigators will collect specimens from your placenta, amniotic fluid and umbilical cord blood. A nasal swab will also be collected from your baby. These procedures will not increase your or your baby's discomfort.

Will there be any significant risk to me or my baby?

No. The study procedures will not interfere with or delay the normal care you and your baby would receive.

Will information about me or my baby be kept confidential?

Yes, all study data and specimens will be marked only with the last four digits of your social security number and a study number. Furthermore, all data will be kept under lock and key by CDR Gregory Gray at NHRC.

What does my participation mean?

By agreeing to participate in this study, you have made a tremendous contribution to the future understanding of how bacterial infections affect pregnancy outcome and the health of the newborn infant.

What if I have further questions?

If you need more information or have further questions please contact:

Gale Schmaltz, RN, MSNc or Paul D. Stamper, M(CLS), MPHc
Microbiology Department
Naval Medical Center San Diego
Tel (619) 532-9242

INVESTIGATORS

Gail H. Cassell, Ph.D.
CDR Gregory C. Gray, MC, USN
CDR Everett F. Magann, MC, USN
Edward W. Hook, III, M.D.
Jane R. Schwebke, M.D.
William W. Andrews, M.D.
John C. Hauth, M.D.
Robert L. Goldenberg, M.D.

IMPORTANT STUDY **PLEASE READ**

Risk Factors for Chorioamnion Infection and Adverse Pregnancy Outcomes Among Active-Duty Military Women and Dependent Women

You have the opportunity to participate in a very important research study being conducted at the Naval Medical Center, San Diego. The purpose of this study is to determine if a certain bacterial infection found in some women's vagina or placenta, is associated with premature delivery and infections of brain and lungs of newborn infants. This is an observational study only, and, will not change your medical care or put you or your baby at any risk.

The Naval Medical Center, San Diego wishes to provide the best obstetrical care possible to military families. For this reason Naval Medical Center is collaborating with the University of Alabama at Birmingham and the Naval Health Research Center to investigate reports that infection causes premature labor. The results of this study may aid in the prevention of poor outcomes of pregnancy (premature birth, sickness and death of infants) among military active-duty and dependent women.

If you choose to participate you will be asked:

At today's visit:

- to read and sign a consent form
- to fill out a data questionnaire
- to give about 2 teaspoons of blood while having your routine prenatal labs drawn

At your 1st obstetrical visit:

- to have vaginal swabs collected by your provider during routine vaginal exam

Upon admission to labor and delivery:

- to give about 2 teaspoons of blood while having your routine admission labs drawn
- to have vaginal swabs collected by a L&D nurse or provider during 1st vaginal exam

At or after delivery you **may have** specimens collected including:

- about 2 teaspoons of amniotic fluid
- placental tissue
- umbilical cord blood
- nasal swab on infant

If your infant should become ill and need to be placed on a breathing machine and/or need a spinal tap, respiratory secretions and spinal fluid specimens will be collected.

There is **no** significant risk for you or your baby by participating in this study. Your participation in this study **will not** interfere with or delay the normal care you and your baby receive. And, these procedures **will not** increase your or your baby's discomfort.

All information about you and your baby **will be** kept confidential. Study data and specimens will be marked only with the last four digits of your sponsor's social security number and an assigned study number.

You will be asked to participate in this important study by study staff. **If you have any questions please ask.** By agreeing to participate in this study, you will make a tremendous contribution to the future understanding of how bacterial infections affect pregnancy outcome and the health of the newborn infant.

**NAVAL MEDICAL CENTER
SAN DIEGO, CALIFORNIA 92134-5000**

**CONSENT BY A SUBJECT FOR VOLUNTARY
PARTICIPATION IN A CLINICAL INVESTIGATION
(RESEARCH) STUDY**

1. I (we), _____, have been asked to voluntarily participate in a research project entitled, "Risk factors for Chorioamnion Infection and Adverse Pregnancy Outcome among Active-Duty Military Women and Dependent Women," being conducted at the Naval Medical Center, San Diego, California (NMCS D), and in collaboration with investigators from the University of Alabama at Birmingham (UAB), the Naval Health Research Center (NHRC), San Diego.

2. The purpose(s) of this research project is designed to determine if pregnant women who have a common bacteria called *Ureaplasma urealyticum* (Uu), or other bacteria in their vagina [bacterial vaginosis (BV)], are more at risk for premature delivery.

3. I (we) understand that my (our) participation in this research project will be for a period of approximately nine months. I understand that the father of my baby will sign this consent form (if he is available), as requested by U.S. Army Medical Research and Materiel Command (USAMRMC) according to the Office Protection of Research Rights, Protection of Human Subjects Code of Federal Regulations 45 CFR 46, paragraph 207(b).

4. The procedures for this project include the following:

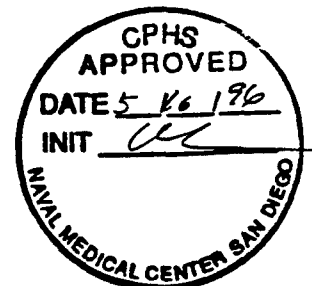
a. Upon my first visit for prenatal care at NMCS D, I will be asked to permit vaginal swabs and a 10-cc blood specimen (2 teaspoons). I will complete Part 1 (four pages) of the Mother's and Infant's Questionnaire. The questions will be similar to ones my physician would normally ask were I not to volunteer for this study. The first four pages of the questionnaire will take approximately 15 minutes of my time to complete. Part 2 of the questionnaire, which is completed by those women who meet the criteria listed in 4a, will be completed by a medical record review done by the study nurse.

The questions regarding drug use and sexually transmitted diseases are of a sensitive and personal nature but could have

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relevance in predicting premature labor or other adverse pregnancy outcomes.

I (we) understand that only women who meet certain criteria will participate in the remainder of the study (study sample will be 1,272 women). These criteria are: delivery by cesarean section with membranes intact at the time of delivery (bag of waters has not broken); selection from a random sample of women who deliver by cesarean section with ruptured membranes at the time of delivery (bag of waters has broken); or selection from a random sample of women who deliver vaginally. At delivery, a further 10-cc blood specimen will be collected along with other admission specimens. A 5- to 10-cc specimen of cord blood will also be taken after the umbilical cord is cut. This cord blood sample poses no inconvenience, danger or discomfort to me or to my infant and is routinely collected for clinical study. I (we) understand that amniotic fluid may be collected at the time of delivery for research purposes only, and that additional vaginal swabs will be performed. I (we) understand that my placenta will be studied with a number of routine cultures performed. I (we) understand that the specimens described in section 4d may be collected from my (our) baby.

b. I (we) understand that the procedures for collecting specimens in this study will not pose any significant risk to me or to my (our) baby. I (we) understand that the study procedures will not interfere with or delay the standard of care for me and for my (our) infant.

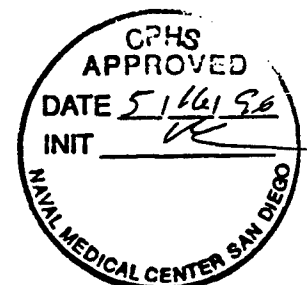
c. I (we) understand that researchers will also study the specimens mentioned above for evidence of chlamydia, yeast, gonorrhoea, Group B streptococcus, and syphilis. Even if I (we) were not volunteering to participate in this research study, physicians would normally conduct these tests.

d. I (we) understand that my (our) infant will have his/her nose cultured by placing a soft swab into his/her nostril for approximately 3 seconds. I (we) also understand that if, at any time, my (our) infant's clinical condition requires that he/she be put on a breathing machine, respiratory secretions will be studied for the bacteria Uu. Also, if my (our) infant becomes sick and a spinal tap (collection of cerebral spinal fluid from the spinal canal) is required, excess fluid will be studied for this bacteria. I (we) understand that cerebral spinal fluid will not be collected routinely on all infants, only those who require

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a spinal tap. Although the specimens collected on my (our) baby are invasive, I (we) will be counseled by trained personnel on any possible risks, and informed consent will be obtained for the spinal tap (if a spinal tap is necessary).

5. All women seeking prenatal care at NMCSO will be invited to participate in the study. Of these, comprehensive placental cultures will be performed on 672 women who have cesarean sections with intact membranes, 300 women who have cesarean sections with ruptured membranes, and 300 women who deliver vaginally.

6. The risks or discomforts which are possibly related to my participation in this study are as follows:

I may feel some mild pain at the site where blood is taken. Sometimes a bruise may occur. Rarely, a small clot may develop or local swelling and bleeding from the puncture site may be seen. The cervical and vaginal cultures will be performed with a soft swab and will be no more uncomfortable than undergoing a Pap smear. I will not feel any discomfort from specimens obtained from my placenta, amniotic fluid, or umbilical cord.

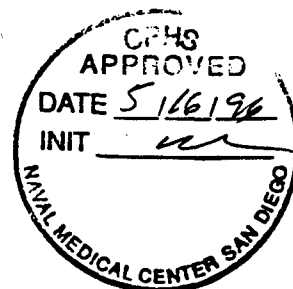
7. I (we) understand that my participation in this research project may or may not be of direct benefit to me or to my (our) infant; however, if I am found to be infected with a known pathogen (bacterium or virus), I will benefit by appropriate treatment. This treatment, in turn, may provide a direct benefit to my (our) infant, as early detection of and treatment for pathogens in the mother, result in improved quality of the gestation and birth periods for the infant. After birth, my (our) infant will continue to receive direct benefits. The neonatologist will have immediate knowledge of whether my (our) infant tests positive for Uu and will be able to administer the necessary treatment in a timely fashion. The results of this study may aid the investigator in gaining important knowledge about the prevention of poor outcomes of pregnancy (i.e., preterm births) among military active-duty and dependent women, and aid in the future medical evaluation and treatment of other patients and their infants.

8. The alternate procedure(s) or course of treatment, should I (we) decide not to participate in this research study, has been explained to me as follows: I (we) understand that I (we) do not have to participate in this research study and can receive the

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standard medical care for prenatal visits and delivery if I (we) do not participate.

9. In all publications and presentations resulting from this research study, information about me or my participation in this project will be kept in the strictest confidence and will not be released in any form identifiable to me personally; however, I (we) realize that authorized personnel from the Navy Medical Department, the Food and Drug Administration (FDA) and USAMRMC, where applicable, may have access to my research file in order to verify that my rights have been adequately protected.

a. The information provided in this study will be analyzed by UAB, NHRC, and NMCSO. The study documents and data files will be maintained by the UAB and NHRC where they will be used to study trends among pregnant military women. Medical research information will be used for analysis and reports by UAB and Departments of the Navy and Defense and other U.S. Government agencies. Use of the information may be granted to non-Government agencies or individuals by the Navy Surgeon General following the provisions of the Freedom of Information Act or contracts and agreements. I (we) voluntarily agree to its disclosure to the agencies or individuals identified above.

b. All responses will be held in confidence by UAB, NHRC, and NMCSO. All samples sent to UAB will be coded with the last four digits of the sponsor's social security number and a study number. Information that I (we) provide will be considered only when statistically summarized with the responses of others and will not be attributed to any single individual in publications.

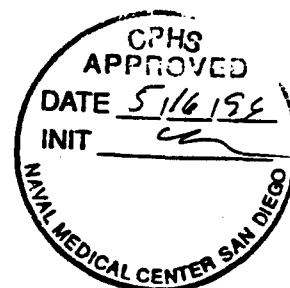
c. I (we) understand that it is the policy of USAMRMC that data sheets are to be completed on all volunteers participating in research for entry into the Command's Volunteer Registry Data Base. The information to be entered into this confidential data base includes my name, address, Social Security number, study name and dates. The intent of the data base is two-fold: first, to readily answer questions concerning my participation in research sponsored by USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure I am adequately warned of risks and to provide me (us) with new information as it becomes available. The information will be stored at USAMRMC for a minimum of 75 years.

9B. If I (we) suffer any injury directly related to my

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participation in this research study, immediate medical attention is available at the Naval Medical Center, San Diego, or at another closer military medical treatment facility, if applicable. I (we) understand that although no financial compensation is available, any injury resulting from my (our) participation in this study will be evaluated and treated in keeping with the benefits or care to which I am (we are) entitled under applicable Navy, other Department of Defense, and other state or Federal regulations.

10. If I (we) have any questions regarding this research study, I may contact Dr. Gray, (619) 553-9967. If I (we) have any questions about my (our) rights as individuals while participating in a research study at the Naval Medical Center, San Diego, I (we) may contact CDR Dean Gubler, MC, USN, Chairman, Committee for the Protection of Human Subjects at (619) 532-8125 or CAPT Charles Gray, MC, USN, Department Head, Clinical Investigation Department at (619) 532-8127. If I (we) believe that I have been injured as a result of my participation in this research study, I (we) may contact CDR K. Allred, JAGC, USN, Naval Medical Center, San Diego, Legal Department, at (619) 532-6475.

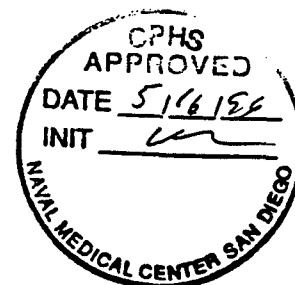
11. I (we) understand that my (our) participation in this project is entirely voluntary and that my (our) decision not to participate will involve no penalty or loss of benefits to which I am (we are) entitled under applicable regulations. If I (we) choose to participate, I am (we are) free to ask questions or to withdraw from the study at any time. If I (we) should decide to withdraw from the research project, I (we) will notify Dr. Gray, (619) 553-9967, to ensure my (our) timely removal from the study. My (our) withdrawal will involve no prejudice to my (our) future health care or any loss of rights or benefits to which I am (we are) otherwise entitled. Any new significant finding developed during the course of this study which might affect my (our) willingness to continue participation will be communicated to me (us).

11B. The investigator may terminate my (our) participation in this study for the following reasons: The investigator or I (we) may terminate my (our) participation in this study at any time. Possible reasons for ending my (our) participation may include my (our) failure to cooperate, or a new finding that continuing the study increases my risk of illness.

Subject's Initials: _____

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Revised 15 May 96



PRIVACY ACT STATEMENT

1. Authority. 5 USC 301

2. Purpose. Medical research information will be collected to enhance basic medical knowledge or to develop tests, procedures, and equipment to improve the diagnosis, treatment, or prevention of illness, injury, or functional impairment.

3. Use. Medical research information will be used for statistical analysis and reports by the Department of the Navy, the Department of Defense, and other U.S. Government agencies, provided this use is compatible with the purpose for which the information was collected. Use of the information may be granted to non-Government agencies or individuals by the Chief, Bureau of Medicine and Surgery in accordance with the provisions of the Freedom of Information Act.

4. Disclosure. I (we) understand that all information contained in this Consent Statement or derived from the medical research study described herein will be retained permanently at Naval Medical Center, San Diego, and salient portions thereof may be entered into my health record. I (we) voluntarily agree to its disclosure to agencies or individuals identified in the preceding paragraph. I (we) have been informed that failure to agree to such disclosure may negate the purposes for which the research study was conducted.

SIGNATURES AND DATE SIGNED:

PRINTED OR TYPED IDENTIFICATION:

Patient / Subject (Date)
(if Applicable)

Name / Status / Sponsor's SSN

Parent / Guardian (Date)
(if Applicable)

Name / Status / SSN

Witness (Date)

Name / Grade or Rank / SSN

Father of Infant (Date)

Name / Grade or Rank / SSN



**NAVAL MEDICAL CENTER
SAN DIEGO, CALIFORNIA 92134-5000**

**CONSENT BY A SUBJECT FOR VOLUNTARY
PARTICIPATION IN A CLINICAL INVESTIGATION
(RESEARCH) STUDY**

**"Risk factors for Chorioamnion Infection and
Adverse Pregnancy Outcome among
Active-Duty Military Women and Dependent Women"**

12. I (we) understand that I am (we are) making a decision whether or not to participate in the research project described in the preceding sections subject to the conditions of participation described above. My (our) signature(s) indicate that I (we) have decided to participate, having read and understood the information presented above and having been given the opportunity to ask any questions that I (we) might have about the research study or my participation in the study. Further, my (our) signature(s) indicate that I (we) have been provided with a copy of this consent document and a copy of a document entitled, "California Experimental Subject's Bill of Rights."

SIGNATURES AND DATE SIGNED: PRINTED OR TYPED IDENTIFICATION:

Patient / Subject (Date) Name / Status / Sponsor's SSN

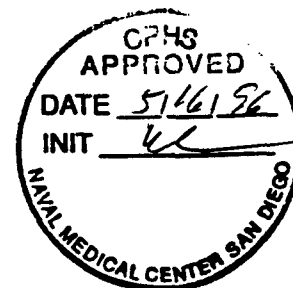
Witness (Date) Name / Grade or Rank / SSN

Researcher/Investigator(Date) Name / Grade or Rank / SSN

Father of Infant (Date) Name / Grade or Rank / SSN

CPHS/IRB Approval Stamp/Seal Required

Revised 15 May 96



EXPERIMENTAL SUBJECTS BILL OF RIGHTS (CA)

Any person who is requested to consent to participate as a subject in a research study involving a medical experiment or who is requested to consent on behalf of another has the right to:

1. Be informed of the nature and purpose of the experiment;
2. Be given an explanation of the procedures to be followed in the medical experiment and any drug or device to be used;
3. Be given a description of any attendant discomforts and risks reasonably to be expected from the experiment;
4. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable;
5. Be given a disclosure of appropriate alternative procedures, drugs, or devices that might be advantageous to the subject and their relative risks and benefits;
6. Be informed of the avenues of medical treatment, if any, available to the subject after the experiment if any complications should arise;
7. Be given an opportunity to ask any questions concerning the experiment or the procedures involved;
8. Be instructed that the consent to participate in the medical experiment may be withdrawn at any time, and the subject may discontinue participation in the medical experiment without prejudice;
9. Be given a copy of a signed and dated written consent form when one is required;
10. Be given the opportunity to decide to consent or not consent to medical experiment without intervention of any element of force, fraud, deceit, duress, coercion, or undue influence on the subject's decision; and
11. Be assured that the subject's confidentiality will be preserved and his/her name will not be released without his/her permission.

Any questions regarding this research study should be directed to the principal investigator or associate investigators. Information is available from the Chairman, Committee for the Protection of Human Subjects, established for the protection of volunteers in research projects at this facility by calling (619) 532-8125 or writing the Chairman, Committee for the Protection of Human Subjects at Naval Medical Center, Clinical Investigation Department (Code AVA), San Diego, CA 92134-5000

CLINICAL INVESTIGATION DEPARTMENT
NAVAL MEDICAL CENTER, SAN DIEGO, CA 92134-5000

6500
AVA
15 May 96

MEMORANDUM

From: Chairman, Committee for the Protection of Human Subjects
To: CDR Gregory Gray, MC, USN

Subj: APPROVAL OF ORIGINAL MODIFIED INFORMED CONSENT FORM

Encl: (1) Consent Form - CIP Study #S-95-077

1. The informed consent document for Clinical Investigation Study Number CIP #S-95-077, entitled "Risk Factors for Chorioamnion Infection and Adverse Pregnancy Outcome among Active-Duty Military Women and Dependent Women," dated 15 May 96 is approved.

2. Approval of your study and consent document(s) may be documented in the combined monthly Minutes of the Scientific Review Committee/Committee for the Protection of Human Subjects. It may also be forwarded, when required, to higher authority (e.g., Naval Health Sciences Education and Training Command or Naval Investigational Drug Review Board) for information or further review.

Date 16 May 96


K. DEAN GUBLER
Commander, Medical Corps
United States Navy
Chairman, Committee for
the Protection of Human Subjects

**NAVAL MEDICAL CENTER
SAN DIEGO, CALIFORNIA 92134-5000**

**CONSENT BY A SUBJECT FOR VOLUNTARY
PARTICIPATION IN A CLINICAL INVESTIGATION
(RESEARCH) STUDY**

1. I (we), _____, have been asked to voluntarily participate in a research project entitled, **"Risk factors for Chorioamnion Infection and Adverse Pregnancy Outcome among Active-Duty Military Women and Dependent Women,"** being conducted at the Naval Medical Center, San Diego, California (NMCS D), and in collaboration with investigators from the University of Alabama at Birmingham (UAB), the Naval Health Research Center (NHRC), San Diego.

2. The purpose(s) of this research project is designed to determine if pregnant women who have a common bacteria called *Ureaplasma urealyticum* (Uu), or other bacteria in their vagina [bacterial vaginosis (BV)], are more at risk for premature delivery.

3. I (we) understand that my (our) participation in this research project will be for a period of approximately nine months. I understand that the father of my baby will sign this consent form (if he is available), as requested by U.S. Army Medical Research and Materiel Command (USAMRMC) according to the Office Protection of Research Rights, Protection of Human Subjects Code of Federal Regulations 45 CFR 46, paragraph 207(b).

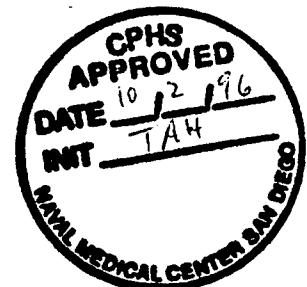
4. The procedures for this project include the following:

a. Upon my first visit for prenatal care at NMCS D, I will be asked to obtain my own vaginal swabs, and permit a physician to obtain vaginal swabs (which is part of the normal procedure of my clinic visit). I will be given written instructions for the self-swabbing technique. I will also be asked to permit a 10-cc blood specimen (2 teaspoons). I will complete Part 1 (four pages) of the Mother's and Infant's Questionnaire. The questions will be similar to ones my physician would normally ask were I not to volunteer for this study. The first four pages of the questionnaire will take approximately 15 minutes of my time to

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complete. Part 2 of the questionnaire, which is completed by those women who meet the criteria listed in 4a, will be completed by a medical record review done by the study nurse. The questions regarding drug use and sexually transmitted diseases are of a sensitive and personal nature but could have relevance in predicting premature labor or other adverse pregnancy outcomes.

I (we) understand that only women who meet certain criteria will participate in the remainder of the study (study sample will be 1,272 women). These criteria are: delivery by cesarean section with membranes intact at the time of delivery (bag of waters has not broken); selection from a random sample of women who deliver by cesarean section with ruptured membranes at the time of delivery (bag of waters has broken); or selection from a random sample of women who deliver vaginally. At delivery, a further 10-cc blood specimen will be collected along with other admission specimens. A 5- to 10-cc specimen of cord blood will also be taken after the umbilical cord is cut. This cord blood sample poses no inconvenience, danger or discomfort to me or to my infant and is routinely collected for clinical study. I (we) understand that amniotic fluid may be collected at the time of delivery for research purposes only, and that additional vaginal swabs will be performed. I (we) understand that my placenta will be studied with a number of routine cultures performed. I (we) understand that the specimens described in section 4d may be collected from my (our) baby.

b. I (we) understand that the procedures for collecting specimens in this study will not pose any significant risk to me or to my (our) baby. I (we) understand that the study procedures will not interfere with or delay the standard of care for me and for my (our) infant.

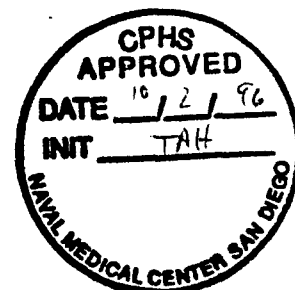
c. I (we) understand that researchers will also study the specimens mentioned above for evidence of chlamydia, yeast, gonorrhoea, Group B streptococcus, and syphilis. Even if I (we) were not volunteering to participate in this research study, physicians would normally conduct these tests.

d. I (we) understand that my (our) infant will have his/her nose cultured by placing a soft swab into his/her nostril for approximately 3 seconds. I (we) also understand that if, at any time, my (our) infant's clinical condition requires that he/she be put on a breathing machine, respiratory secretions will be

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studied for the bacteria Uu. Also, if my (our) infant becomes sick and a spinal tap (collection of cerebral spinal fluid from the spinal canal) is required, excess fluid will be studied for this bacteria. I (we) understand that cerebral spinal fluid will not be collected routinely on all infants, only those who require a spinal tap. Although the specimens collected on my (our) baby are invasive, I (we) will be counseled by trained personnel on any possible risks, and informed consent will be obtained for the spinal tap (if a spinal tap is necessary).

5. All women seeking prenatal care at NMCSO will be invited to participate in the study. Of these, comprehensive placental cultures will be performed on 672 women who have cesarean sections with intact membranes, 300 women who have cesarean sections with ruptured membranes, and 300 women who deliver vaginally.

6. The risks or discomforts which are possibly related to my participation in this study are as follows:

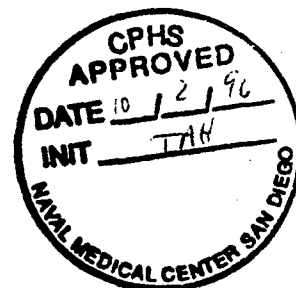
I may feel some mild pain at the site where blood is taken. Sometimes a bruise may occur. Rarely, a small clot may develop or local swelling and bleeding from the puncture site may be seen. The cervical and vaginal cultures will be performed with a soft swab and will be no more uncomfortable than undergoing a Pap smear. I will not feel any discomfort from specimens obtained from my placenta, amniotic fluid, or umbilical cord.

7. I (we) understand that my participation in this research project may or may not be of direct benefit to me or to my (our) infant; however, if I am found to be infected with a known pathogen (bacterium or virus), I will benefit by appropriate treatment. This treatment, in turn, may provide a direct benefit to my (our) infant, as early detection of and treatment for pathogens in the mother, result in improved quality of the gestation and birth periods for the infant. After birth, my (our) infant will continue to receive direct benefits. The neonatologist will have immediate knowledge of whether my (our) infant tests positive for Uu and will be able to administer the necessary treatment in a timely fashion. The results of this study may aid the investigator in gaining important knowledge about the prevention of poor outcomes of pregnancy (i.e., preterm births) among military active-duty and dependent women, and aid in the future medical evaluation and treatment of other patients and their infants.

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8. The alternate procedure(s) or course of treatment, should I (we) decide not to participate in this research study, has been explained to me as follows: I (we) understand that I (we) do not have to participate in this research study and can receive the standard medical care for prenatal visits and delivery if I (we) do not participate.

9. In all publications and presentations resulting from this research study, information about me or my participation in this project will be kept in the strictest confidence and will not be released in any form identifiable to me personally; however, I (we) realize that authorized personnel from the Navy Medical Department, the Food and Drug Administration (FDA) and USAMRMC, where applicable, may have access to my research file in order to verify that my rights have been adequately protected.

a. The information provided in this study will be analyzed by UAB, NHRC, and NMCSO. The study documents and data files will be maintained by the UAB and NHRC where they will be used to study trends among pregnant military women. Medical research information will be used for analysis and reports by UAB and Departments of the Navy and Defense and other U.S. Government agencies. Use of the information may be granted to non-Government agencies or individuals by the Navy Surgeon General following the provisions of the Freedom of Information Act or contracts and agreements. I (we) voluntarily agree to its disclosure to the agencies or individuals identified above.

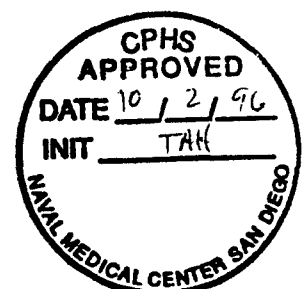
b. All responses will be held in confidence by UAB, NHRC, and NMCSO. All samples sent to UAB will be coded with the last four digits of the sponsor's social security number and a study number. Information that I (we) provide will be considered only when statistically summarized with the responses of others and will not be attributed to any single individual in publications.

c. I (we) understand that it is the policy of USAMRMC that data sheets are to be completed on all volunteers participating in research for entry into the Command's Volunteer Registry Data Base. The information to be entered into this confidential data base includes my name, address, Social Security number, study name and dates. The intent of the data base is two-fold: first, to readily answer questions concerning my participation in research sponsored by USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure I am adequately warned of risks and to provide me (us) with new information as it

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becomes available. The information will be stored at USAMRMC for a minimum of 75 years.

10. If I (we) suffer any injury directly related to my participation in this research study, immediate medical attention is available at the Naval Medical Center, San Diego, or at another closer military medical treatment facility, if applicable. I (we) understand that although no financial compensation is available, any injury resulting from my (our) participation in this study will be evaluated and treated in keeping with the benefits or care to which I am (we are) entitled under applicable Navy, other Department of Defense, and other state or Federal regulations.

11. If I (we) have any questions regarding this research study, I may contact Dr. Gray, (619) 553-9967. If I (we) have any questions about my (our) rights as individuals while participating in a research study at the Naval Medical Center, San Diego, I (we) may contact CDR Dean Gubler, MC, USN, Chairman, Committee for the Protection of Human Subjects at (619) 532-8125 or CAPT Charles Gray, MC, USN, Department Head, Clinical Investigation Department at (619) 532-8127. If I (we) believe that I have been injured as a result of my participation in this research study, I (we) may contact CDR K. Allred, JAGC, USN, Naval Medical Center, San Diego, Legal Department, at (619) 532-6475.

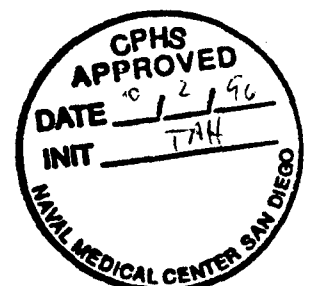
12. I (we) understand that my (our) participation in this project is entirely voluntary and that my (our) decision not to participate will involve no penalty or loss of benefits to which I am (we are) entitled under applicable regulations. If I (we) choose to participate, I am (we are) free to ask questions or to withdraw from the study at any time. If I (we) should decide to withdraw from the research project, I (we) will notify Dr. Gray, (619) 553-9967, to ensure my (our) timely removal from the study. My (our) withdrawal will involve no prejudice to my (our) future health care or any loss of rights or benefits to which I am (we are) otherwise entitled. Any new significant finding developed during the course of this study which might affect my (our) willingness to continue participation will be communicated to me (us).

13. The investigator may terminate my (our) participation in this study for the following reasons: The investigator or I (we)

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may terminate my (our) participation in this study at any time. Possible reasons for ending my (our) participation may include my (our) failure to cooperate, or a new finding that continuing the study increases my risk of illness.

14. I (we) understand that I am (we are) making a decision whether or not to participate in the research project described in the preceding sections subject to the conditions of participation described above. My (our) signature(s) indicate that I (we) have decided to participate, having read and understood the information presented above and having been given the opportunity to ask any questions that I (we) might have about the research study or my participation in the study. Further, my (our) signature(s) indicate that I (we) have been provided with a copy of this consent document and a copy of a document entitled, "California Experimental Subject's Bill of Rights."

SIGNATURES AND DATE SIGNED: PRINTED OR TYPED IDENTIFICATION:

Patient / Subject (Date)

Name / Status / Sponsor's SSN

Witness (Date)

Name / Grade or Rank / SSN

Researcher/Investigator(Date)

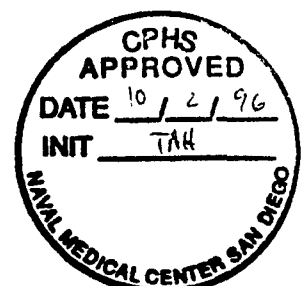
Name / Grade or Rank / SSN

Father of Infant (Date)

Name / Grade or Rank / SSN

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PRIVACY ACT STATEMENT

1. Authority. 5 USC 301

2. Purpose. Medical research information will be collected to enhance basic medical knowledge or to develop tests, procedures, and equipment to improve the diagnosis, treatment, or prevention of illness, injury, or functional impairment.

3. Use. Medical research information will be used for statistical analysis and reports by the Department of the Navy, the Department of Defense, and other U.S. Government agencies, provided this use is compatible with the purpose for which the information was collected. Use of the information may be granted to non-Government agencies or individuals by the Chief, Bureau of Medicine and Surgery in accordance with the provisions of the Freedom of Information Act.

4. Disclosure. I (we) understand that all information contained in this Consent Statement or derived from the medical research study described herein will be retained permanently at Naval Medical Center, San Diego, and salient portions thereof may be entered into my health record. I (we) voluntarily agree to its disclosure to agencies or individuals identified in the preceding paragraph. I (we) have been informed that failure to agree to such disclosure may negate the purposes for which the research study was conducted.

SIGNATURES AND DATE SIGNED: PRINTED OR TYPED IDENTIFICATION:

Patient / Subject (Date)
 (if Applicable)

Name / Status / Sponsor's SSN

Parent / Guardian (Date)
 (if Applicable)

Name / Status / SSN

Witness (Date)

Name / Grade or Rank / SSN

Father of Infant (Date)

Name / Grade or Rank / SSN

EXPERIMENTAL SUBJECTS BILL OF RIGHTS (CA)

Any person who is requested to consent to participate as a subject in a research study involving a medical experiment or who is requested to consent on behalf of another has the right to:

1. Be informed of the nature and purpose of the experiment;
2. Be given an explanation of the procedures to be followed in the medical experiment and any drug or device to be used;
3. Be given a description of any attendant discomforts and risks reasonably to be expected from the experiment;
4. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable;
5. Be given a disclosure of appropriate alternative procedures, drugs, or devices that might be advantageous to the subject and their relative risks and benefits;
6. Be informed of the avenues of medical treatment, if any, available to the subject after the experiment if any complications should arise;
7. Be given an opportunity to ask any questions concerning the experiment or the procedures involved;
8. Be instructed that the consent to participate in the medical experiment may be withdrawn at any time, and the subject may discontinue participation in the medical experiment without prejudice;
9. Be given a copy of a signed and dated written consent form when one is required;
10. Be given the opportunity to decide to consent or not consent to medical experiment without intervention of any element of force, fraud, deceit, duress, coercion, or undue influence on the subject's decision; and
11. Be assured that the subject's confidentiality will be preserved and his/her name will not be released without his/her permission.

Any questions regarding this research study should be directed to the principal investigator or associate investigators. Information is available from the Chairman, Committee for the Protection of Human Subjects, established for the protection of volunteers in research projects at this facility by calling (619) 532-8125 or writing the Chairman, Committee for the Protection of Human Subjects at Naval Medical Center, Clinical Investigation Department (Code AVA), San Diego, CA 92134-5000

CLINICAL INVESTIGATION DEPARTMENT
NAVAL MEDICAL CENTER, SAN DIEGO, CA 92134-5000

6500
AVA
2 Oct 96

MEMORANDUM

From: Chairman, Committee for the Protection of Human Subjects
To: CDR Gregory Gray, MC, USN

Subj: APPROVAL OF ORIGINAL MODIFIED INFORMED CONSENT FORM

Encl: (1) Consent Form - CIP Study #S-95-077

1. The informed consent document for Clinical Investigation Study Number CIP #S-95-077, entitled "Risk Factors for Chorioamnion Infection and Adverse Pregnancy Outcome among Active-Duty Military Women and Dependent Women," dated 24 Sep 96 is approved.
2. Approval of your study and consent document(s) may be documented in the combined monthly Minutes of the Scientific Review Committee/Committee for the Protection of Human Subjects. It may also be forwarded, when required, to higher authority (e.g., Naval Health Sciences Education and Training Command or Naval Investigational Drug Review Board) for information or further review.
3. Approval is only for the consent form. Initiation and enrollment of patients must be deferred until formal approval by the Head, Clinical Investigation Department, is forwarded to you.

Date 02 Oct 96


K. DEAN GUBLER
Commander, Medical Corps
United States Navy
Chairman, Committee for
the Protection of Human Subjects

APPENDIX 1 MOTHER'S AND INFANT'S DATA QUESTIONNAIRE

(Epidemiological Study of Pregnancies: Medical Record Abstraction Form and Questionnaire)

This questionnaire will help us provide the best medical care to mothers and their infants. **WE NEED YOUR HELP.** Note that this information will remain confidential and is obtained for investigational purposes only.

IMPORTANT INSTRUCTIONS

- * USE NO. 2 PENCIL ONLY.
- * Do NOT use ink, ballpoint, or felt tip pens.
- * Erase cleanly and completely any changes you make.

- * Make black marks that fill the circle.
- * Do NOT make any stray marks on the form.

SECTION A: Maternal Demographics

1. What is today's date?

MO.	DAY	YR.
0	0	0
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9

2. What is your SPONSOR'S Social Security Number:

SOCIAL SECURITY NUMBER								
0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4	4
5	5	5	5	5	5	5	5	5
6	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	8
9	9	9	9	9	9	9	9	9

3. What is your birthdate?

MO.	DAY	YR.
0	0	0
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9

4. What is your current age?

YRS
0
1
2
3
4
5
6
7
8
9

5. What are your Initials?

First	Middle	Last
A	A	A
B	B	B
C	C	C
D	D	D
E	E	E
F	F	F
G	G	G
H	H	H
I	I	I
J	J	J
K	K	K
L	L	L
M	M	M
N	N	N
O	O	O
P	P	P
Q	Q	Q
R	R	R
S	S	S
T	T	T
U	U	U
V	V	V
W	W	W
X	X	X
Y	Y	Y
Z	Z	Z

6. What is your home address?

Street

City

State

Zip Code

7. What are your phone numbers?

Work

MO.	DAY	YR.
0	0	0
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9

Home

MO.	DAY	YR.
0	0	0
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9

8. If the father of your baby did not sign the consent form, please blacken this circle ☐ and state the reason:

9. What is your CURRENT marital status?

- ☐ Never married
- ☐ Married
- ☐ Separated
- ☐ Divorced
- ☐ Widowed
- ☐ Other

10. Which is the BEST descriptor of your ethnic/racial background?

- ☐ White/Caucasian
- ☐ Black/African-American
- ☐ Asian or Pacific Islander
- ☐ Eskimo, Aleut, or American Indian
- ☐ Spanish/Hispanic
- ☐ Other (specify) _____

11. What is the HIGHEST level of education you have completed?

- ☐ 11th grade or less
- ☐ 12th grade or GED
- ☐ 1-2 years of college/trade school
- ☐ 3 or more years of college/trade school
- ☐ 1 year or more of graduate or professional school

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Please complete questions 12 and 13 regarding your and the baby's father's occupation.

12. What is your and the father's CURRENT occupation/military status?

You Father

- | | | |
|-----------------------|-----------------------|--|
| ☐ | ☐ | Active Duty - Navy |
| ☐ | ☐ | Active Duty - Marine Corps |
| ☐ | ☐ | Active Duty - Army |
| ☐ | ☐ | Active Duty - Air Force |
| ☐ | ☐ | Reservist |
| ☐ | ☐ | National Guard |
| ☐ | ☐ | Retired Military - list current occupation below |
| | | You _____ |

Father _____

- | | | |
|-----------------------|-----------------------|---------------------------------|
| ☐ | ☐ | Civilian, list occupation below |
| | | You _____ |

Father _____

13. If MILITARY, what is your AND/OR the father's CURRENT rank/paygrade?

You

- | | | | | | |
|-----------------------|-----|-----------------------|------|-----------------------|----------------|
| ☐ | E-1 | ☐ | W-1 | ☐ | O-1 |
| ☐ | E-2 | ☐ | W-2 | ☐ | O-2 |
| ☐ | E-3 | ☐ | W-3 | ☐ | O-3 |
| ☐ | E-4 | ☐ | W-4 | ☐ | O-4 |
| ☐ | E-5 | ☐ | W-5 | ☐ | O-5 |
| ☐ | E-6 | | | ☐ | O-6 |
| ☐ | E-7 | ☐ | O-1E | ☐ | O-7 or above |
| ☐ | E-8 | ☐ | O-2E | | |
| ☐ | E-9 | ☐ | O-3E | ☐ | Not applicable |

Father

- | | | | | | |
|-----------------------|-----|-----------------------|------|-----------------------|----------------|
| ☐ | E-1 | ☐ | W-1 | ☐ | O-1 |
| ☐ | E-2 | ☐ | W-2 | ☐ | O-2 |
| ☐ | E-3 | ☐ | W-3 | ☐ | O-3 |
| ☐ | E-4 | ☐ | W-4 | ☐ | O-4 |
| ☐ | E-5 | ☐ | W-5 | ☐ | O-5 |
| ☐ | E-6 | | | ☐ | O-6 |
| ☐ | E-7 | ☐ | O-1E | ☐ | O-7 or above |
| ☐ | E-8 | ☐ | O-2E | | |
| ☐ | E-9 | ☐ | O-3E | ☐ | Not applicable |

SECTION B: GENERAL HEALTH AND SUBSTANCE ABUSE

1. Do you have a history of any of the following:

- | | | | |
|------------------------------|-----------------------|-----------------------|-----------------------|
| a. Cancer | ☐ | ☐ | ☐ |
| b. Heart disease | ☐ | ☐ | ☐ |
| c. Diabetes | ☐ | ☐ | ☐ |
| d. Hypertension | ☐ | ☐ | ☐ |
| e. Asthma | ☐ | ☐ | ☐ |
| f. Thyroid problems | ☐ | ☐ | ☐ |
| g. Gastrointestinal problems | ☐ | ☐ | ☐ |
| h. Epilepsy | ☐ | ☐ | ☐ |
| i. Other (specify _____) | ☐ | ☐ | ☐ |

2. Before your pregnancy, when was your last routine physical exam?

- ☐ Within the previous year
☐ 1-2 years before
☐ 3-5 years before
☐ Over 5 years before
☐ Never

3. Do you currently smoke?

- ☐ No (continue at question 4)
☐ Yes

If Yes, indicate the average number of cigarettes you smoke per day then skip to question 5.

- ☐ 1-5
☐ 6-10
☐ 11-20
☐ greater than 1 pack, but less than 3 packs
☐ greater than 3 packs

4. Are you exposed to anyone's tobacco smoke for at least 30 minutes total per day?

No Yes

- | | | |
|--------------|-----------------------|-----------------------|
| a. At home | ☐ | ☐ |
| b. At work | ☐ | ☐ |
| c. Elsewhere | ☐ | ☐ |

5. Approximately how many servings (e.g., cups, glasses, cans) of caffeinated beverage (e.g., coffee, tea, soft drink) do you drink per day on average?

- | | | |
|-------------------------|-------------------------|------------------------------------|
| ☐ 0 | ☐ 4 | ☐ 8 |
| ☐ 1 | ☐ 5 | ☐ 9 |
| ☐ 2 | ☐ 6 | ☐ 10 |
| ☐ 3 | ☐ 7 | ☐ More than 10 |

6. Are you currently taking any:

a. prescription drugs?

- ☐ No
☐ Yes, specify: _____

b. other medication (e.g., vitamins, over-the-counter pain relievers, sinus medication)?

- ☐ No
☐ Yes, specify: _____

7. Approximately how many drinks containing alcohol do you consume, on average? (Count a drink as 1 can/bottle (about 12 ounces) of beer, 1 glass (about 4 ounces) of wine, or 1 shot (about 1.5 ounces) of liquor.)

- ☐ 10 or more drinks per week
☐ 3-9 drinks per week
☐ 1-2 drinks per week
☐ 2-3 drinks per month
☐ 1 drink per month
☐ None at all
☐ Do not know

8. Do you have a history of drug abuse?

- ☐ No
☐ Yes, specify which drugs: _____

SECTION C: DENTAL HISTORY

- How many times per week do you brush your teeth?
 - ☐ 0
 - ☐ 1-6
 - ☐ 7 (meaning once a day)
 - ☐ 8-13
 - ☐ 14 (meaning twice a day)
 - ☐ More than 14
- Do you routinely floss your teeth?
 - ☐ No (continue at question 3)
 - ☐ Yes
 If Yes, please indicate how often:
 - ☐ Daily
 - ☐ Weekly
 - ☐ Monthly
- Prior to pregnancy, did your gums bleed from brushing, flossing, and/or spontaneously?
 - ☐ No
 - ☐ Yes
- Have you had any cavities within the last year?
 - ☐ No (continue at question 5)
 - ☐ Yes
 If Yes, please indicate how many:
 - ☐ 1-3
 - ☐ 4-6
 - ☐ 7-10
 - ☐ More than 10
- Have you lost any teeth within the last year (not due to injury)?
 - ☐ No
 - ☐ Yes
- Have you been told by a dentist that you have gum disease and/or any type of dental disease?
 - ☐ No
 - ☐ Yes

SECTION D: GYNECOLOGICAL/ OBSTETRICAL HISTORY

- What method of birth control were you using before your recent pregnancy? (Mark all that apply.)
 - ☐ None
 - ☐ Diaphragm
 - ☐ IUD (Intrauterine Device)
 - ☐ Foam
 - ☐ Condom
 - ☐ Rhythm/any other natural family planning
 - ☐ The Pill
 - ☐ Norplant®
 - ☐ Depo-Provera®
 - ☐ Hypodermic injections
 - ☐ Withdrawal
 - ☐ Other (specify _____)

- Are any of the following included in your medical reproductive history?

- | | Yes | No | Don't know |
|---|-----------------------|-----------------------|-----------------------|
| a. Surgery involving fallopian tube(s) | ☐ | ☐ | ☐ |
| b. Congenital (born with) tubal abnormality | ☐ | ☐ | ☐ |
| c. Other congenital reproductive abnormality | ☐ | ☐ | ☐ |
| d. Fertility treatment(s) | ☐ | ☐ | ☐ |
| e. Identified chromosomal abnormality | ☐ | ☐ | ☐ |
| f. Positive (abnormal) pap smear | ☐ | ☐ | ☐ |
| g. Endometriosis | ☐ | ☐ | ☐ |
| h. Endometritis | ☐ | ☐ | ☐ |
| i. Abnormal estrogen/progesterone level | ☐ | ☐ | ☐ |
| j. Blood-group incompatibility with fetus (Rh or other blood incompatibility) | ☐ | ☐ | ☐ |
| k. Bleeding during pregnancy | ☐ | ☐ | ☐ |
| l. "Bag of waters" breaking before labor | ☐ | ☐ | ☐ |
| m. Other reproductive complication(s) OR problems, specify: _____ | ☐ | ☐ | ☐ |

- Are any of the following sexually transmitted diseases included in your medical history?

- | | Yes | No | Don't know |
|--------------------------------------|-----------------------|-----------------------|-----------------------|
| a. Chlamydia infection | ☐ | ☐ | ☐ |
| b. Gonorrhea | ☐ | ☐ | ☐ |
| c. Syphilis | ☐ | ☐ | ☐ |
| d. Genital warts | ☐ | ☐ | ☐ |
| e. Genital herpes | ☐ | ☐ | ☐ |
| f. PID (Pelvic Inflammatory Disease) | ☐ | ☐ | ☐ |
| g. Bacterial vaginosis | ☐ | ☐ | ☐ |
| h. Trichomoniasis | ☐ | ☐ | ☐ |
| i. Vaginal infection | ☐ | ☐ | ☐ |
| j. Yeast infection | ☐ | ☐ | ☐ |
| k. Chronic discharge and/or odor | ☐ | ☐ | ☐ |
| l. HIV | ☐ | ☐ | ☐ |
| m. Other, specify: _____ | ☐ | ☐ | ☐ |

- How many sexual partners have you had:

In the last 30 days?

In the last 6 months?

1	2
3	4
5	6
7	8
9	10
11	12
13	14
15	16
17	18
19	20
21	22
23	24
25	26
27	28
29	30
31	32
33	34
35	36
37	38
39	40
41	42
43	44
45	46
47	48
49	50
51	52
53	54
55	56
57	58
59	60
61	62
63	64
65	66
67	68
69	70
71	72
73	74
75	76
77	78
79	80
81	82
83	84
85	86
87	88
89	90
91	92
93	94
95	96
97	98
99	100

1	2
3	4
5	6
7	8
9	10
11	12
13	14
15	16
17	18
19	20
21	22
23	24
25	26
27	28
29	30
31	32
33	34
35	36
37	38
39	40
41	42
43	44
45	46
47	48
49	50
51	52
53	54
55	56
57	58
59	60
61	62
63	64
65	66
67	68
69	70
71	72
73	74
75	76
77	78
79	80
81	82
83	84
85	86
87	88
89	90
91	92
93	94
95	96
97	98
99	100

- How frequently do you have intercourse?

- ☐ Every day
- ☐ 2-6 times a week
- ☐ Once a week
- ☐ Once a month
- ☐ Less than once a month
- ☐ Other

6. Have you ever used a douche?

- ☐ No (continue at Section E, question 1)
☐ Yes

If Yes, when?

- ☐ As regular hygiene
☐ After sexual relations
☐ As a result of vaginal itching or discharge (and/or fishy vaginal odor)

If Yes, what product was used?
 (Mark all that apply.)

- ☐ Water
☐ Vinegar
☐ Store bought
☐ Other

SECTION E: PREGNANCY HISTORY AT ENROLLMENT

1. What is your current height?

Feet Inches

0	1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---	---

2. What was your pre-pregnancy weight?

Pounds

0	1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---	---

3. What is your current weight?

Pounds

0	1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---	---

4. What is your estimated delivery date?

MO.	DAY	YR.
0	1	2
3	4	5
6	7	8
9	0	1
2	3	4
5	6	7
8	9	0
1	2	3
4	5	6
7	8	9
0	1	2
3	4	5
6	7	8
9	0	1
2	3	4
5	6	7
8	9	0
1	2	3
4	5	6
7	8	9
0	1	2
3	4	5
6	7	8
9	0	1

5. What was the start date of your last menstrual period?

MO.	DAY	YR.
0	1	2
3	4	5
6	7	8
9	0	1
2	3	4
5	6	7
8	9	0
1	2	3
4	5	6
7	8	9
0	1	2
3	4	5
6	7	8
9	0	1
2	3	4
5	6	7
8	9	0
1	2	3
4	5	6
7	8	9
0	1	2
3	4	5
6	7	8
9	0	1

6. Was an ultrasound performed?

- ☐ No (continue at question 7)
☐ Yes

If Yes, when?

If Yes, estimated date of conception:

MO.	DAY	YR.
0	1	2
3	4	5
6	7	8
9	0	1
2	3	4
5	6	7
8	9	0
1	2	3
4	5	6
7	8	9
0	1	2
3	4	5
6	7	8
9	0	1
2	3	4
5	6	7
8	9	0
1	2	3
4	5	6
7	8	9
0	1	2
3	4	5
6	7	8
9	0	1

MO.	DAY	YR.
0	1	2
3	4	5
6	7	8
9	0	1
2	3	4
5	6	7
8	9	0
1	2	3
4	5	6
7	8	9
0	1	2
3	4	5
6	7	8
9	0	1
2	3	4
5	6	7
8	9	0
1	2	3
4	5	6
7	8	9
0	1	2
3	4	5
6	7	8
9	0	1

7. How many weeks pregnant are you?

Weeks

0	1	2	3	4	5	6	7	8	9
---	---	---	---	---	---	---	---	---	---

8. How many times have you been pregnant (including current pregnancy)?

9. Of these pregnancies, how many were:

- a. live births (children born alive)?
 b. still births (fetal deaths occurring after 22 weeks' gestation)?
 c. full term births?
 d. premature births (less than 37 weeks' gestation)?
 e. low birth weight births (less than 2,500 grams)?
 f. miscarriages/spontaneous abortions (fetal death before 22 weeks' gestation)?
 g. abortions (pregnancy surgically terminated)?

One	Two	Three	Four	Five	Six	Seven	Eight	Nine	Ten	More than 10
0	1	2	3	4	5	6	7	8	9	0
1	2	3	4	5	6	7	8	9	0	1
2	3	4	5	6	7	8	9	0	1	2
3	4	5	6	7	8	9	0	1	2	3
4	5	6	7	8	9	0	1	2	3	4
5	6	7	8	9	0	1	2	3	4	5
6	7	8	9	0	1	2	3	4	5	6
7	8	9	0	1	2	3	4	5	6	7
8	9	0	1	2	3	4	5	6	7	8
9	0	1	2	3	4	5	6	7	8	9

10. If any of the previous pregnancies resulted in either premature births or abortions, please specify result and reason:

APPENDIX 1

MOTHER'S AND INFANT'S DATA QUESTIONNAIRE

(Epidemiological Study of Pregnancies: Medical Record Abstraction Form and Questionnaire)

IMPORTANT INSTRUCTIONS

- * USE NO. 2 PENCIL ONLY.
- * Do NOT use ink, ballpoint, or felt tip pens.
- * Erase cleanly and completely any changes you make.

SECTION G: First Prenatal Lab Results

1. What Is the SPONSOR'S Social Security Number:

SOCIAL SECURITY NUMBER									
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9

2. What Is the SPONSOR'S birthdate?

MO.		DAY		YR.	
0	1	0	1	0	0
0	1	0	1	0	0
0	1	0	1	0	0
0	1	0	1	0	0
0	1	0	1	0	0
0	1	0	1	0	0
0	1	0	1	0	0
0	1	0	1	0	0
0	1	0	1	0	0
0	1	0	1	0	0

3. What are the SPONSOR'S Initials?

First	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
Middle	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
Last	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z

Cervical Exam (Items 1-4 to be filled out by the medical provider)

- | | No | Yes |
|---|-----------------------|-----------------------|
| 1. Mucopurulent Cervical Discharge | ☐ | ☐ |
| 2. Easily Induced Endocervical Bleeding | ☐ | ☐ |
| 3. Edematous Inflammation | ☐ | ☐ |

4. % ectopy:

%
0
1
2
3
4
5
6
7
8
9

Clinical Microbiology (Items 5-11 to be filled out by the microbiologist)

5. Bacterial Vaginosis Slide:

Score (#): ☐ 1 ☐ 5 ☐ 8
☐ 2 ☐ 6 ☐ 9
☐ 3 ☐ 7 ☐ 10
☐ 4

No Yes

6. Yeast ☐ ☐
 7. Unsat ☐ ☐
 8. Sperm ☐ ☐

9. WBC: ☐ absent
☐ few
☐ moderate
☐ many

Comments: _____

10. Wet Prep:

No Yes

- a. Trichomonas ☐ ☐
 b. Yeast ☐ ☐
 c. Clue Cells ☐ ☐
 d. Blood In Vagina ☐ ☐
 e. Vaginal DC present ☐ ☐
 Description:
☐ clumpy
☐ homogenous
☐ frothy

f. Whiff test (KOH) ☐ ☐

Negative Positive

g. InPouch® T.V. ☐ ☐

11. pH:

pH
0
1
2
3
4
5
6
7
8
9

00058

VOLUNTEER REGISTRY DATA SHEET

THIS FORM IS AFFECTED BY THE PRIVACY ACT OF 1974

1. AUTHORITY: 5 USC 301; 10 USC 1071-1090; 44 USC 3101; EO 9397
2. Principal and Routine Purposes: To document participation in research conducted or sponsored by the U.S. Army Medical Research and Development Command. Personal information will be used for identification and location of participants.
3. Mandatory or Voluntary Disclosure: The furnishing of the SSN is mandatory and necessary to provide identification and to contact you if future information indicates that your health may be adversely affected. Failure to provide the information may preclude your participation in the research study.

PART A-INVESTIGATOR INFORMATION

(To Be Completed By Investigator)

PLEASE PRINT, USING INK OR BALLPOINT PEN

1. Study NR: _____ 2. Protocol Title: _____
3. Contractor (Laboratory/Institute Conducting Study): _____
4. Study Period: From: 01/____/____ To: 15/____/____
(DA/MO/YR) (DA/MO/YR)
5. Principal/Other Investigator(s) Names(s) 6. Location/Laboratory
- (1) _____ / _____
(Last) (First) (MI)
- (2) _____ / _____
- (3) _____ / _____

PART B-VOLUNTEER INFORMATION

(To Be Completed By Volunteer)

PLEASE PRINT, USING INK OR BALLPOINT PEN

7. SSN: ____/____/____ 8. Name: _____
(Last) (First) (MI)
9. Sex: M__F__ 10. Date of Birth: ____/____/____ 11. *MOS/Job Series: ____ 12. *Rank/Grade: ____
13. Permanent Home Address (Home of Record) or Study Location Address:
- _____
(Street) (P.O. Box/Apartment No.)
- _____
(City) (Country) (State) (Zip Code)
- (_____) - _____
(Perm Home Phone No)
14. *Local Address (If Different From Permanent Address):
- _____
(Street) (P.O. Box/Apartment No.)
- _____
(City) (Country) (State) (Zip Code)
- (_____) - _____
(Local Phone No)
15. *Military Unit: _____ Zip Code: _____
- Organization: _____ Post: _____ Duty Phone No. (____) - _____

PART C-ADDITIONAL INFORMATION
(To Be Completed By Investigator)

PLEASE PRINT, USING INK OR BALLPOINT PEN

16. Location of Study:

17. Is Study Completed: Y___ N___

Did volunteer finish participation: Y___ N___ If YES, Date finished: ____/____/____
(DA/MO/YR)

If NO, Date withdrawn: ____/____/____ Reason withdrawn: _____
(DA/MO/YR)

18. Did Any Serious or Unexpected Adverse Incident or Reaction Occur: Y___N___ If YES, Explain:

19.*Volunteer Followup: _____

Purpose: _____

Date: ____/____/____ Was contact made: Y___N___ If No action taken, explain:
(DA/MO/YR)

20.*Hard Copy Records Retired: Place: _____ File NR: _____

21.*Product Information:

Product: _____

Manufacturer: _____

Lot NR: _____ Expiration Date: _____

NDA NR: _____ IND/IDE NR: _____

*Indicates that item may be left blank if information is unavailable or does not apply.
Entries must be made for all other items.

SELF-SWABBING INSTRUCTIONS

Self swabbing can be performed by standing with one foot propped on the toilet seat or by sitting on toilet with knees apart.

You have been given 6 sterile swabs (2 per package) and 1 sterile tube.

1. Wash hands well with soap and water. Rinse well.
2. Wash outer vaginal area as instructed for "clean catch" urine specimen.
3. Very carefully remove 2 swabs from their packet (without touching the actual swab).
4. With one hand, gently hold open the skin surrounding the vaginal opening.
5. Swab the side walls of the vagina.
6. Rotate the swabs against the side walls of the vagina so that both swabs absorb secretions.
7. Carefully place swabs in the sterile tube without touching them to any other surface.
8. Repeat steps 3 through 7 for swabs 3 and 4.
9. Repeat steps 3 through 7 for swabs 5 and 6.
10. Replace blue cap on tube when all 6 swabs have been placed inside.
11. Return tube to study nurse.

APPENDIX 4

INSERVICE LECTURE

History

Dr Gail Cassell, Professor and Chairman of the Department of Microbiology, at the University of Alabama, Birmingham, has been studying *Ureaplasma* and *Mycoplasmas* for 20+ years and has done extensive research in this area. Her work has brought these organisms to the attention of maternal-child health practitioners.

Recent data indicates that:

- U.S. military women may be at increased risk for delivery of preterm births.
- *Ureaplasma urealyticum* (*Uu*) infection of the chorioamnion is associated with births less than 37 weeks
- infection is inversely related to gestational age and birth weight
- *Uu* infection is a significant cause of pneumonia, meningitis and death in very low birth weight infants

Dr. Cassell has received a grant from the Department of Defense to study in cooperation with Naval Medical Center, San Diego, and the Naval Health Research Center "Risk Factors for Chorioamnion Infection and Adverse Pregnancy Outcomes Among Active-duty and Dependent Military Women."

Eligibility

All women who report for prenatal care at NMCS D and plan to deliver their baby at NMCS D will be offered the opportunity to participate in this study

Objectives

1. To determine whether *Uu* chorioamnion colonization is associated with preterm birth in both active duty military personnel and dependents of active duty military personnel.
2. To identify factors that may predict chorioamnion invasion and premature birth, specifically, if the presence of bacterial vaginosis (BV) is a risk factor for ureaplasma invasion of the chorioamnion.

Methodology

Pre-registration:

- patients will be identified and asked to participate
- a consent form will be read and signed by each participant
- a data questionnaire will be filled out by each participant
- an extra red top will be drawn to look for *Uu* antibodies

First prenatal visit:

- six vaginal swabs will be collected via sterile technique by participant's provider during pelvic examination
- one swab will be used to do a wet-prep in the clinic looking for yeast and *Trichomonas*
- one swab will be used for gram -stain looking for BV
- two swabs will be placed in transport media and frozen at -70 degree centigrade and shipped to UAB to look for *Uu* and *Mycoplasmas*
- one swab for PCR for *Uu*
- one swab for pH, whiff test and mucosal antibodies

Delivery:

- an extra red top will be collected upon admission to L&D to look for *Uu* antibodies
- four vaginal swabs will be collected by participant's provider during first pelvic exam (one for gram-stain for BV, others shipped at -70 degree C to UAB for study)
- placenta, cord blood, amniotic fluid and respiratory secretions (infant) will be collected and processed for *Uu* culture
- specimens will be collected and processed by UAB Study personnel 24 hours per day, 7 days per week, to ensure recovery of fastidious organisms and to avoid enrollment bias

NICU:

- if infant requires intubation ETT secretions will be collected and cultured here at NMCSO by UAB study staff for *Uu* and *Mycoplasmas*
- if infant requires a lumbar puncture; CSF will be collected and cultured here at NMCSO by UAB study staff for *Uu* and *Mycoplasmas*
- UAB study personnel will be available to do blood cultures for *Uu* and *mycoplasmas* also

Autopsy (Infant):

- upon request of the medical officer, brain and lung tissue as well as blood, pleural fluid and CSF can be cultured by UAB study staff for *Uu* and *Mycoplasmas*

UAB STUDY

OB CLINIC STAFF PROCEDURE FOR FIRST PRENATAL VISIT

During the 1st prenatal visit each study participant must have 6 vaginal swabs collected during her routine pelvic examination. **These swabs should be collected at the same time in the vaginal vault.** It is acceptable to inoculate the swabs in a pool of secretions or with discharge. **DO NOT COLLECT FROM CERVIX.**

One swab will be used to do a wet prep in the clinic by study personnel to identify *Trichomonas* and yeast. One swab will be used for PCR, another for pH, and the others will be used to test for bacterial vaginosis, *Ureaplasma urealyticum* and *mycoplasmas*. **Study participants will be known to staff by UAB STUDY sticker on the front cover of the chart.**

1. Collect 6 vaginal swabs during pelvic examination via sterile technique.
 - **use dacron swabs only**
 - place swabs in vaginal vault, but not cervical
 - rotate 360 degrees in order to apply vaginal secretions to all swabs
 - remove swabs from vagina
2. Place all 6 swabs in sterile glass vial.
3. Replace sterile cap on vial.
4. Place patient's stamped label on vial.
5. Place specimens in UAB Study collection rack.
6. "Stand-by" will place vial in UAB Study collection rack in Check-out for collection by UAB Study personnel.
7. **Providers:** Please fill in Cervical Exam information (items 1-4), on Appendix 1 (middle of page, shaded area) with #2 pencil after "New OB" examination completed.
"Stand-by" to place Appendix 1 with swabs in "check-out" for collection by UAB Study staff

UAB STUDY

LABOR AND DELIVERY STAFF PROCEDURE

Study participants will be known to staff by UAB STUDY sticker found on the front cover of the patient's chart. All specimens collected on study participants will be cultured for *Ureaplasma urealyticum* and *mycoplasmas*.

1. Draw an extra red top (total of 2: 1 for UAB Study and one for routine L/D lab work per normal routine) on each participating woman during screening blood work.
 - a. place participant's stamper label on red top for UAB Study
 - b. place UAB Study red top in laboratory refrigerator
 - c. UAB Study personnel will collect vials from refrigerator several times throughout the day
2. 4 vaginal swabs should be collected on **all** study participants during their first pelvic examination by their provider, via sterile technique.
 - a. **use dacron swabs only**
 - b. place all 4 swabs in vaginal vault at same time, not cervical
 - c. rotate 360 degrees to apply vaginal secretions to all swabs
 - d. it is acceptable to inoculate swabs in a pool of secretions or with discharge
 - e. remove swabs from vagina
 - f. place all 4 swabs in sterile glass vial
 - g. replace cap on vial
 - h. label all specimens with patient's stamper plate and place in laboratory refrigerator
 - i. UAB study personnel will collect these from refrigerator several times throughout the day
3. **Page UAB Study personnel upon delivery of participants delivering by c-section with intact membranes or participants with the last 2 digits of sponsor's social security numbers that are __, __, __, __, __, who deliver via c-section with ruptured membranes or vaginal delivery.**
4. All study participants who will be delivered by **cesarean section** who have **intact membranes** should have:
 - a. approximately 5 to 10 cc of amniotic fluid, collected in a sterile syringe, via trans-uterine amniocentesis, just prior to making the uterine incision, or after the uterus is entered, but before the membranes are ruptured (cap syringe after collection)
 - b. approximately 5 to 10 cc cord blood collected via sterile technique and placed in a red top vacutainer, marked "Cord"
 - c. placenta placed in 2 sterile bags, via sterile technique, upon delivery
 - d. label all specimens with patient's stamper plate
5. All **randomly selected participants** who deliver by **c-section with ruptured membranes** who deliver by **c-section with ruptured membranes** should have:
 - a. approximately 5 to 10 cc, or whatever is available, of amniotic fluid collected with a sterile syringe, via sterile technique, cap syringe after collection (if possible, collect amniotic fluid via trans-uterine amniocentesis, just prior to making the uterine incision, or after the uterus is entered, but before the membranes are ruptured)
 - b. approximately 5 to 10 cc cord blood collected via sterile technique and placed in a red top vacutainer, marked "Cord"
 - c. placenta placed in 2 sterile bags, via sterile technique, upon delivery
 - d. label all specimens with patient's stamper plate
6. All **randomly selected participants** who deliver by **vaginal delivery** should have:

- a. approximately 5 to 10 cc, or whatever is available, of amniotic fluid collected with a sterile syringe, via sterile technique (cap syringe after collection)
 - b. approximately 5 to 10 cc cord blood collected via sterile technique and placed in a red top vacutainer, marked "Cord"
 - c. placenta placed in 2 sterile bags, via sterile technique, upon delivery
 - d. label all specimens with patient's stamper plate
7. All infants of study participants (including those that might expire shortly after delivery) from categories #4, 5, or 6 should have a nasal swab taken. If infant requires immediate transport to transition nursery or NICU, please notify appropriate nursery staff that infant is a study participant and requires a nasal swab.
 - a. utilizing sterile technique, place sterile **dacron** swab into infant's nare and rotate 360 degrees
 - b. place swab in **room temperature** transport media
 - c. Swish swab around in media well
 - d. Express excess fluid from swab by placing swab to side of container, above fluid, and applying gentle pressure, rotate swab a few times repeating this step to assure the majority of fluid is removed from the swab
 - e. Label container with patient's stamper plate, marked "Infant NP"
8. Please flag all charts/paperwork of infants from category #4, 5, or 6 with UAB STUDY sticker as a reminder to NICU staff that should this infant require intubation and/or L/P further specimens should be collected.
9. UAB study personnel will pick up all specimens collected after delivery to process per study protocol.

UAB STUDY

NICU STAFF PROCEDURES

In order to complete this study "full circle" infants that require admission to the NICU need to be cultured. **Study participants will be known to the NICU staff by the UAB STUDY sticker placed on the infant's chart/paperwork.**

1. If infant is brought immediately to transition nursery or NICU after delivery: infant's nasal swab should be collected by nursery staff by sterile technique as soon as possible, preferably within 1 hour of delivery and/or prior to repeated placement of suction either by bulb or catheter. **Swab should be collected prior to antibiotic therapy.**
 - a. Utilizing sterile technique, place sterile **dacron** swab into infant's nare and rotate 360 degrees
 - b. Place swab in **room temperature** transport media
 - c. Swish swab around in media well
 - d. Express excess fluid from swab by placing swab to side of container, above fluid, and applying gentle pressure, rotate swab a few times repeating this step to assure the majority of fluid is removed from the swab
 - e. Label container with patient's stamper plate
 - f. **DO NOT** send this specimen to the lab with other laboratory specimens. UAB study personnel will pick this specimen up ASAP.
2. If any participating infant requires intubation, respiratory secretions should be collected.
 - a. Remove transport media from refrigerator so that it can warm to room temperature.
 - b. Suction via ETT by sterile technique utilizing sterile suction catheter, sterile gloves, and sterile specimen trap.
 - c. If needed use approx. 0.5 cc sterile (unopened bullet) non-bacteriostatic saline to wash secretions into specimen trap.
 - d. Please ensure that sterile (unopened bullet) non-bacteriostatic saline is utilized if lavage is necessary for suctioning purposes.
 - e. Via sterile technique, utilizing a sterile TB syringe, place approx. 0.2 to 0.5 cc (preferably 0.5 cc) of secretions into **room temperature** transport media.
 - f. Label vial with infant's stamper plate.
 - g. Mark label with "ETT."
 - h. If infant has been receiving antibiotics prior to intubation, please note on specimen label, the type of antibiotics the infant is on (i.e. Ampicillin and Gentamicin).
 - i. Return inoculated vial to the laboratory refrigerator.
 - j. UAB study personnel will pick up these vials daily from the refrigerator.
3. If any participating infant requires L/P:
 - a. Remove transport media from laboratory refrigerator to warm to room temperature.
 - b. Utilizing sterile technique drop approximately 0.2 to 0.5cc (preferably 0.5 cc) CSF into **room temperature** transport media vial.
 - c. Label vial with infant's stamper plate.
 - d. Mark label with "CSF."
 - e. If infant has been receiving antibiotics prior to L/P, please note on specimen label, the type of antibiotics the infant is on (i.e. Ampicillin and Gentamicin).
 - f. Return inoculated vial to the laboratory refrigerator.
 - g. UAB study personnel will pick up the vials daily from the refrigerator.
4. If the medical officer from the NICU would like **any baby** (not necessarily a study participant's) to be cultured for *Ureaplasma* or *Mycoplasmas*, please follow above procedures and the UAB study personnel will perform these cultures also.

5. If the medical officer from the NICU would like **any baby (both study participant and non participant)** to have blood cultures checked for *Ureaplasma* or *Mycoplasmas* the UAB study personnel will perform these cultures also. **Blood cultures should be drawn before placing infant on antibiotics.**
 - a. Remove transport media from laboratory refrigerator to warm to room temperature.
 - b. Draw blood specimen via sterile technique.
 1. Wash skin with 3 sterile betadine preps
 2. Wipe with sterile 2x2 or sterile alcohol prep
 3. Utilize sterile butterfly and sterile syringe to draw blood
 4. Withdraw 0.2 to 0.5 cc blood (preferably 0.5 cc)
 5. Use sterile 2x2 to place over top of butterfly when removing from skin
 6. Place blood via sterile technique into **room temperature** transport media
 - c. Label inoculated vial with infant's stamper plate.
 - d. Mark label with "blood."
 - e. Return inoculated vial to laboratory refrigerator.
 - f. UAB study personnel will pick up specimens daily from laboratory refrigerator.
6. If an infant expires and an autopsy is requested, the UAB study personnel will be available to culture brain and lung tissue, CSF, pleural fluid and blood for *Ureaplasma* and *Mycoplasmas* at the medical officer's request.

APPENDIX 5

Summary of First 100 Questionnaires

DATABASE MANAGEMENT PROCEDURES

- FoxPro - data entry
- SAS - descriptive statistics
- For official study - questionnaires will be scanned into a database

Maternal Demographic Characteristics

- *AGE* Range: 16 - 39 years, Mean: 26.04 years,
Median: 25 years
- *MARITAL STATUS* 86% married
- *RACE* 58% white, 16% black
- *MILITARY STATUS* 57% civilian, 33% Navy (of
these, 81% E2 - E6 rank)
- *EDUCATION* 34% - high school, 36% - 1-2 years
of college

Maternal Health-Related Behavior

- **SMOKING** 93% non-smokers
- **ALCOHOL** 85% - no alcohol
- **BRUSH TEETH** 41% - 2 times/day
11% - once/day
- **FLOSS TEETH** 60% - floss (most floss daily)
- **DOUCHING** 52% - ever douched (of these,
65% for regular hygiene, 28% for vaginal itch/
discharge)

Maternal Health-Related Behavior

- *SEXUAL ACTIVITY* 67% - 2-6 times/week, 23% - once/week
- *NUMBER OF SEXUAL PARTNERS* 94% - one partner (last 30 days), 91% - one partner (last 6 months)
- *BIRTH CONTROL USE* 59% before recent pregnancy (32% - oral contraceptives, 12% - condoms)

Pregnancy History

BIRTH OUTCOMES

- 75% previously pregnant
- 56% previous live birth

Of these: 14% pre-term delivery
 12.5% low birth weight

- 4% previous still birth
- 29% previous spontaneous abortion/miscarriage
- 27% previous induced abortion

Maternal Reproductive History

- **REPRODUCTIVE HISTORY** 55% had a reproductive health care problem
 - 20% abnormal Pap
 - 13% bleeding during pregnancy
 - 14% membrane rupture
- **STD** 12% chlamydia, 5% gonorrhea, 6% genital herpes, 1% syphilis
- **INFECTIONS** 53% yeast infection, 8% vaginal infection, 5% PID, 5% genital warts, 3% bacterial vaginosis, 2% chronic discharge and/or odor

Maternal Medical History

- *ASTHMA* 13%
- *DIABETES* 10%
- *HYPERTENSION* 6%
- *GASTROINTESTINAL PROBLEMS* 5%
- *HEART DISEASE* 4%
- *THYROID PROBLEMS* 4%
- *CANCER* 3%
- *EPILEPSY* 2%

Preliminary Laboratory Results

CERVICAL EXAM RESULTS

- 78% negative - mucopurulent cervical discharge
- 83% negative - endocervical bleeding
- 91% negative - edematous inflammation

Preliminary Laboratory Results

WET PREP RESULTS

- 99% negative - Trichomonas
- 95% negative - Yeast
- 80% negative - Clue cells
- 73% negative - Blood in the vagina
- 72% negative - Vaginal discharge (when present, 85%
was homogenous)
- 80% negative - Whiff test (KOH)
- 96% negative - InPouch T.v.

Preliminary Laboratory Results

06000

BACTERIAL VAGINOSIS SLIDE RESULTS

- 21% positive for BV
- > Of 68 slides read using the new Gram stain criteria, 14 were positive.

CERVICOVAGINAL CULTURE RESULTS

- 62% positive for Uu
- > Of 145 cultures, 90 were positive.



DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

26 Aug 02

MEMORANDUM FOR Administrator, Defense Technical Information
Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir,
VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

Phyllis M. Rinehart
PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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