VITA

Statistical Analysis Plan

Final Version 1.0
(04 MAR 2020)

Based on Protocol version 1.0 (dated 29 JUN 2017)

Trial registration: ISRCTN14161293

The following people have reviewed the Statistical Analysis Plan and are in agreement with the contents				
Name	**Job title**	**Trial Role**	**Signature**	**Date**
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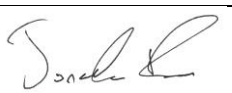
Trish Hepburn	Senior Medical Statistician	Senior Trial Statistician		
Prof. Jonathan Ross	Professor of Sexual Health and HIV	Chief Investigator		4.3.20

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Abbreviations

Abbreviation	Description
Adverse Event (AE)	Any untoward medical occurrence in a participant or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. Comment: An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of an investigational medicinal product, whether or not related to the investigational medicinal product.
BV	Bacterial Vaginosis
DMC	Data Monitoring Committee
SAP	Statistical Analysis Plan
Serious Adverse Event (SAE)	Any untoward medical occurrence or effect that: 1 Results in death **2** Is life-threatening* **3** Requires hospitalisation or prolongation of existing hospitalisation **4** Results in persistent or significant disability or incapacity **5** Is a congenital anomaly/birth defect **6** Or is otherwise considered medically significant by the Investigator** Comments: The term severe is often used to describe the intensity (severity) of a specific event. This is not the same as serious, which is based on participant's/event outcome or action criteria. * Life threatening in the definition of an SAE refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe. ** Medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered serious.
SHC	Sexual Health Clinic
STI	Sexually Transmitted Infection
TMG	Trial Management Group
TSC	Trial Steering Committee

Changes from protocol

The table below details changes to the planned analyses in the SAP compared to the trial protocol which after discussion with the TMG are not considered to require a protocol amendment.

Protocol version and section	Protocol text	SAP version and section	SAP text	Justification
V1.0 13.2.4	Assuming that 80% of participants receiving oral metronidazole achieve resolution of symptoms, 1710 participants (855 in each treatment arm) are required for analysis to detect a 6% increase in response rate to 86% in participants receiving lactic acid gel (risk ratio 1.08) at the 5% SL (2-sided) with 90% power. To allow for non-collection of the primary outcome from up to 10%, (e.g. due to loss to follow up) a total of **1900** participants will be recruited – 950 to each treatment arm.	V1.0 section 2.1	Following the April 2019 DMC closed meeting and further analyses of the data, the sample size at that time (463 participants) was considered sufficient to provide an answer to the primary question of the trial, hence it was agreed to stop recruitment to the trial. Recruitment stopped on June 27th 2019.	The trial was stopped early following the recommendation of the DMC.
v1.0 section 13.2.1	-	V1.0 section 7.4	In addition, by the following subgroups which were not specified in the protocol: d) number of episodes of BV in 12 months before baseline (0, 1-3, >3) e) total time with BV in 12 months before baseline (<2 weeks, >=2 weeks &	Request from CI: to assist in interpretation of the trial primary endpoint. These are additional subgroup analyses of the primary outcome.

Protocol version and section	Protocol text	SAP version and section	SAP text	Justification
			<3 months, >=3 months)	
V1.0	(Not in protocol)	V1.0 section 7.1	"as there is only one GP site, site type will not be included as covariate)"	There was only one GP centre in the study; a decision was made to stop recruiting GP centres, therefore it was not appropriate to include this as a covariate.
V1.0	The primary analyses for symptom resolution will be investigated to determine whether treatment effectiveness differs according to the following sub-groups: a) Presence of concomitant STI (sexually transmitted infection)(yes/no) b) BV confirmed by positive microscopy (yes/no) c) Type of centre participant presented at (sexual health clinic vs GP/other clinics)	V1.0 section 7.4	The primary analyses for symptom resolution will be investigated to determine whether treatment effectiveness differs according to the following sub-groups, as specified in the protocol: a) Presence of concomitant STI (yes/no) b) BV confirmed by positive microscopy (yes/no) The plan was to also look at the following sub-group: c) Type of centre participant presented at (sexual health clinic vs GP/other clinics) however, there was only one non-sexual health clinic (SHC) so this will not be done.	There was only one GP centre in the study; a decision was made to stop recruiting GP centres, therefore it was not appropriate to include this as a covariate.
V1.0; various sections	"SF-12 questionnaire at baseline, 2 weeks, and 6 months"	V1.0 sections 1.7 and 2.4	"SF-12 questionnaire at baseline, 2 weeks, 3 months and 6 months"	Collection of the 3 month SF-12 data was suggested by the HTA funding committee, but the protocol was

Protocol version and section	Protocol text	SAP version and section	SAP text	Justification
				not updated in error.
V1.0, objectives and outcomes in sections 1, 2.1, 2.2, 13.1.2,	[missing]	V1.0, sections 2.4, 4.4, 7.5	**Secondary objective:** To compare the time to resolution of BV symptoms **Secondary outcome:** Time to resolution of BV symptoms	Omitted in error from objectives/outcomes

Amendments to versions

Version	Date	Change/comment	Statistician

Additional contributors to the SAP (non-signatory)

Name	Trial role	Job Title	Affiliation
Prof. Alan Montgomery	Co-Investigator	Professor of Medical Statistics and Clinical Trials Director of Nottingham Clinical Trials Unit	NCTU, University of Nottingham
Prof. Siobhan Creanor	Data Monitoring Committee statistician	Professor of Medical Statistics and Clinical Trials	University of Plymouth

1. INTRODUCTION & PURPOSE

This document details the rules proposed and the presentation that will be followed, as closely as possible, when analysing and reporting the main results from the NIHR funded VITA trial.

The purpose of the plan is to:

1. Ensure that the analysis is appropriate for the aims of the trial, reflects good statistical practice, and that interpretation of a priori and post hoc analyses respectively is appropriate.

2. Explain in detail how the data will be handled and analysed to enable others to perform or replicate these analyses.

Additional exploratory or auxiliary analyses of data not specified in the protocol may be included in this analysis plan.

This analysis plan will be made available if required by journal editors or referees when the main papers are submitted for publication. Additional analyses suggested by reviewers or editors will be performed if considered appropriate. This should be documented in a file note.

Amendments to the statistical analysis plan will be described and justified in the final report of the trial and where appropriate in publications arising from the analysis.

Health economic and qualitative analysis plans are beyond the scope of this document.

2. SYNOPSIS OF STUDY DESIGN AND PROCEDURES

[Synopsis from protocol V1.0]

1.1 Title	A randomised controlled trial to assess the clinical and cost effectiveness of topical lactic acid gel for treating second and subsequent episodes of bacterial vaginosis
Acronym	VITA
Short title	Metronidazole **V**ersus lactic ac**I**d for **T**reating bacterial v**A**ginosis
1.2 Trial Design	Open-label, multicentre, parallel group, randomised controlled trial.
1.3 Objectives	**Primary objective:** to determine whether intravaginal lactic acid gel is better than oral metronidazole for symptomatic resolution of recurrent bacterial vaginosis (BV). **Secondary objectives:** a. To compare the time to first recurrence of BV symptoms; b. To compare the frequency of BV episodes over 6 months; c. To compare the frequency of BV treatments required over 6 months; d. To compare microbiological resolution of BV on microscopy 2 weeks after presentation; e. To compare the tolerability profiles of lactic acid gel and metronidazole; f. To compare adherence to lactic acid gel versus metronidazole tablets; g. To compare acceptability of use of lactic acid gel versus metronidazole tablets; h. To determine comparative presence of concurrent sexually transmitted infections at baseline and at week 2; i. To compare quality of life (measured using the SF-12); j. To compare cost effectiveness of using intravaginal lactic acid gel versus oral metronidazole tablets. In addition, samples for additional microbiological analysis, including gene sequencing, will be collected for future investigation into the factors associated with successful treatment.
1.4 Participant Population	Women with symptoms of bacterial vaginosis (BV) and a history of one or more episodes of BV within the previous 2 years which resolved with treatment.
1.5 Key Eligibility Criteria	**Inclusion criteria:** 1. Age 16 years or over. 2. Clinical diagnosis of bacterial vaginosis – based on patient reported symptoms of discharge with an unpleasant (typically fishy) odour (with or without positive microscopy according to local site practice).

	3. History of at least one previous episode of bacterial vaginosis within the past two years (clinically diagnosed or patient reported) which resolved with treatment. 4. Willing to use either intravaginal lactic acid gel or oral tablets for the management of BV. 5. Willing to take their own vaginal samples. 6. Willing to avoid vaginal douching during treatment. 7. Willing to provide contact details and be contacted for the purpose of collecting follow-up information. 8. Willing to avoid sexual intercourse or use effective contraception for the 7-day duration of study treatment. (Condoms are not considered to be effective contraception due to a potential interaction with lactic acid gel). 9. Access to the internet, email and willing to complete web based follow up questionnaires in English. 10. Written informed consent. **Exclusion criteria:** 1. Contra-indications or allergy to lactic acid gel or metronidazole tablets. 2. Pregnant or breastfeeding. 3. Patients currently trying to conceive and not willing to avoid sexual intercourse or use effective contraception for the 7-day duration of study treatment. 4. Use of oral antibiotics (other than the study treatment) or antifungal agents; concurrently, within the last 2 weeks or planned use within the next 2 weeks. 5. Use of topical vaginal antibiotics, antifungals or acidifying products (other than the study treatment); concurrently, within the last 2 weeks, or planned use within the next 2 weeks. 6. Previous participation in this study. 7. Current participation in another clinical trial involving an investigational medicinal product.
1.6 Sample Size	Assuming that 80% of participants receiving oral metronidazole achieve resolution of symptoms, 1710 participants (855 in each treatment arm) are required for analysis to detect a 6% absolute increase in response rate to 86% in participants receiving lactic acid gel (risk ratio 1.08) at the 5% SL (2-sided) with 90% power. To allow for non-collection of the primary outcome from up to 10% of randomised participants, (e.g. due to loss to follow up) a total of **1900** participants will be recruited – 950 to each treatment arm.
1.7 Outcome Measures	**Primary outcome measure:** The primary outcome is resolution of bacterial vaginosis based on participant reported resolution of symptoms at week 2 (14 days from randomisation). **Secondary outcome measures:** a. Time to first recurrence of BV;

	b. Number of participant reported BV episodes over 6 months; c. Number of participant reported BV treatment courses over 6 months; d. Microbiological resolution of BV on microscopy of vaginal smears at week 2; e. Comparative tolerability of lactic acid gel and metronidazole assessed by participant reporting of side effects (including nausea, vomiting, taste disturbance, vaginal irritation, diarrhoea and abdominal pain) and via participant interviews; f. Participant reported adherence to treatment; g. Acceptability of treatments via qualitative assessment in a subgroup of participants; h. Prevalence of concurrent sexually transmitted infections (gonorrhoea, chlamydia and trichomoniasis) at baseline and at week 2; i. Quality of life assessed by SF-12 questionnaire at baseline, 2 weeks, 3 months and 6 months; j. Comparative cost effectiveness of using intravaginal lactic acid gel versus oral metronidazole tablets via NHS Service use questionnaire.
1.8 Intervention	Participants will be randomised to receive either intravaginal lactic acid gel used once daily for 7 days (intervention group) OR oral metronidazole tablets, 400mg twice daily for 7 days (control group).

2.1. Sample size and justification

Assuming that 80% of participants receiving oral metronidazole achieve resolution of symptoms, 1710 participants (855 in each treatment arm) are required for analysis to detect a 6% increase in response rate to 86% in participants receiving lactic acid gel (risk ratio 1.08) at the 5% SL (2-sided) with 90% power.

To allow for non-collection of the primary outcome from up to 10%, (e.g. due to loss to follow up) a total of **1900** participants will be recruited – 950 to each treatment arm.

A planned review of unblinded data by the DMC in May 2019 led the DMC to conclude that the sample size at that time (463 participants) was sufficient to provide an answer to the primary research question (resolution of BV symptoms at week 2), and hence recruitment should be stopped. There were no safety concerns. Recruitment continued while further analyses were conducted by the trial team and presented to the DMC, following which the TSC and the HTA agreed that recruitment to the trial should stop. Recruitment ceased on June 28th 2019, with a total of 518 participants randomised.

2.2. Blinding and breaking of blind

This is an open-label trial. There will be no blinding of the participant, investigator, site research team or NCTU trial management, data and IT personnel to treatment allocation. The site research team are only likely to have contact with the participant at baseline; the post-baseline data are collected via participant completed questionnaire and a sample sent by the participant direct to the laboratory.

However, the central laboratory staff performing BV microscopy and STI testing will be blinded to participants' treatment allocation, and all analyses that present data separately by treatment arm or that estimate between-group effects will be conducted by a statistician blinded to treatment allocation until after the database has been locked and this statistical analysis plan signed-off. The CI will also remain blinded until after database lock.

2.3. Trial committees

A Trial Management Group (TMG), Trial Steering Committee (TSC) and Data Monitoring Committee (DMC) will be assembled to oversee the trial. The general purpose, responsibilities and structure of the committees are described in the protocol. Further details of the roles and responsibilities of the TSC and DMC can be found in their charters agreed prior to the start of recruitment to the trial.

2.4. Outcome measures

Table 1: Summary of the outcome measures

Outcome measures	Scale, description and source	Derivation of scores	Time point (Weeks/Months)			
Primary outcome			**0**	**2w**	**3m**	**6m**
Resolution of bacterial vaginosis based on participant reported resolution of symptoms at Week 2 (14 days from randomisation).	**Scale:** Participant answers 'Yes' or 'No' to question "Have your BV symptoms cleared and stayed cleared following your study treatment?" **Source**: participant completed online questionnaires at Week 2 and 3 months (if not on week 2), week 2 telephone calls.	Combination of resolution from questionnaire(s) and telephone calls. See 4.4 on derived variables for further detail.		✓		
Secondary outcomes						
Time to first recurrence of BV symptoms	**Scale:** Time in days from date of resolution of BV symptoms, for those resolving within 2 weeks. Both resolution and recurrence are participant self-reported. **Source:** resolution (clearance) dates from week 2 questionnaire. Recurrence (new episode) dates from 3 and 6 month questionnaires.	Date of first new episode (if any), in those who have resolved within 2 weeks, minus date of resolution. See 4.4 for further detail.			✓	✓
Number of participant reported BV episodes over 6 months	**Scale:** Number of new episodes if symptoms resolved within 2 weeks. **Source:** Data from 3 month and 6 month questionnaires, plus 6 month telephone calls.	Sum of number of new episodes from 3 month questionnaire: "How many new episodes of BV type symptoms have you experienced?" and 6 month questionnaire and telephone calls: "How many new episodes of bacterial vaginosis type symptoms have you experienced (in the last 3 months)?" IF they have first resolved. See 4.4 for further detail. Note that			✓	✓

Outcome measures	Scale, description and source	Derivation of scores	Time point (Weeks/Months)			
		this will be on a subset of participants who have completed all 3 questionnaires.				
Number of participant reported BV treatment courses over 6 months	**Scale:** Number of additional courses of treatment received for BV (in addition to trial medication). **Source:** from week 2, 3 and 6 month questionnaires.	Sum of number of courses of each of the additional treatments (including 'other'). See 4.4 for further detail.		✓	✓	✓
Microbiological resolution of BV on microscopy of vaginal smears at week 2	**Scale:** The number with microbiological resolution is the number of negative results at week 2 out of those with positive results at week 0. **Source:** Data (baseline and week 2) received from Birmingham central lab.	A negative result is 0 (no bacteria), 1 (normal flora), 2 (intermediate BV) or U (gram positive cocci). A positive result is 3 (confirmed BV) on the Ison-Hay scale. A result of 'NI' (slide result is not interpretable) will be treated as missing result.		✓		
Comparative tolerability of treatments (participant reported side-effects)	**Scale:** For each of the side effects in the questionnaire (nausea, vomiting, taste changes, vaginal irritation, abdominal pain, diarrhoea) participant recorded: presence, time from starting treatment, severity, duration, resolution. **Source:** Data from week 2 questionnaire.	Presented as per categories on week 2 questionnaire. Duration is in hours (number of days multiplied by 24). See 4.4 for further detail.		✓		
Participant reported adherence to treatment	**Scale:** Any taken/used, course completed, percentage received, reason if not completed, time to starting treatment. **Source:** Data from lactic acid/metronidazole page on week 2 questionnaire, apart from randomisation date.	**Any taken/used**: at least one time ticked yes. **Course completed**: participant answers "yes" to 'Did you complete your course of…", whichever days are ticked. **Percentage received:** number of times taken out of number of times should been taken, summarised by mean, SD, median, IQR, min, max.		✓		

Outcome measures	Scale, description and source	Derivation of scores	Time point (Weeks/Months)			
		Also number and proportion with at least 85% adherence. **Reason if not completed:** categories as per questionnaire. **Time to starting treatment** – difference in days between randomisation date and date started treatment (on questionnaire), summarised by median, IQR, min, max, n; participant-reported treatment start dates which are not feasible are estimated where possible (usually by randomisation date). See 4.4 for further detail.				
Acceptability of treatments	**Scale:** How easy to take **Source:** week 2 questionnaire.	**How easy to take:** categories as per questionnaire.		✓		
Prevalence of concurrent sexually transmitted infections at baseline and at week 2	**Scale:** Chlamydia, gonorrhoea, trichomoniasis: positive or negative at baseline and week 2. **Source:** central lab dataset.	Scored as either P for positive, N for negative, I for indeterminate, E for equivocal. Combine with kit number to match participant number and week.	✓	✓		
Quality of life (measured using the SF-12)	See health economics analysis plan.		✓	✓	✓	✓
Comparative cost effectiveness of using lactic acid gel versus oral metronidazole tablets	See health economics analysis plan.		✓	✓	✓	✓
Time to resolution of BV	Time in days from randomisation to resolution. Source: resolution date from questionnaires.	Resolution date minus randomisation date (in days).		✓	✓	✓

Additional qualitative data collection

A qualitative sub-study will consist of semi-structured telephone interviews to explore acceptability and adherence to therapies with participants, and how these can be optimised. A sub-group of

participants will be contacted by researchers from the University of Warwick. Interviews will be audio recorded, and transcribed. The results are published[1].

3. INTERIM ANALYSIS

There is no planned formal interim analysis of treatment efficacy, although the DMC will be provided with descriptive outcome data separately by trial arm in closed reports. However, an assessment of recruitment and adherence to treatment will be performed using data from the first 6 months of participant recruitment. This is to determine how feasible it is that the trial is able to adequately address its primary and secondary objectives.

The TSC and DMC will use the following criteria as a guide to determine whether the trial should progress:

Number of participants:

a) Reviewing the number of participants, out of those randomised, completing their Week 2 assessment against the following targets:

- >90% - continue the trial
- 65-90% - review recruitment and retention procedures to identify underlying problems and put in place strategies to address these, with review in 6 months
- 35-65% - review recruitment and retention procedures to identify underlying problems and put in place strategies to address these. Ongoing review over 6 months and terminate the trial if the recruitment trajectory does not indicate that full recruitment can occur within an acceptable recruitment period.
- <35% - terminate the trial

Adherence to treatment

b) Reviewing adherence to lactic acid gel and metronidazole against the following pre-defined targets:

- Median adherence 5-7 days per week - continue the trial
- Median adherence 3-4 days per week - review data from the qualitative interviews on adherence and tolerability to identify underlying problems and put in place strategies to address these, with review in 6 months
- Median adherence < 3 days per week - terminate the trial

4. GENERAL ANALYSIS CONSIDERATIONS

4.1. Analysis sets

Outcome	Analysis set
Primary outcome (resolution of BV symptoms) *Secondary outcomes*	Participants will be analysed according to randomised group regardless of adherence with the allocated intervention.
Safety outcomes	All participants who have received at least one dose of metronidazole/used one tube of lactic acid gel (self-reported) will be summarised according to the treatment they received; those not given the randomised treatment are documented at baseline. Participants who were randomised, but received no treatment will be summarised separately, if data are available.

4.2. Timing of final analysis

All quantitative outcome data will be analysed at the end of the trial after final database lock.

4.3. Statistical software

Analyses will be performed using Stata version 15 or above.

4.4. Derived variables

See table 1, section 2.4. In addition:

The following variables derived within the database will be checked by the statistician, and any anomalies reported to the data team who will resolve together with the TMG if necessary:

1. Consent age will be checked against the age calculated at date of consent, using date of birth (participant dataset)
2. The number of BV episodes in 12 months prior to randomisation ("past 12 months") will be checked against the equivalent categorical variable (0, 1-3, >3 episodes. Both variables are in the participant dataset).

Date checks (anomalies to be reported to the data team; and statistical programming to be added if needed):

1. Completion date to be compared for all datasets from the questionnaires to see if these are the same (if different, to check that most relevant ones are used).
2. The following dates to be checked to ensure they are in the correct order: date of birth, consent, randomisation, baseline, treatment start, questionnaire, week 2 questionnaire, 3 month questionnaire, recurrence (new episodes), 6 month questionnaire. Resolution (clearance) dates on all 3 questionnaires must be appropriate to questionnaire and start dates.

3. If 3 and 6 month questionnaire responses indicate that BV resolved with additional treatment, then check that additional treatment was recorded, and vice versa.

Variable(s)	Derivation
Age (at randomisation) (years)	The integer part of (randomisation date in days – date of birth in days)/365.25
Length of time with current / most recent sexual partner (months)	Time = (years x 12) + months + (weeks x 12/52) + (days x 12/365.25) If any fields are missing then assumed zero, unless all missing in which case this length of time is set to missing.
Approximate time since most recent sexual intercourse (days)	Time = (years x 365.25) + (months x 365.25/12) + (weeks x 7) + (days) If any fields are missing then assumed zero, unless all missing in which case this time is set to missing.
Treatment adherence	• Did participant take/use any of randomised treatment? = yes if have ticked any of the 'yes' boxes to say have taken/used treatment; no if have ticked 'no' for all of them, and missing if there are not any 'yes' and not all 'no' boxes are ticked. • Was trial treatment completed? (yes/no) This is a participant response, and will NOT be amended to reflect their other responses eg only a few occasions ticked, but ticked 'yes' to treatment completed. • Percentage of course used. For metronidazole = 100*(number of doses)/14. For lactic acid = 100*(number of doses)/7. Summary statistics will be presented along with the number and proportion with at least 85% adherence to treatment. • Time from randomisation to start of treatment = (treatment start date in days)-(randomisation date in days). If the date given for starting treatment is before randomisation, or after the date of questionnaire completion, the treatment start will be taken to be the same as randomisation date. Any such changes made will be documented in the tables. • Reasons why treatment course is not completed will be categorised by the unblinded members of the TMG with wording for the categories agreed by the CI (in unblinded fashion), and tabulated.
Resolution of BV symptoms (clearance) at 2 weeks	Percentage resolved = (number with resolution ("cleared")/total number with response to question about resolution) • Calculated for questionnaire responses and telephone responses separately and combined. • If yes/no is missing, but date of resolution (clearance) is provided, then this will be assumed as resolved. • If resolution (yes/no) and date are both missing on the week 2 questionnaire and the primary outcome is also not available from a telephone call, but it is available on the 3 month questionnaire

<table>
<tr><td></td><td>then the 3 month response will be used
• Hence, the order of priority for obtaining the primary outcome is: 1. Yes/no on week 2 questionnaire 2. Date of resolution on week 2 questionnaire 3. Telephone call 4. Result from 3 month questionnaire.</td></tr>
<tr><td>Time to first recurrence of BV</td><td>Number experiencing a recurrence = number with a new episode (either ‘yes’ to new episode or number of new episodes >0 on 3 month or 6 month questionnaires) after first resolving by week 2.
• If it is not clear whether they have first resolved, then recurrence is missing.
Time to first recurrence = time of first new episode THAT COUNTS AS RECURRENCE (at either 3 or 6 months) minus time of resolution (for those resolving before week 2)
• set to missing if date of first episode is before date of resolution</td></tr>
<tr><td>Number of new episodes of BV</td><td>• Add number of episodes from 3 and 6 month questionnaires
• For those who have resolution AND have 3 month AND 6 month data (complete case), OR just resolution and 3 month data only (a separate variable – episodes up to 3 months).
• Episodes will not be counted where there is no resolution at 2 weeks.
• Number of episodes will be missing if just have resolution and 6 month data as number of episodes at 3 months is unknown.</td></tr>
<tr><td>Number of courses of additional medications for BV.</td><td>These are added up for each of the additional medications (including ‘other’), from all three questionnaires
• Calculated for week 2 only, week 2 & 3 month, all three questionnaires
• When number of courses is considered to be impossibly high the changes below will be followed:

<table>
<tr><td>Questionnaire</td><td>Given courses</td><td>Replace by</td></tr>
<tr><td>Week 2</td><td>0-2</td><td>No change</td></tr>
<tr><td></td><td>3-7</td><td>1</td></tr>
<tr><td></td><td>>=8</td><td>2</td></tr>
<tr><td>Months 3 & 6</td><td>0-9</td><td>No change</td></tr>
<tr><td></td><td>10-14</td><td>2 courses</td></tr>
<tr><td></td><td>15-21</td><td>3 courses</td></tr>
<tr><td></td><td>22-28</td><td>4 courses</td></tr>
<tr><td></td><td>29-30</td><td>5 courses</td></tr>
</table></td></tr>
<tr><td>Side effects (adverse events)</td><td>• Presence of side effect/adverse event:
o If =”no” or missing, but details given, then assume a “yes”
o If = “yes” but no details still include, eg if details (severity etc) are missing.
• Duration of side effect (in hours): = (24*days) + hours (either or both fields may be completed).
• Where duration is given in days, but also 24 hours, this is</td></tr>
</table>

	assumed to mean x days 24hrs a day, so the 24 hours will not be added.
Conception date	This will be estimated using the following in order of preference, depending on available data • 38 weeks before expected date of delivery (if given) • 38 weeks before live born healthy baby (outcome date) • 4 weeks before date pregnancy confirmed (very approximate) • Using dates of questionnaire completion, and whether participant had answered 'yes' or 'no' to pregnancy.
Questionnaire completion	For questionnaire completion table, completion defined as entering at least one item of data in the entire questionnaire.
General	Where 'no' or missing is given, but there are details present (eg for adverse events, STIs, additional medications), a 'yes' will be assumed. This does not apply to taking a complete course of trial treatment.

4.5. Procedures for missing data

The analysis covariates in this trial are those variables used for minimisation (female partners in 12 months before baseline, episodes of BV in 12 months before baseline, site) except site type (there was only one GP site), plus vaginal douching (yes/no). The only missing value amongst these covariates is for douching for one participant, which will be substituted by 'No', the much more frequent response.

Primary outcome (resolution of symptoms at week 2): sensitivity analyses will include the following imputations for missing primary outcome data: missing substituted by resolved, missing substituted by not resolved, and multiple imputation. The multiple imputation will use chained equations, with the following covariates: female partners in 12 months before baseline, episodes of BV in 12 months before baseline, site, vaginal douching, plus any other variables that are imbalanced between allocated groups at baseline (visual inspection).
For secondary outcomes, imputation will only happen as detailed in the table above (4.4) i.e. where data are missing, but can be assumed as 'yes' or 'no' from other data.

For laboratory data results 'I' (indeterminate), 'E' (equivocal), or 'NI' (not interpretable) are treated as missing.

See section 4.4 (derived variables) for situations where missing values can be assumed from other variables.

5. DESCRIPTION OF PARTICIPANT CHARACTERISTICS

5.1. Participant flow

The flow of participants through the study will be shown in a CONSORT flow chart. This will include the number of participants approached, the number who did not participate (reasons will be tabulated), numbers consented, numbers excluded/ineligible prior to randomisation but post-consent (by major reason and overall), the number of participants randomised to each of the two treatment groups, the number not receiving allocated treatment, number withdrawn post-randomisation, and the numbers with primary outcome data (Week 2) and with at least somedatareturning the questionnaires at 3 and 6 months. The number and percentage of post-randomisation withdrawals by treatment group and overall and by major reason will be summarised in a separate table.

5.2. Baseline characteristics

Continuous variables will be summarised in terms of the mean, standard deviation, median, lower and upper quartiles, minimum, maximum and number of observations, as appropriate for normally distributed or skewed data. Categorical variables will be summarised in terms of frequency counts and percentages. Summaries will be by allocated treatment group, and overall at baseline. Baseline characteristics include demographics (age, ethnicity), relevant medical history (including BV, contraception), sexual history, vaginal douching, sexually transmitted diseases (STIs), BV symptoms, microscopy for BV (central and local).

6. ASSESSMENT OF STUDY QUALITY

6.1. Randomisation

Missing randomisation numbers will be reported in the tables together with reasons.

The number of participants randomised by minimisation variable and treatment group will be tabulated. The minimisation variables are: site, type of site (SHC/GP), female partners in the 12 months before baseline (yes/no), number of episodes of BV in the 12 months before baseline (0, 1-3, >3).

Participants randomised in error (e.g. subsequently found to be not eligible at the time of randomisation) will be indicated with reasons in the CONSORT flow diagram. Any such participants will be analysed as randomised.

6.2. Adherence

Adherence to treatment will be assessed by whether the participant took any of the trial treatment, participant reported treatment course completion, percentage of course received (summary statistics including at least 85% adherence), reasons for not completing treatment course, ease of use of treatment, and time from randomisation to starting treatment. It will also be investigated qualitatively in interviews with a sub-group of participants. See details in sections 2.4 and 4.4.

6.3. Follow-up and withdrawals

The number and percentage of participants completing the primary outcome (resolution of symptoms at Week 2) will be presented for Week 2 questionnaire completion, telephone call completion, and overall completion (i.e. all primary outcome data, whichever source, including from 3 month questionnaire); by treatment group and overall.

The numbers (and percentages) returning each questionnaire will be presented by treatment group and overall, as will the time from randomisation to questionnaire return or to telephone data collection.

Withdrawals will be summarised by treatment group and reason: withdrawal of consent for trial, withdrawal from trial due to AE, lost-to-follow-up, other. Details for 'other' will be given where available. Non-return of questionnaires is counted as missing data not withdrawal.

Questionnaires are available for completion at 14-28 days from randomisation for the week 2 questionnaire, 3-4 months for the 3 month questionnaire, and 6-7 months for the 6 month questionnaire. Questionnaires cannot be completed outside these times without request to the trial team. Not completing a questionnaire will be regarded as missing data rather than loss to follow-up.

Missing primary outcome data were followed up by a telephone call. Not answering a telephone call, to obtain missing data, is not considered as loss to follow-up. Later in the trial missing 6 month recurrence data (recurrence and number of episodes) were followed up by telephone.

6.4. Non-compliance (protocol deviations)

Recorded incidences of non-compliance (not routinely documented in this trial, and expected to be few because of the trial design) will be listed and tabulated by treatment group, using the following categories: Inclusion / Exclusion Criteria, Informed Consent, Treatment Randomisation Error, Wrong value of minimisation variable entered onto randomisation system, Did not receive randomised treatment, Other.

Participants not receiving their randomised treatment will be included in the primary analysis as randomised, but a sensitivity analysis will include them as treatment received (apart from those not receiving any treatment who will not be included). Where a participant is assigned an incorrect value for one of the minimisation variables (identified in the protocol deviation dataset), it is not possible to change the value of the variable once the participant has been randomised, and if the variable is not collected anywhere else the baseline value will be incorrect. As this is unlikely to occur often, and is equally likely to occur in either trial arm, data will be analysed as in the database.

7. ANALYSIS OF EFFECTIVENESS

Analyses will be reported using 2-sided 95% confidence intervals. Secondary outcomes will be considered supportive to the primary analysis.

7.1. Primary analysis

Numbers (and percentages) resolved (cleared) at week 2 will be presented by treatment group. The analysis will include data obtained from both questionnaires and telephone calls.

The risk difference and risk ratio will be used to compare the difference in resolution rate at week 2 between treatment groups using a mixed effects model for binary outcome including minimisation variables as covariates (site, female partners in the 12 months before baseline, number of episodes in the 12 months before baseline; as there is only one GP site, site type will not be included as covariate) and vaginal douching. Site will be declared a panel variable within a generalised estimating equation model.

If the proposed analysis model does not converge, the odds ratio will be calculated using a mixed effects logit model with the same covariates.

7.2. Sensitivity analysis of primary outcome

Sensitivity analyses for missing primary outcome are given in section 4.5 (procedures for missing data). Risk differences and 95% confidence intervals will be presented.

If any prognostic variables are imbalanced at baseline (between treatment groups, by visual inspection), further adjustment will be made for these variables in a sensitivity analysis.

A forest plot will be used to present these different analyses.

7.3. Secondary analysis of primary outcome

Numbers (and percentages) resolving, using questionnaire data and telephone data separately, will be summarised by treatment group.

Resolution will be further reported by trial arm and use of additional medication post-randomisation.

7.4. Subgroup analysis of primary outcome

The primary analysis for symptom resolution will be further investigated, irrespective of the primary analysis results, to determine whether treatment effectiveness differs according to the following sub-groups as specified in the protocol:

a) Presence of concomitant STI (yes/no)
b) BV confirmed by positive microscopy (yes=central lab grade 3 Ison-Hay` /no)

The following sub-group analysis was also planned:

c) Type of centre participant presented at (sexual health clinic vs GP/other clinics). However as there was only one non-SHC centre this analysis will not be conducted.

In addition, the following subgroups not specified in the protocol will be conducted:

d) Number of episodes of BV in 12 months before baseline (0, 1-3, >3)

e) Total time with BV in 12 months before baseline (<2weeks, >=2 weeks & <3 months, >=3 months)

Data will be summarised by treatment group and subgroup.
Between-group treatment effects will be provided for each subgroup, but interpretation of any subgroup effects will be based on the treatment-subgroup interaction and 95% confidence interval, estimated by fitting an appropriate interaction term in the regression models. Since the trial is powered to detect overall differences between the groups rather than interactions of this kind, these subgroup analyses will be regarded as exploratory.

Resolution of BV symptoms at week 2, split by additional treatment (with or without) will be summarised by proportions, unadjusted differences and 95% confidence intervals. Additional treatment was taken post-baseline hence this is not treated as a formal subgroup analysis.

7.5. Secondary outcomes

All outcomes will be summarised by treatment group as appropriate: continuous variables will be summarised in terms of the mean, standard deviation, median, lower and upper quartiles, minimum, maximum and number of observations, as appropriate for normally distributed or skewed data. Categorical variables will be summarised in terms of frequency counts and percentages.

Numbers of episodes, for those resolving with 2 weeks, will be tabulated by allocated treatment group for within 3 months, and within 6 months (to include those within 3 months). The median time to first recurrence (first new episode) with 95% confidence interval will be presented for each treatment group; data will also be presented in a Kaplan Meier plot. Where time is not known (either not occurred or data missing) the time will be censored at the last time for which the status is known. Treatment groups will not be formally compared because the starting point is resolution, so the comparison would not be between randomised groups.
Median times from resolution to first recurrence of symptoms- (and 95% confidence intervals) will also be presented by additional medication and treatment group, without formal comparison.

The following post baseline data will be summarised and compared, using regression methods with minimisation variables and douching as covariates, between allocated treatment groups:

- Number of new episodes (for those who resolve within 2 weeks).
- Number of additional (participant reported) treatment courses for BV over 6 months; also up to 3 months, and 3-6 months separately.
- Microbiological resolution: negative for BV at week 2 in those positive for BV at baseline.
- STIs at week 2 (positive/ negative/ indeterminate/ equivocal), allowing for baseline presence of STIs. Summaries will also be made for each STI separately (gonorrhoea, chlamydia, trichomoniasis), but separate STIs will not be analysed.
- Time to resolution of symptoms (at any time in the 6 months), numbers resolved by 2 weeks, 3 months, 6 months will be presented for those taking additional treatment, and those not taking additional treatment, as well as both with and without additional treatment (together). Where resolution is not known (either not occurred or data missing) the time will censored at the last time for which the status is known. Cox regression will be used for the comparison between treatment groups of median time to resolution of BV symptoms. Kaplan Meier plots by treatment, and also by additional treatment if numbers allow.

7.6. Other outcomes

These data will be summarised by allocated treatment group:

- Time with BV recurrence after resolution of symptoms (<1 week, 1-2 weeks, >2-4 weeks, >4 weeks); recurrences up to 3 months, 3-6 months.
- Status of symptoms (better, but not cleared/ improved initially, but worsened again/no change/worse) for those without resolution of BV at week 2 (participant reported.
- Symptom assessment at week 2 (yes/no for each of genital discharge, offensive vaginal smell and vaginal irritation).
- Recurrence symptoms compared to typical symptoms (2 weeks – 3 months, 3-6 months). Were symptoms typical of usual symptoms, with categories: always, sometimes, seldom.
- Additional medication for BV: in first 2 weeks, 2 weeks – 3 months, 3-6 months, with numbers taking metronidazole tablets, metronidazole gel, lactic acid gel, clindamycin cream, other treatments.
- Antibiotics for other conditions/ illness (over 6 months: 2 weeks – 3 months, 3-6 months): numbers taking amoxicillin, flucloxacillin, doxycycline, other treatments.
- Vaginal thrush post randomisation: occurrence yes/no and number of episodes within 2 weeks, 2 weeks – 3 months, 3-6 months; treatments: clotrimazole, fluconazole, itraconazole, other.
- Sexual contact, for each questionnaire: yes/no, use of condoms, new sexual partners; 2 week questionnaire only: summary of time to having sex from start of treatment.
- Vaginal douching post randomisation (yes/no) for each of 0-2 weeks, 2 weeks-3 months, 3-6 months.
- Participant reported STIs diagnosed from 2 weeks post-baseline (week 2- 3 months and 3-6 months): number of episodes for gonorrhoea, chlamydia, trichomoniasis, pelvic inflammatory disease.
- Other reasons why participants did not take week 2 samples (using the categories: had period, travelling abroad, misunderstood instructions, unknown).

8. ANALYSIS OF SAFETY

8.1. Adverse events

In order to provide secondary outcome data to compare tolerability of the two treatments, only specified adverse events experienced during treatment with either lactic acid gel or metronidazole will be reported. The following are regarded as expected for the purpose of this trial and will be reported on the week 2 follow-up questionnaire completed by the participant:

- Nausea
- Vomiting
- Taste changes
- Vaginal irritation (e.g. redness, stinging, itching, swelling, burning)
- Abdominal pain
- Diarrhoea

Presence, time from starting treatment, severity, duration, and resolution, plus severity for those not resolving, of each of these adverse events will be summarised by treatment received. No formal statistical analysis of these data are planned. Vaginal irritation will also be summarised for those without baseline vaginal irritation.

Serious Adverse Events (SAEs) are not anticipated in this low risk trial, but there is a reporting procedure specified in the protocol. Participant's attendance at an A&E department or unplanned hospital admission relating to their BV or BV treatment since the date of randomisation are prompted in the week 2 questionnaire, and they will (together with the 'other' category) be checked by the CI to determine whether the admission indicated a serious adverse event. Planned hospital admissions are not to be reported as SAEs. If there are any SAEs these will be summarised by the treatment the participant received. Hospital admissions (for BV or BV treatment) will be summarised.

8.2. Pregnancies

Any pregnancies occurring during the trial will be reported according to the information on the non-databased pregnancy notification form and any additional information provided to the NCTU where appropriate. This will include approximate time from randomisation to conception (see section 4.4) and pregnancy outcome.

9. OTHER ANALYSIS

Health economics data (SF-12™ Health Survey and costs) will be analysed by the health economist, with details in a separate health economics analysis plan.

10. EXPLORATORY ANALYSIS

None.

11. FINAL REPORT TABLES AND FIGURES

Dummy tables are appended to this SAP.

12. PUBLICATIONS

1. Anstey Watkins J, Ross JDC, Thandi S, Brittain C, Kai J, Griffiths F. Acceptability of and treatment preferences for recurrent bacterial vaginosis-Topical lactic acid gel or oral metronidazole antibiotic: Qualitative findings from the VITA trial. *PLoS One*. 2019;14(11):e0224964. Published 2019 Nov 15. doi:10.1371/journal.pone.0224964