binx health ltd

binx health *io*® CT/NG Assay

Single-use Cartridge for the qualitative detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Instructions For Use – CLIA-Waived

1.002.101 · MOB-ART-0700 v4.0 · MAY 2022 · English

Rx Only IVD

General Information

A Certificate of Waiver is required to perform this test in a CLIA-waived setting. To obtain a Certificate of Waiver, please contact your state health department.

Failure to follow the manufacturer's instructions, or changing the instructions, will mean that the test will not meet the requirements for CLIA waiver (42 CFR 493.15(e)(1)).

The binx *io* CT/NG Assay must be run using the binx *io* Instrument positioned on a level surface between 10-35°C, 0-80% relative humidity.

Intended Use

The binx health *io* CT/NG Assay, when tested using the binx health *io* Instrument, is a fully automated, rapid, qualitative test intended for use in point-of-care or clinical laboratory settings for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA by polymerase chain reaction. The binx health *io* CT/NG Assay is intended for use with female vaginal swab specimens, collected either by a clinician or self-collected by a patient in a clinical setting, or male urine specimens, as an aid in the diagnosis of symptomatic or asymptomatic *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* infection. For a symptomatic male patient with a chlamydia negative test result, further testing with a laboratory-based molecular test is recommended.

CLIA Waiver

A Certificate of Waiver is required to perform this test in a CLIA waived setting. Failure to follow the manufacturer's instructions, or changing the instructions, will mean that this test will not meet the requirements for CLIA waiver. Users of this test should refer to the Quick Reference Guide. You can enroll your laboratory in the CLIA program by completing an application (Form CMS-116) available on the CMS CLIA website (https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/index?redirect=/CLIA/) or from your local State Agency. Send your completed application to the address of the local State Agency for the State in which your laboratory is located. Additionally, check with your State Agency for any other state-specific requirements. If you do not have online access and do not have information about your State Agency, you may contact the CLIA program at 410-786-3531 for the address and phone number of your State Agency.

Summary and Explanation of the Assay

Chlamydia trachomatis (CT) infection is one of the most common Sexually Transmitted Infections (STI) globally [1]. Many women infected with CT are asymptomatic (without symptoms). If left untreated, this infection can cause Pelvic Inflammatory Disease (PID) in women, which can lead to complications including infertility, chronic pelvic pain and ectopic pregnancy. In men, if untreated, CT can cause urethritis or epididymitis. Many infected people are asymptomatic (without symptoms) [2, 3].

The CT genomic DNA target detected in the binx health *io* CT/NG Assay is in the genome sequence of *C. trachomatis.* There are currently 20 known serovars of CT (A, B, Ba, C, D, D', E, F, G, H, I, Ia, J, K, L1, L2, L2b, L3, nvCT and FI-nvCT). The Assay has been evaluated against a panel of 16 serovars, including the new or Swedish variant (nvCT).

Neisseria gonorrhoeae (NG) is a Gram-negative diplococcus and is the causative agent of gonorrhea in both males and females. Women with an NG infection is a often asymptomatic [4]. If untreated, this infection can lead to PID, infertility, chronic pelvic pain and ectopic pregnancy. NG infection in males is generally accompanied by symptoms such as penile discharge, pain or a burning sensation when urinating, or inflammation of the foreskin [5].

The *io* CT/NG Assay uses two distinct genetic targets to detect NG, both of which are located in the NG genome. The detection of NG is dependent on both genetic targets being identified in the specimen. This Assay has been evaluated against a panel of 32 different strains of NG, including strains from WHO and other characterized strains, geographically diverse clinical isolates and strains with known mutations.

Principles of the Procedure

The binx health *io* CT/NG Assay comprises an assay-specific single-use Cartridge containing all the components required to perform the *io* CT/NG Assay, which is processed on the binx health *io* Instrument. Following loading of the specimen on to the Cartridge and insertion of the Cartridge into the Instrument, the user follows a few simple on-screen prompts to start the Assay. The Assay process is then carried out under complete control of the Instrument, requiring no further user interaction until the *io* CT/NG Assay is complete.

The Assay follows three sequential processes that take place inside the Cartridge:

1. Cells in the specimen are lysed to release the DNA. This DNA is extracted and purified to remove any cellular debris and other unwanted materials;
2. Primers specific to *Chlamydia trachomatis* and two distinct *Neisseria gonorrhoeae* targets are used to amplify sections of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA using the Polymerase Chain Reaction (PCR); and
3. Complementary and specific electrochemically-labeled DNA probes hybridize to the amplified DNA. Any double-stranded DNA-probe complexes are digested by an enzyme, which releases the electrochemical label. A voltage is applied to a carbon electrode, oxidizing any released electrochemical label. This discharge of electrons generates a current which is measured to indicate the presence of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* DNA. As the enzyme only possesses double-stranded DNA activity, when there is no amplified DNA present, as in negative specimens, the probes remain as single-stranded DNA and are not digested, the electrochemical label is not released, and no current is measured.

An Internal Process Control (IPC) is present on the Cartridge which undergoes the same process steps as the specimen. The detection of the IPC at the end of an Assay when the DNA target has not been detected verifies that the Assay has functioned correctly. If the IPC is not detected within an acceptable range the Assay will be defined as invalid and no result will be recorded. If the DNA target is detected, the IPC will be disregarded as the detection of the DNA target verifies that the Assay has functioned correctly.

Reagents and Materials

Materials Provided

binx health *io* CT/NG Single Use Assay Kit contains:

- One (1) *io* CT/NG Cartridge
- One (1) Sample Transfer Pipet
- One (1) desiccant pouch

The Instructions For Use (IFU), this document, is supplied with each carton of ten Single Use Assay kits.

The *io* CT/NG Cartridge contains the following reagents:

- 1.4 mL DNA extraction solution *(contains >50% guanidine thiocyanate)*
- 0.7 mL wash solution *(contains >70% ethanol)*
- 0.405 mL elution solution
- Stabilized dry deposition of IPC *(contains bacterial genomic DNA)*
- Stabilized dry depositions of reagents for the amplification of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and IPC DNA *(contains recombinant enzyme from a non-infectious bacterial source)*
- Stabilized dry depositions of reagents for the detection of amplified *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and IPC DNA *(contains recombinant enzyme from a non-infectious bacterial source)*

Materials Required But Not Provided

binx health *io* Instrument (Cat. No. 3.001.001)

binx health *io* Vaginal Swab Specimen Collection Kit, US Version (Cat. No. 5.012.001)

binx health *io* Male Urine Specimen Collection Kit, US Version (Cat 5.002.001)

ZeptoMetrix NATtrol™ CT External Run Controls (Cat No. NATCT(434)-6MC) as a CT-positive, NG-negative control

ZeptoMetrix NATtrol™ NG External Run Controls (Cat No. NATNG-6MC) as an NG-positive, CT-negative control

Warnings and Precautions

1. For *in vitro* diagnostic use.
2. This Assay is for use only with female vaginal swabs collected using the binx health Vaginal Swab Specimen Collection Kit or male urine collected using the binx health Male Urine Specimen Collection Kit.
3. Do not use expired *io* CT/NG Cartridges.
4. Do not freeze *io* CT/NG Cartridges. Freezing Cartridges may cause an incorrect result to be obtained.
5. Do not use if the Cartridge packaging has been damaged, ruptured or opened for an indeterminate period prior to use. Dispose of any unused Cartridges as hazardous waste.
6. Cartridge contains guanidine thiocyanate (H302: harmful if swallowed, H312: harmful for contact with skin, H332: harmful if inhaled, H412: harmful to aquatic organisms; long-term effects, P273: do not disperse in environment).
7. Cartridge contains ethanol (H225: highly flammable liquid and vapor, P210: keep away from heat/sparks/open flames/hot surfaces, no smoking).
8. Specimens and used Cartridges may be infectious. Wear appropriate personal protective equipment (PPE) when handling these. Wash hands thoroughly afterwards. Wear disposable gloves and change gloves between specimens. Keep the bench area clean by wiping with a 10% bleach solution. Wipe all spills and change gloves afterwards. Appropriate precautions for disposal of biohazardous materials should be established in line with local or national policies and practices.
9. Male urine samples should not be frozen. Freezing may degrade the sample and could lead to false negative results.
10. The *io* Instrument should be placed on a level surface. False negative results may be obtained if the *io* Instrument is not on a level surface.
11. Ensure vaginal swab samples are correctly shaken after the sample is collected and again prior to running to ensure all material is eluted from the swab. Inadequate shaking could lead to false negative results.
12. For additional warnings and precautions relating to the use of the *io* Instrument and for cleaning and decontamination procedures, please refer to the binx health *io* Instrument Operator Manual.

Key To Symbols Used On Product Labeling

IVD For *in vitro* diagnostic use	User to consult the Instructions For Use	Store Cartridges between these temperatures (°C)
LOT Manufacturer's lot number	The expiry date in the format YYYY-MM-DD	Warning! Refer to the Instructions For Use
Details of the manufacturer of the device	Cartridges are single use only. Do not attempt to re-use	Rx For Prescription Use Only

binx health *io* CT/NG Cartridges

Storage of Cartridges

binx health *io* CT/NG Cartridges ("Cartridges") and the Sample Transfer Pipet are provided in a sealed foil pouch containing a desiccant sachet.

Cartridges must be stored between 2-8°C. They will not run on the binx health *io* Instrument when the expiration date marked on the labeling has passed. The performance of the *io* CT/NG Assay cannot be guaranteed if stored outside this temperature range. Do not freeze *io* CT/NG Cartridges. Freezing Cartridges may cause an incorrect result to be obtained.

Handling of Cartridges and Specimen Transfer Pipet

Only open the Cartridge pouch when ready to perform an *io* CT/NG Assay. Once opened, a Cartridge must be used within six (6) hours. The specimen can be loaded on to the Cartridge at any time during this six-hour period.

It is recommended the Cartridge is handled by the grooved areas located at each side of the Cartridge and the Sample Transfer Pipet is handled by the bulb to avoid contamination of the tip.

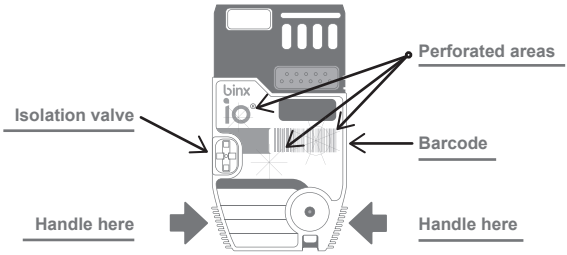

Figure 1. Cartridge Handling

Avoid handling, labeling or writing on the Cartridge label in the area around the barcode. If the barcode is perforated, damaged, defaced or covered, the *io* Instrument will not allow an *io* CT/NG Assay to be performed using this Cartridge.

Do not press in the perforated circular areas of the Cartridge label as the liquid reagent pouches are housed beneath. This could lead to these being compressed, the reagent being released and the Assay not functioning correctly.

Do not damage or attempt to depress the isolation valve. This could lead to the Cartridge not functioning correctly.

binx health *io* Instrument

Please refer to the binx health *io* Instrument Operator Manual for a full description and details of:

- Installation
- Operation
- Performance
- Characteristics
- QC procedures
- Precautions
- Hazards
- Service and maintenance

Specimens

Specimen Collection

binx health *io* Vaginal Swab Specimen Collection Kits (Cat. No. 5.012.001) and binx health *io* Male Urine Specimen Collection Kits (Cat. No. 5.002.001) are not provided with the *io* CT/NG Cartridges and must be purchased separately.

The *io* CT/NG Assay has been validated for use in clinical settings with clinician- and self-collected vaginal swab specimens, and male urine specimens, collected and stored in the above collection kits in accordance with the Instructions for Use provided with the collection kits.

The performance of this Assay with other specimen types and specimen collection devices has not been evaluated. Please refer to the Instructions For Use provided with the binx health *io* Specimen Collection Kits.

Specimen Identification

Once a specimen has been collected the container should be labeled with a unique identifier in accordance with local procedures for specimen traceability and identification.

If a barcode is to be used for specimen identification, a handheld USB barcode scanner may be used (Cat. No. 3.000.020). Alternatively, the specimen identifier can be entered on to the Instrument manually or the Instrument can assign a unique specimen identifier.

Specimen Storage

Following collection of a specimen, the *io* CT/NG Assay must be performed on the specimen within:

- 24 hours if stored at room temperature (2-25°C) after being transferred into the collection medium.
- Seven days if stored refrigerated (2-8°C) after being transferred into the collection medium.

The performance of the Assay with specimens stored outside these temperature ranges and periods has not been evaluated.

Male urine samples should not be frozen. Freezing may degrade the sample and could lead to false negative results.

Specimen Transport

Specimens can be transported ensuring that the temperatures described in the Specimen Storage section are maintained and the combined transport and storage period does not exceed those shown for each temperature range.

Procedure

Procedural Notes

1. Prior to performing an *io* CT/NG Assay on the *io* Instrument you may be required to log in, entering a User ID and password.

   User access policy on the Instrument is defined by your local administrator. In order to access and use the *io* Instrument, the administrator will need to grant users access. Please refer to the *io* Instrument Operator Manual for instructions on how to log in.

2. All components of the Cartridge are **single use only**. The *io* Instrument will recognize attempts to re-use a Cartridge and prevent an Assay being performed.

3. Do not cover, damage or deface the barcode on the Cartridge.

4. When transferring the specimen to the Cartridge, avoid spillage of the specimen on to the Cartridge. This will assist in preventing user or run-to-run contamination. If a specimen is spilled on the Cartridge, carefully remove with clean tissue prior to loading the Cartridge into the Instrument and dispose of the tissue as potentially contaminated waste.

5. Specimen addition must be carried out using the Sample Transfer Pipet provided as this delivers the volume required to perform the Assay.

6. There is no need to allow a Cartridge to equilibrate to ambient temperature prior to use.

7. Avoid leaving a completed Cartridge in the *io* Instrument once the Assay has finished.

Assay Procedure

This procedure provides the principal steps to performing an *io* CT/NG Assay to accompany the on-screen instructions. For a fully detailed procedure, with pictorial guide and screenshots of the *io* Instrument, please refer to the *io* Instrument Operator Manual.

Instrument set-up

1. From the **Main Menu** screen press the **Run/Cancel Test** key on the touchscreen.

2. Enter a unique specimen identification by either (a) entering the Specimen ID manually via the **Specimen ID** box on the touchscreen and enter using the keypad, (b) scanning a barcode on the specimen collection tube using a handheld barcode scanner, or (c) allowing the Instrument to automatically assign a unique Specimen ID. Press **Next.**

3. The administrator may configure the *io* Instrument so that some of these options for entering the Specimen ID are not available.

4. If the administrator has configured the *io* Instrument so that specific patient details are required to be entered, the **Enter Patient Details** screen will appear; if not the *io* Instrument will go to Step 5. To enter patient details, press the relevant box on the touchscreen, enter the details using the keypad, then press **Enter.**

5. The *io* Instrument will prompt the loading of the specimen on to the Cartridge. Please note: the *io* Instrument drawer will open automatically at this prompt screen.

Running an Assay

1. Take a sealed Cartridge pouch, tear open at the nick and retain the Cartridge and Sample Transfer Pipet in the pouch until required.

2. Ensure the work area is clean before removing the Cartridge from the foil pouch and placing on a clean, flat surface. At this point the Cartridge can be labelled with a barcode or patient information can be handwritten on to the Cartridge, if required (Figure 2). Barcodes and any written information should be contained within the space provided.

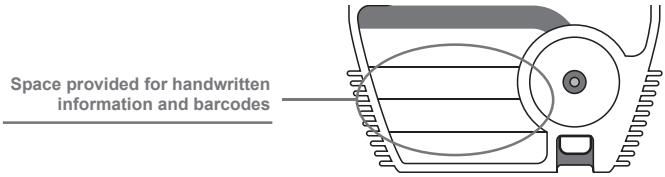

Figure 2. Space provided for Cartridge labeling

3. It is essential to fully mix the specimen prior to use. Inadequate mixing of the specimen prior to transfer of the specimen to the Cartridge may result in false negative results due to uneven distribution of organisms or inefficient release of organisms from the swab.

For vaginal swab specimens:

Hold the transport medium tube by the cap making sure that it is closed tightly. Shake the tube four (4) times downwards with rapid movements of the wrist.

NOTE: Inverting the tube up and down is not recommended. If the sample appears too viscous, the specimen may remain attached to the swab. In these circumstances it is recommended that the shaking time be extended in order to break down any solid material and to release the sample from the swab.

For male urine specimens:

Invert the transport medium tube a minimum of four (4) times to mix the urine and transport medium thoroughly.

If samples are very foamy following mixing, please wait for the foam to disperse before proceeding.

4. Remove the Sample Transfer Pipet from the pouch, holding the bulb. Avoid touching the tip to minimize the risk of contamination.

5. Remove the cap of the specimen collection tube (in the case of vaginal swab specimens, the swab will remain attached to the inside of the cap).

6. Squeeze the upper (larger) bulb of the Sample Transfer Pipet and submerge the tip in the liquid specimen (Figure 3). Keeping the tip submerged, slowly release the bulb to draw the liquid into the Sample Transfer Pipet. Ensure the shaft of the Sample Transfer Pipet is full of liquid. The shaft provides the exact volume required to perform the Assay. Excess specimen volume will flow into the lower overflow bulb and will be retained in the pipet when the sample is expelled.

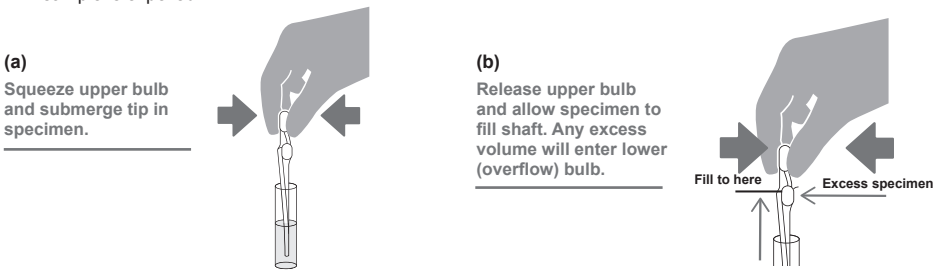

Figure 3. Use of Sample Transfer Pipet

7. Keeping the pipet vertical, insert the tip into the center of the sample port of the Cartridge (Figure 4a). Push the pipet into the sample port until the tip touches the base of the port. Squeeze the upper bulb gently until all the sample in the shaft is expelled into the Cartridge.

8. Prior to starting the *io* CT/NG Assay confirm that the sample has been loaded correctly by observing the sample fill indicator window (Figure 4b). After the sample has been correctly loaded, the white area will turn black. If the sample fill indicator window does not turn black, discard the Cartridge and re-load the sample onto a new Cartridge.

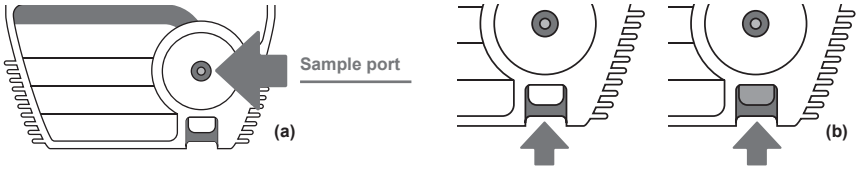

Figure 4. Sample fill indicator window

If you are unsure of correct sample loading after the first attempt, please note the *io* Cartridge can accept a second sample volume without impacting function or the Assay result. However, if you believe you have attempted to load the sample on to the Cartridge twice **DO NOT ATTEMPT TO LOAD A SAMPLE ONTO THE CARTRIDGE A THIRD TIME.** Discard this Cartridge and start the Assay procedure again with a new Cartridge.

Once you are satisfied the sample has been loaded correctly, either press the **Next** button on the touchscreen or proceed directly to Step 9.

9. Insert the Cartridge into the drawer, ensuring the Cartridge is seated correctly in the recess and slowly push the drawer closed.
10. The *io* Instrument will read the barcode on the front of the Cartridge and recognize the Assay type. The details of the Assay type about to be run including Specimen ID and any patient information will be displayed. Confirm this information is correct and press the **Run Test** button.
11. The Test Running screen will appear and remain while the Assay is in progress (about 30 minutes).
12. Following completion of the Assay the screen will update to a Test Complete summary and the Instrument drawer will open.
13. Dispose of any unwanted specimen, the Sample Transfer Pipet and the used Cartridge. These should be disposed of as biohazardous materials in accordance with local or national policies and practices.
14. The Assay result can be viewed immediately by pressing the **View Results** button on the touchscreen.
15. To immediately print or save this Assay result, select **Print,** then either **Print Hard Copy** or **Print to File** to save to a USB storage device which will require an optional USB printer, or a USB storage device connected to a USB port at the rear of the Instrument.

Retrieval of *io* CT/NG Assay Results

When logged in with a user account, only *io* CT/NG Assay results generated by that user are visible. When logged in as an administrator, Assay results generated by all users are visible.

1. From the Main Menu screen press either the **My Results** button (from a user account) or **View Data** button (from an administrator account) on the touchscreen.
2. The Available Test Results screen appears. Use the scroll buttons to find the Assay result you are searching for.

   The Assay results can be sorted in ascending or descending order by any of the column headings. Press the heading of the column you wish to sort by until the arrow points upwards (for ascending) or downwards (for descending).

   Alternatively, press the **Search** button on the touchscreen. The Search Test Results screen will appear. A search can be performed on one or multiple fields by pressing the appropriate search field box and either entering the search criteria using the keypad or by selecting from the available options in the dropdown box. Once the search criteria have been entered press the **Search** button.
3. Assay results will be displayed with the Specimen ID and Assay type. To maintain patient confidentiality, the Patient ID (if one has been entered) will not be displayed on the same screen as the Assay result.
4. To print or save this Assay result, select **Print**, then either **Print Hard Copy** or **Print to File** to save to a USB storage device. You will need the optional USB printer, or a USB storage device (both not included) connected to a USB port at the rear of the Instrument to do so.
5. Select **Exit** to return to the Main Menu screen.

Table 1.
Interpretation of Assay Results

io CT/NG Assay Result	Explanation of the Assay Results
CT Not Detected	*Chlamydia trachomatis* target DNA was **not detected** in the patient specimen. The IPC passed specification. This is a valid result.
CT Detected	*Chlamydia trachomatis* target DNA **was detected** in the patient specimen. This is a valid result.
NG Not Detected	At least one of the DNA targets for *Neisseria gonorrhoeae* was **not detected** in the patient specimen. The IPC passed specification. This is a valid result.
NG Detected	Both DNA targets for *Neisseria gonorrhoeae* **were detected** in the patient specimen. This is a valid result.
Test Invalid	The presence or absence of the DNA targets for *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* **could not be ascertained** in the patient specimen. If a CT or NG Not Detected result is obtained, and the IPC was outside the acceptable range, Test Invalid indicates a failure in the Assay process. The Assay should be repeated using the same patient specimen.
User Aborted	A user cancelled the Assay. No result is given.
Error	An internal fault occurred that terminated the Assay before it finished.

In the event of a **Test Invalid** result the specimen should be re-tested. A specimen can be re-tested up to three (3) times.

If repeated invalid results are obtained, the specimen may be incompatible with the Assay or the Instrument may have developed a fault. In these cases, contact binx technical support or their representative.

If an **Error** occurs, the screen instructs the user to shut down the Instrument and to restart and repeat the test or to contact binx technical support. An error code is reported at the bottom of the screen and should be provided to technical support.

Limitations of the *io* CT/NG Assay

- The *io* CT/NG Assay has only been validated for use with vaginal swab specimens collected by clinicians or self-collected in a clinical setting, collected and stored using the binx health *io* Vaginal Swab Specimen Collection Kit or male urine specimens, collected and stored using the binx health Male Urine Specimen Collection Kit. The use of other specimen types and/or collection devices has not been evaluated; therefore, Assay performance and specimen integrity cannot be guaranteed.
- Male urine samples should not be frozen. Freezing may degrade the sample and could lead to false negative results.
- Reliable results are dependent on adequate specimen collection, transport, storage, and processing. Failure to follow proper procedures in any one of these steps can lead to incorrect results.
- The Assay provides qualitative information regarding the presence or absence of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* DNA in a patient specimen. Results provided by this Assay should be considered in conjunction with other clinical and laboratory data available to the clinician to ascertain the presence or extent of a *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* infection.

- As with other nucleic acid-based assays, DNA from non-viable *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* organisms and free *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* DNA can be detected by this Assay. In instances where a patient has received therapeutic treatment a CT and/or NG Detected result cannot be interpreted as an indication of the presence of viable *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* organisms.
- A negative test result does not preclude the possibility of infection.
- False positive results may arise if the specimen added to the Cartridge has been contaminated with either a *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* positive specimen or amplified *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* DNA from a previous positive Assay.
- *Neisseria sicca* and Herpes Simplex virus 2 may be cross-reactive with *Chlamydia trachomatis* and *Corynebacterium xerosis* may be cross-reactive with *Neisseria gonorrhoeae.*
- The presence of leukocytes at a concentration of 1 x 10^6 cells/mL in male urine specimens may produce a false negative result for *Neisseria gonorrhoeae*.
- Inadequate mixing of the specimen prior to transfer of the specimen to the Cartridge may result in false negative results due to uneven distribution of organisms or inefficient release of organisms from the swab.
- A confirmatory assay, using an alternative method, should be used in cases where it is suspected the reporting of a positive result may have a severe adverse psychological effect on the patient.
- The performance of the Assay was not evaluated in female patients younger than 16 years of age or male patients younger than 17 years of age.

Quality Control

An internal process control (IPC) which verifies all aspects of the *io* CT/NG Assay process have functioned as expected, is performed automatically on every *io* Cartridge, requiring no additional user input. In an Assay where CT and/or NG is not detected the IPC is measured by the *io* Instrument to ensure it is within an acceptable range. If it is outside the acceptable range the *io* Instrument will return a **Test Invalid** message and no result will be displayed or recorded against that specimen. If it is within the acceptable range the **CT Not Detected** and/or **NG Not Detected** result will be displayed and recorded by the *io* Instrument. The IPC result will not be displayed, but the return of a **CT** and/or **NG Not Detected** result is conditional on the IPC measurement being within the acceptable range. Where CT and/or NG DNA is detected, the IPC will be disregarded as the detection of the CT and/or NG DNA target will verify that the Assay has functioned correctly. The **CT Detected** and/or **NG Detected** result will be displayed and recorded by the Instrument.

External quality control materials are not provided with the test and need to be ordered separately. Recommended external quality control samples are manufactured by ZeptoMetrix Corp., Buffalo, NY. Two controls are available: a CT positive control (Cat. No. NATCT(434)-6MC) which also acts as a NG negative control and a NG positive control (Cat. No. NATNG-6MC) which also acts as a CT negative control. Controls should be shaken vigorously for five seconds before use. A control can be tested with an *io* CT/NG Cartridge as if it were a patient sample, using the Sample Transfer Pipet. Controls must be run whenever a new User is introduced to the testing process or whenever a new lot of *io* CT/NG Cartridges is received. If the controls do not perform as expected, please contact binx on 1-844-692-4691 or support@mybinxhealth.com. Please refer to the *io* Instrument Operator Manual for performing quality control tests.

Expected Values

The positivity rates of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in patient populations is variable and dependent on factors such as age, gender, the presence or absence of symptoms, behavioural and other risk factors, and can vary between different clinic types and by geographical region.

The positivity rates of the binx *io* CT/NG Assay observed during the clinical study were 9.0% and 3.1% for CT and NG, respectively, in vaginal swab specimens and 12.7% and 7.8% for CT and NG, respectively, in male urine specimens. The positivity rates obtained at each site during the clinical study are shown in Table 2a for CT and Table 2b for NG.

Table 2a.

Positivity rates obtained at each collection site for the binx health *io* CT/NG Assay for *C. trachomatis* with female vaginal swab and male urine specimens

Site	Female			Male		
	N	No. of binx positive	Positivity	N	No. of binx positive	Positivity
1	74	5	6.8%	89	22	24.7%
2	39	2	5.1%	125	11	8.8%
3	85	16	18.8%	258	38	14.7%
4	1053	99	9.4%	127	19	14.7%
5	73	3	4.1%	33	4	12.1%
6	154	11	7.1%	59	9	15.3%
7	14	0	0.0%	9	0	0.0%
8	9	1	11.1%	N/A	N/A	N/A
9	22	0	0.0%	29	1	3.4%
10	N/A	N/A	N/A	12	2	16.7%
11	N/A	N/A	N/A	181	11	6.1%
TOTAL	**1523**	**137**	**9.0%**	**922**	**117**	**12.7%**

Table 2b.

Positivity rates obtained at each collection site for the binx health *io* CT/NG Assay for *N. gonorrhoeae* for female vaginal swab and male urine specimens

Site	Female			Male		
	N	No. of binx positive	Positivity	N	No. of binx positive	Positivity
1	74	8	10.8%	89	16	18.0%
2	39	1	2.6%	125	12	9.6%
3	85	0	0.0%	258	18	7.0%
4	1053	26	2.5%	127	6	4.7%
5	73	1	1.4%	33	1	3.0%
6	154	9	5.8%	59	7	11.9%
7	14	2	14.3%	9	2	22.2%
8	9	0	0.0%	N/A	N/A	N/A
9	22	0	0.0%	29	1	3.4%
10	N/A	N/A	N/A	12	0	0.0%
11	N/A	N/A	N/A	181	9	5.0%
TOTAL	**1523**	**47**	**3.1%**	**922**	**72**	**7.8%**

Positive and Negative Predictive Values for Hypothetical Prevalence Rates

The sensitivity and specificity of the binx health *io* CT/NG Assay was used to calculate the positive predictive values (PPV) and negative predictive values (NPV) with vaginal swab specimens (Table 3a) and male urine specimens (Table 3b) at a range of hypothetical prevalence rates.

Table 3a.

Hypothetical PPV and NPV for the binx *io* CT/NG Assay with vaginal swab specimens

	***Chlamydia trachomatis* (CT)**				***Neisseria gonorrhoeae* (NG)**			
Prevalence %	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**
1	96.1%	99.1%	51.9%	100.0%	100.0%	99.9%	91.0%	100.0%
5	96.1%	99.1%	84.9%	99.8%	100.0%	99.9%	98.1%	100.0%
10	96.1%	99.1%	92.2%	99.6%	100.0%	99.9%	99.1%	100.0%
15	96.1%	99.1%	95.0%	99.3%	100.0%	99.9%	99.4%	100.0%
20	96.1%	99.1%	96.4%	99.0%	100.0%	99.9%	99.6%	100.0%
25	96.1%	99.1%	97.3%	98.7%	100.0%	99.9%	99.7%	100.0%

Table 3b.

Hypothetical PPV and NPV for the binx *io* CT/NG Assay with male urine specimens

	***Chlamydia trachomatis* (CT)**				***Neisseria gonorrhoeae* (NG)**			
Prevalence	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
1%	92.5%	99.3%	57.2%	99.9%	97.3%	100.0%	100.0%	100.0%
5%	92.5%	99.3%	87.4%	99.6%	97.3%	100.0%	100.0%	99.9%
10%	92.5%	99.3%	93.6%	99.2%	97.3%	100.0%	100.0%	99.7%
15%	92.5%	99.3%	95.9%	98.7%	97.3%	100.0%	100.0%	99.5%
20%	92.5%	99.3%	97.1%	98.1%	97.3%	100.0%	100.0%	99.3%
25%	92.5%	99.3%	97.8%	97.5%	97.3%	100.0%	100.0%	99.1%

Performance Characteristics of the *io* CT/NG Assay

Clinical Performance

Clinical Study

The clinical performance characteristics of the binx health *io* CT/NG Assay were determined in a prospective multi-site study by comparing the *io* CT/NG Assay results to a Composite Infected Status (CIS) derived from an algorithm based on the results of three currently commercially available CT/NG nucleic acid amplification tests (NAATs) using clinician-collected vaginal swab and male urine specimens.

Vaginal swab specimens were prospectively collected from consenting participants across nine clinical sites (comprising 11 collection sites) and male urine specimens were prospectively collected from consenting participants across 10 collection sites in the United States. Clinical sites included OB/GYN, sexually transmitted disease clinics, family planning clinics and HIV clinics. Participants included symptomatic and asymptomatic males and females. At each test site, the personnel used to conduct the *io* CT/NG Assay were, in the vast majority (95% overall), point-of-care users (individuals trained in the use of the binx health *io* CT/NG System, but not trained or experienced in general laboratory testing procedures).

For females, a single vaginal swab specimen (either self-collected or clinician collected) was obtained and used to perform the binx *io* CT/NG Assay and three additional clinician-collected vaginal swabs were obtained from each participant to perform the three reference NAATs. All comparator samples were collected by healthcare professionals in accordance with their respective instructions for use, following a randomization protocol regarding the order of collection. For self-collected specimens, the binx vaginal swab sample was collected first and for clinician-collected specimens, the binx vaginal swab sample was collected last. For males, a first-catch urine sample was obtained and aliquoted into four collection kits: one aliquot was used to perform the binx *io* CT/NG Assay and three additional aliquots were used to perform the reference NAATs. A study participant was classified as infected, using the CIS algorithm, if at least two of the three reference NAATs returned a positive result and uninfected if at least two of the three reference NAATs returned a negative result.

There were 2,791 total participants enrolled into the study (1,634 female, 1,157 male) of which 29 were ineligible or withdrew consent and 316 participants were excluded due to deviations to the study protocol. Of the 2,446 specimens for which a CIS could be determined, one female specimen generated a final Test Invalid result after re-test with the *io* CT/NG Assay and had to be excluded from analysis.

A total of 1,523 vaginal swab specimens were fully evaluable. Of these, 736 were self-collected vaginal swabs (SCVS) and 787 were clinician-collected vaginal swabs (CCVS). Of the total number of vaginal specimens collected, 706 were from asymptomatic participants and 817 from symptomatic participants. A total of 922 male urine specimens were fully evaluable. Of the total number of male urine specimens collected, 614 were from asymptomatic participants and 308 were from symptomatic participants.

For females, a total of 129 eligible specimens were classified as infected for CT, of which 62 were symptomatic and 67 were asymptomatic. A total of 1,394 participants were classified as not infected for CT, of which 755 were symptomatic and 639 were asymptomatic. A total of 45 participants were classified as infected for NG, of which 29 were symptomatic and 16 were asymptomatic. A total of 1,478 participants were classified as not infected for NG, of which 788 were symptomatic and 690 were asymptomatic. The median age of participants was 27, ranging from 16 to 74 years.

For males, a total of 120 eligible specimens were classified as infected for CT, of which 60 were symptomatic and 60 were asymptomatic. A total of 802 participants were classified as not infected for CT, of which 248 were symptomatic and 554 were asymptomatic. A total of 74 participants were classified as infected for NG, of which 62 were symptomatic and 12 were asymptomatic. A total of 848 participants were classified as not infected for NG, of which 246 were symptomatic and 602 were asymptomatic. The median age of participants was 28, ranging from 17 to 76 years.

Invalid or Indeterminate Results

In cases where the *io* CT/NG Assay returned a **Test Invalid** result, the patient specimen was retested. The result of the retest was used in the analysis. **A Test Invalid** result was reported in 32 out of the 2,478 Cartridges tested (or 1.29%) in this study. If three successive **Test Invalid** results were reported for a specimen this was recorded as Indeterminate (IND). One final **Test Invalid** result by the *io* CT/NG Assay was recorded in the study and the specimen was excluded from the final data analysis.

If an **Error, Invalid** or **Indeterminate** result was returned from a reference NAAT, the Assay was repeated in accordance with the manufacturers' instructions. The result of the retest was used in the analysis. If the retest returned an **Error, Invalid** or **Indeterminate** result this was recorded as indeterminate. If the results from the other two reference NAATs were concordant, the CIS was determined using these results and included in the clinical performance analysis. If results from the other two reference NAATs were discordant, the CIS could not be determined, and these results are not included in the clinical performance analysis.

Clinical Performance Results: *Chlamydia trachomatis* (CT)

Results from the *io* CT/NG Assay were compared to the CIS for CT for the determination of sensitivity and specificity. Sensitivity and specificity for CT by symptom status are shown in Table 4.

Table 4.
Clinical performance of the binx health *io* CT/NG Assay against CIS for *Chlamydia trachomatis* with female vaginal swab and male urine specimens

	Female							Male						
Symptom Status	Total N	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)	Total N	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)
Asymptomatic	706	65	2	634	5	97.0% (89.8% - 99.2%)	99.2% (98.2% - 99.7%)	614	56	4	549	5	93.3% (84.1% - 97.4%)	99.1% (97.9% - 99.6%)
Symptomatic	817	59	3	747	8	95.2% (86.7% - 98.3%)	98.9% (97.9% - 99.5%)	308	55	5	247	1	91.7% (81.9% - 96.4%)	99.6% (97.8% - 99.9%)
Total	1523	124	5	1381	13	96.1% (91.2% - 98.3%)	99.1% (98.4% - 99.5%)	922	111	9	796	6	92.5% (86.4% - 96.0%)	99.3% (98.4% - 99.7%)

N= Number of specimens TP = True Positive FN = False Negative TN = True Negative FP = False Positive
Confidence Intervals (CI) for sensitivity and specificity: Wilson Score Method

Clinical Performance Results: *Neisseria gonorrhoeae* (NG)

Results from the *io* CT/NG Assay were compared to the CIS for NG for the determination of sensitivity and specificity. Sensitivity and specificity for NG by symptom status are shown in Table 5.

Table 5.
Clinical performance of the binx health *io* CT/NG Assay against CIS for *Neisseria gonorrhoeae* with female vaginal swab and male urine specimens

	Female							Male						
Symptom Status	Total N	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)	Total N	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)
Asymptomatic	706	16	0	689	1	100.0% (80.6% - 100.0%)	99.9% (99.2% - 100.0%)	614	11	1	602	0	91.7% (64.6% - 98.5%)	100.0% (99.4% - 100.0%)
Symptomatic	817	29	0	787	1	100.0% (88.3% - 100.0%)	99.9% (99.3% - 100.0%)	308	61	1	246	0	98.4% (91.4% - 99.7%)	100.0% (98.5% - 100.0%)
Total	1523	45	0	1476	2	100.0% (92.1% - 100.0%)	99.9% (99.5% - 100.0%)	922	72	2	848	0	97.3% (90.7% - 99.3%)	100.0% (99.5% - 100.0%)

N= Number of specimens TP = True Positive FN = False Negative TN = True Negative FP = False Positive
Confidence Intervals (CI) for sensitivity and specificity: Wilson Score Method

The CT Not Infected (NI) or Infected (I) outcome for each combination of the three comparator tests used to form the CIS, along with the *io* CT/NG Assay result, are shown in Table 6a for vaginal swab specimens and Table 6b for male urine specimens.

Table 6a.

Analysis of Composite Infected Status (CIS) for *Chlamydia trachomatis* by symptom status for vaginal swab specimens

CIS	Comparator System			binx *io*	Symptom Status		Total
	NAAT1	NAAT2	NAAT3		Sx	Asx	
NI	-	-	-	-	731	620	1351
NI	-	-	+	-	2	3	5
NI	-	+	-	-	5	7	12
NI	+	-	-	-	0	0	0
NI	-	-	IND	-	2	1	3
NI	-	IND	-	-	1	2	3
NI	IND	-	-	-	6	1	7
NI	-	-	-	+	8	5	13
NI	-	-	+	+	0	0	0
NI	-	+	-	+	0	0	0
NI	+	-	-	+	0	0	0
NI	-	-	IND	+	0	0	0
NI	-	IND	-	+	0	0	0
NI	IND	-	-	+	0	0	0
Total not Infected					**755**	**639**	**1394**
I	+	+	+	+	56	63	119
I	+	+	-	+	0	1	1
I	+	-	+	+	0	1	1
I	-	+	+	+	1	0	1
I	+	+	IND	+	0	0	0
I	+	IND	+	+	1	0	1
I	IND	+	+	+	1	0	1
I	+	+	+	-	1	2	3
I	+	+	-	-	2	0	2
I	+	-	+	-	0	0	0
I	-	+	+	-	0	0	0
I	+	+	IND	-	0	0	0
I	+	IND	+	-	0	0	0
I	IND	+	+	-	0	0	0
Total Infected					**62**	**67**	**129**

CIS = Comparator Infected Status NI = Not Infected I = Infected IND = Indeterminate
Sx = Symptomatic Asx = Asymptomatic

Table 6b.
Analysis of Composite Infected Status (CIS) for *Chlamydia trachomatis* by symptom status for male urine specimens

CIS	Comparator System			binx *io*	Symptom Status		Total
	NAAT1	NAAT2	NAAT3		Sx	Asx	
NI	-	-	-	-	243	542	785
NI	-	-	+	-	0	3	3
NI	-	+	-	-	0	0	0
NI	+	-	-	-	0	1	1
NI	-	-	IND	-	2	1	3
NI	-	IND	-	-	1	0	1
NI	IND	-	-	-	1	2	3
NI	-	-	-	+	1	5	6
NI	-	-	+	+	0	0	0
NI	-	+	-	+	0	0	0
NI	+	-	-	+	0	0	0
NI	-	-	IND	+	0	0	0
NI	-	IND	-	+	0	0	0
NI	IND	-	-	+	0	0	0
NI	-	-	-	IND	0	0	0
NI	-	-	+	IND	0	0	0
NI	-	+	-	IND	0	0	0
NI	+	-	-	IND	0	0	0
NI	-	-	IND	IND	0	0	0
NI	-	IND	-	IND	0	0	0
NI	IND	-	-	IND	0	0	0
Total not Infected					**248**	**554**	**802**
I	+	+	+	+	54	56	110
I	+	+	-	+	0	0	0
I	+	-	+	+	0	0	0
I	-	+	+	+	0	0	0
I	+	+	IND	+	0	0	0
I	+	IND	+	+	0	0	0
I	IND	+	+	+	1	0	1
I	+	+	+	-	3	3	6
I	+	+	-	-	1	0	1
I	+	-	+	-	1	0	1
I	-	+	+	-	0	1	1
I	+	+	IND	-	0	0	0
I	+	IND	+	-	0	0	0
I	IND	+	+	-	0	0	0
I	+	+	+	IND	0	0	0
I	+	+	-	IND	0	0	0
I	+	-	+	IND	0	0	0
I	-	+	+	IND	0	0	0
I	+	+	IND	IND	0	0	0
I	+	IND	+	IND	0	0	0
I	IND	+	+	IND	0	0	0
Total Infected					**60**	**60**	**120**

CIS = Comparator Infected Status NI = Not Infected I = Infected IND = Indeterminate
Sx = Symptomatic Asx = Asymptomatic

The NG Not Infected (NI) or Infected (I) outcome for each combination of the three comparator tests used to form the CIS, along with the *io* CT/NG Assay result, are shown in Table 7a for vaginal swab specimens and Table 7b for male urine specimens.

Table 7a.

Analysis of Composite Infected Status (CIS) for *Neisseria gonorrhoeae* by symptom status for vaginal swab specimens

CIS	Comparator System			binx *io*	Symptom Status		Total
	NAAT1	NAAT2	NAAT3		Sx	Asx	
NI	-	-	-	-	776	681	1457
NI	-	-	+	-	3	4	7
NI	-	+	-	-	0	0	0
NI	+	-	-	-	0	0	0
NI	-	-	IND	-	2	1	3
NI	-	IND	-	-	0	2	2
NI	IND	-	-	-	6	1	7
NI	-	-	-	+	0	0	0
NI	-	-	+	+	0	0	0
NI	-	+	-	+	0	0	0
NI	+	-	-	+	1	0	1
NI	-	-	IND	+	0	0	0
NI	-	IND	-	+	0	1	1
NI	IND	-	-	+	0	0	0
Total not Infected					**788**	**690**	**1478**
I	+	+	+	+	24	15	39
I	+	+	-	+	1	0	1
I	+	-	+	+	2	1	3
I	-	+	+	+	1	0	1
I	+	+	IND	+	0	0	0
I	+	IND	+	+	0	0	0
I	IND	+	+	+	1	0	1
I	+	+	+	-	0	0	0
I	+	+	-	-	0	0	0
I	+	-	+	-	0	0	0
I	-	+	+	-	0	0	0
I	+	+	IND	-	0	0	0
I	+	IND	+	-	0	0	0
I	IND	+	+	-	0	0	0
Total Infected					**29**	**16**	**45**

CIS = Comparator Infected Status
NI = Not Infected
I = Infected
IND = Indeterminate
Sx = Symptomatic
Asx = Asymptomatic

Table 7b.

Analysis of Composite Infected Status (CIS) for *Neisseria gonorrhoeae* by symptom status for male urine specimens

CIS	Comparator System			binx *io*	Symptom Status		Total
	NAAT1	NAAT2	NAAT3		Sx	Asx	
NI	-	-	-	-	241	594	835
NI	-	-	+	-	1	4	5
NI	-	+	-	-	0	0	0
NI	+	-	-	-	1	2	3
NI	-	-	IND	-	2	1	3
NI	-	IND	-	-	1	0	1
NI	IND	-	-	-	0	1	1
NI	-	-	-	+	0	0	0
NI	-	-	+	+	0	0	0
NI	-	+	-	+	0	0	0
NI	+	-	-	+	0	0	0
NI	-	-	IND	+	0	0	0
NI	-	IND	-	+	0	0	0
NI	IND	-	-	+	0	0	0
NI	-	-	-	IND	0	0	0
NI	-	-	+	IND	0	0	0
NI	-	+	-	IND	0	0	0
NI	+	-	-	IND	0	0	0
NI	-	-	IND	IND	0	0	0
NI	-	IND	-	IND	0	0	0
NI	IND	-	-	IND	0	0	0
Total not Infected					**246**	**602**	**848**
I	+	+	+	+	59	10	69
I	+	+	-	+	0	0	0
I	+	-	+	+	0	0	0
I	-	+	+	+	0	0	0
I	+	+	IND	+	0	0	0
I	+	IND	+	+	0	0	0
I	IND	+	+	+	2	1	3
I	+	+	+	-	0	1	1
I	+	+	-	-	0	0	0
I	+	-	+	-	1	0	1
I	-	+	+	-	0	0	0
I	+	+	IND	-	0	0	0
I	+	IND	+	-	0	0	0
I	IND	+	+	-	0	0	0
I	+	+	+	IND	0	0	0
I	+	+	-	IND	0	0	0
I	+	-	+	IND	0	0	0
I	-	+	+	IND	0	0	0
I	+	+	IND	IND	0	0	0
I	+	IND	+	IND	0	0	0
I	IND	+	+	IND	0	0	0
Total Infected					**62**	**12**	**74**

CIS = Comparator Infected Status NI = Not Infected I = Infected IND = Indeterminate
Sx = Symptomatic Asx = Asymptomatic

Analytical Sensitivity (Limit of Detection)

An analytical limit of detection (LoD) of the binx *io* CT/NG Assay was carried out independently in vaginal swab matrix in eNAT buffer and in male urine in eNAT buffer. Genomic DNA was extracted from CT serovars E (ATCC-VR-348B) and F (ATCC-VR-346) and from two NG strains (ATCC 49226 and ATCC 700825). These were then quantified using qPCR against a reference standard in genome equivalents (GE/mL). Probit analyses were carried out using whole CT and NG cells that had been quantified in this manner.

At least five separate input concentrations as a minimum were used to cover a wide range (0.01-99%) of detection rates and each input concentration was tested with at least 20 replicates for each dilution. A probit regression analysis was used to model the *io* CT/NG Assay **CT Detected/NG Detected** rate and identify the spiked concentration level that demonstrated a detection rate of 95%. The LoD study was carried out on two distinct Cartridge lots. The highest value is the claimed LoD for that serovar/strain. The LoD was then verified for each CT serovar and each NG strain, using a further total of 40 Cartridges per serovar/strain per Cartridge lot using two further preparations of the claimed LoD generated by two different operators for both vaginal swab specimens (Table 8a) and male urine specimens (Table 8b).

Table 8a.
LoD for two CT serovars and two NG strains in vaginal swab specimens

Organism	GE/mL	IFU/mL	CFU/mL
CT serovar E (ATCC-VR-348B)	407.4	5.6	N/A
CT serovar F (ATCC-VR-346)	755.5	0.3	N/A
NG strain ATCC 49226	245.6	N/A	2.1
NG strain ATCC 700825	206.1	N/A	2.5

GE = Genome Equivalents
IFU = Inclusion Forming Units
CFU = Colony Forming Units

Table 8b.
LoD for two CT serovars and two NG strains in male urine specimens

Organism	GE/mL	IFU/mL	CFU/mL
CT serovar E (ATCC-VR-348B)	485.3	6.6	N/A
CT serovar F (ATCC-VR-346)	769.3	0.3	N/A
NG strain ATCC 49226	125.6	N/A	1.1
NG strain ATCC 700825	212.3	N/A	2.5

GE = Genome Equivalents
IFU = Inclusion Forming Units
CFU = Colony Forming Units

Assay Inclusivity

The LoDs for the additional 14 CT serovars (that had been similarly quantified) were determined in negative vaginal swab and pooled male urine matrices by testing replicates of 20.

In vaginal swab matrix, CT serovars B, Ba, C, D, G, H, J, K, L2, nvCT were detected at 377.8 GE/mL. Serovars A, I, L1, L3 were detected at 755.5 GE/mL in ≥19/20 replicates. An additional 30 NG strains (including two fluoroquinolone resistant isolates) were spiked into vaginal swab matrix and tested. The reported detectable level was confirmed by testing replicates of three at or near the LoD. Of the 30 NG strains tested, 16 strains were detected at 245.6 GE/mL in three out of three replicates. The remaining 14 strains were further tested, and all were detected at 1,228.0 GE/mL in ≥19/20 replicates.

In pooled male urine, CT serovars A, B, Ba, C, D, I, J, L2, nvCT were detected at 384.7 GE/mL. Serovars G, H, K, L1, L3 were detected at 769.3 GE/mL in ≥19/20 replicates. An additional 30 NG strains (including two fluoroquinolone resistant isolates) were spiked into pooled male urine and tested. The reported detectable level was confirmed by testing replicates of three at or near the LoD. Of the 30 NG strains tested, 16 strains were detected at 212.3 GE/mL in three out of three replicates. Thirteen strains were detected at 1,061.0 GE/mL in ≥19/20 replicates. The remaining strain (NG California 201304 #1) was detected in 17/20 replicates and was therefore subjected to a Probit LoD study which yielded an LoD of 553.38 GE/mL, confirmed by a verification study (40/40 replicates detected).

Analytical Specificity (Cross-Reactivity)

A panel of 62 species were investigated for cross-reactivity (Table 9). A panel of microorganisms and *H. sapiens* were assessed using cultured organisms at a concentration of 1 x 10^6 CFU/mL for bacteria or 1 x 10^5 PFU/mL for viruses,

or at a concentration of 2 ng/mL of genomic DNA generated by reverse transcription as available. Two further species were evaluated *in silico* by bioinformatic analysis of the genetic targets used in the binx health *io* CT/NG Assay against the published genome sequences for these organisms. *In silico* analysis concluded that neither of these organisms would be detected by the Assay.

For *Chlamydia trachomatis*, an expected result was CT Detected, NG Not Detected. For *Neisseria gonorrhoeae*, an expected result was CT Not Detected, NG Detected. For all other organisms, an expected result was CT Not Detected, NG Not Detected. One strain of *Neisseria sicca* gave a single CT positive result from 20 replicates in vaginal swab matrix and may therefore be cross-reactive with the CT analyte. *Corynebacterium xerosis* gave a single NG positive result and Herpes Simplex virus 2 gave a single CT positive result from 20 replicates in male urine and may therefore be cross-reactive with the NG and CT analytes, respectively.

Table 9.

Panel of organisms tested for cross-reactivity for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Organism	Input	Organism	Input
*Bacteriodes fragilis**	3.5 x 10^5 GE/mL	*Neisseria meningitidis* Serogroup D*	8.2 x 10^5 GE/mL
*Bacteriodes ureolyticus**	1.1 x 10^6 GE/mL	*Neisseria meningitidis* Serogroup W135*	8.2 x 10^5 GE/mL
*Clostridium perfringens**	5.7 x 10^5 GE/mL	*Neisseria meningitidis* Serogroup Y*	8.2 x 10^5 GE/mL
Corynebacterium genitalium	1.0 x 10^6 CFU/mL	*Neisseria cinerea*	1.0 x 10^6 CFU/mL
Corynebacterium xerosis	1.0 x 10^6 CFU/mL	*Neisseria denitrificans*	1.0 x 10^6 CFU/mL
Escherichia coli	1.0 x 10^6 CFU/mL	*Neisseria elongata* (4)	1.0 x 10^6 CFU/mL
*Gardnerella vaginalis**	1.1 x 10^6 GE/mL	*Neisseria gonorrhoeae**	1.2 x10^8 GE/mL
*Haemophilus ducreyi**	1.1 x 10^6 GE/mL	*Neisseria flava*	1.0 x 10^6 CFU/mL
Herpes simplex virus 1*	1.2 x 10^7 GE/mL	*Neisseria flavescens* (3)	1.0 x 10^6 CFU/mL
*Homo sapiens**	5.7 x 10^2 GE/mL	*Neisseria lactamica* (3)	1.0 x 10^6 CFU/mL
Human papilloma virus 16*	1.8 x 10^6 GE/mL	*Neisseria mucosa* (4)	1.0 x 10^6 CFU/mL
Kingella denitrificans	1.0 x 10^6 CFU/mL	*Neisseria perflava* (2)	1.0 x 10^6 CFU/mL
Kingella kingae	1.0 x 10^6 CFU/mL	*Neisseria polysaccharea*	1.0 x 10^6 CFU/mL
Lactobacillus acidophilus	1.0 x 10^6 CFU/mL	*Neisseria sicca* (4)*	6.7 x 10^5 GE/mL
Lactobacillus brevis	1.0 x 10^6 CFU/mL	*Neisseria subflava* (2)	1.0 x 10^6 CFU/mL
Lactobacillus jensenii	1.0 x 10^6 CFU/mL	*Trichomonas vaginalis*	1.0 x 10^6 CFU/mL
Lactobacillus lactis	1.0 x 10^6 CFU/mL	*Ureaplasma urealyticum**	2.0 x 10^6 GE/mL
Moraxella lacunata	1.0 x 10^6 CFU/mL	*Ureaplasma parvum**	2.5 x 10^6 GE/mL
Staphylococcus epidermidis	1.0 x 10^6 CFU/mL	*Atopobium vaginae**	1.3 x 10^6 GE/mL
Streptococcus agalactiae	1.0 x 10^6 CFU/mL	*Bifidobacterium longum**	8.2 x 10^5 GE/mL
Candida albicans	1.0 x 10^6 CFU/mL	BVAB-2†	*in silico*
Candida glabrata	1.0 x 10^6 CFU/mL	*Enterococcus faecalis*	1.0 x 10^6 CFU/mL
Candida parapsilosis	1.0 x 10^6 CFU/mL	Herpes Simplex Virus 2*	1.2 x 10^7 GE/mL
*Chlamydia pneumoniae**	1.5 x 10^6 GE/mL	*Klebsiella pneumoniae*	1.0 x 10^6 CFU/mL
*Chlamydia psittaci**	1.6 x 10^6 GE/mL	*Megasphaera* type 1†	*in silico*
*Mycoplasma genitalium**	3.2 x 10^6 GE/mL	*Mobiluncus curtisii**	8.6 x 10^5 GE/mL
*Mycoplasma hominis**	2.8 x 10^6 GE/mL	*Mobiluncus mulieris**	7.6 x 10^5 GE/mL
Neisseria meningitidis Serogroup A*	8.2 x 10^5 GE/mL	*Peptostreptococcus anaerobius**	8.8 x 10^5 GE/mL
Neisseria meningitidis Serogroup B*	8.2 x 10^5 GE/mL	*Proteus mirabilis*	1.0 x 10^6 CFU/mL
Neisseria meningitidis Serogroup C*	8.2 x 10^5 GE/mL	*Pseudomonas aeruginosa*	1.0 x 10^6 CFU/mL
*Chlamydia trachomatis**	2.3 x 10^7 GE/mL	*Staphylococcus aureus*	1.0 x 10^6 CFU/mL

(n) number of strains tested *Organisms tested with genomic DNA (2 ng/mL) † *In silico* analysis

Neisseria sicca may be cross-reactive with *Chlamydia trachomatis* in vaginal swab specimens. In male urine specimens, *Corynebacterium xerosis* may be cross-reactive with *Neisseria gonorrhoeae* and Herpes Simplex virus 2 may be cross-reactive with *Chlamydia trachomatis.*

Microbial Interference

Microbial interference with the *io* CT/NG Assay was evaluated by testing 2x LoD of both CT serovar F (ATCC VR-346) and NG strain ATCC 49226 spiked into negative vaginal and male urine matrices, aliquots of which were subsequently spiked with a panel of ten microorganisms (see Table 10) at a concentration of 1 x 10^5 CFU/mL. No interference was observed and an expected result of **CT Detected, NG Detected** was obtained in all cases.

Table 10.
Panel of organisms used for microbial interference testing with vaginal swab and male urine specimens

Organism
Corynebacterium xerosis
Escherichia coli
Lactobacillus acidophilus
Lactobacillus brevis
Lactobacillus jensenii
Lactobacillus lactis
Staphylococcus epidermidis
Streptococcus agalactiae
Candida albicans
Candida glabrata

Carryover Contamination Study

A study was conducted to demonstrate that the *io* CT/NG Cartridge prevents run-to-run cross contamination. Negative samples containing pooled vaginal matrix were run following very high CT/NG double positive samples (containing 2.26 x 10^6 GE/mL CT and 1.18 x 10^7 GE/mL NG). The study used four separate *io* Instruments, with 50 Cartridges run per Instrument, alternating between negative samples and very high CT/NG double positive samples (200 Cartridges run across all instruments, comprising 100 negative, 100 very high positive). Each of the 100 negative specimen runs were correctly detected as **CT Not Detected, NG Not Detected** and each of the 100 positive specimen runs were correctly identified as **CT Detected, NG Detected.**

Reproducibility

The reproducibility of the binx health *io* CT/NG Assay was evaluated at point-of care settings at three U.S. locations using two non-laboratorians as operators at each site.

Vaginal Swab Matrix Reproducibility Study

CT and NG organisms were seeded into pooled vaginal swab matrix at concentrations representing low positive (1x LoD), moderate positive (3x LoD) and high positive (2.26 x 10^5 GE/mL CT or 1.18 x 10^6 GE/mL NG) samples. Negative (non-seeded) pooled vaginal swab samples were also included. The resulting panel of 11 swab matrix samples were tested twice per day for seven consecutive days by two operators at three sites (11 specimens x 2 replicates x 7 days x 3 sites x 2 operators). *io* CT/NG Assays were performed according to the Assay procedure. The rate of agreement for vaginal swab matrix samples with expected CT and NG results for each panel member is shown in Table 11a.

Table 11a.
Summary of reproducibility testing results by site: vaginal swab matrix specimens

Panel No.	Sample	Analyte	Site 1 % agreement	Site 2 % agreement	Site 3 % agreement	% Total Agreement
1	**CT: Neg**	CT	92.9% (26/28)	100.0% (28/28)	92.9% (26/28)	**95.2% (80/84)**
	NG: High Positive	NG	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**
2	**CT: Neg**	CT	89.3% (25/28)	96.4% (27/28)	92.9% (26/28)	**92.9% (78/84)**
	NG: Mod. Positive	NG	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**
3	**CT: Neg**	CT	100.0% (28/28)	100.0% (28/28)	92.9% (26/28)	**97.6% (82/84)**
	NG: Low Positive	NG	100.0% (28/28)	100.0% (28/28)	96.4% (27/28)	**98.8% (83/84)**
4	**CT: High Positive**	CT	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**
	NG: Neg	NG	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**
5	**CT: Mod. Positive**	CT	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**
	NG: Neg	NG	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**
6	**CT: Low Positive**	CT	96.4% (27/28)	92.9% (26/28)	100.0% (28/28)	**96.4% (81/84)**
	NG: Neg	NG	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**
7	**CT: High Positive**	CT	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**
	NG: High Positive	NG	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**
8	**CT: High Positive**	CT	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**
	NG: Low Positive	NG	100.0% (28/28)	96.4% (27/28)	100.0% (28/28)	**98.8% (83/84)**
9	**CT: Low Positive**	CT	100.0% (28/28)	96.4% (27/28)	96.4% (27/28)	**97.6% (82/84)**
	NG: High Positive	NG	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**
10	**CT: Low Positive**	CT	100.0% (28/28)	92.9% (26/28)	100.0% (28/28)	**97.6% (82/84)**
	NG: Low Positive	NG	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**
11	**CT: Neg**	CT	92.9% (26/28)	100.0% (28/28)	92.9% (26/28)	**95.2% (80/84)**
	NG: Neg	NG	100.0% (28/28)	100.0% (28/28)	100.0% (28/28)	**100.0% (84/84)**

Neg = Negative Low Positive = 1x LoD Mod. Positive = 3x LoD CT High Positive = 2.26 x 10^5 GE/mL NG High Positive = 1.18 x 10^6 GE/mL

Male Urine Matrix Reproducibility Study

CT and NG organisms were seeded into male urine matrix at concentrations representing low positive (1x LoD), moderate positive (3x LoD) and high positive (4.15 x 10^6 GE/mL CT or 8.4 x 10^5 GE/mL NG) samples. Negative (non-seeded) pooled male urine samples were also included. The resulting panel of 11 pooled male urine samples were tested three times per day for five consecutive days by two operators at three sites (11 specimens x 3 replicates x 5 days x 3 sites x 2 operators). *io* CT/NG Assays were performed according to the Assay procedure. The rate of agreement for male urine matrix samples with expected CT and NG results for each panel member is shown in Table 11b.

Table 11b.
Summary of reproducibility testing results by site: male urine matrix specimens

Panel No.	Sample	Analyte	Site 1 % agreement	Site 2 % agreement	Site 3 % agreement	% Total Agreement
1	**CT: Neg**	CT	100.0% (30/30)	100.0% (30/30)	96.7% (29/30)	**98.9% (89/90)**
	NG: High Positive	NG	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	**100.0% (90/90)**
2	**CT: Neg**	CT	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	**100.0% (90/90)**
	NG: Mod. Positive	NG	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	**100.0% (90/90)**
3	**CT: Neg**	CT	96.7% (29/30)	100.0% (30/30)	100.0% (30/30)	**98.9% (89/90)**
	NG: Low Positive	NG	93.3% (28/30)	96.7% (29/30)	93.3% (28/30)	**94.4% (85/90)**
4	**CT: High Positive**	CT	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	**100.0% (90/90)**
	NG: Neg	NG	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	**100.0% (90/90)**
5	**CT: Mod. Positive**	CT	96.7% (29/30)	100.0% (30/30)	100.0% (30/30)	**98.9% (89/90)**
	NG: Neg	NG	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	**100.0% (90/90)**
6	**CT: Low Positive**	CT	93.3% (28/30)	83.3% (25/30)	96.7% (29/30)	**91.1% (82/90)**
	NG: Neg	NG	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	**100.0% (90/90)**
7	**CT: High Positive**	CT	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	**100.0% (90/90)**
	NG: High Positive	NG	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	**100.0% (90/90)**
8	**CT: High Positive**	CT	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	**100.0% (90/90)**
	NG: Low Positive	NG	96.7% (29/30)	93.3% (28/30)	96.7% (29/30)	**95.6% (86/90)**
9	**CT: Low Positive**	CT	100.0% (30/30)	100.0% (30/30)	93.3% (28/30)	**97.8% (88/90)**
	NG: High Positive	NG	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	**100.0% (90/90)**
10	**CT: Low Positive**	CT	86.7% (26/30)	100.0% (30/30)	93.3% (28/30)	**93.3% (84/90)**
	NG: Low Positive	NG	90.0% (27/30)	100.0% (30/30)	93.3% (28/30)	**94.4% (85/90)**
11	**CT: Neg**	CT	93.3% (28/30)	100.0% (30/30)	100.0% (30/30)	**97.8% (88/90)**
	NG: Neg	NG	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	**100.0% (90/90)**

Neg = Negative Low Positive = 1x LoD Mod. Positive = 3x LoD CT High Positive = 4.15 x 10^6 GE/mL NG High Positive = 8.40 x 10^5 GE/mL

Interfering Substances

The performance of the binx health *io* CT/NG Assay was evaluated in the presence of a panel of potentially interfering substances. The substances were diluted to the concentrations shown in Tables 12a and 12b and spiked into negative vaginal and male urine matrices, respectively. The substances were tested in the presence (2x LoD) and absence of CT and NG. No interference was observed in the presence of these substances at the concentrations tested in negative vaginal swab or urine matrices. One substance, leukocytes, produced one false negative result for *Neisseria gonorrhoeae* out of 20 replicates tested in the male urine matrix.

Table 12a.
Panel of potential interfering substances tested with vaginal swab specimens

Substance	Concentration
Human blood	10.0% (v/v)
Contraceptive Jelly	0.25% (v/v)
Mucin	1.0% (w/v)
Seminal fluid	5.0% (v/v)
Vaginal Moisturiser	0.25% (w/v)
Anti-fungal cream (Canesten)	0.25% (v/v)
Anti-fungal cream (Daktarin)	0.25% (v/v)
Vaginal lubricant	0.25% (v/v)
Feminine anti-itch cream (2% lidocaine)	0.25% (v/v)
Leukocytes	1 x 10^6 cells/mL
Progesterone	7 mg/mL
β-Estradiol (Estrace cream)	0.25% (v/v)
Anti-viral cream (Acyclovir)	0.25% (v/v)
Hemorrhoidal cream	0.25% (v/v)

Table 12b.
Panel of potential interfering substances tested with male urine specimens

Substance	Concentration
Human blood	1.0% (v/v)
Seminal fluid	5.0% (v/v)
Mucin	0.5% (w/v)
Albumin (BSA)	10 mg/mL
Glucose	10 mg/mL
Bilirubin	0.2 mg/mL
Leukocytes	1x10^6 cells/mL
Progesterone	7 mg/mL
β-Estradiol (Estrace cream)	0.25% (v/v)
Paracetamol	3.2 mg/mL
Aspirin	40 mg/mL
Acidic Urine	pH 4.0
Alkaline Urine	pH 9.0
Azithromycin	0.8 mg/mL
Doxycycline	3.6 mg/mL

CLIA Waiver Field Study

The performance of the *io* CT/NG Assay, when used by untrained users, was evaluated at three CLIA waived sites (or sites representative of CLIA waived sites) by nine users (three per site) who had no laboratory testing experience nor training on the *io* CT/NG Assay. The field study consisted of testing 250 contrived samples (115 female, 135 male) comprising female vaginal swab matrix or male urine matrix spiked with 2x, 5x and 20x LoD of CT serovar F and NG strain ATCC 700825, and negative (unspiked) samples. Testing was carried out over a four-week period and was integrated into the users' daily workflow to demonstrate that untrained users can perform testing of vaginal swab samples and urine samples using the binx CT/NG Assay accurately and consistently, amidst busy patient-focused environments. Because urine samples and vaginal swab samples require a different workflow after sample collection, two sets of samples were used to demonstrate both workflows. Female vaginal swab samples were contrived using pooled vaginal swab matrix in preservative using the binx Vaginal Swab Specimen Collection Kit. The users processed the female vaginal swab samples according to the test procedure by mixing and pipetting the sample into the Cartridge. In contrast to female vaginal swab samples, testing of male urine samples requires the user to add the patient's urine into the Male Urine Specimen Collection Kit tube prior to running the test. Therefore, the study was designed to accommodate this step into the workflow to mimic the real-world use of the *io* CT/NG Assay. The test samples were contrived by spiking CT and NG organisms directly into the preservative in the Male Urine Specimen Collection Kit tubes. Users were required to add urine to the sample tube containing preservative, using the 2 mL pipets supplied in the Male Urine Specimen Collection Kits. Once urine was added to the sample collection tubes, users followed the sample preparation instructions (mixing by inversion and pipetting into the Cartridge). The performance of the binx *io* CT/NG Assay in the hands of untrained users testing vaginal swab samples and urine male samples is shown below in Tables 13a and 13b, respectively.

Table 13a.
CLIA Waiver field study: vaginal swab matrix specimens

Sample	Low (2x LoD)		Med (5x LoD)		High (20x LoD)	
	No. Detected/ No. Tested (% Agreement with Expected Results)		No. Detected/ No. Tested (% Agreement with Expected Results)		No. Detected/ No. Tested (% Agreement with Expected Results)	
	CT	NG	CT	NG	CT	NG
CT Pos/NG Neg	20/20 (100%)	0/20 (100%)	10/10 (100%)	0/10 (100%)	5/5 (100%)	0/5 (100%)
NG Pos/CT Neg	0/20 (100%)	20/20 (100%)	1/10* (90%)	10/10 (100%)	0/5 (100%)	5/5 (100%)
	Un-spiked					
Negative	0/45 (100%)	0/45 (100%)	N/A			

*One Medium Positive NG sample gave a false positive result for CT (CT Detected).

Table 13b.
CLIA Waiver field study: male urine matrix specimens

Sample	Low (2x LoD)		Med (5x LoD)		High (20x LoD)	
	No. Detected/ No. Tested (% Agreement with Expected Results)		No. Detected/ No. Tested (% Agreement with Expected Results)		No. Detected/ No. Tested (% Agreement with Expected Results)	
	CT	NG	CT	NG	CT	NG
CT Pos/NG Neg	20/20 (100%)	0/20 (100%)	10/10 (100%)	0/10 (100%)	5/5 (100%)	0/5 (100%)
NG Pos/CT Neg	0/20 (100%)	20/20 (100%)	0/10 (100%)	10/10 (100%)	0/5 (100%)	5/5 (100%)
CT Pos/NG Pos	10/10 (100%)	10/10 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)
	Un-spiked					
Negative	0/45 (100%)	0/45 (100%)	N/A			

Reporting Assay or Test System Problems

In the first instance please report any assay or instrument problems to binx health using the contact details on the back page of this Instructions for Use booklet to allow binx health to track any device problems. Issues can also be reported to FDA as part of the Medwatch Product Safety Reporting Program at **www.fda.gov/medwatch**

References

1. World Health Organisation website. WHO Fact Sheet Sexually Transmitted Infections (STI). Feb 28 2019 https://www.who.int/health-topics/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis)
2. UK National Health Service (NHS) website. www.nhs.uk/Conditions/Chlamydia
3. US Centers for Disease Control website. www.cdc.gov/std/chlamydia
4. US Centers for Disease Control website. Sexually Transmitted Diseases Treatment Guidelines 2015 https://www.cdc.gov/std/tg2015/tg-2015-print.pdf
5. UK National Health Service (NHS) website. www.nhs.uk/Conditions/Gonorrhoea

Contact and Support

binx health limited, Derby Court, Epsom Square, White Horse Business Park,
Trowbridge, Wiltshire, BA14 0XG, United Kingdom.

www.mybinxhealth.com

Sales and Product Technical Support

Telephone: 1-844-MYBINX-1 (1-844-692-4691)

E-mail: support@mybinxhealth.com

Ordering Information

binx health *io* CT/NG Cartridges, US (x10)	Cat. No. 1.002.110
binx health *io* CT/NG Cartridges, US (x50)	Cat. No. 1.002.150
binx health *io* Instrument	Cat. No. 3.001.001
binx health *io* Vaginal Swab Specimen Collection Kit, US (x50)	Cat. No. 5.012.050
binx health *io* Vaginal Swab Specimen Collection Kit, US (x500)	Cat. No. 5.012.500
binx health *io* Male Urine Specimen Collection Kit, US (x50)	Cat. No. 5.002.050
binx health *io* Male Urine Specimen Collection Kit, US (x500)	Cat. No. 5.002.500
USB Barcode Scanner	Cat. No. 3.000.020

binx