Cluster randomized crossover trial to evaluate point-of-care testing and treatment of sexually transmitted infections to improve birth outcomes in high-burden, low-income settings

STATISTICAL ANALYSIS PLAN

Trial Sponsor
Papua New Guinea Institute of Medical Research

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Statement of Compliance
This study will be conducted in accordance with Good Clinical Practice (GCP) as required by ICH GCP E6

Version	Author	Date	Reason for revision
1.0	Handan Wand	29 Nov 2016	Initial draft
2.0	Handan Wand	19 Mar 2017	Revised in response to feedback on v1.0 from DSMB Chair and CIs
3.0	Handan Wand	09 Nov 2017	Revised in response to feedback on v2.0 from DSMB Chair
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8.0	Handan Wand	18 May 2021	Revised in response to feedback from DSMB
9.0	Handan Wand	29 Oct 2022	Revised to include planned subgroup analyses

PROTOCOL SUMMARY

Title:	**Cluster randomised crossover trial to evaluate point-of-care testing and treatment of sexually transmitted infections to improve birth outcomes in high-burden, resource-limited settings**
Project name:	WANTAIM: Women and Newborn Trial of Antenatal Interventions and Management
Population:	4600 antenatal women and their newborns in Papua New Guinea.
Trial Design:	Cluster randomised crossover trial. The unit of randomisation is a primary health care clinic and its catchment communities. Each participating cluster has been randomised to participate in either the intervention or the control arm of the trial in the first phase of the study, and following a short washout period, will then cross over to participate in the alternative trial arm in the second phase of the study.
Research Sites:	Ten geographically distinct clusters selected from sites in East New Britain, Madang, Milne Bay Provinces.
Study Duration:	Four years
Subject Duration:	Each pregnant woman will be enrolled at their first antenatal clinic visit and followed up until one week after birth (approx. 5-6 months per participant). Primary outcome data will be censored at 72 hours postpartum A subset of up to 2000 mothers and their newborns will be followed up until 4-6 weeks postpartum
Aim:	To measure the effectiveness, health system implementation requirements, cost-effectiveness and acceptability of antenatal point-of-care testing and immediate treatment of sexually transmitted infections (STI) to improve birth outcomes in high-burden, resource-limited settings.

INTRODUCTION

Background

Every year there are an estimated 2.6 million stillbirths and 2.6 million neonatal deaths globally; the majority are in low- and middle-income countries (LMIC), primarily in remote and rural communities.[1] Papua New Guinea (PNG) has among the highest neonatal mortality ratios worldwide, with an estimated 25 per 1000 live births in 2013, compared with a global figure of 18.[2,3] Preterm birth and low birth weight are closely linked, as well as being independent and major contributors to neonatal mortality. Together, they affect around 20% of newborns in PNG.[4] The causes of preterm birth and low birth weight are diverse, but a range of infections including malaria, HIV, syphilis, and other sexually transmitted and genital infections (STI) have been implicated.[5-10] In many resource-limited countries, poor access to antenatal care means that opportunities for early diagnosis and clinical intervention of such infections are missed.[11]

Research studies among antenatal women in a number of LMIC have found extremely high rates of infection with genital STIs, particularly gonorrhoea, chlamydia, trichomonas and bacterial vaginosis, which are readily curable with cheap antibiotics.[5] In PNG, pregnant women have among the highest prevalence of these infections of any developing country.[12] In the country's first bio-behavioural survey of STIs in pregnancy, we found that the prevalence of chlamydia was 23%, gonorrhoea 14%, and trichomonas 22%, with 44% of women having at least one of these infections.[13] Similar levels of infection were found in a pilot study of antenatal point-of-care STI testing and treatment conducted by our group[14] in which 54% of women had one or more of chlamydia, gonorrhoea, trichomonas or BV, and the prevalence of each of these STIs was 19%, 11%, 38%, and 18%, respectively. Similar STI prevalences were also observed in an earlier randomised trial of malaria prevention in pregnancy.[15] In all of these studies, between 65-80% of infections among antenatal women were asymptomatic.

STIs and adverse birth outcomes

Gonorrhoea, chlamydia, trichomonas and bacterial vaginosis have been linked to adverse birth outcomes. The precise pathogenesis remains unclear, with postulated mechanisms including direct infection of the foetus; stimulation of foetal inflammatory responses; excessive maternal immunogenic reactions; or a combination of factors.[16,17] There is evidence that gonorrhoea and chlamydia are associated with both preterm birth and low birth weight,[5,8,9,18-24] as well as miscarriage,[5,22] stillbirth,[5,25] premature rupture of membranes,[5,18,26] postpartum endometritis[5] and ophthalmia neonatorum.[5] The reported strength of association varies across studies and endpoints. In a meta-analysis, we found that trichomonas is associated with a relative risk of 1.4 (95%CI: 1.1-1.7) for preterm birth,[27] and is also linked to low birth weight and premature rupture of membranes. Bacterial vaginosis is strongly associated with preterm birth and other adverse outcomes.[18,21,28] The population attributable risk of these infections as causes of adverse birth outcomes depends on their underlying population prevalence. For preterm birth, estimates have ranged from 15% for gonorrhoea, chlamydia, trichomonas and bacterial vaginosis individually, and up to 42% for a combination of one or more of these infections.[9,20,21]

Management of STIs in pregnancy

Diagnosis of genital infections has traditionally relied upon microscopy, culture, and/or serology, all of which require technical expertise and laboratory services which are not widely available in most resource-limited settings. Accordingly, in the 1990s, the World Health Organization (WHO) developed a syndromic management strategy for diagnosing genital infections that uses clinical presentation without laboratory confirmation to make treatment decisions. Syndromic management, however, cannot identify asymptomatic infections, which might contribute to its limited impact on disease prevalence.[29,30] The development and introduction of nucleic acid amplification test (NAAT) technologies in the 1990s provided commercially available laboratory systems, which are highly accurate, robust for the detection of *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis* in both urine and genital swabs, and have relatively short turnaround time. The costs and technical requirements of these platforms however have meant that they are not routinely available in resource-limited settings.

Following the successful implementation of point-of-care devices for the rapid diagnosis of syphilis and HIV, there was an effort to use similar strategies, generally based on lateral flow technology, for chlamydia, gonorrhoea and trichomonas testing, but the results were disappointing, largely due to poor sensitivity.[31-36] In the past 5 years, there has been a major breakthrough in rapid diagnosis of STIs. A fully automated portable NAAT platform (GeneXpert, Cepheid, Sunnyvale, CA) can perform tests for *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis* that are as accurate as laboratory-based NAATs. This platform has substantially improved the diagnosis and management of tuberculosis in many low- and middle-income settings, including PNG. Disposable cartridges hold the reagents including primers and probes for the simultaneous detection of chlamydia and gonorrhoea, with a separate cartridge used for trichomonas detection. Test results are available in approximately 90 minutes for Xpert CT/NG and 60 minutes for the Xpert TV test. Building on the experience from the Test Treat ANd GO (TTANGO) Trial in remote Aboriginal communities in Australia,[37] we showed the feasibility of point-of-care STI testing and treatment in a pilot study in selected antenatal settings in PNG.[14]

The diagnosis of bacterial vaginosis has until recently relied on Amsel's clinical criteria, or on highly skilled, time-consuming microscopic examination of Gram stained vaginal smears using the Nugent score[38-40]. The BVBlue test (OSOM BVBlue Test, Gryphus Diagnostics, USA) is a chromogenic test based on the detection of increased vaginal fluid sialidase activity.[41] It is the first point-of-care test available for the diagnosis of bacterial vaginosis and, in previous evaluations, had high sensitivity (90%) and specificity (95%) compared with clinical and laboratory criteria.[41,42]

Women and Newborn Trial of Antenatal Interventions and Management (WANTAIM)

The WANTAIM Trial aims to evaluate the effect of point-of-care testing and immediate treatment of curable STIs and bacterial vaginosis in pregnancy on adverse birth and neonatal outcomes in Papua New Guinea. If WANTAIM shows a beneficial effect of antenatal point-of-care STI testing and treatment, our findings could hasten access to these technologies and lead to improved maternal and neonatal health. The trial protocol is available at:
https://wellcomeopenresearch.org/articles/4-53/v2

PURPOSE AND SCOPE OF THIS DOCUMENT

This Statistical Analysis Plan (SAP) describes the statistical methods that will be used in the WANTAIM Trial to evaluate the effects of the intervention on adverse birth and neonatal outcomes. This document has been prepared in accordance with International Council on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines.[43]

The SAP is applicable to the statistical analysis of data generated during the WANTAIM Trial.

The Trial Statistician is responsible for conducting the statistical analysis of trial data according to the approved trial protocol and the SAP.

MATERIALS AND EQUIPMENT

Desktop personal computers with MS Windows Network, SAS version 9.4 and STATA 14.0 (or newer versions) will be used in the preparation of tables and in the analysis of trial data.

RESEARCH OBJECTIVES

Primary Objective

1. Evaluate whether point-of-care testing and immediate treatment of curable STIs in pregnancy leads to a reduction in preterm birth and/or low birth weight compared with standard antenatal care.

In this trial, 'curable STIs' includes *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis* and bacterial vaginosis, all of which will be tested for and treated at point-of-care in the intervention arm. Antenatal syphilis and HIV counselling, testing and treatment will be provided in both trial arms.

Secondary Objectives

1. Evaluate whether point-of-care STI testing and treatment in pregnancy leads to an increase in mean birth weight compared with standard antenatal care;
2. Evaluate whether point-of-care STI testing and treatment in pregnancy leads to a reduction in premature rupture of membranes compared with standard antenatal care;
3. Evaluate whether point-of-care testing in pregnancy increases the diagnosis and treatment of STIs compared with symptom-based 'syndromic' management;
4. Evaluate the cost-effectiveness of point-of-care STI testing and treatment in pregnancy compared with standard antenatal care;
5. Evaluate the health system implementation requirements of point-of-care STI testing and treatment in pregnancy compared with standard antenatal care;
6. Evaluate the acceptability of antenatal point-of-care STI testing and treatment compared with standard care;
7. Evaluate whether point-of-care STI testing and treatment in pregnancy leads to a reduction in neonatal eye infection and/or pneumonia compared with standard antenatal care (*among a sub-set of 2000 participants only*);
8. Evaluate mother-to-child transmission of *C. trachomatis* and *N. gonorrhoeae* (*among a sub-set of 2000 participants only*);
9. Evaluate the performance of the Xpert^{TM} CT/NG Test for the diagnosis of neonatal eye infection and pneumonia using ocular and nasopharyngeal specimens (*among a sub-set of 2000 participants only*).

TRIAL OUTCOME MEASURES

Primary outcome

The primary outcome is a **composite measure** of two events, the proportion of women and their newborns in each trial arm who experience either:

a) preterm birth (live birth before 37 weeks' gestational age as estimated by ultrasound examination at 26 weeks' gestational age or earlier, adjusted according to reported date of last menstrual period in accordance with trial standard operating procedures); **and/or**

b) low birth weight (birth weight <2500 g measured as soon as possible after birth using calibrated, medical-grade infant weighing scales accurate to within 10 g; birth weights measured within 72 hours of birth only will be included in the primary outcome).

From these data a composite primary outcome measure will be calculated, which will be the proportion of women in each trial arm experiencing either preterm birth or low birth weight. The effect of the intervention on preterm birth and low birth weight will also be reported separately.

Secondary outcomes

1. Mean birth weight among newborns in the control and intervention arms of the trial;
2. Proportion of women who experience premature rupture of membranes (defined as membrane rupture before the onset of labour);
3. Number of curable STIs diagnosed and treated (women in the intervention arm diagnosed and treated at point-of-care; women in the control arm with a laboratory-confirmed STI, based on testing of stored urine specimens, who receive curative syndromic management treatment);
4. Incremental cost effectiveness ratios (cost per preterm birth and/or low birth weight case averted; cost per STI diagnosed and treated; cost per life year saved; cost per disability-adjusted life year (DALY) averted);

5. Health system implementation requirements;
6. Acceptability of antenatal point-of-care STI testing and treatment among (a) women and (b) health workers.
7. Proportion of newborns with an eye infection or moderate/severe pneumonia by 4-6 weeks postnatal (*among a sub-set of 2000 participants only*);
8. Incidence of mother to child transmission of *C. trachomatis* or *N. gonorrhoeae* as indicated by positive newborn eye (*C. trachomatis* or *N. gonorrhoeae*) or nasopharyngeal (*C. trachomatis*) swabs by 4-6 weeks postnatally (*among a sub-set of 2000 participants only*);
9. Evaluate the performance (sensitivity, specificity, positive and negative predictive values) of the Xpert CT/NG Test for the diagnosis of neonatal eye infection and pneumonia using ocular and nasopharyngeal specimens (*among a sub-set of 2000 participants only*).

Data capture, management, and analysis for measurement of secondary outcome 4 (health economics), outcome 5 (health systems), and outcome 6 (acceptability) will be described in detail separately.

TRIAL DESIGN AND SETTING

WANTAIM is a cluster-randomised controlled crossover trial,[44] being conducted in 10 clusters in two provinces in PNG. The unit of randomisation is a primary health care facility and its catchment communities. Trial clusters were selected in consultation with staff in provincial health authorities, church health services, health facilities and with other local stakeholders using pre-defined criteria (e.g. antenatal attendance data, geographical location of health facility and catchment communities in relation to other potential trial clusters).

Ten geographically distinct clusters were assigned in a 1:1 ratio to control and intervention arms in the first phase of the trial. Following a washout period of 2-3 months at the end of the first phase, each cluster will cross over to participate in the alternative trial arm in the second phase of the study (a so-called ‘A-B / B-A’ trial design; Figure 1).

A summary of the trial intervention and control arms and visits is shown in Figure 2, and a summary of trial procedures, in Figure 3.

ELIGIBILITY AND EXCLUSION CRITERIA

Eligibility criteria

- Aged 16 years and over;
- Attending first antenatal clinic visit;
- Estimated gestational age 26 weeks or earlier, based on obstetric ultrasound examination at first antenatal clinic visit;
- Able to complete study informed consent procedures; to understand why the study is being carried out, and the potential risks and benefits associated with study participation;
- Willing to undergo a clinical assessment including ultrasound examination, to provide self-collected vaginal swabs and a venepuncture specimen, to comply with study follow-up schedule, including a postnatal visit within 48h of birth;
- Live within a one-hour drive of participating study clinic;
- Able to provide reliable contact details to facilitate future community tracing and postnatal follow-up.

Exclusion criteria

- Severe, symptomatic anaemia identified during the enrolment visit that requires hospitalisation (Hb <6 g/dl accompanied by symptoms requiring urgent treatment);
- Permanent disability that prevents or impedes study participation and/or comprehension (such that it is not possible to obtain informed consent to participate).

All women excluded on health grounds will be referred to the appropriate clinical specialist at each site, where they will be treated according to PNG national treatment guidelines.

Figure A1. Crossover design: trial arm and phases

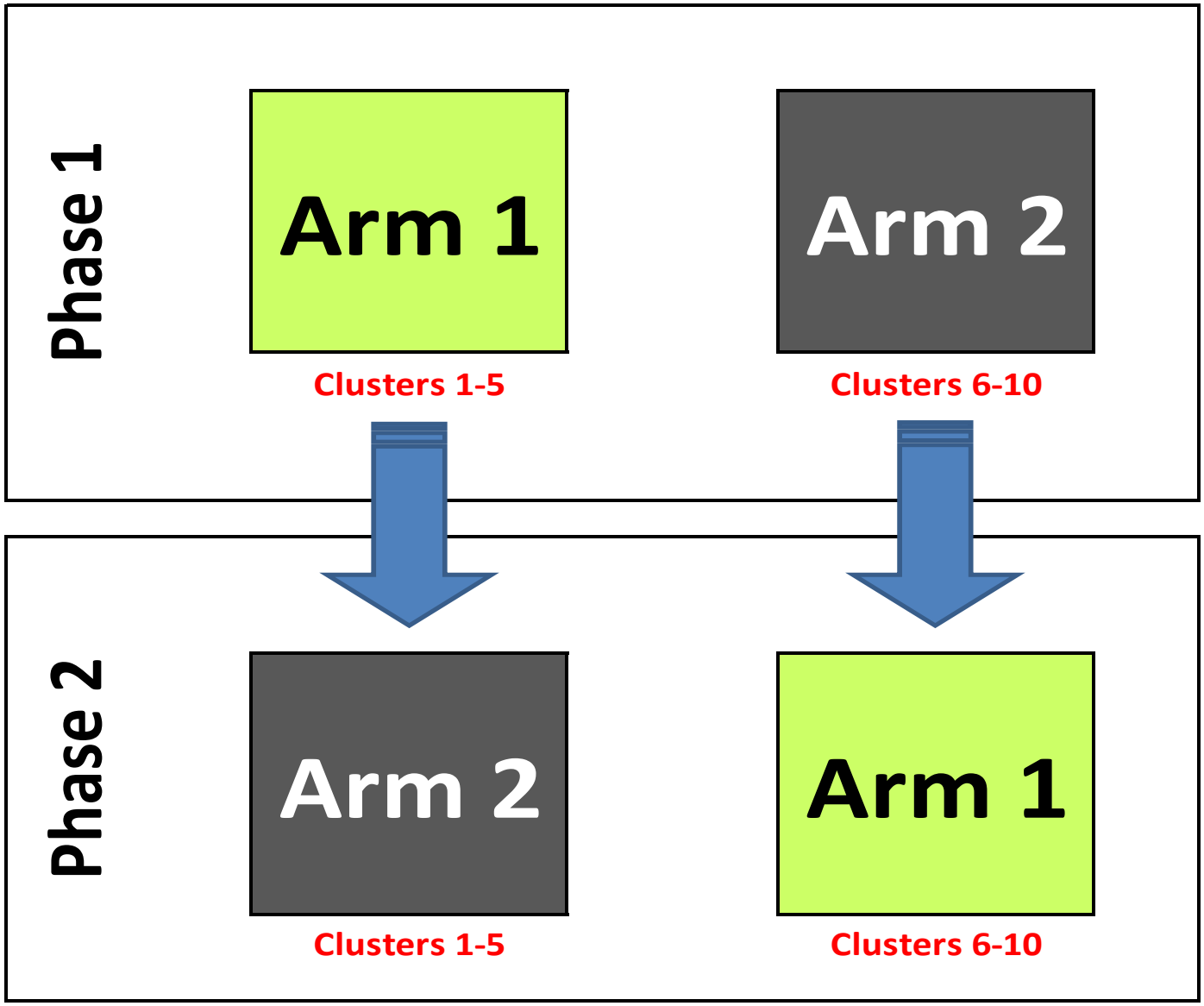
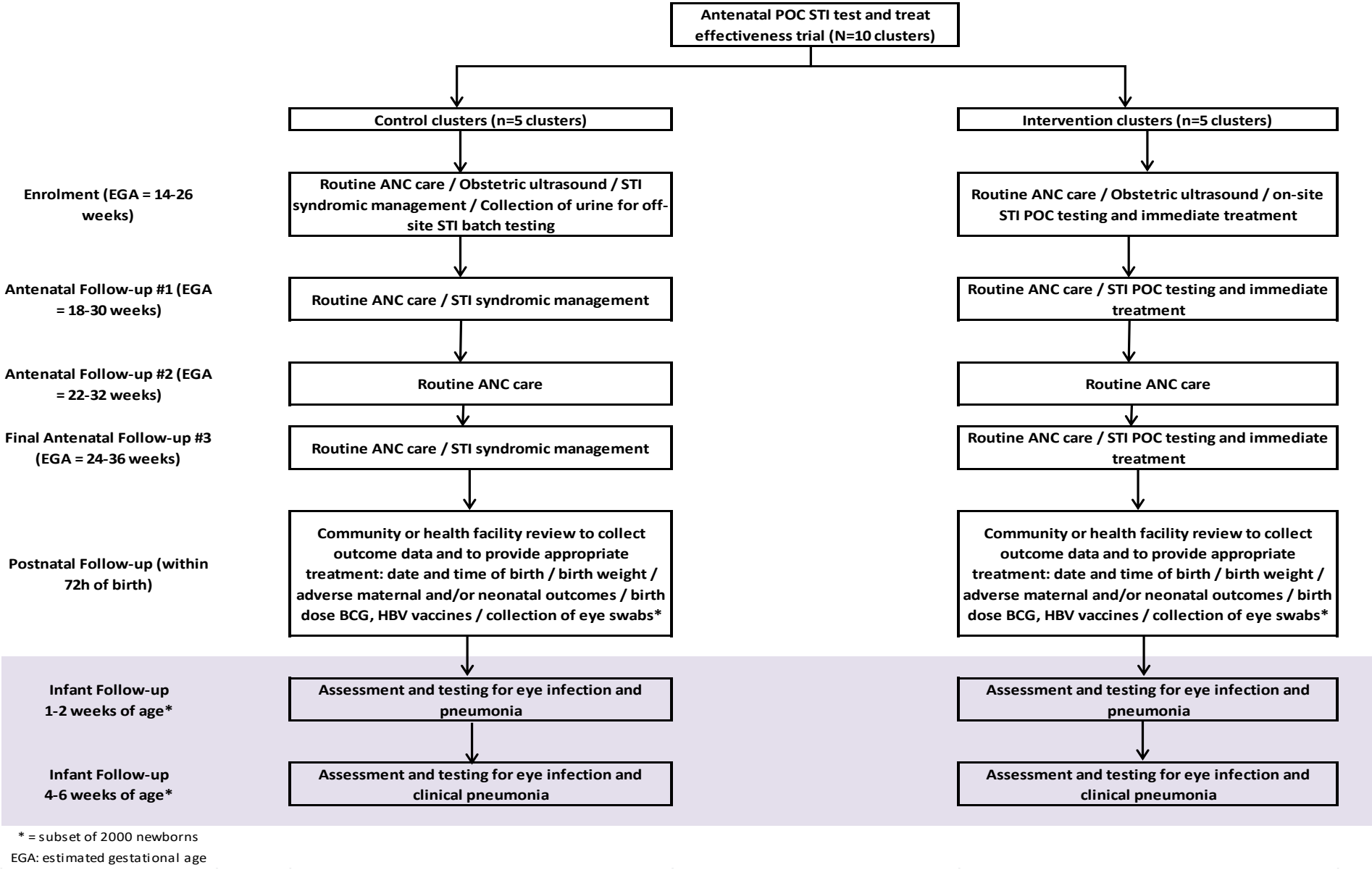


Figure 2. Summary of trial interventions and visits



* = subset of 2000 newborns

EGA: estimated gestational age

Figure 3. Summary of trial procedures.

	Antenatal interview and examination	Routine antenatal tests	Obstetric ultrasound scan	Syndromic STI diagnosis and treatment	POC STI testing and treatment	Residual urine for off-site STI testing	Dried blood spot for off-site malaria qPCR	Routine antenatal prophylaxis	Socioeconomic, cost and acceptability data	Cost diaries	Semi-structured interviews (cost, acceptability)	Collection of trial outcome data	Postnatal care: mother and newborn	Assessment and testing for eye infection, pneumonia	
ENROLMENT VISIT (EGA = 14 - 26 weeks)															
CONTROL	+	Syp, HIV, Hb, RDT*, urine	+	+		+	+	FeFol, SP	+	SS					
INTERVENTION	+	Syp, HIV, Hb, RDT*, urine	+		+		+	FeFol, SP	+	SS					
FIRST ANTENATAL FOLLOW-UP VISIT (EGA = 18 - 30 weeks)															
CONTROL	+	Hb, RDT*, urine		+		+		FeFol, SP	+	SS					
INTERVENTION	+	Hb, RDT*, urine			+			FeFol, SP	+	SS					
SECOND ANTENATAL FOLLOW-UP VISIT (EGA = 22 - 32 weeks)															
CONTROL	+	Hb, RDT*, urine						FeFol, SP	+	SS	SS				
INTERVENTION	+	Hb, RDT*, urine						FeFol, SP	+	SS	SS				
FINAL ANTENATAL FOLLOW-UP VISIT (EGA 34 - 36 weeks)															
CONTROL	+	Hb, RDT*, urine		+		+		FeFol	+	SS					
INTERVENTION	+	Hb, RDT*, urine			+			FeFol	+	SS					
POSTNATAL FOLLOW-UP VISIT (within 72 h of birth)															
CONTROL	+								+	SS	SS	birth w t, time, ABO	+	SS	
INTERVENTION	+								+	SS	SS	birth w t, time, ABO	+	SS	
POSTNATAL FOLLOW-UP VISIT #2 (1-2 weeks of birth)															
CONTROL													SS	SS	
INTERVENTION													SS	SS	
POSTNATAL FOLLOW-UP VISIT #3 (4-6 weeks of birth)															
CONTROL													SS	SS	
INTERVENTION													SS	SS	

* = if clinically indicated; SS = sub-set to be invited to participate

POC: point of care; Syp: syphilis rapid test at POC; HIV: HIV rapid test at POC; Hb: haemoglobin estimation at POC; RDT: rapid diagnostic test for malaria at POC: urine: urinalysis for protein, glucose at POC

ABO: adverse birth outcomes; FeFol: oral iron and folate supplementation; SP: oral suphaddoxine-pyrimethamine; maximum of three doses each at least 4 w eeks apart

RANDOMISATION

In each province close to the start of recruitment, members of the local community advisory board, representatives from local health authorities, and staff from clinical research sites will be invited to a launch event in which the randomisation sequence will be selected by a senior independent stakeholder e.g. the Provincial Administrator. The trial statistician will prepare different randomisation sequences that will be put into separate identical opaque envelopes. These will be placed in a traditional woven *bilum* string bag. The independent stakeholder will select an envelope from the *bilum* and open it to reveal the sequence to the audience. An example of possible randomisation sequences is provided below (Figure 4).

Figure 4: Example of possible randomisation sequences for East New Britain Province

	A	B	C	D	E	F	G	H	I	J
Kerevat	1	1	1	1	1	1	2	2	2	2
Paparatava	1	1	1	2	2	2	1	1	1	2
Gelegele	1	2	2	1	1	2	1	1	2	1
Warangoi	2	1	2	1	2	1	1	2	1	1
Napapar	2	2	1	2	1	1	2	1	1	1

1=intervention arm in first phase of the trial

2= intervention arm in second phase of the trial

A subgroup of up to 2000 newborns will be followed up at 1-2 weeks and 4-6 weeks postnatal to collect secondary outcome data (secondary objectives 7-9). Five of the 10 trial clusters will be randomly selected to participate in this part of the study. The randomisation sequence will be computer-generated and allocated by the trial statistician. Each of the clusters selected to participate in the extended postnatal follow-up component will do so during both the intervention and the control phase of the trial. Each selected cluster will thereby contribute around 200 newborns in each trial phase (2000 in total).

The intervention cannot be blinded but, where possible, outcome assessment will be blinded, and statistical analyses will be blinded. All trial investigators will be blinded to participants' outcomes broken down by study arms until after the analysis plan is signed off and the database is locked.

POWER AND SAMPLE SIZE CONSIDERATIONS

Primary outcome

Sample size requirements were based on our earlier findings about the incidence of the primary outcome in PNG.[13-15,45] The proportion of pregnancies resulting in preterm birth and low birth weight were estimated to be 15% each, and 18% combined. Research findings on the relationship between STIs and birth outcomes suggest that effective STI treatment could reduce this combined proportion by up to 45%[27,46] in those with STIs. This reduction would translate into a relative reduction of around 23% in a population in which nearly half of all pregnant women have chlamydia, gonorrhoea, trichomonas and/or bacterial vaginosis, as is the case in PNG.[13-15]

To measure this effect size with α=0.05, β=0.20 (80% power), and an estimated intra-cluster correlation coefficient (ICC) of 0.003, 16 clusters of 200 women would be required in a cluster randomised crossover trial (8 clusters per phase; 3200 women in total). In case one or more trial cluster does not successfully complete the trial, two additional clusters will be added (10 clusters of 200 women in each phase; 4000 women in total). Based on our earlier work in this setting,[45,47,48] we anticipate loss to follow-up for the primary outcome of around 15%, so we will recruit a total of 4600 women from 10 clusters in two phases (230 women per cluster in each phase of the trial).

Secondary outcomes, including neonatal outcomes

The resources of the trial allow for outcome data to be collected from a subset of the study population (2000 newborns from five randomly selected clusters in each trial phase), who will be invited to participate in extended follow-up visits at 1-2 weeks and 4-6 weeks after birth. We have defined three secondary outcomes that will be investigated:

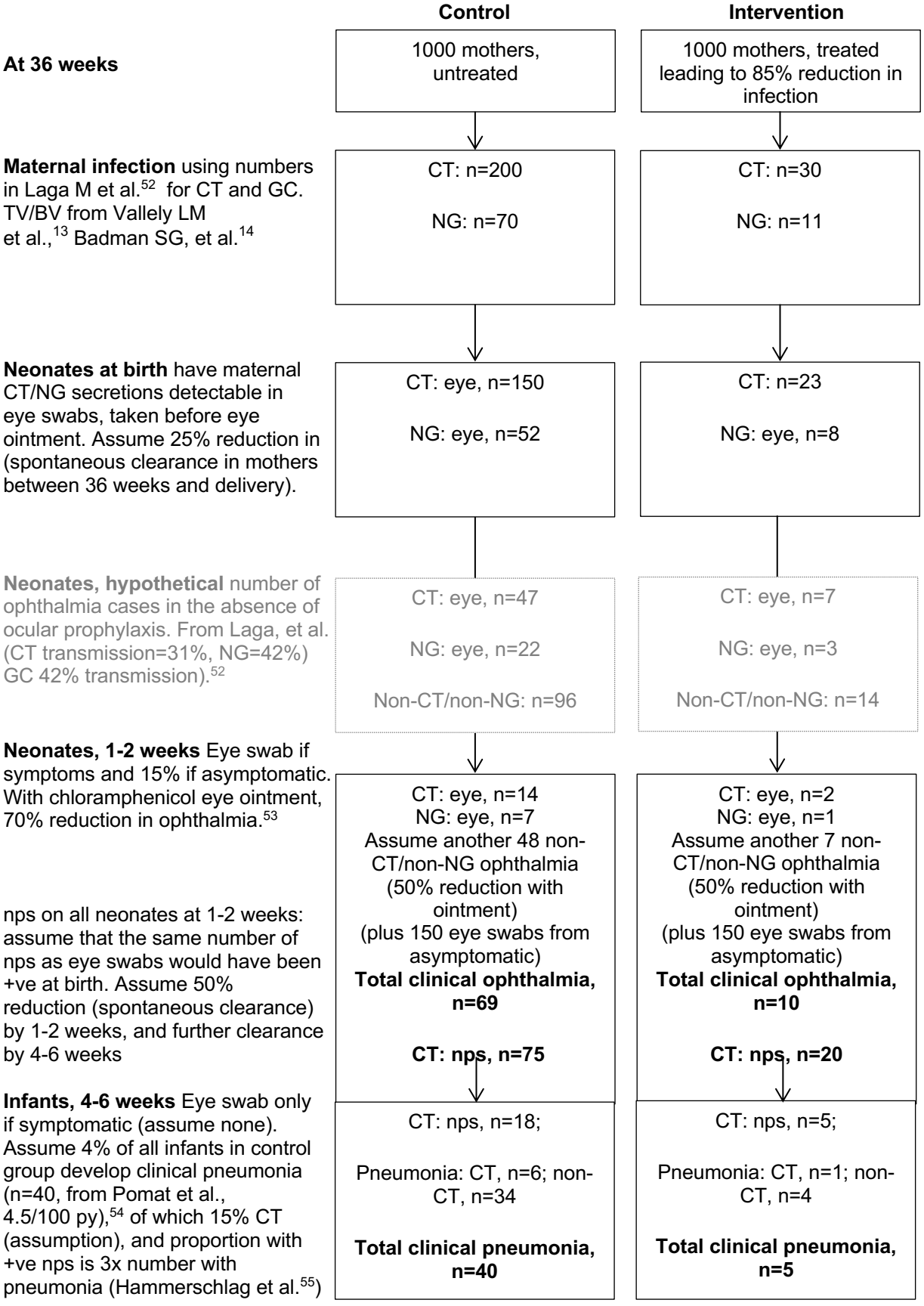
7. Proportion of newborns with a clinical diagnosis of either eye infection or moderate/severe pneumonia by 4-6 weeks postnatal;
8. Incidence of mother to child transmission of *C. trachomatis* or *N. gonorrhoeae* as indicated by positive newborn eye (*C. trachomatis* or *N. gonorrhoeae*) or nasopharyngeal (*C. trachomatis*) swabs by 4-6 weeks postnatally;
9. Performance (sensitivity, specificity, positive and negative predictive values) of the Xpert CT/NG Test for the diagnosis of neonatal eye infection and pneumonia using ocular and nasopharyngeal specimens compared with laboratory-based polymerase chain reaction (PCR) assays.

The statistical power for the above secondary objectives is based on the following assumptions, which are shown in Figure 5:

a. 30% of women in the control group are infected with either *C. trachomatis* or *N. gonorrhoeae* (assumes syndromic management has no impact on prevalence).[13]
b. The intervention will reduce exposure of newborns to the specified genital STIs by 85% (assumes that all diagnosed infections are treated at point-of-care but undiagnosed infection, re-infection and/or antimicrobial resistance result in some neonatal infections). The antibiotics used to treat specific infections will also cure some other pathogens that cause ophthalmia neonatorum or newborn respiratory infection.
c. Eye swabs are taken at birth, before ocular prophylaxis is given. We assume that 25% of maternal CT and NG infections in both intervention and control groups clear spontaneously between 36 weeks and birth.
d. Numbers of cases of ophthalmia in the control group will be similar to Laga et al, in which 21% of mothers had CT and 6.6% had NG at delivery.[52] Ophthalmia neonatorum cases caused by CT (n=60), NG (n=25), or other causes (n=96) in babies of 781 mothers by 28 days. For simplicity, we use the exact numbers (Table III) and assume these numbers would occur in infants of 1000 mothers by 1-2 weeks. Transmission of ophthalmia was 31% (63/201) for CT and 42% (28/67) for NG.
e. Neonatal ocular prophylaxis given after the first eye swabs are taken will reduce the risk of CT and NG ophthalmia neonatorum by 70%[51] (and non-CT, non-NG ophthalmia by 50%) in control and intervention groups.
f. Neonatal ocular prophylaxis will have no effect on nasopharyngeal acquisition of *C. trachomatis* e.g. as a result of nasolacrimal transfer.

Figure 5 shows the estimated number of cases of neonatal eye infection, nasopharyngeal carriage, and neonatal pneumonia under these assumptions. These numbers are used to calculate statistical power for outcome #7 and statistical precision for outcomes #8 and #9.

Figure 5: Flow chart - neonatal outcomes and number of swabs available



Abbreviations: CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; nps, nasopharyngeal swab

Outcome 7: neonatal eye infection or moderate/severe pneumonia by 4-6 weeks (Figure 4)

	Ophthalmia neonatorum	Pneumonia	Total (%)
Control (n=1000)	73	40	113 (11.3%)
Intervention (n=1000)	12	5	17 (1.7%)

For a relative risk reduction of 85% (risk ratio 0.15, 95% CI 0.09, 0.25), with α=0.05, β=0.10 (90% power), assuming a design effect of 3 would require a total sample size of 942 participants; and with a design effect of 4, a total of 1256 participants. If the event rate in the control arm is lower, e.g. 9% and the effect size smaller, e.g. risk ratio 0.19 (95% CI 0.11, 0.31), with α=0.05, β=0.10, assuming a design effect of 3 would require a total sample size of 1350 participants; and with a design effect of 4, a total of 1800 participants.

Outcome 8: mother to child transmission of *C. trachomatis* or *N. gonorrhoeae* (Figure 4)

This outcome is measured in the control group only. The application of eye ointment at birth means that transmission of *C. trachomatis* and *N. gonorrhoeae* and establishment of infection in the conjunctiva cannot be established. The proportion of infants who had positive results in eye swabs at birth can be measured. For *C. trachomatis*, transmission resulting in infant nasopharyngeal colonisation or infection can be measured at 1-2 weeks and 4-6 weeks. The small expected number of nasopharyngeal swabs with *C. trachomatis* detected by 4-6 weeks makes these estimates imprecise.

C. trachomatis in newborn conjunctiva (assumed to be maternal transfer)

	Maternal CT at 36 weeks gestation	Newborn CT in eye swab at birth	Proportion (95% CI)
Control (n=1000)	200	150	0.75 (0.69, 0.80)

C. trachomatis in nasopharyngeal swabs (assumed to be infection)

	Maternal CT at 36 weeks gestation	Newborn CT in nps at 1-2 weeks	Proportion (95% CI)	Infant CT in nps at 4-6 weeks	Proportion (95% CI)
Control (n=1000)	200	75	0.38 (0.31, 0.45)	18	0.09 (0.05, 0.14)

N. gonorrhoeae in newborn conjunctiva (assumed to be maternal transfer)

	Maternal NG at 36 weeks gestation	Newborn NG in eye swab at birth	Proportion (95% CI)
Control (n=1000)	70	52	0.74 (0.62, 0.84)

Outcome 9: diagnostic performance of Xpert CT/NG (Figure 4*)*

The specimens obtained in the trial will be used to estimate assay performance for the detection of *C. trachomatis* and *N. gonorrhoeae* in eye swabs and of *C. trachomatis* in nasopharyngeal swabs. Based on numbers in Figure 4, there will be a maximum of 2321 eye swabs (collected at birth and at 1-2 weeks) and a maximum of 4000 nasopharyngeal swabs. Assuming the GeneXpert test has 90% sensitivity or specificity results in the following 95% confidence intervals:

	CT eye swab	CT nasopharyngeal swab	NG eye swab
Number of swabs	2321	4000	2321
Number positive by PCR	251	118	90
Number negative by PCR	2070	3882	2231
GeneXpert sensitivity 90%	85.6 to 93.4%	82.9 to 94.6	81.9 to 95.3
GeneXpert specificity 90%	88.6 to 91.3	86.6 to 88.7	88.7 to 91.2

DATA MANAGEMENT AND PRE-ANALYSIS STATISTICAL REVIEW

Data management

Data will be collected using paper-based Case Report Forms (CRFs) at each visit (enrolment, antenatal follow-up and postnatal visits). All CRFs will be designed in TeleForm Elite version 10.5 and uploaded into a secure centrally located clinical trials database created in ORACLE.

Completed CRFs will be checked on the day of completion and any errors, discrepancies or out of range values will be corrected by study staff. Any corrections or alterations to the original CRF will be initialled by the member of staff. Original copies of the CRF will be held at the clinic in a locked filing cabinet accessible only by the clinic staff and the dedicated trial Data Manager. For the purposes of ICH GCP, completed CRFs will be considered source documents. Completed CRFs will be electronically scanned, either at the clinic or at a central facility, on a weekly basis (e.g. the day following new antenatal enrolments) using a portable scanner into a computer using a tagged image file format (TIFF). Data will be stored in a dedicated electronic folder prior to verification using TeleForm. A copy of the locally-held data will then be uploaded using TeleForm into a dedicated study-specific ORACLE database located on a secure, off-site server at UNSW Sydney, Australia. ORACLE is compliant with ICH GCP E6 and with United States Food and Drugs Authority (FDA) guidance[56] (Title 21 of the Code of Federal Regulations (CFR) Part 11;Available at: http://www.fda.gov/RegulatoryInformation/Guidances/ucm125067.htm). UNSW Sydney will provide IT and technical support to the study team for the duration of the trial.

Following successful data upload, locally held data (TIFF scans) will be moved to a dedicated folder indicating completed data entry. Data entry and review will be ongoing activities during the entire trial period and will be conducted in accordance with study-specific SOPs. A random sub-set of electronic records from the master data base will be checked for accuracy against the scanned electronic TIFF files of participant CRFs every three months by the in-country trial Data Manager and Trial Coordinator. Electronic versions of the locked datasets and TIFF files for this study will be maintained as part of the PNG Institute of Medical Research (PNGIMR) research studies database. Electronic STI test results will be uploaded monthly into the ORACLE database as per study-specific SOPs, using a program developed at UNSW that enables test data to be extracted by Study ID Number from the .GXX data files generated by the GeneXpert platform. Electronic data held on portable ultrasound machines will also be regularly extracted for off-site back-up, as per study-specific SOPs.

The electronic trial database will be backed up both onsite (at PNGIMR) and off-site (at UNSW Sydney) at regular intervals as specified in study-specific data management SOPs. Copies of the databases generated in PNG and interim datasets will also be sent to the study CIs at regular intervals. At the end of the trial, the database will be locked following final data entry and database cleaning, and will then be provided to the Trial Statistician for data analyses. After completion of data analyses, copies of the final database and analytical datasets (neither of which will contain any subject identifying information) will be maintained on secure servers located at the PNGIMR and UNSW Sydney.

Pre-analysis statistical review

Prior to the analysis, data will be checked for outlying values and logical inconsistencies. We will conduct a pre-analysis statistical review to make decisions regarding the participants to be included in the analysis, identifying outliers and how to handle them in analysis. We will also specify the final forms of the tables as well as the covariates that we will present in these tables. After importing each database from ORACLE to SAS format, we will present simple descriptive distributions tables and/or figures for each variable.

BASELINE ANALYSIS

Baseline measurements will be presented using descriptive statistics across trial clusters by study period; continuous measurements will be presented using the mean and standard deviation (SD) and the median and interquartile range (IQR) (Tables X1, X2). Binary and categorical variables will be presented using counts and percentages. There will be no formal comparison of baseline characteristics across the study periods.

DESCRIPTION OF PATIENT FLOW AND ANALYSIS DATASETS

We will prepare a flow chart to show the participation and flow of participants during the trial and follow-up in accordance with the CONSORT guidelines, including additional guidance provided for cluster randomised trials.[57] This diagram will describe the number of women at each stage of the trial. This diagram will also describe "per-protocol" and "intention to treat" datasets. Tabulated versions of these data will also be presented.

STATISTICAL METHODS

All analyses will account for the effects of clustering, incorporating sources of variation between individuals within a period, between clusters and between periods. Data will be analysed using an adaptation of the statistical methods described by Hayes and Moulton[58] for a standard cluster randomised crossover trial and add a period effect into an ANOVA model on log(p), where p= proportion of outcome of interest so that the cluster periods are treated as "units" in the analysis, and the data are cluster summaries (e.g. log of the proportion of the primary outcome in each cluster period).

Analysis of the primary outcome

The primary outcome variable is the proportion of women and their newborns who experience preterm birth and/or low birth weight. This will be calculated for each trial cluster. The primary analysis will be unadjusted.

a) Unadjusted (crude) estimates for primary outcome

The main analysis of the primary outcome will be conducted by means of a cluster-level analysis. The observed risk of the primary outcome will be calculated as proportions in the intervention and control periods for each cluster (please see in Table 1a and 1b). Briefly, we will present the risk ratios for intervention versus control (Table 1a) for primary and secondary outcome variables; 95% CIs will be presented by incorporating any variability due to the variation between clusters in intervention effects, as well as intrinsic "binomial variation" in the cluster-period proportions. We will present the differences in means for continuous outcomes and their cluster-adjusted 95% CIs. We will use a standard approach for a two-period two-treatment crossover trial design which is an ANOVA with treatment, period and cluster effects (see Table 1a and 1b).

The investigators do not expect substantial differences in individual level variables between the two phases for any of the clusters so the planned primary analysis will report unadjusted effects.

b) Adjusted estimates

Adjusted analysis will be considered if the characteristics of women change substantially over time in two steps. This assessment will be conducted in two-steps: Step 1: we will fit a logistic regression model for the primary outcome of interest. This model will incorporate a term for the cluster, period and covariates of interest (please see below) except the intervention (treatment) effect. This analysis will use individual level data. Step 2: we will obtain observed (O) and expected (E) numbers of outcomes for each cluster and phase. This step will produce O/E (total of 20). These cluster-specific proportions/summaries will be analysed following the methodology described in Table 1a and 1b.

c) Covariates of interest

Besides key demographic and socio-economic characteristics including maternal age, marital status, geographical location, education level and employment status, we also consider parity, a history of pregnancy, past STI diagnosis. Other potentially important factors are age at sexual debut, number of sexual partners past 12 months and condom use.

Analysis of secondary outcomes

Data capture, management, and analysis for measurement of secondary outcome 4 (health economics), outcome 5 (health systems), and outcome 6 (acceptability) will be described in detail separately.

a) The following secondary outcome measures will be analysed using the same statistical approach as the primary outcome measure (i.e. analysing the cluster-level means/proportions; Table 1b):

   1. Mean birth weight
   2. Proportion of women who experience premature rupture of membranes;
   3. Number of curable STIs diagnosed and appropriately treated;

Those that are measured quantitatively (mean birth weight) will be analysed as continuous as well as binary variables, using standard cut-off points where available (e.g. low birth weight <2500 grams) before they are converted into cluster-level summaries.

b) The following secondary outcome measures will be analysed with standard survival analysis techniques using the sub-set of individual level participant data. In this analysis, treatment and period will be considered as fixed effects while cluster effects will be considered as a random component to account "within cluster" correlations (Table 1c):

   7. Proportion of newborns with an eye infection or moderate/severe pneumonia by 4-6 weeks postnatal (*among a sub-set of 2000 participants only*);
   8. Incidence of mother to child transmission of *C. trachomatis* or *N. gonorrhoeae* as indicated by positive newborn eye (*C. trachomatis* or *N. gonorrhoeae*) or nasopharyngeal (*C. trachomatis*) swabs by 4-6 weeks postnatally (*among a sub-set of 2000 participants only*);

c) The sensitivity, specificity, positive and negative predictive value of Xpert CT/NG for the detection of newborn *C. trachomatis* or *N. gonorrhoeae* infection using eye and nasopharyngeal swabs, compared with laboratory-based PCR will be calculated using standard methods (secondary outcome 9)

Missing data

Only available data will be analysed. There will be no data imputation for missing data for the primary and the secondary endpoints.

Intention to treat (ITT) analysis set

The analysis of the primary outcome will be by ITT at the cluster level, meaning that all participants with a recorded outcome will be included in the analysis, and will be analysed according to the treatment group to which they were randomised, and all clusters will be included in the analysis according to the treatment group to which they were randomised.[59]

Per-protocol (PP) analysis set

We will also contact PP analyses, which will include all women and their children with an interpretable result. Study participants will be followed up until they drop-out or experience a major deviation from the protocol.

Interim analysis

There is no planned interim analysis.

Subgroup analyses

The following additional subgroup analyses will be conducted. Proportions will be calculated for each trial cluster. Unadjusted and adjusted analyses will be conducted as described above (page 16).

1. Proportion of women who experience spontaneous abortion/miscarriage or stillbirth or early neonatal death;
2. Proportion of women who experience low/birth weight or premature birth or spontaneous abortion/miscarriage or stillbirth or early neonatal death;
3. Proportion of women who experience low/birth weight or premature birth or spontaneous abortion/miscarriage or stillbirth or early neonatal death or premature rupture of membranes;
4. Among women who tested positive for chlamydia: Proportion of women and their newborns who experienced “preterm birth” or “low birth weight”;
5. Among women who tested positive for gonorrhoea: Proportion of women and their newborns who experienced “preterm birth” or “low birth weight”;
6. Among women who tested positive for TV: Proportion of women and their newborns who experienced “preterm birth” or “low birth weight”;
7. Among women who tested positive for syphilis: Proportion of women and their newborns who experienced “preterm birth” or “low birth weight”;
8. Among women who tested chlamydia/gonorrhoea/TV: Proportion of women and their newborns who experienced “preterm birth” or “low birth weight”;
9. Among women who tested chlamydia/gonorrhoea/TV/syphilis: Proportion of women and their newborns who experienced “preterm birth” or “low birth weight”;
10. Among women who tested positive for chlamydia: Proportion of women and their newborns who experience spontaneous abortion/miscarriage or stillbirth or early neonatal death;
11. Among women who tested positive for gonorrhoea: Proportion of women and their newborns who experience spontaneous abortion/miscarriage or stillbirth or early neonatal death;
12. Among women who tested positive for TV: Proportion of women and their newborns who experience spontaneous abortion/miscarriage or stillbirth or early neonatal death;
13. Among women who tested positive for syphilis Proportion of women and their newborns who experience spontaneous abortion/miscarriage or stillbirth or early neonatal death;
14. Among women who tested chlamydia/gonorrhoea/TV: Proportion of women and their newborns who experience spontaneous abortion/miscarriage or stillbirth or early neonatal death;
15. Among women who tested chlamydia/gonorrhoea/TV/syphilis: Proportion of women and their newborns who experience spontaneous abortion/miscarriage or stillbirth or early neonatal death;
16. Among women who tested positive for chlamydia: Proportion of women and their newborns who had low/birth weight or premature birth or spontaneous abortion/miscarriage or stillbirth or early neonatal death or premature rupture of membranes;
17. Among women who tested positive for gonorrhoea: Proportion of women and their newborns who had low/birth weight or premature birth or spontaneous abortion/miscarriage or stillbirth or early neonatal death or premature rupture of membranes;
18. Among women who tested positive for TV: Proportion of women and their newborns who had low/birth weight or premature birth or spontaneous abortion/miscarriage or stillbirth or early neonatal death or premature rupture of membranes;
19. Among women who tested positive for syphilis: Proportion of women and their newborns who had low/birth weight or premature birth or spontaneous abortion/miscarriage or stillbirth or early neonatal death or premature rupture of membranes;

20. Among women who tested positive for one or more of chlamydia/gonorrhoea/TV: Proportion of women and their newborns who had low/birth weight or premature birth or spontaneous abortion/miscarriage or stillbirth or early neonatal death or premature rupture of membranes;
21. Among women who tested positive for one or more of chlamydia/gonorrhoea/TV/syphilis: Proportion of women and their newborns who had low/birth weight or premature birth or spontaneous abortion/miscarriage or stillbirth or early neonatal death or premature rupture of membranes.

Table 1a: Primary/secondary outcome variables (1/0) (as a binary outcome variable) (overall and subgroup analyses)

	Study period #1	**Study period #2**		
	Intervention	**Control**	**Crude Risk Ratio (95% CI)**	h_j
Cluster 1	$p_{11} = n_{11}/m_{11}$	$p_{01} = n_{01}/m_{01}$	$p_{11}/p_{01} = r_1$	$h_1 = log(p_{11}/p_{01})$
Cluster 2	$p_{12} = n_{12}/m_{12}$	$p_{02} = n_{02}/m_{02}$	$p_{12}/p_{02} = r_2$	$h_2 = log(p_{12}/p_{02})$
Cluster 3	$p_{13} = n_{13}/m_{13}$	$p_{03} = n_{03}/m_{03}$	$p_{13}/p_{03} = r_3$	$h_3 = log(p_{13}/p_{03})$
Cluster 4	$p_{14} = n_{14}/m_{14}$	$p_{04} = n_{04}/m_{04}$	$p_{14}/p_{04} = r_4$	$h_4 = log(p_{14}/p_{04})$
Cluster 5	$p_{15} = n_{15}/m_{15}$	$p_{05} = n_{05}/m_{05}$	$p_{15}/p_{05} = r_5$	$h_5 = log(p_{15}/p_{05})$
	Control	**Intervention**		
Cluster 6	$p_{06} = n_{06}/m_{06}$	$p_{16} = n_{16}/m_{16}$	$p_{16}/p_{06} = r_6$	$h_6 = log(p_{16}/p_{06})$
Cluster 7	$p_{07} = n_{07}/m_{07}$	$p_{17} = n_{17}/m_{17}$	$p_{17}/p_{07} = r_7$	$h_7 = log(p_{17}/p_{07})$
Cluster 8	$p_{08} = n_{08}/m_{08}$	$p_{18} = n_{18}/m_{18}$	$p_{18}/p_{08} = r_8$	$h_8 = log(p_{18}/p_{08})$
Cluster 9	$p_{09} = n_{09}/m_{09}$	$p_{19} = n_{19}/m_{19}$	$p_{19}/p_{09} = r_9$	$h_9 = log(p_{19}/p_{09})$
Cluster 10	$p_{010} = n_{010}/m_{010}$	$p_{110} = n_{110}/m_{110}$	$p_{110}/p_{010} = r_{10}$	$h_{10} = log(p_{110}/p_{010})$
Overall	**#1** arithmetic mean for intervention= $\frac{n_{11}+\cdots+n_{110}}{m_{11}+\cdots+m_{110}} = \overline{X}_1$ **#2** arithmetic mean for control= $\frac{n_{01}+\cdots+n_{010}}{m_{01}+\cdots+m_{010}} = \overline{X}_0$		**#5** Geometric means of cluster risk ratios= $(r_1 + \cdots + r_{10} = \overline{R}_G)$	#8 Arithmetic mean of logarithm of the pair-wise risk ratios: $\overline{h} = (h_1 + \cdots + h_{10})/10$ $s_m^2 = \frac{1}{c-1}\sum_j (h_j - \overline{h})^2$

Means of cluster summaries	**#3** Means of cluster summaries for intervention: $\overline{P}_1$ = ($\overline{p}_{11}$ + $\cdots + \overline{p}_{110}$)/10 **#4** Means of cluster summaries for intervention: $\overline{P}_0$ = ($\overline{p}_{01}$ + $\cdots + \overline{p}_{010}$)/10	**#6** Geometric means of cluster summaries for intervention: $\overline{P}_{G1}$ **#7** Geometric means of cluster summaries for intervention: $\overline{P}_{G0}$

Comparing intervention & control groups:

#1 Estimated risk difference (i.e. absolute reduction): Difference between means of cluster summaries $\overline{P_1} - \overline{P_0}$

Where

$p_{ij} = n_{ij}/m_{ij}$; $i = 1$ (intervention) and $i = 0$ (control), proportion of outcome of interest for i^{th}study arm for the j^{th} cluster

n_{ij} = number of outcome of interest in the j^{th}cluster,$j = 1,\ldots,10$ and the i^{th}study arm, i.e. intervention ($i = 1$) or control ($i = 0$)

m_{ij}= sample size of each cluster

Calculate pairwise risk ratio: p_{1j}/p_{0j}

 # 2 Estimated risk ratio (in original scale): $\overline{P}_1/\overline{P}_0$

#3 Estimated risk ratio (in logarithmic scale): Inspect the distribution of the observed summary measures (i.e. logarithmic transformation of if they are "skewed" then use a logarithmic transformation:

Logarithm of the pairwise risk ratios and calculate: $h_j = log(p_{1j}/p_{0j})$

Estimated risk ratio: $exp(\overline{h})$ where $\overline{h} = (h_1 + \cdots + h_{10})/c$, $h_j = log(p_{1j}/p_{0j})$, c= total number of clusters

Test statistics:

$$t_m = \frac{\overline{h}}{\sqrt{\frac{s_m^2}{c}}}$$

Where $\overline{h}$ is the mean of the differences across matched pairs and s_m^2 is the empirical variance of these differences:

$$s_m^2 = \frac{1}{c-1}\sum_j (h_j - \overline{h})^2$$

The computed values of t_m is referred to tables of the t distribution with $\gamma = (c-1)$ degrees of freedom. A 95% CI for the log(rate ratio) will be:

$$\overline{h} \pm t_{\gamma,0.025} \times \frac{s_m}{\sqrt{c}}$$

ADJUSTED ANALYSIS: If we observe imbalance between the study arms with regard to the demographic, socioeconomic and clinical endpoints, we will also conduct an adjusted analysis:

ANOVA model:

$$\boldsymbol{log(p_{ijk}) = \alpha + \beta_i + \gamma_j + \delta_k + \varepsilon_{ijk}}$$

where $\boldsymbol{\alpha}$ is the grand mean, $\boldsymbol{\beta_i}$ is the intervention effect ($\beta_0 = 0$), $\boldsymbol{\gamma_j}$ is the cluster effect, $\boldsymbol{\delta_k}$ is the period effect and $\boldsymbol{\varepsilon_{ijk}}$ is the residual with 8 d.f. Also, $\boldsymbol{p_{ijk}}$ is the proportion with the outcome in the intervention ($i = 1$) or control ($i = 0$) condition, cluster j and period k.

Table 1b: Primary and secondary outcomes and intervention effect estimates

	Summary statistics Prevalence of binary and mean of continuous outcomes	
	Intervention (95% CI)	**Control (95% CI)**
Primary outcome (composite)		
Preterm birth or low birth weight (0/1)	$\overline{p_1}$ (95% CI)	$\overline{p_0}$ (95% CI)
Preterm birth (0/1)	$\overline{p_1}$ (95% CI)	$\overline{p_0}$ (95% CI)
Low birth weight (0/1)	$\overline{p_1}$ (95% CI)	$\overline{p_0}$ (95% CI)
Secondary outcomes		
Premature rupture of membranes (0/1)	$\overline{p_1}$ (95% CI)	$\overline{p_0}$ (95% CI)
Birth weight (grams)	$\bar{x}_1$(SD)	$\bar{x}_0$(SD)

Table 1c: Neonatal related secondary outcomes and intervention effect estimates

Neonatal outcomes	**Summary statistics** Prevalence of the binary outcomes	
	Intervention (95% CI)	**Control (95% CI)**
Neonatal eye infection and/or pneumonia (0/1) (**composite end point**)	$\overline{p_1}$ (95% CI)	$\overline{p_0}$ (95% CI)
Proportion of babies with *C. trachomatis*/ and/or *N. gonorrhoeae* (0/1) (mother to child transmission of *C. trachomatis* and/or *N. gonorrhoeae*) **(individual and composite)**	$\overline{p_1}$ (95% CI)	$\overline{p_0}$ (95% CI)

ANOVA model:

$$\boldsymbol{log(p_{ijk}) = \alpha + \beta_i + \gamma_j + \delta_k + \varepsilon_{ijk}}$$

where $\boldsymbol{\alpha}$ is the grand mean, $\boldsymbol{\beta_i}$ is the intervention effect ($\beta_0 = 0$), $\boldsymbol{\gamma_j}$ is the cluster effect, $\boldsymbol{\delta_k}$ is the period effect and $\boldsymbol{\varepsilon_{ijk}}$ is the residual with 8 d.f. Also, $\boldsymbol{p_{ijk}}$ is the proportion with the outcome of interest in the intervention ($i = 1$) or control ($i = 0$) condition, cluster j and period k.

Table 1d: Evaluate the performance of the Xpert CT/NG Test for the diagnosis of neonatal eye infection and pneumonia using ocular and nasopharyngeal specimens

	Sensitivity (95% CI)	Specificity (95% CI)	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
Ocular specimen				
Nasopharyngeal specimen				

Table X1: Selected socio-demographic characteristics of women in study periods 1 and 2

	Study period #1		**Study period #2**		**Overall**	
	Intervention	Control	Intervention	Control	Intervention	Control
Maternal age in years, mean ± SD						
Marital Status, % (n)						
Single						
Married						
Separated/divorced/widow						
Province of birth, n (%)						
East New Britain Province						
Madang Province						
Other						
Education level						
None						
Attended primary school						
Attended high school						
Attended tertiary level education						
Employment						
Household duties						
Substance farming						
Market selling						
Self employed						
Formal employment						
Student						
Other						
Husband/partner education level						
None						
Attended primary school						
Attended high school						
Attended tertiary level education						
Husband/partner employment						
Household duties						
Subsistence farming						
Market selling						
Self employed						
Formal employment						
Student						
Other						

Total household expenditure (past 2 wk)						
PNG Kina						
Household assets						
Mobile phone						
Radio						
Bicycle						
Cooking stove						
Other						
Source of drinking water						
Unprotected well (public/private)						
Protected well (public/private)						
Water tank (public/private)						
Piped water into home						
Piped water into community (public tap)						
Other						
Toilet facilities						
Water flush toilet						
Pit latrine (public/private)						
Other						

Table X2: Selected behavioural and clinical characteristics of women in study periods 1 and 2

	Study period #1		**Study period #2**		**Overall**	
	Intervention	Control	Intervention	Control	Intervention	Control
Parity						
0						
1						
2						
3						
4						
≥5						
Previous pregnancy loss						
Miscarriage						
Induced abortion						
Stillbirth						
Past STI diagnosis (any)						
Yes						
No						
Past history of syphilis						
Yes						
No						
Age at sexual debut						
Mean (SD)						
Median (IQR)						
Number of life time sexual partners						
Mean (SD)						
Median (IQR)						
Number of partners in past 12 months						
1 partner						
2 partners						
3 partners						
4 partners						
5+ partners						
Mean (SD)						
Median (IQR)						
Vaginal sex in past 4 weeks						
Yes						
No						
Condom use at last vaginal sex						

Yes						
No						
Risk factors at enrolment						
STIs (CT, NG, TV and BV)						
Anaemia						
Malaria						
HIV						
Syphilis						
Hypertension						
Pre-eclampsia						
Gestational diabetes						
Currently smokes tobacco						
Currently drinks alcohol						
Currently chews betel nut						
Other						

REFERENCES

1. Blencowe H, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet* 2012; **379**(9832): 2162-72.

2. Wang H, et al. Global, regional, and national levels of neonatal, infant, and under-5 mortality during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014; **384**(9947): 957-79.

3. Kassebaum NJ, et al. Global, regional, and national levels and causes of maternal mortality during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014; **384**(9947): 980-1004.

4. NHIS Maternal Health Data. Port Moresby: National Department of Health, Government of Papua New Guinea, 2012.

5. Mullick S, et al. Sexually transmitted infections in pregnancy: prevalence, impact on pregnancy outcomes, and approach to treatment in developing countries. *Sex Transm Infect* 2005; **81**(4): 294-302.

6. Minkoff H, et al. Risk factors for prematurity and premature rupture of membranes: a prospective study of the vaginal flora in pregnancy. *Am J Obstet Gynecol* 1984; **150**(8): 965-72.

7. Donders GG, et al. The association of gonorrhoea and syphilis with premature birth and low birthweight. *Genitourin Med* 1993; **69**(2): 98-101.

8. Johnson HL, et al. Sexually transmitted infections and adverse pregnancy outcomes among women attending inner city public sexually transmitted diseases clinics. *Sex Transm Dis* 2011; **38**(3): 167-71.

9. Rours GI, et al. Chlamydia trachomatis infection during pregnancy associated with preterm delivery: a population-based prospective cohort study. *Eur J Epidemiol* 2011; **26**(6): 493-502.

10. Silveira MF, et al. Chlamydia trachomatis infection during pregnancy and the risk of preterm birth: a case-control study. *Int J STD AIDS* 2009; **20**(7): 465-9.

11. Ministerial Task Force on Maternal Health in Papua New Guinea. Port Moresby: National Department of Health, Government of Papua New Guinea, 2009.

12. Vallely A, et al. The prevalence of sexually transmitted infections in Papua New Guinea: A systematic review and meta-analysis. *PLoS ONE* 2010; **5**(12): e15586.

13. Vallely LM, et al. Prevalence and risk factors of Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis and other sexually transmissible infections among women attending antenatal clinics in three provinces in Papua New Guinea: a cross-sectional survey. *Sex Health* 2016.

14. Badman SG, et al. A novel point-of-care testing strategy for sexually transmitted infections among pregnant women in high-burden settings: results of a feasibility study in Papua New Guinea. *BMC Infect Dis* 2016; **16**: 250.

15. Wangnapi RA, et al. Prevalence and risk factors for Chlamydia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis infection in pregnant women in Papua New Guinea. *Sex Transm Infect* 2015; **91**(3): 194-200.

16. Coleman JS, et al. Trichomonas vaginalis vaginitis in obstetrics and gynecology practice: new concepts and controversies. *Obstet Gynecol Surv* 2013; **68**(1): 43-50.

17. Romero R, et al. The preterm parturition syndrome. *Bjog* 2006; **113 Suppl 3**: 17-42.

18. Gravett MG, et al. Independent associations of bacterial vaginosis and Chlamydia trachomatis infection with adverse pregnancy outcome. *JAMA* 1986; **256**(14): 1899-903.

19. Association of Chlamydia trachomatis and Mycoplasma hominis with intrauterine growth retardation and preterm delivery. The John Hopkins Study of Cervicitis and Adverse Pregnancy Outcome. *Am J Epidemiol* 1989; **129**(6): 1247-57.

20. Elliott B, et al. Maternal gonococcal infection as a preventable risk factor for low birth weight. *J Infect Dis* 1990; **161**(3): 531-6.

21. French JI, et al. Readily treatable reproductive tract infections and preterm birth among black women. *Am J Obstet Gynecol* 2006; **194**(6): 1717-26; discussion 26-7.

22. Silva MJ, et al. Perinatal morbidity and mortality associated with chlamydial infection: a meta-analysis study. *Braz J Infect Dis* 2011; **15**(6): 533-9.

23. de Borborema-Alfaia AP, et al. Chlamydia trachomatis infection in a sample of northern Brazilian pregnant women: prevalence and prenatal importance. *Braz J Infect Dis* 2013; **17**(5): 545-50.

24. Folger AT. Maternal Chlamydia trachomatis Infections and Preterm Birth: The Impact of Early Detection and Eradication During Pregnancy. *Matern Child Health J* 2013.

25. Kupka R, et al. Predictors of stillbirth among HIV-infected Tanzanian women. *Acta Obstet Gynecol Scand* 2009; **88**(5): 584-92.

26. Silverman RK, Wojtowycz M. Risk factors in premature rupture of membranes. *Prim Care Update Ob Gyns* 1998; **5**(4): 181.

27. Silver B, et al. Perinatal Morbidity Associated With Trichomonas Vaginalis: A Meta-Analysis. STI & AIDS World Congress. Vienna; 2013.

28. McGregor JA, et al. Prevention of premature birth by screening and treatment for common genital tract infections: results of a prospective controlled evaluation. *Am J Obstet Gynecol* 1995; **173**(1): 157-67.

29. Vallely LM, et al. Performance of syndromic management for the detection and treatment of genital Chlamydia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis among women attending antenatal, well woman and sexual health clinics in Papua New Guinea: a cross-sectional study. *BMJ Open* 2017; **7**(12): e018630.

30. van Gemert C, et al. Syndromic management of sexually transmissible infections in resource-poor settings: a systematic review with meta-analysis of the abnormal vaginal discharge flowchart for Neisseria gonorrhoea and Chlamydia trachomatis. *Sex Health* 2018; **15**(1): 1-12.

31. Sabido M, et al. Clinic-based evaluation of a rapid point-of-care test for detection of Chlamydia trachomatis in specimens from sex workers in Escuintla, Guatemala. *J Clin Microbiol* 2009; **47**(2): 475-6.

32. Pearce DM, et al. A new rapid molecular point-of-care assay for Trichomonas vaginalis: preliminary performance data. *Sex Transm Infect* 2013; **89**(6): 495-7.

33. Nunez-Forero L, et al. Diagnostic accuracy of rapid tests for sexually transmitted infections in symptomatic women. *Sex Transm Infect* 2016; **92**(1): 24-8.

34. Hegazy MM, et al. Performance of rapid immunochromatographic assay in the diagnosis of Trichomoniasis vaginalis. *Diagn Microbiol Infect Dis* 2012; **74**(1): 49-53.

35. Gaydos CA, et al. Rapid Diagnosis of Trichomonas vaginalis by Testing Vaginal Swabs in an Isothermal Helicase-Dependent AmpliVue Assay. *Sex Transm Dis* 2016; **43**(6): 369-73.

36. Benzaken AS, et al. Diagnosis of gonococcal infection in high risk women using a rapid test. *Sex Transm Infect* 2006; **82 Suppl 5**: v26-8.

37. Guy RJ, et al. A randomised trial of point-of-care tests for chlamydia and gonorrhoea infections in remote Aboriginal communities: Test, Treat ANd GO- the "TTANGO" trial protocol. *BMC Infect Dis* 2013; **13**: 485.

38. Amsel R, et al. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983; **74**(1): 14-22.

39. Mengistie Z, et al. Comparison of clinical and gram stain diagnosis methods of bacterial vaginosis among pregnant women in Ethiopia. *J Clin Diagn Res* 2013; **7**(12): 2701-3.

40. Thomason JL, et al. Statistical evaluation of diagnostic criteria for bacterial vaginosis. *Am J Obstet Gynecol* 1990; **162**(1): 155-60.

41. Bradshaw CS, et al. Evaluation of a point-of-care test, BVBlue, and clinical and laboratory criteria for diagnosis of bacterial vaginosis. *J Clin Microbiol* 2005; **43**(3): 1304-8.

42. Myziuk L, et al. BVBlue test for diagnosis of bacterial vaginosis. *J Clin Microbiol* 2003; **41**(5): 1925-8.

43. ICH. Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice. E6(R2): Internataional Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), 2016.

44. Crespi CM. Improved Designs for Cluster Randomized Trials. *Annu Rev Public Health* 2016; **37**: 1-16.

45. Unger HW, et al. Sulphadoxine-pyrimethamine plus azithromycin for the prevention of low birthweight in Papua New Guinea: a randomised controlled trial. *BMC Med* 2015; **13**: 9.

46. Gray RH, et al. Randomized trial of presumptive sexually transmitted disease therapy during pregnancy in Rakai, Uganda. *Am J Obstet Gynecol* 2001; **185**(5): 1209-17.

47. Pomat WS, et al. Safety and immunogenicity of neonatal pneumococcal conjugate vaccination in Papua New Guinean children: a randomised controlled trial. *PLoS One* 2013; **8**(2): e56698.

48. Pomat WS, et al. Safety and immunogenicity of pneumococcal conjugate vaccines in a high-risk population: a randomised controlled trial of 10-valent and 13-valent PCV in Papua New Guinean infants. *Clin Infect Dis* 2018.

49. Justel M, et al. Vertical transmission of bacterial eye infections, Angola, 2011-2012. *Emerg Infect Dis* 2015; **21**(3): 471-3.

50. Pourabbas B, et al. Prevalence of Chlamydia trachomatis and Neisseria gonorrhoeae infections among pregnant women and eye colonization of their neonates at birth time, Shiraz, Southern Iran. *BMC Infect Dis* 2018; **18**(1): 477.

51. WHO. Web annex D: Evidence profiles and evidence-to-decision framework. WHO guidelines for the treatment of Neisseria gonorrhoeae. Geneva: World Health Organization, 2016.

52. Laga M, et al. Epidemiology of ophthalmia neonatorum in Kenya. *Lancet* 1986; **2**(8516): 1145-9.

53. World Health Organization. WHO Guidelinesfor the Treatment of Chlamydia trachomatis. Geneva, 2016.

54. Pomat WS, et al. Safety and immunogenicity of neonatal pneumococcal conjugate vaccination in Papua New Guinean children: a randomised controlled trial. *PLoS One* 2013; **8**(2): e56698.

55. Hammerschlag MR. Chlamydial infections in infants and children. In: Holmes KK, Mardh PA, Sparling PF, et al., eds. Sexually Transmitted Diseases. New York: McGraw-Hill; 1999: 593.

56. FDA. Guidance for Industry Part 11, Electronic Records; Electronic Signatures — Scope and Application, 2003.

57. Campbell MK, et al. Consort 2010 statement: extension to cluster randomised trials. *Bmj* 2012; **345**: e5661.

58. Hayes RJ, Moulton LH. Cluster Randomised Trials. Second ed. Boca Raton, Florida, USA: Chapman and Hall/CRC Press; 2017.

59. White IR, et al. Strategy for intention to treat analysis in randomised trials with missing outcome data. *Bmj* 2011; **342**: d40.