

Protocol Title: *Establishing Baseline Sysmex UF-5000 Flow Cytometer Results in Healthy Men*

IU Protocol #26774

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Abbreviations

GC	<i>Neisseria gonorrhoeae</i>
CT	<i>Chlamydia trachomatis</i>
CDC	Centers for Disease Control
DCF	Data Collection Form
EMR	Electronic Medical Record
US	United States
BFC	Bell Flower Clinic
NAAT	Nucleic Acid Amplification Test
NGU	Nongonococcal urethritis
LE	Leukocyte esterase
STI	Sexually Transmitted Infections
WBC	White blood cells

1.0 Background and Rationale

Acute urethritis (i.e., inflammation of the male urethra) affects millions of men within the United States annually [1]. In young sexually active men, urethritis is most commonly caused by the sexually transmitted infections (STI) *Neisseria gonorrhoeae* (GC, causes gonorrhea) and *Chlamydia trachomatis* (CT, causes chlamydia), but can also be caused by a lengthening list of known and emerging pathogens that cause “nongonococcal urethritis” (NGU) [2]. STI management has proven challenging due to the prevalence of asymptomatic infections and because men often fail to seek STI care until they experience pronounced symptoms. However, even without urethral symptoms, most men with a urethral STI have measurable evidence of urethritis [3]. Thus, a cheap, simple, and non-invasive point of care (POC) test (i.e., results available within 30 minutes) that accurately detects urethritis could be a game changer in STI management as asymptomatic infections could be detected and treated, potentially decreasing the risk of spreading STIs to sexual partners.

Currently, the only widely used POC test available for diagnosis of urethritis in men in the US is Gram stain microscopy of a urethral swab smear [4]. This approach has been shown to be highly effective for identifying men who have urethritis and has 95% sensitivity for diagnosing GC in symptomatic men [4]. Although the urethral swab remains the gold standard test to diagnose urethritis at the POC, it is rarely used outside of specialized STI clinics [5] given its many limitations, which include: (1) it often elicits severe pain, and in rare cases can permanently damage the urethra [6]; (2) it requires specialized instrumentation (an inverted microscope with a 1000x objective) and Gram stain reagents; (3) it must also be administered and interpreted by trained personnel that have different inter-operator accuracy in reporting results, and (4) anxiety associated with this procedure is a common reason men cite for delaying STI care and failing to return for follow-up visits [6]. In many other clinical settings, POC screening for STIs would be highly desirable, for example during annual wellness exams of high-risk male adolescents; screening with specific STI nucleic acid amplification tests (NAATs) is not cost-effective in low-risk populations and the urethral swab procedure is unacceptable in these populations. As an alternative approach, a few investigators have evaluated Leukocyte esterase (LE) testing as part of a routine urinalysis using first-catch urine to diagnose urethritis, but the low sensitivity and specificity of this approach has limited its use to resource-limited settings.

Flow cytometry is routinely used in clinical settings to identify populations of cells (e.g., neutrophils, red blood cells for whole blood counts) from blood and urine [7]. Modern flow cytometers are highly automated, rapid, and inexpensive to run, which makes them an optimal assay for screening for urethritis by detecting elevated neutrophils in asymptomatic men. Interestingly, several recent publications have shown that flow cytometric analysis of urine can be used to diagnose urethritis with comparable sensitivity and specificity to the urethral Gram stain smear [8, 9]. However, most of these studies did not use state-of-the-art flow cytometers,

did not calibrate Gram stain to flow cytometric results directly, and did not diagnose the sensitivity/specificity of this approach for detection of a broad section of urethral STI. We hypothesize that the state-of-the-art flow cytometer analyzer, Sysmex UF-5000, may be able to similarly provide a non-invasive and cost-effective approach for urethritis diagnosis in men using first-catch urine when compared to Gram stain and urinalysis. A prior version, the Sysmex UF-1000i, has been shown to be promising in detection of urinary tract infections, but has not yet been studied in STIs. The UF-5000 could be a powerful screening approach for urethral STI that could be employed in a broad range of clinical settings, not just specialized STI clinics. Separately, if urine flow cytometry could be optimized to yield similar sensitivity to the Gram stain smear, we suspect this approach would rapidly replace the Gram stain smear in clinical practice which in turn would improve patient capture and retention.

2.0 Objective(s)

2.1 Aim 1: Establish the baseline number of neutrophils (WBC/uL) in urine and LE by urinalysis in 20 healthy controls without gonorrhea and chlamydia in FCU collected with a sterile urine cup (~30-60mL) or a 10mL Colli-Pee device (~10mL).

Hypothesis: Our hypothesis is that the 10mL Colli-Pee will increase both accuracy of both urine flow cytometry and LE by urinalysis and sensitivity (a higher number of WBC/uL will be recorded and a higher level of LE, compared to the standard-of-care urine cup (30-60mL).

2.2 Aim 2: Determine the day-to-day variation in baseline neutrophil and LE levels, comparing Colli-Pee and sterile urine cups.

Hypothesis: We hypothesize that the 10mL Colli-Pee will increase precision of both urine flow cytometry and LE (averaged across 3 days) by urinalysis compared to the standard-of-care urine cup (30-60mL).

2.3 Aim 3: Characterize the decrease in LE and Sysmex WBC/uL urine levels over time (time 0, 1hr, 2hr, 4hr, and 8 hr.) by urinary pH.

Hypothesis: We hypothesize that urines with a lower pH (measured by urinalysis) will have a more rapid loss of WBCs/uL and LE over time, compared to urine with a higher pH.

3.0 Outcome Measures/Endpoints

3.1 Primary Outcome Measures

The primary outcomes to be measured will be the outputs of the urinalysis (leukocyte esterase, LE) and the Sysmex UP-5000. For the urinalysis, the categorical results will be “neg”, “1+”, “2+”, “3+”, and “4+” leukocyte esterase. The UF5000 results will be the

continuous variable of “WBCs per microliter” of urine. We will then compare these results across (1) device type (Aim 1); (2) repeat sampling (Aim 2); and (3) over time (Aim 3), comparing specimens from 0 hours, 1 hours, 2 hours, 4 hours, and 8 hours.

4.0 Eligibility Criteria

4.1 Inclusion Criteria

- Men aged 18 to 50.
- No urethral symptoms (discharge, dysuria)

4.2 Exclusion Criteria

- History of genital surgery or known urethral abnormalities
- Testing positive for gonorrhea and chlamydia
- Diagnosis of a sexually transmitted infection within the last year.
- Not available to collect a urine specimen in the morning 3x/week for 2 weeks

5.0 Study Design

5.1 Study Population

We will recruit 20 men aged 18 to 50 from within Indiana University-Indianapolis and Indiana University School of Medicine who have no urethral symptoms and have not been diagnosed with an STI within the last year. Participants will be recruited using posted flyers.

5.2 Study Procedures

We will recruit healthy men aged 18 to 50 who, after consenting to participate, will be assigned to start collecting a urine specimen three times week (typically, Monday, Wednesday, and Friday) for two weeks (**Table**). If participants prefer, they may either start urine collection the day they consent or the next convenient morning (whichever aligns best with their schedule). The device used to collect urine will be either a Colli-Pee or a standard-of-care 60ml sterile urine cup. Participants with an odd birth year will use the Colli-Pee during the first week; even birth year participants will use the Colli-Pee during the second week). Participants will be asked to not urinate within the hour prior to collecting their urine specimen. To accommodate participant schedules, the date/time that they collect their urine is flexible but will need to occur during the work week (Mon-Fri) and in the morning due to the time-series experiments in aim 3.

Table: Anticipated Study Visits (N=6) and Devices for Collecting First-Catch Urine (N = 10 healthy controls)											
	Enroll	Week 1					Week 2				
	Any Day	Mon	Tues	Wed	Thurs	Fri	Mon	Tues	Wed	Thurs	Fri
Odd DOB	\$*	CP		CP		CP+\$	UC		UC		UC+\$
Even DOB	\$*	UC		UC		UC+\$	CP		CP		CP+\$

**Enrollment and First Urine Collection may occur on the same day if participant prefers*

CP, Colli-Pee

UC, Urine Cup

\$, Compensation
(Enrollment, 3rd
Collection)

Each collection day (3x per week), participants will present to MS224E and complete questions on the DCF about time of last urination and any symptoms they are currently

having. They will then be provided with a study-coded (i.e., HCU01) sterile collection device and asked to collect their specimen in the MS224 bathroom (approx. 30 ft from MS224E). Upon collection, participants will secure the cup/tube, drop it into a biohazard bag, seal the bag, wash their hands, and then deliver the specimen to MS224E. The specimen will then be aliquoted and subjected to flow cytometry and urinalysis. Residual. Any residual specimens will be stored at -80C for future studies.

Participants will be compensated \$30 on study enrollment and then \$30 each week after their 3rd urine collection (typically on Friday), for a total of \$90/participant.

Clinical characteristics. Clinical characteristics for each participant will be recorded in the DCF. Variables include date of birth (year only); race; ethnicity; symptoms (dysuria, discharge, testicular pain, etc.), prior STI diagnosis (gonorrhea, chlamydia, trichomonas etc.), current symptoms, and time of last urination (See appendix for DCF).

Treatment and Laboratory testing. Should any participant test positive for a sexually transmitted infection, treatment will be provided for free by scheduling a treatment visit at the Bell Flower STD Clinic. This will be facilitated by Dr. Jordan.

6.0 Enrollment/Randomization

Over 2 months, we will collect 6 urine specimens from 20 men aged 18 to 50 (total N = 120 specimens). Enrollment will occur without regard to race or ethnicity and we anticipate our enrollment population will be similar to the population that attends the Bell Flower Clinic. In 2023, BFC clients were approximately 55% African- American, 15% Hispanic, 30% White and 15% other. In 2023, there were 11,386 unique client visit to BFC, of which 65% were male (N = 7342) and 85% were symptomatic. The type of device used during the first week will be assigned based on the birth year (odd years will use a Colli-Pee during the first week, even years will use a Colli-Pee during their second week).

7.0 Study Procedures

Consent

Subjects will provide written consent following a detailed explanation of the study.

Potential benefits of the proposed research to subjects and others

There is no direct benefit to research participants. The knowledge gained from this project will allow us to identify novel diagnostics to detect urethral inflammation and potentially detect more GC/CT infections in the future.

Protection against risk

Confidentiality is protected by omission of individual identifiers and assigning a study number to each specimen. Separate password-protected files containing individual identifiers will be maintained for administrative purposes to allow for longitudinal tracking. Study-coded data will be stored in Microsoft excel on IU servers using password-protected, IU-owned and operated computers. Our final protection against retrospective discovery will be to destroy linkages between individual identifiers and study identification

numbers at the conclusion of the data analysis. Thus, data—including the data collection form—will be identified only by research identification numbers and no linkage to individual identifiers will exist. Computer files are maintained within the offices of the principal investigator. Access to any files is limited to pre-approved key research personnel only.

Importance of knowledge to be gained

The importance of this study is that we will understand whether the Colli-Pee or standard-of-care urine cup is the optimal device for collecting urine for measuring neutrophils using the non-invasive Sysmex urethral flow cytometer or LE by urinalysis. We will also be able to know which analysis method and collection device is the most precise across different collection days and how quickly the urine results (WBCs/uL or LE) change over time. This knowledge may help us assess the potential of the Sysmex UF-5000 to replace the Gram stain in the future.

Participant reimbursement

Participants will be compensated a total of \$90 for participating in this study. Compensation will be provided on study enrollment (\$30) and each Friday, after they have completed 3 urine collections. Compensation will be given as a \$30 Liat Gift Card.

8.0 Study Calendar

In this 4-month study, we will collect 60 urine specimens from 20 participants in the first 2 months. In the later 2 months, we will then analyze the results and consolidate findings in an R01/R21 application to be submitted in mid-2025.

9.0 Reportable Events

Adverse Events

The proposed research constitutes minimal risk to study participants. Adverse events are expected to be mild and infrequent and involve possible loss of patient confidentiality and embarrassment at collecting a urine specimen.

10.0 Data Safety Monitoring

The use of a data and safety monitoring committee is not proposed, since the study represents basic research using non-invasively-collected de-identified human specimens and there is no intervention arm.

11.0 Study Withdrawal/Discontinuation

Participants may withdraw or discontinue involvement at any time. Participants who wish to formally withdraw must notify the Principal Investigator for this study **in writing** at Stephen Jordan, MD, Van Nuys Med Sci Building, Suite 224, 635 Barnhill Drive, MS 224, Indianapolis, IN 46202. However, the research team, research sponsor(s) and/or the research organizations may still use samples that were collected as part of the research

project between the date the informed consent form was signed and the date the withdraw notice was received. This is to protect the quality of the research results.

If the participant wishes to cease participation in the study in the middle of sample collection, the participant can verbally tell the practitioner, sample collection will stop, and all data and specimens will be immediately destroyed.

12.0 Statistical Considerations

For aim 1, we will use descriptive statistics to characterize the study populations (median age, race, ethnicity, etc.) and univariate tests to assess for differences in the populations. For Aim 2, we will evaluate the performance (sensitivity, specificity, PPV/NPV) of the non-invasive assays (UF5000 and urinalysis) to the urinalysis LE and determine the percent agreement to identify the optimal collection device. For Aim 3, we will use univariate tests to assess for differences in cell counts over time.

13.0 Statistical Data Management

De-identified data will be kept on the secure IU department server in a Microsoft Excel file and merged with other data as needed. Statistical analyses of de-identified data will be performed using Prism 10 and involve collaboration with the Indiana University Department of Biostatistics (Dr. Jie Ren).

14.0 Privacy/Confidentiality Issues

Subject information is protected by HIPAA and study participants will be assigned a unique code that identifies them. Only Dr Jordan (PI) will know the identity of the participants. The identity of these participants will be kept on a password-protected file on Dr. Jordan's IU-owned and IU IT-maintained computer. All other key personnel will only have access to the study-coded de-identified specimens and study-coded DCFs.

15.0 Follow-up and Record Retention

DCF records will be kept for 10 years and then archived indefinitely. However, after 10 years, the key which allows re-identification of subjects (Kept by the PI) will be destroyed. All data will be stored on secure IU servers. The paper data collection form will be kept in a locked cabinet located in a badge access area.

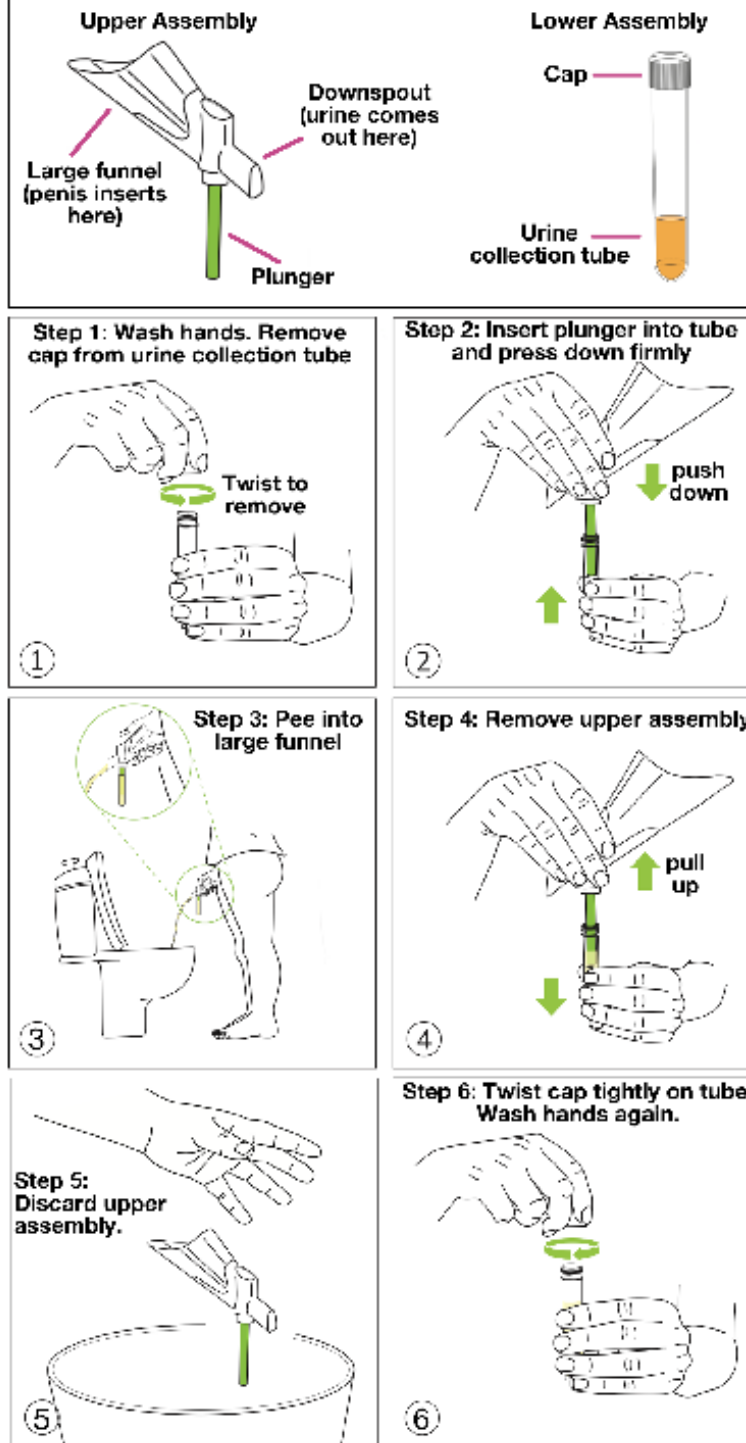
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17.0 Appendix

Colli-Pee Instructions



Data Collection Form

Controls for Sysmex UF5000 Urinary Flow Cytometer Study

v1.0 (02/19/2025)

Study ID

Study Criteria

Consent form signed ☐ Yes ☐ No
Age > 18 ☐ Yes ☐ No
No genital symptoms ☐ Yes ☐ No
>1 year since STI Dx ☐ Yes ☐ No

*If no, do not continue. Participant not eligible.***Sex at Birth**

☐ Male ☐ Female ☐ Other ☐ Unknown

Gender of sex partners (last year)

☐ Cis male ☐ Cis female
☐ Trans male ☐ Trans. female
☐ Other ☐ Not sexually active

Genitourinary Symptoms

Discharge ☐ Yes ☐ No ☐ Unknown
Dysuria ☐ Yes ☐ No ☐ Unknown
Other ☐ Yes ☐ No ☐ Unknown Describe:

Date of Visit (Enrollment)

/ /
Mo Day Year

DOB (Year only)**Gender Identity**

☐ Cis male ☐ Cis female
☐ Trans male ☐ Trans. female
☐ Other ☐ Non-binary ☐ Unknown

Race

☐ White ☐ Am. Indian/Alaskan
☐ Black ☐ Hawaiian/Pac. Island
☐ Asian ☐ Multi-racial
☐ Other ☐ Unknown

Hispanic

☐ No ☐ Yes ☐ Unknown

Visit and Specimen Information

	Device (CP/UC)	Collection Date	Collection Time	Time Last Urinated	Symptoms Today?	Specimen Collected
Visit 1						
Visit 2						
Visit 3						
Visit 4						
Visit 5						
Visit 6						

Odd DOB, collect CP during first week. Even DOB, collect UC during first week