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An optimized diagnostic screening tool and GeneXpert pooling algorithm for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* to reduce cost of molecular STI screening in resource-limited settings

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BS, University of Florida, 2012
PhD, Emory University, 2020

Thesis Committee Chair: Susan Allen, MD, MPH

An abstract of
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Abstract

An optimized diagnostic screening tool and GeneXpert pooling algorithm for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* to reduce cost of molecular STI screening in resource-limited settings

By Sarah Connolly

Background: Sexually transmitted infections (STIs) such as chlamydia (CT) and gonorrhea (NG) have been shown to increase the risk of heterosexual HIV-1 transmission as well as other reproductive tract comorbidities such as pelvic inflammatory disease, infertility, and pregnancy complications. In women, CT and NG are often asymptomatic and undetected by syndromic management. Molecular testing for these STIs is highly sensitive, but time and cost restraints preclude implementation of these technologies in resource-limited settings.

Methods: Pooling samples for simultaneous testing in GeneXpert cartridges is one strategy for reducing the cost per individual tested. The current study describes a pooling strategy based on identification of social and demographic factors associated with CT/NG prevalence in a high-risk cohort of HIV-uninfected Zambian female sex workers or single mothers conducted from 2016-2019.

Results: Factors significantly ($p<0.05$) associated with CT/NG via logistic regression included city, younger age, lower education, *Trichomonas vaginalis*, bacterial vaginosis, and syphilis infection. The cost per test with unguided pooling was $12.96. However, the cost per test can be further reduced to as low as $9.43 per sample by strategically pooling women with similar CT/NG factors together and testing those at highest risk individually.

Conclusions: The checklist tool developed and pooling approach described can be used in a variety of different of treatment algorithms in order to strategically manage limited resources, while also maximizing the number of women receiving STI screening and treatment.

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An optimized diagnostic screening tool and GeneXpert pooling algorithm for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* to reduce cost of molecular STI screening in resource-limited settings

Introduction

Inflammatory sexually transmitted infections (STIs) such as *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are associated with HIV viral shedding(1) and acquisition(2-4). The prevalence of CT and NG is high among women at elevated risk of HIV infection, thus further increasing their risk. In a Zambian cohort of high-risk women (HRW), including female sex workers (FSW) and single mothers with a child under age five (SM)(5-7), the prevalence of CT and/or NG infection was 17%. The population attributable risk of genital ulceration and inflammation in either or both partners was 63% for male-to-female and 80% for female-to-male HIV transmission in Zambian discordant couples(8). Therefore, curable STIs are an important target for HIV prevention.

In sub-Saharan Africa, where the HIV burden is greatest, syndromic management is the World Health Organization (WHO) recommended approach to detecting STIs, although WHO acknowledges that the sensitivity and specificity of this method are low for CT and NG infections(9). In similar settings, syndromic management has performed poorly compared to molecular diagnostic methods(2), but is still preferred due to wide-spread lack of financial resources, laboratory equipment, and trained laboratory technicians.

Syndromic management relies on clinical presentation for diagnosis and treatment. CT and NG are often asymptomatic(2, 10) but when clinical manifestations are present, they may resemble other common vaginal dysbioses (discharge, odor, discomfort) which can complicate diagnosis and result in improper treatment. Suboptimal or incorrect therapy for a vaginal infection can deplete healthy flora, worsen other vaginal

conditions such as candida(11), or contribute to antibiotic resistance. Asymptomatic cases, therefore undetected and untreated, can lead to pelvic inflammatory disease, ectopic pregnancy, infertility, and increased risk of HIV infection(12, 13).

Strategies for overcoming the low sensitivity of syndromic management include periodic presumptive treatment of extremely high-risk populations(14, 15) or testing for biomarkers, such as inflammatory cytokines(16). Another approach is to reduce the cost-per-sample tested using highly sensitive molecular methods through specimen pooling(17-22). In this study, we sought to strategically guide sample pooling through the development of an easy-to-use risk categorization checklist to maximize cost savings.

Materials and Methods

Population: This study examines data from a 2016 cross-sectional study adding CT/NG testing in a cohort of HIV-uninfected women from Lusaka or Ndola, the two largest cities in Zambia(5). Participants were either FSW recruited from community sex work hot spots or SM referred to the Zambia-Emory HIV Research Project (ZEHRP) from their local post-natal care provider.

At the study visit, each participant completed a sociodemographic and risk behavior questionnaire, received a pelvic examination, and was tested for HIV, syphilis, candida, bacterial vaginosis (BV), *Trichomonas vaginalis* (TV), CT, and NG. If a bacterial STI was detected, the woman received free treatment at ZEHRP. If HIV was detected, the woman was referred for antiretroviral treatment at the government clinic. There were 825 unique clinic visits where molecular CT/NG testing was performed on the GeneXpert. On several occasions, the same individual was observed at two different visits, however these data were not excluded because sexual behaviors and test results vary over time.

Variables considered: Samples were collected from September 2016 to January 2019. The outcome of interest is a positive CT/NG GeneXpert result, meaning the sample contains either CT, NG, or both.

A composite variable for reported symptoms was created to capture participants who reported one or more of the following: cystitis, dysuria, vaginal itching, vaginal discharge, dyspareunia, lower abdominal pain, or acute/chronic/recurrent genital ulcer. If the individual did not report any of these symptoms, even when specifically prompted by

the medical provider, she was considered asymptomatic. Another composite variable was created to capture clinical signs observed on pelvic exam. This variable included anyone who had on either the external or internal genitalia: inguinal adenopathy, inflammation, ulceration, condyloma or warts, cervicitis, cervical discharge or pus, vaginal discharge, erosion or friability of the cervix or vagina, non-menstrual bleeding, or adnexal tenderness or mass.

Other clinical covariates were based on laboratory diagnostics, including whether TV, sperm, or candida were observed on wet mount microscopy, HIV rapid test from follow-up visits to document new infections(23), or treatment for an incident syphilis infection determined by rapid plasma regain (RPR) and previous syphilis serology(24, 25). BV was classified as a composite variable of KOH whiff test and presence of clue cells.

Sociodemographic variables included self-report of: illiteracy, education, year of birth, city of residence, unprotected sex in the past 1-3 months, history of transactional sex, number of partners in the past 1-3 months, pregnancy, and verified current long-acting reversible contraception (LARC) usage. Illiteracy was classified as unable to easily read English, Nyanja, or Bemba (the most common languages spoken in Lusaka and Ndola, respectively). Education was divided into two categories based on completing secondary school or college, versus no school or only primary school. Year of birth from the participant questionnaire was used to calculate age in 2016 and create age groups (18-24, and 25+). LARC included the copper intrauterine device (IUD) and the hormonal implant.

Determining optimal pool size: The CT/NG GeneXpert assay is a highly-sensitive, highly-specific (sensitivity CT 99.5%, NG 100%; specificity CT 99.1%, NG 99.9%) automated system to qualitatively detect genomic DNA of CT or NG in a clinical specimen via real-time polymerase chain reaction (26). At the time of study, each GeneXpert cartridge cost approximately $18 United States Dollars (USD) and is intended to test a specimen from a single individual yielding a result in 90 minutes. Serial dilutions up to 1:10 of known-positive specimens, simulating sample pooling, maintain 80%-100% sensitivity (not shown). The proportion of positive pools was calculated based on the previously published equation, $s=[1-(1-p)^c]$, where s is the proportion of positive pools, p is the prevalence of disease, and c is the number of samples in each pool(18, 19).

In the event that the pool tests negative, all samples included are considered CT/NG negative. If the pool tests positive, it is deconvoluted by re-testing each specimen individually. The optimal pool size is achieved when the cost per sample, given frequency of deconvolution and prevalence of disease, reaches a minimum.

Logistic regression modeling of CT/NG: The probability that the GeneXpert outcome is positive follows a binomial distribution. Thus, logistic regression modeling was used to predict the probability that an individual was infected with CT and/or NG.

To select variables for model inclusion, frequencies of each predictor among the infected and uninfected groups were compared, overall as well as stratified by city, by chi-square or Fisher's exact test. Variables for which the chi-square or Fisher's exact test p-value was below 0.05 in at least one city or overall were candidates for inclusion in the full multivariate logistic regression (MLR) model. Correlation coefficients between each

of the predictors were all less than 0.5 and all variance inflation factors were below 10, therefore no variables were removed due to collinearity.

For simple translation to a screening checklist, interaction terms were excluded and relevant variables were included in the MLR as main effects. Before mathematical model selection, each predictor was pragmatically evaluated by time required to collate information necessary to inform pooling decisions. Model selection was then performed on the variables remaining in the MLR model using the R stepAIC function in both directions.

A composite score, weighted 1 point each, was created based on the risk factors included in the final reduced MLR model and categorized into high, middle, and low scoring groups. These score categories were tested for their ability to predict the odds of CT/NG infection in a bivariate logistic regression model.

Assessing diagnostic performance: Sensitivity and specificity of syndromic management, based on symptoms only or signs and symptoms together, was calculated. Sensitivity was determined by dividing the number of true positives by the total number of individuals with either CT and/or NG. Specificity was calculated by dividing the number of true negatives by the total number of individuals who were CT and NG uninfected.

To assess the performance of the screening checklist developed in this study, positive predictive value (PPV) and negative predictive value (NPV) were calculated. NPV was calculated for the low-scoring group by dividing the number of CT/NG uninfected low-scoring women by the total number of low-scoring women. PPV was

calculated for the high-scoring group by dividing the number of CT and/or NG infected high-scoring women by the total number of high-scoring women.

Ethics: The data used in this study were derived from a cross-sectional study which was approved by Institutional Review Board of Emory University and the Research Ethics Committee of the University of Zambia. Written informed consent from each participant was completed before any study materials were gathered.

Results

Syndromic management fails to detect the majority of CT/NG cases in this population

Of the 825 total data entries with GeneXpert test results, there were 124 instances of either CT and/or NG infection. Information on reported symptoms was available for 559 of these women and a pelvic examination was performed on 530 women. The GeneXpert detected 68 cases of only CT, 34 cases of only NG, and 22 CT/NG co-infections. Neither clinical signs (chi-square $p=0.48$) nor reported symptoms (Fisher's exact $p=0.43$) were statistically significantly associated with CT/NG (Table 1). Prevalence of either CT and/or NG in this population was 15%, and all but three of the infected women (91/94, 97%) reported no symptoms and would have been missed under traditional syndromic management.

Of the women who reported symptoms, one infected with NG only reported vaginal itching (likely due to co-infection with TV), which is not a characteristic symptom of NG and would have gone undetected. Two women with CT reported symptoms. One reported painful urination and painful intercourse, but only after being prompted specifically about those symptoms; she was treated for TV, CT, and NG despite only being positive for CT, and represents an instance where syndromic management can lead to over-treatment. The other woman reported lower abdominal pain and field notes state it was suspected she had TV and a urinary tract infection. These three cases, in addition to the 91 asymptomatic cases, illustrate CT/NG cases that would have been missed, incompletely diagnosed, or over-treated under traditional syndromic management.

Setting the GeneXpert CT/NG result as the gold standard(27), we calculated the sensitivity and specificity of syndromic detection in this population (Table 2). Under traditional syndromic management, diagnosis is inferred based on reported symptoms. In this population, the sensitivity by symptoms only is 0.02, however the specificity is 0.98. A pelvic examination was performed on all women, allowing us to consider clinical signs, in addition to reported symptoms, to evaluate the sensitivity and specificity of syndromic management plus exam. The sensitivity of using reported symptoms and/or clinical signs to infer CT/NG infection is 0.68, a dramatic increase to using reported symptoms alone. This increase in sensitivity is accompanied by a sacrifice in specificity to 0.29, which could result in truly CT/NG negative women being prescribed unnecessary antibiotic therapy.

Sociodemographic factors and other STIs are associated with CT/NG infection

Given that transmission of CT and/or NG is related to sexual risk-taking, we expect that testing positive for CT/NG would be associated with testing positive for other STIs or sociodemographic factors that may also be associated with this risk. TV and incident syphilis infection were found to be associated with testing positive for CT and/or NG (chi-square p=<0.01, 0.02, respectively), however HIV was not associated even though twenty-four women seroconverted and were HIV+ at the time of sample collection (Fisher's exact p=0.77) (Table 3). Candida was not associated with CT/NG in this study (Fisher's exact p=0.41), but BV, another vaginal dysbiosis typically only identified in sexually active women, was found to be statistically significantly associated with CT/NG (chi-square p=0.02). Observation of sperm on wet mount microscopy, an

indicator of recent unprotected sexual intercourse, was not associated with CT/NG (chi-square p=0.37).

Several sociodemographic factors were found to be associated with CT/NG in at least one of the cities. These include age 18-24 (Ndola chi-square p=<0.01), not completing secondary school (Lusaka chi-square p=0.02), reporting unprotected sex in the past 1-3 months (Lusaka chi-square p=0.02), and using LARC (Ndola chi-square p=0.02) (Table 4).

Factors associated with CT and/or NG infection in at least one city were added to an MLR model of the probability of CT/NG infection. Due to missing data, the unprotected sex in the last 1-3 months variable was pragmatically eliminated. The variables city, age group, education, LARC usage, TV, BV, and incident syphilis infection comprised the full MLR. Following bidirectional model selection, no variables were recommended for elimination.

Based on the final MLR model controlling for city, education, LARC usage, TV, BV, and incident syphilis infection, women between the ages of 18 and 24 had 1.97 (95% CI: 1.13, 3.45) higher odds of having a positive CT and/or NG result than women age 25 years or older (Table 5). Women who had not completed secondary school (Adj. OR: 2.19, 95% CI: 1.20, 4.03) or women who live in Lusaka (Adj. OR: 2.07, 95% CI: 1.04, 4.13) had more than twice the odds of testing positive for CT and/or NG when adjusting for these other factors. Individuals currently using the IUD or implant as contraception also had higher odds of testing positive (Adj. OR: 1.65, 95% CI: 0.99, 2.75). Finally, women with TV (Adj. OR: 3.96, 95% CI: 1.53, 10.28) or an incident syphilis infection

(OR: 4.58, 95% CI: 1.63, 12.93) had the highest odds of testing positive for either CT and/or NG, holding all other sociodemographic factors and laboratory results constant.

Development of a diagnostic screening checklist

The seven risk factors contained in the MLR model were then built into a screening checklist to guide GeneXpert sample pooling (Figure 1). The checklist layout was tailored for use in the ZEHRP clinics and is meant to fit into the existing clinic flow, beginning in reception. The demographic/clinic checkboxes are easy to complete from the participant file and do not require nurse consultation or interview about recent risk factors, which could delay a pooling decision and thus time to CT/NG GeneXpert result. Each checkmark is worth 1 point which the receptionist tallies (total of 4) and sends with the diagnostic test requisition form to the lab.

The lab tests included in the laboratory portion of the checklist are low-cost with short time-to-result and do not demand highly-advanced technical skill. These tests would be performed upon first receiving the participant sample and the technician would then tally the laboratory portion of the checklist (total of 3) and sum it to the reception total (overall total of 7 possible). Based on the overall score, the sample is then categorized and tested according to the pooling algorithm, also described on the bottom of the checklist. Initial lines are included at each step for quality control purposes.

In the ZEHRP HRW population, the distribution of scores is roughly normally distributed with a median of 2 and standard deviation of 1 (Figure 2). While scores could range from 0 to 7, the actual scores of the participants only ranged from 0 to 5. The scores were divided into categories representing low, middle, and high-risk populations

where scores 0 and 1 were considered low-risk, scores 2 and 3 were considered middle-risk, scores 4 or greater were categorized as high-risk. These risk categories were statistically significantly associated with testing positive for CT/NG on the GeneXpert in a bivariate logistic regression model. Women with a low score (0 or 1) had 0.44 (95% CI: 0.21, 0.87) times the odds of testing positive compared to mid-scoring women, whereas high-scoring women (4+) had 4.85 (95% CI: 2.49, 9.44) times the odds of testing positive, relative to mid-scoring women (Table 6).

Furthermore, the checklist score successfully stratifies the population into groups with statistically significantly different CT/NG prevalence, a key element to sample pooling. The prevalence of either CT and/or NG in the mid-scoring group, 16% (Table 6), is reflective of the CT/NG prevalence in the overall population, 15%. Low-scoring category CT/NG prevalence (8%), mid-scoring, and high-scoring category prevalence (48%) differ significantly when compared by chi-square test (χ^2=38.50, p=<0.01).

Projected cost savings using a guided sample pooling strategy

The cost of pooling is driven by the prevalence of disease in the population because all samples in a positive pool must be re-tested individually in a new GeneXpert test cartridge. By grouping the samples based on their probability of testing positive, samples can be pooled strategically in order to maximize savings. The cost of a single unpooled sample is approximately $18 USD, and based on the prevalence of CT and/or NG for each risk category shown in Table 6, the maximum cost savings is achieved when low category samples are pooled in groups of 4 ($8.57 saved per sample) and middle category samples in pools of 3 ($4.73 saved per sample) (Figure 3). There is actually a

cost increase when pooling high-risk category samples due to the high probability of testing positive, therefore these samples should be tested individually.

In a hypothetical STI clinic with similar risk factors and CT/NG prevalence to that of this study, and which tests approximately 500 participants each quarter, the cost savings associated with the checklist assessment varies based on subsequent clinical and testing decisions. After one year, more than $10,000 could be saved by testing all women in groups of 3, and over $10,600 saved if all women are tested following the proposed algorithm (Figure 4).

The risk checklist approach has the potential to further aid low-resource clinics when faced with the decision of only offering tests to those at highest risk for CT/NG. Combining GeneXpert testing with presumptive treatment, or only testing certain risk groups, aids strategic management of limited resources. More dramatic cost-savings can be achieved when the checklist is used to guide treatment and testing decisions beyond sample pooling. For example, if low-scoring women are not screened and high-scoring women are presumptively treated, leaving only the middle-scoring women to be tested on the GeneXpert, the annual cost savings exceeds $18,800. If used in this manner to guide testing and treatment decisions, it is important to consider the positive and negative predictive values of the checklist score. In this study population, a low score had a NPV of 0.92, while a high score had a PPV of 0.48 (Table 6).

Discussion

The screening algorithm proposed here has the potential to extend molecular diagnostic technology for STIs to limited-resource settings. We have shown that in this population syndromic management performs inadequately for detecting CT/NG. The WHO suggests that in some populations, such as adolescents, it may be preferable to tailor detection algorithms based on risk factors and patterns of sexual behavior within the specific community(9). Here, we examined a variety of social and demographic characteristics in order to identify relationships with CT/NG prevalence among HRW in Zambia. A critical element to our algorithm is including low-cost, rapid laboratory results that are also associated with CT/NG. Together, these methods can be used to develop more efficient clinical screening tools targeted to specific populations.

It is important to note that the women included in our study were part of a sub-study within an overarching prospective cohort study on HIV prevention. These women largely did not report STI signs or symptoms, explaining why syndromic management was not sensitive. This also highlights the high prevalence of asymptomatic CT/NG in this population that would have gone undetected in the absence of routine screening. Due to widespread perceptions that a lack of symptoms denotes health, it is important to increase awareness about the asymptomatic nature of STIs, their long-term health effects, and the importance of testing for those at risk.

Score-based approaches in similar populations have been shown to predict CT/NG with higher sensitivity and specificity compared to syndromic management(28, 29). The proposed algorithm differs in that it is designed as a tool to better manage limited resources yet still provide the maximum number of women with a highly-

sensitive molecular test for a lower cost per sample. Depending on the availability of resources, it may only be feasible to test certain risk groups. Ideally, all women would receive a molecular CT/NG test, however funding limitations may force providers to triage participants for CT/NG GeneXpert testing. The screening checklist could offer a strategic approach to making these difficult decisions, whether that includes not testing women at low-risk of CT/NG infection, or presumptively treating women at highest risk without conducting molecular testing. In this way, a working compromise might be reached to conserve scarce resources while still offering GeneXpert testing to women at greatest risk of CT/NG infection.

By providing a strategy to lower the cost per sample tested, clinics around the world may be able to incorporate these more sophisticated point-of-care tools in their screening protocols. Increasing accessibility and improving diagnostic capacity in developing countries can help identify and treat individuals infected with CT/NG, thereby also reducing their risk of acquiring HIV and limiting the overuse of antibiotics which drives pathogen drug-resistance.

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Table 1. Prevalence of Signs and Symptoms by CT and/or NG Status and City.

	Overall					Lusaka	Ndola
	Either CT and/or NG (n=124)		CT/NG Uninfected (n=701)		Fisher's p-value Either CT and/or NG vs Uninfected	Fisher's p-value Either CT and/or NG vs Uninfected	Fisher's p-value Either CT and/or NG vs Uninfected
	N	(%)	N	(%)			
Signs (n=530)					0.48[a]	0.57	0.77
Clinical signs observed	60	(48)	310	(44)			
No clinical signs observed	30	(24)	130	(19)			
Pelvic exam not performed (Missing)	34	(27)	261	(37)			
Symptoms (n=559)					0.43	0.61	0.59
Symptoms reported	3	(2)	9	(1)			
Asymptomatic/Reported no symptoms	91	(73)	456	(65)			
No symptoms reported (Missing)	30	(24)	236	(34)			

CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*.

[a]$\chi 2$ p-value.

Table 2. Prevalence of Signs and Symptoms Combined by CT/NG Status.

	CT Only (n=68)		NG Only (n=34)		CT & NG Co-infected (n=22)		CT/NG Uninfected (n=701)	
	N	(%)	N	(%)	N	(%)	N	(%)
Asymptomatic, No clinical signs observed	20	(29)	6	(18)	2	(9)	125	(18)
Asymptomatic, Clinical signs observed	30	(44)	16	(47)	12	(55)	304	(43)
Symptoms reported, No clinical signs observed	0	(0)	0	(0)	0	(0)	4	(1)
Symptoms reported, Clinical signs observed	1	(1)	1	(3)	0	(0)	5	(1)
(Missing symptoms or signs)	17	(25)	11	(32)	8	(36)	263	(38)
Syndromic management by symptoms only								
Sensitivity	0.02							
Specificity	0.98							
Syndromic management by symptoms and/or clinical signs								
Sensitivity	0.68							
Specificity	0.29							

CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*.

Table 3. Prevalence of Lab Results by CT and/or NG Status and City.

	Overall					Lusaka	Ndola
	Either CT and/or NG (n=124)		CT/NG Uninfected (n=701)		Fisher's p-value Either CT and/or NG vs Uninfected	Fisher's p-value Either CT and/or NG vs Uninfected	Fisher's p-value Either CT and/or NG vs Uninfected
	N	(%)	N	(%)			
Trichomonas vaginalis (microscopy)					<0.01[a]	0.02	0.02
Positive	13	(10)	23	(3)			
Negative	106	(85)	651	(93)			
(Missing)	5	(4)	27	(4)			
HIV rapid test					0.77	0.69	0.41
Positive or Discrepant	4	(3)	20	(3)			
Negative	119	(96)	675	(96)			
(Missing)	1	(1)	6	(1)			
Sperm (microscopy)					0.37[a]	1.00	0.44[a]
Positive	5	(4)	43	(6)			
Negative	109	(88)	609	(87)			
(Missing)	10	(8)	49	(7)			
Candida (microscopy)					0.41	1.00	0.56
Positive	2	(2)	24	(3)			
Negative	100	(81)	555	(79)			
(Missing)	22	(18)	122	(17)			
BV (Both KOH+ & Clue Cells)					0.02[a]	1.00	<0.01[a]
Positive	37	(30)	147	(21)			
Negative	62	(50)	425	(61)			
(Missing)	25	(20)	129	(18)			
New syphilis infection (based on RPR)					0.02	1.00	0.01
Yes	8	(6)	13	(2)			
No	88	(71)	454	(65)			
(Missing)	28	(23)	234	(33)			

BV, bacterial vaginosis; CT, *Chlamydia trachomatis*; KOH, potassium hydroxide; NG, *Neisseria gonorrhoeae*; RPR, rapid plasma reagin.
[a]$\chi 2$ p-value.

Table 4. Prevalence of Demographic Factors and Risk Behaviors by CT and/or NG Status and City.

	Overall					Lusaka	Ndola
	Either CT and/or NG (n=124)		CT/NG Uninfected (n=701)		χ2 p-value Either CT and/or NG vs Uninfected	χ2 p-value Either CT and/or NG vs Uninfected	χ2 p-value Either CT and/or NG vs Uninfected
	N	(%)	N	(%)			
Age Group					<0.01	0.09	<0.01
Age 18-24	91	(73)	412	(59)			
Age 25+	30	(24)	284	(41)			
(Missing)	3	(2)	5	(1)			
Literacy					0.11	0.77	0.06
Reads English, Bemba, or Nyanja	74	(60)	361	(51)			
Illiterate	49	(40)	328	(47)			
(Missing)	1	(1)	12	(2)			
Education					0.13	0.02	0.39
No School or Primary School Only	85	(69)	426	(61)			
Secondary School or Higher	38	(31)	261	(37)			
(Missing)	1	(1)	14	(2)			
Sex in Exchange for Money					0.80	0.93	0.80
Never	59	(48)	339	(48)			
Ever	64	(52)	350	(50)			
(Missing)	1	(1)	12	(2)			
Unprotected Sex (Last 1-3 months)					0.06	0.02	0.29
None	29	(23)	201	(29)			
At least once	62	(50)	272	(39)			
(Missing)	33	(27)	228	(33)			
Pregnant					0.33[a]	0.56[a]	0.42[a]
Not Pregnant	90	(73)	453	(65)			
Pregnant	4	(3)	12	(2)			
(Missing)	30	(24)	236	(34)			
Uses LARC Method					0.15	0.29	0.02
No LARC	72	(58)	449	(64)			
Uses Implant or IUD	48	(39)	224	(32)			
(Missing)	4	(3)	28	(4)			

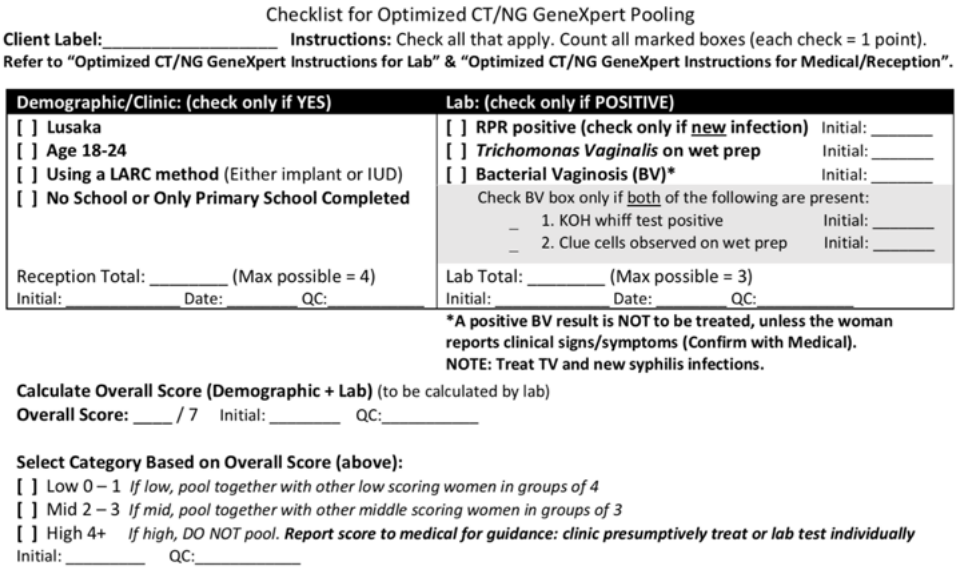
CT, *Chlamydia trachomatis*; IUD, intrauterine device; LARC, long-acting reversible contraception; NG, *Neisseria gonorrhoeae*.
[a]Fisher's exact p-value.

Table 5. Logistic Regression Model of Factors Associated With Either CT and/or NG.

			Multivariate Final Model (n=496)			
Predictor of CT and/or NG	N	(%)	Adj. Odds Ratio	Lower 95% CI	Upper 95% CI	Adj. p-value
City						
Ndola	88	(14)	*ref*			
Lusaka	36	(18)	2.07	1.04	4.13	0.04
Age Group						
Age 18-24	91	(18)	1.97	1.13	3.45	0.02
Age 25+	30	(10)	*ref*			
Education						
No School or Primary School Only	85	(17)	2.19	1.20	4.03	0.01
Secondary School or Higher	38	(13)	*ref*			
Unprotected Sex (Last 1-3 months)[a]						
None	29	(13)				
At least once	62	(19)				
Uses LARC Method						
No LARC	72	(14)	*ref*			
Uses Implant or IUD	48	(18)	1.65	0.99	2.75	0.06
Trichomonas vaginalis (microscopy)						
Positive	13	(36)	3.96	1.53	10.28	<0.01
Negative	106	(14)	*ref*			
BV (Both KOH+ & Clue Cells)						
Positive	37	(20)	1.87	1.05	3.34	0.03
Negative	62	(13)	*ref*			
New syphilis infection (based on RPR)						
Yes	8	(38)	4.58	1.63	12.93	<0.01
No	88	(16)	*ref*			

Adj, adjusted; CI, confidence interval; CT, *Chlamydia trachomatis*; IUD, intrauterine device; KOH, potassium hydroxide; LARC, long-acting reversible contraception; NG, *Neisseria gonorrhoeae*; ref, reference group; RPR, rapid plasma reagin.

[a]Pragmatically eliminated from the multivariate model.



Checklist for Optimized CT/NG GeneXpert Pooling

Client Label:_____ **Instructions:** Check all that apply. Count all marked boxes (each check = 1 point).
Refer to "Optimized CT/NG GeneXpert Instructions for Lab" & "Optimized CT/NG GeneXpert Instructions for Medical/Reception".

Demographic/Clinic: (check only if YES)	Lab: (check only if POSITIVE)
[ ] **Lusaka**	[ ] **RPR positive (check only if new infection)** Initial: _____
[ ] **Age 18-24**	[ ] ***Trichomonas Vaginalis* on wet prep** Initial: _____
[ ] **Using a LARC method** (Either implant or IUD)	[ ] **Bacterial Vaginosis (BV)*** Initial: _____
[ ] **No School or Only Primary School Completed**	Check BV box only if both of the following are present: _ 1. KOH whiff test positive Initial: _____ _ 2. Clue cells observed on wet prep Initial: _____
Reception Total: _____ (Max possible = 4) Initial: _____ Date: _____ QC: _____	Lab Total: _____ (Max possible = 3) Initial: _____ Date: _____ QC: _____

***A positive BV result is NOT to be treated, unless the woman reports clinical signs/symptoms (Confirm with Medical).**
NOTE: Treat TV and new syphilis infections.

Calculate Overall Score (Demographic + Lab) (to be calculated by lab)
Overall Score: ____ / 7 Initial: _____ QC:_____

Select Category Based on Overall Score (above):
[ ] Low 0 – 1 *If low, pool together with other low scoring women in groups of 4*
[ ] Mid 2 – 3 *If mid, pool together with other middle scoring women in groups of 3*
[ ] High 4+ *If high, DO NOT pool.* ***Report score to medical for guidance: clinic presumptively treat or lab test individually***
Initial: _____ QC:_____

Figure 1: Checklist for Optimized CT/NG GeneXpert Pooling

This guided pooling strategy was designed for use in the ZEHRP clinics. It fits seamlessly into the existing clinic flow and is based on factors that do not require a nurse consultation or extensive laboratory wait times, which could delay time to GeneXpert result.

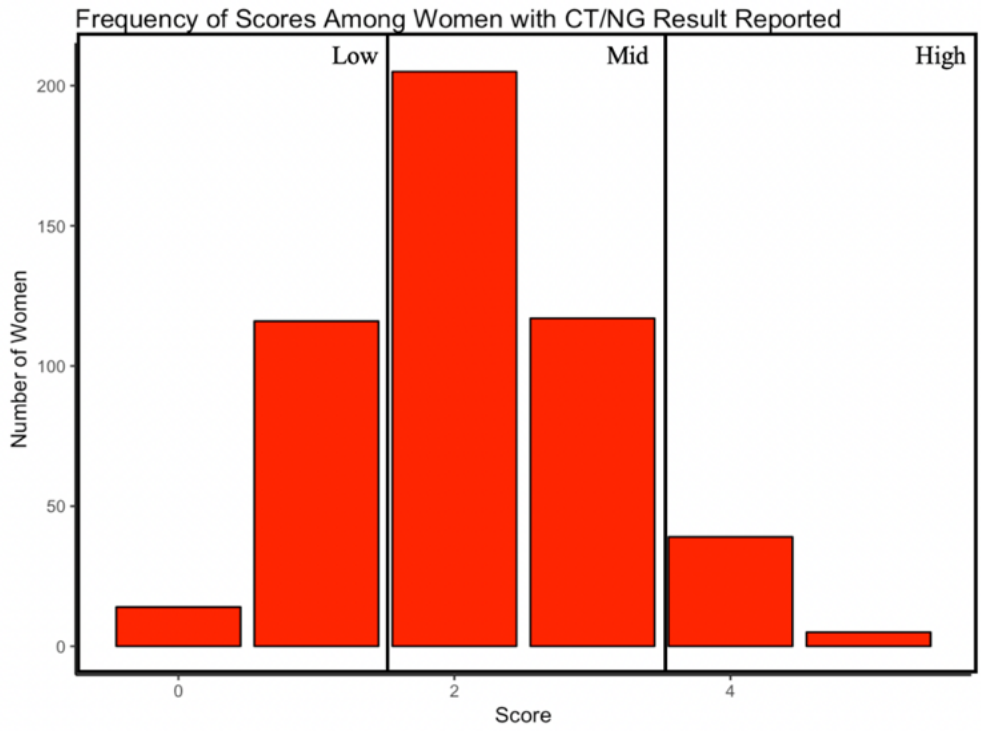


Figure 2: Distribution of Checklist Scores with CT/NG Result Reported (N=496)

The checklist scores are roughly normally distributed (mean = 2.13, std dev = 0.99). They are categorized into low (0-1), mid (2-3), and high (4+) risk groups. Only women with non-missing values for each score predictor were included.

Table 6. Bivariate Logistic Regression Model of Composite Score Category and Either CT and/or NG (n=496).

Predictor of CT and/or NG	N	(%)	Odds Ratio	Lower 95% CI	Upper 95% CI	p-value
Score Category						
Low (0-1)	10	(8)	0.44	0.21	0.87	0.02
Mid (2-3)	51	(16)	*ref*			
High (4+)	21	(48)	4.85	2.49	9.44	<0.01
NPV (Low score)	0.92					
PPV (High score)	0.48					

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; ref, reference group

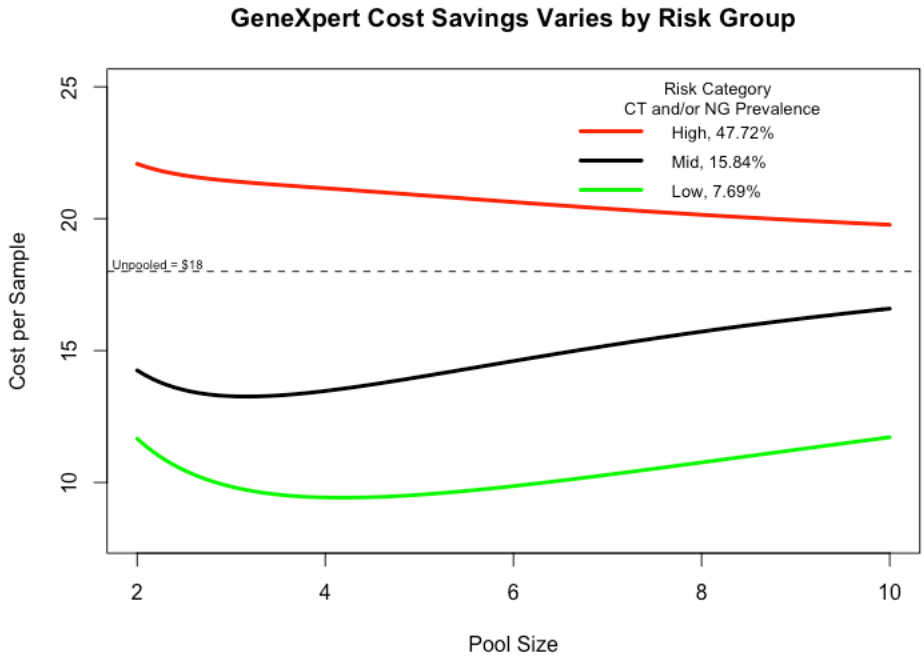


Figure 3: Pooling Low and Mid Risk Group Samples Results in Cost Savings per Sample

The average cost per sample for pooled mid- and low-risk category samples is lower than the unpooled cost per sample, $18 USD. Based on the prevalence of CT and/or NG in the various risk groups, the optimum pool size for low-risk category samples is 4 ($9.43 per sample) and the optimum pool size for mid-risk category samples is 3 ($13.27 per sample). There is no cost savings for pooling samples in the high-risk category.

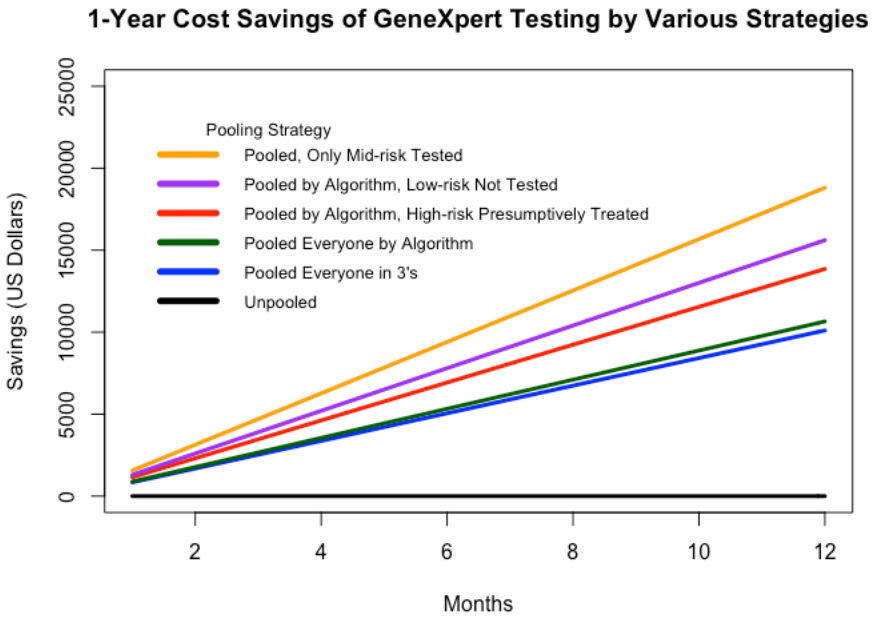


Figure 4: Cost Savings Over Time is Greatest with Score-Guided Pooling Strategy

Pooling all women in groups of 3 regardless of risk category results in a dramatic cost savings relative to testing every woman individually. However, when the score-guided screening strategy is used to determine who should be tested, there is only a modest increase in cost savings. Potential screening options that further augment cost savings might include presumptively treating those in the high-risk category and testing mid- and low-risk participants, testing only mid- and high-risk participants and not testing asymptomatic low-risk participants, or a combination of the two in which only the mid-risk group is tested and the high-risk group is offered presumptive treatment.

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