CLIA Waiver by Application Approval Determination

Decision Summary

A. Document Number

CW200012

B. Parent Document Number(s)

K191352 and K200533

C. CLIA Waiver Type:

CLIA Waiver by Application

D. Applicant

binx health, Inc.

E. Proprietary and Established Names

binx *io* CT/NG Assay and binx *io* System

F. Measurand (analyte)

1. Chlamydia trachomatis genomic DNA
2. *Neisseria gonorrhoeae* genomic DNA

G. Sample Type(s)

- Female Vaginal swabs
- Male Urine

H. Type of Test

Nucleic acid amplification assay, qualitative; cartridge based.

I. Test System Description

1. Overview

The binx health *io* CT/NG Assay (the "Assay") is a qualitative *in vitro* diagnostic test system consisting of a table-top instrument and test cartridges containing all the necessary reagents to perform the test. The test is designed for use in near-patient settings to deliver results in approximately 30 minutes.

This device was previously cleared under K191352 (with female vaginal swabs) and K200533 (with male urine) for use in POC settings. The current application for CLIA waiver is to expand the use of the test to CLIA waived testing facilities.

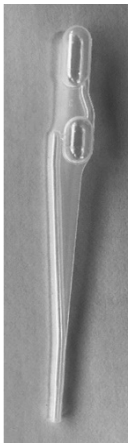
2. Test System Components

The binx health *io* CT/NG Assay System consists of the following components:

- The binx *io* Instrument for running the Cartridge (the "Instrument");
- The binx *io* CT/NG Cartridge (or "Cartridge"), which contains all the necessary reagents to perform the binx *io* CT/NG Assay on the binx *io* Instrument, packaged in cartons of 10 single use units;
- A single-use, fixed-volume transfer pipet (packaged with the Cartridge) for transferring the sample to the Cartridge;
- The Vaginal Swab Specimen Collection Kit consisting of a sterile flocked swab and a sample Collection Tube containing preservation medium;
- The Male Urine Collection Kit consisting of a sample collection tube containing preservation medium and a single 2 mL transfer pipet.

binx *io* Instrument

binx *io* CT/NG Cartridge



Sample Transfer Pipet

The binx *io* Cartridge is assay-specific and is intended for a single-use on a single patient sample.

Sample collection kits and the Quality Controls are available separately from the assay kit.

The assay uses a vaginal swab collected in a preservation medium (eNAT) or male urine added to eNAT. After the sample (0.5 mL) is added to the port of the Cartridge using the included sample transfer pipet, the Cartridge is inserted into the Instrument and the assay begins.

Image showing loading of the Cartridge.

Each Cartridge label contains a barcode that includes the test type, batch information, and expiration date, which is automatically scanned by the Instrument. At the start of the assay, the instrument initiates the release of liquid reagents. After DNA extraction and purification, the sample is divided between two PCR chambers allowing for CT and NG DNA amplification by thermal cycling to be run separately. While only one genomic DNA target is used for the detection of CT, two genomic DNA targets are amplified for the NG and both must be present to generate a positive result for NG. In the detection step, complementary electrochemically labeled DNA probes hybridize to the amplified DNA followed by enzymatic digestion of the double stranded DNA-probe complexes which release the electrochemical label. Application of a voltage oxidizes the released label at the electrode, which generates a measurable current in nA and indicates the presence of CT and/or NG DNA. In negative specimens, the probes remain as single-stranded DNA and cannot be digested by the enzyme; no release of the electrochemical label occurs and no current is generated.

The assay incorporates an Internal Process Control (IPC) which undergoes all the analytical processing along with each patient sample, from the DNA extraction and purification through the detection. If the IPC measurement is outside of the specified range, the instrument will return an “Assay Invalid” message. A negative result for CT and/or NG will only be returned if the IPC measurement is 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 functions as expected.

The following table shows the possible assay outcomes.

Assay Result	Interpretation of 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. No result is given.

3. Quality Controls

The external control materials recommended for use with the Assay are manufactured by ZeptoMetrix Corporation, Buffalo, NY. The controls are supplied in boxes of six 1 mL single-use aliquots. The device labeling states that external controls must be run every time a new User is introduced to the testing process, or whenever a new lot of the Assay is received.

The lot-to-lot reproducibility and stability data for the NATtrol *Chlamydia trachomatis* (CT) Positive Control (which acts as a NG-negative control) and NATtrol *Neisseria gonorrhoeae* (NG) Positive Control (which acts as a CT-negative control) was generated by the manufacturer (Zeptometrix) and was included in the documents submitted for this application. The testing was conducted using an FDA-cleared CT/NG Assay according to the instructions for use and the data was collected retrospectively on three consecutive lots of each control over the course of 30 months. Controls were stored at between 2-8°C prior to testing. Although the acceptance criteria were qualitative, the Ct values obtained

were monitored for assessment by the manufacturer. The data showed that results at all timepoints met the acceptance criteria (Detected or Not Detected), supporting the claim of a 12-month shelf life for these materials.

J. Demonstrating "Simple"

- The Instrument is fully automated, the Assay is self-contained and does not need any calibration by the User.
- All reagents are enclosed in the test cartridge.
- Uses unprocessed vaginal swab and first-catch male urine specimens that are added to a transport tube containing a preservation solution; the tube is mixed by hand and the specimen is transferred into the cartridge which is then loaded onto the *io* Instrument.
- To avoid measuring of liquids, a fixed volume pipet is provided for adding the sample to the cartridge.
- There are no technique dependent steps in performing the test. The mixing of the samples is performed by manually shaking the tube.
- All analytical steps are performed by the Instrument, invisible to the user.
- No calibration of the Instrument is required.
- There is no intervention during the analysis.
- The Instrument is simple to use; and no training is required.
- No troubleshooting is required.
- The maintenance is limited to wiping the outer surfaces of the Instrument with isopropanol.
- The results are displayed on the screen in an easy to understand format.
- The error codes are simplified, with a clear message on the follow up action.
- Final results are displayed on the Instrument screen, as "Positive", "Negative" or "Invalid.".
- Contains a Quick Reference Instruction sheet that is written is simple words with graphics.
- The Graphical User Interface offers features that make the system user-friendly, such as:
    a. The progress of the test/time to completion is presented visually by clock-like icon on the screen.
    b. The user is prompted to take the correct step in the procedure for the assay to proceed.
    c. For proper sample loading, the user is prompted to check the sample indicator window on the Cartridge to ensure that a patient specimen was added to the Cartridge.
    d. Error messages to the user are displayed at the bottom of the screen (footer area) and provide guidance on the next steps, e.g., *The instrument detected a fault. Please re-start the instrument and repeat the test or contact support.*

K. Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-safe Mechanisms

1. Risk Analysis

A comprehensive risk analysis of the binx CT/NG Assay on the binx *io* instrument has been conducted in accordance with ISO 14971:2019. The sponsor utilized the Device Hazard Analysis and the Failure Mode Effects Analysis (FMEA) methods to assess the risks of failure that may occur during use or misuse of the device. The identified risks were assigned a severity score (1-4, negligible to catastrophic) which was then multiplied by the probability of occurrence score (1-5, improbable to frequent). Estimated risk scores of ≤ 6 were classified as 'low' and acceptable risks; risk scores of 8 and 9 were classified as 'moderate' and risks of ≥ 10 were classified as 'high' and therefore unacceptable. All risks were reduced as far as possible through design iterations and mitigations.

Based on the hazard analysis, all possible sources of error that might be at an increased likelihood of occurring when the test system is used in CLIA waived settings by untrained users, were evaluated in flex studies. The considered risks included operator errors (human factors), sample and device handling and storage, and environmental factors.

2. Fail-Safe and Failure Alert Mechanisms

The binx CT/NG Assay was designed to include numerous features and fail-safe mechanisms built into the system to prevent erroneous results.

Design Features

- The test is designed for single use, where each cartridge along with the transfer pipet is individually packaged in a foil pouch. The individual packaging maintains the integrity of the reagents and prevents contamination.
- The provided transfer pipet is designed to deliver the required sample volume to the Cartridge, without measurement.
- The reagents are integrated into the cartridges as a combination of liquid reagents in blister packs and dried reagents to maximize their stability and to minimize contamination.
- The Cartridge label contains a barcode that includes the test type, batch information and expiry date; the encoded expiry date prevents the use of the cartridges beyond their stability date.
- Because the barcode is destroyed after the automatic processing begins, the cartridge cannot be reused.
- The instrument drawer is designed so that the Cartridge can only be loaded in the correct orientation.
- The *io* Instrument touchscreen is designed to facilitate easy and intuitive operation and guides the user to load the Cartridge into the Instrument.
- The touchscreen prompts the user to take the correct steps in cartridge processing.

- The Cartridge has a visual sample addition indicator window which turns from light to dark to indicate to the User that a sample has been added to the Cartridge.
- As part of the Cartridge loading process, the user is prompted to check the sample indicator window on the Cartridge, to ensure that a patient specimen was added to the Cartridge.
- Test results are interpreted automatically by the Instrument; a qualitative result is provided on the *io* Instrument screen.
- External controls must be run when a new User is introduced to the testing process or whenever a new lot of CT/NG Cartridges is received.

Built-in Fail-safe Measures

- The Internal Process Control monitors for all steps of analytical processing, including extraction, amplification and detection of the targets. Failure of the IPC may be caused by:
  - Compromised reagent integrity
  - Inhibitory substances in specimens
  - Failed cartridge processing
- Predetermined assay parameters require that the generated electrochemical peak signal must fall within specifications, otherwise an invalid result is returned.
- After each Cartridge run is completed, the *io* Instrument actuates an isolation valve on the Cartridge, sealing it to prevent carryover contamination.
- Amplicon carryover contamination is minimized by enzymatic degradation of any amplicon generated from previous Assay runs.
- A backup copy of stored data is available in case the data becomes corrupted.
- An electronic self-check occurs before each cartridge run is initiated, during which functionality of a multitude of sensors is assessed.
- If an unexpected firmware state is encountered, the default reaction is to cancel operation of the Instrument and shut down. If an Assay run is in process, an "error" result is reported.
- If power loss occurs, the instrument shuts down.

3. Flex Studies

The systematic review of the risk analysis identified the conditions where there was a low but potential risk of obtaining an incorrect result. These conditions were evaluated in flex studies described below.

The flex studies focused on three areas of potential risks:

a. Specimen and reagent handling
b. Operator errors in performing the test
c. Environmental conditions

The test samples used in the flex studies were contrived in pooled vaginal swab matrix and pooled male urine, both collected from healthy donors and screened for negativity prior to use. Positive samples were prepared by co-spiking with CT serovar F and a strain of NG organisms at 3x LoD concentration. Negative samples consisted of un-spiked matrix. For some studies, as indicated below, the positive vaginal swab samples were prepared by spiking the organisms directly onto the swab such that the final concentration, when placed into the 2 mL tube of eNAT buffer and diluted, would be 3x LoD. Each positive and negative sample was tested in 5 replicates for each condition being evaluated. Each flex study included testing the samples under control (normal) conditions, according to the test procedure, as a point of reference. The users were blinded as to the positivity status of the samples. There were 27 *io* Instruments in total used across the flex studies.

Specimen and Reagent Handling

a. Improper Storage of Collection Kits

The study evaluated the effect of exposing the collection buffer (eNAT) outside of the specified temperature of 5°C-25°C.

i. The collection buffer tubes were placed at 40°C for 7 days prior to testing. Positive and negative samples in both matrices (as above) were tested in 5 replicates for each condition. All tests returned expected results, demonstrating that storing the collection kits outside of the recommended temperature had no effect on the performance of the binx CT/NG Assay.

ii. The collection kits were placed at -20°C for 40 hours prior to use. After being brought to room temperature, the buffer tubes were used to test low positive CT/NG swabs and urine and negative swabs and urine, each in 5 replicates. All positive swabs generated expected results, i.e., positive for CT and for NG. Among the negative swabs, all but one test returned the expected results; one negative swab gave a false positive NG result. Five additional swabs were tested using the previously frozen buffer and all generated the expected negative results for a total of 9/10 correct results. The false positive result was attributed to a low level of contamination during sample preparation and not due to the control condition. All urine samples both positive and negative generated the expected results.

b. Freezing of Cartridges

This study evaluated the effect of freezing on the cartridges in a case of inadvertent storage at -20°C. For this study, CT/NG Assay Cartridges were frozen at -20°C for a minimum of 18 hours prior to testing. Low positive and negative samples were run using the cartridges that were previously frozen. Although all tests returned expected results, the following was noted:

a. An audible crunching sound was heard as the lysis buffer actuator engaged to release the lysis buffer into the cartridge. This was attributed to the fact that the lysis buffer was still frozen inside the blister.
b. During post-run inspection it was observed that cartridges that had been stored frozen had a significant increase in the volume of liquid remaining in the sample input well; normally, only a minimal volume of liquid is left in the sample well.
a. The review of the electrochemical peak height data for the positive samples showed that all target peak heights (CT, NG1 and NG2) are lower by approximately 40% for the frozen cartridge condition compared to the control cartridge (unfrozen) condition.

In conclusion, although experimentation has not shown erroneous results, the residual liquid remaining in the sample well could indicate the possibility of an incorrect (false negative) result because not all of the sample may have been extracted for use in the assay. To mitigate against this risk, the labeling for the CT/NG Assay includes a warning: *Do not freeze io CT/NG Cartridges. Freezing Cartridges may cause an incorrect result to be obtained.*

c. Exposing Cartridges to High Temperatures during Storage

The kit labeling specifies that the Cartridges should be stored at 2-8°C. For this study, the cartridges were stored at 40°C for seven days prior to use. For both swab and urine, all low positive and negative samples tested using these cartridges returned expected results, showing that exposing the Cartridges to elevated temperatures up to 40°C does not affect the test outcome for either positive or negative samples.

d. Susceptibility to Damage upon Dropping

This study evaluated the effect of a mechanical shock on the cartridges when dropped on the floor from a height of three feet. After the drop, the cartridges were used to test the low positive and negative samples in each matrix. All tests returned expected results showing that running vaginal swab and male urine samples using a Cartridge that had been dropped onto a hard surface does not affect obtaining the correct test outcome for either positive or negative samples.

e. Freezing of Samples

The test procedure specifies that samples can be stored at room temperature (25°C) for 24 hours or at 2-8°C for seven days. For this study, the eluted swab samples in the collection buffer were placed at -20°C for 7 days prior to testing. Similarly, the urine samples in the collection buffer were placed at -20°C for 7 days prior to testing.

All female vaginal swab sampled returned correct test results when tested after being frozen at -20°C, indicating that freezing of the swab samples does not affect test outcome.

However, all low positive male urine samples that had been frozen prior to testing returned false negative results. Additionally, two target-negative samples returned invalid results. The sponsor added explicit warnings in the labeling to guard against freezing of urine specimens: *Male urine samples should not be frozen. Freezing may degrade the sample and could lead to false negative results.*

f. Prolonged Storage of Samples in the Collection Buffer

The test procedure specifies that urine and swab samples may be stored at room temperature (25°C) for 24 hours or at 2-8°C for seven days. For this study, vaginal swab and male urine samples were tested at t=0 and then again following incubation at (a) 25°C for 24, 48, 72 and 96 hours, and (b) 2-8°C for 7, 12, 16 and 20 days. The following results were obtained:

- One positive vaginal swab sample returned an invalid result after incubation for 24 hrs at 25°C, generating a valid positive result upon repeat.
- One negative vaginal swab sample returned an invalid result after incubation for 20 days at 2-8°C, generating a valid negative result upon repeat.
- One positive male urine sample returned an invalid result after incubation for 72 hrs at 25°C, generating a valid positive result upon repeat.

- One positive male urine sample returned an invalid result after incubation for 20 days at 2-8°C, generating a valid positive result upon repeat.
- One negative vaginal swab sample returned a false positive CT result after incubation for 48 hrs at 25°C.

The false positive CT result was attributed to a low-level contamination of the negative swab matrix and is discussed below, under *Notes on Flex Studies*.

In summary, the results of the study demonstrated that prolonged storage of vaginal swab samples and urine samples in the collection medium at the claimed storage temperatures stated in the labeling, i.e., 25°C and 2-8°C, up to 96 hours and up to 20 days, respectively, does not pose a risk of erroneous results. Nevertheless, the binx CT/NG assay is intended to be used at the point-of-care to deliver an actionable result while the patient is waiting, therefore, storing of samples for prolonged periods of time is not anticipated.

User Errors

g. Elution of the Target from Swab

The test procedure specifies that female vaginal swab samples should be mixed by four rapid downward wrist shakes at the point of collection. For this study, the contrived swab samples (spiked directly) were left in the buffer for 1 minute, 5 minutes and 10 minutes without shaking, then removed, and the sample was transferred onto the cartridge for testing. The results showed that shaking of the sample is critical and that failure to properly mix the swab samples will generate false negative results; all negative results yielded expected results. This condition is mitigated by including an explicit caution in the labeling: *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.*

h. Mixing of Urine Samples

The test procedure specifies that male samples should be mixed by four inversions after addition of the urine to the collection buffer, and prior to testing. For this study, the urine samples were added to the buffer and left in the buffer for 1 minute, 5 minutes and 10 minutes without mixing, then the sample was transferred onto the cartridge for testing. All tests returned expected results, demonstrating that failure to mix the urine sample prior to testing does not affect the performance of the binx CT/NG Assay.

i. Varied Sample Volume

The nominal sample volume that is added using the fixed volume pipet is 500 µL (0.5 mL). In this study, low positive and negative samples were added to the cartridge at various volumes to determine the minimum and maximum volume of the sample that is required to obtain accurate results. Female vaginal swab samples and male urine samples were evaluated at volumes ranging from ¼ to 2x the nominal volume (i.e., 125 µL to 1.0 mL). The results showed, for both sample types, that the assay is insensitive to variations in the input volume down to 1/2 of the nominal assay input, i.e., 250 µL. However, sample volume <250 µL may result in false negative results when testing samples at a low bacterial concentration. Overfilling of the sample port did not have effect on the results. All negative samples yielded expected results. One invalid result was obtained which gave a valid result upon the repeat.

The risk of the user adding less than optimal volume of sample is mitigated through the use of the fixed volume pipet provided with each cartridge. The clear instructions on how to use the pipet further mitigate errors in sample transfer.

j. Delay in Testing after Sample Transferred onto Cartridge

The IFU states that the user must run the Cartridges within six hours of loading a sample into a Cartridge. In this study, low positive and negative vaginal swab and male urine samples were loaded onto Cartridges which were then left for eight hours before being run on the Instrument. All samples yielded expected results, indicating that a delay in processing a loaded cartridge up to eight hours does not affect the test outcome.

k. Excessive Shaking of Samples Causing Foaming

The test instructions direct the user to mix the vaginal swab specimens by shaking with a rapid downward movement of the wrist four times and the male urine samples by inverting four times. For this study, low positive and negative vaginal swab and male urine samples were shaken vigorously 10 times prior to loading, causing some foaming. The samples were then loaded on Cartridges for testing. All tests returned expected results showing that vigorous shaking of samples and generating foam does not affect the final assay outcome.

l. Movement during analysis

The effect of movement of the Instrument during a test run was evaluated by processing each sample and moving the instrument while the testing was ongoing. The instrument was picked up and moved three feet, while each test was in progress. All tests subjected to movement returned expected results, demonstrating that the test system is not sensitive to movement during analysis.

Operational Environment

m. Operational Temperature and Humidity

The labeling specifies optimum operational conditions to be 10°C to 35°C and 0% to 80% relative humidity (RH). The sponsor notes that the tolerance to humidity decreases linearly at 35°C to 59% RH.

- 5°C/5% RH
- 5°C/95% RH
- 40°C/5% RH
- 40°C/95% RH
- 40°C/80% RH
- Room Temperature control (15-30°C/10-80% RH)

Environmental chambers were used to simulate the test conditions. Positive and negative vaginal swab or male urine samples were used as test inputs for each condition above.

During testing at 40°C and 95% RH condition, a built-in humidity sensor caused the instrument to shut down due to water vapor condensing on the Instrument. This feature is designed to protect the user from an electric shock. Further testing at 40°C and 80% RH (still outside of the specifications) also resulted in the instrument shutdown due to an internal temperature sensor. The functionality of this lock-out feature was further evaluated by removing the sensor mechanism and repeating testing at 40°C and 80% RH, resulting in uninterrupted operation. All samples generated expected results when subjected to all the various temperature and humidity combinations, with an exception of one negative vaginal swab that yielded a false positive CT result at 40°C and 5% RH. The one positive CT result was attributed to a low-level contamination with the CT target (discussed below) and not to the condition being evaluated. Overall, the data demonstrated that the built-in sensors mitigate the risks of erroneous results by shutting down the system when extremes of temperature and humidity occur in the environment.

n. Vibrations

To evaluate the effect of vibrations on the test system, two *io* instruments were positioned one foot (12 inches) on either side of a microcentrifuge operating at 9,500 rpm. Low positive and negative vaginal swabs and urine samples were run, for a total of 20 runs. All test samples returned expected results demonstrating that the test system is not affected by vibrations that may be caused by other nearby instrumentation.

o. Poor Ventilation

The Instrument Operator Manual directs the user to position the instrument such that there is a small gap (> 50 mm) between the Instrument and other objects/walls for ventilation purposes. For this study, ten *io* instruments were positioned in pairs, side-by-side, such that there was no space between them. Low positive and negative vaginal swab and male urine samples were used as test inputs. All tests returned expected results. There were two samples that generated invalid results, returning valid results upon repeat; those were not attributed to the test condition.

p. Non-level Work Space

The Instrument Operator Manual directs the user to position the instrument on a stable, flat surface with an incline of no more than 2.5°. In this study, *io* Instruments were placed on tilted tables set at 15° inclines forwards, backwards, left, and right. Low positive and negative samples in swab and urine matrix were tested. All tests returned expected results, except for one unexpected NG false negative in male urine. The false negative NG result was not attributed to the stress condition and is discussed below. Because the false negative result was returned for only one out of a total of 40 low positive samples tested when the instrument was tilted in any direction, suggests it is not a systematic Instrument failure due to the test condition. Nevertheless, the sponsor added a warning to the labeling: *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 during test processing*.

Notes on Flex Studies

Of the 917 Cartridges used in the flex studies, a total of three unexpected CT false positive results, one false negative NG result and 17 invalid results were observed during the flex studies.

The false positive CT results were investigated, and the quantitative peak heights generated for the three samples were determined to be low compared to the signal generated by the population of positive swab test samples used in the flex studies. It was determined that the observed false positive results were likely due to a low level of contamination with the target during the preparation of the test samples, rather than caused by the conditions being evaluated in the flex studies.

The one unexpected false negative NG result observed during the “tilt” study was due to one of the NG targets falling just below the assay detection threshold of 50.0 nA. The quantitative peak heights generated by all the positive samples tested in this flex study were examined. Based on the analysis, there was no associated trend from the CT, IPC and NG2 targets to suggest that the false negative result was due to the condition tested. Considering that no other tilt position generated false negative results, it was concluded that this result was due to stochastic distribution of the NG target in this specimen and not the result of the flex study tilt condition. However, as noted above, the sponsor included a caution in the labeling indicating that the instrument must be placed on a level surface during test processing.

Conclusions from Flex Studies

The flex studies carried out in support of this CLIA waiver submission have effectively and comprehensively demonstrated the robustness of the System to generate correct results even when operated under conditions of stress. The combination of the device design and the built-in fail-safe features, along with the clear test instructions which include relevant cautions, the risks of erroneous results are minimal. As such, the benefits offered by the availability of reliable results within one patient visit, facilitating immediate treatment for these sexually transmitted infections outweigh any potential hazards.

4. Verification of the fail-safe mechanisms

Many of the failsafe and failure alert mechanisms have been tested as part of the instrument and cartridge validation and verification process but some were tested at the "software level" (i.e. as part of simulations during software verification). The following features have been evaluated with Cartridges loaded with samples.

a. Internal Process Control (IPC)

The IPC is designed to guard against a false negative test result due to failures during the analytical steps. For samples with no target detected, an absence of correct detection of the IPC, the instrument will return an "Invalid result." For positive samples, where a signal (peak) from the amplification is detected, the result will be reported as "positive" irrespective of the status of the internal control. Any invalid result invalidates the whole Cartridge result.

The functionality of the IPC was evaluated by testing low positive and negative samples in 3 replicates with cartridges manufactured with, and without, the internal control DNA. All tests returned expected results as shown below showing that the system will not return a negative result if the IPC does not generate expected signal. The study also demonstrated that successful amplification of targets present in the sample will generate positive results independent of the signal generated by the IPC.

Sample	**IPC DNA Present**	**IPC DNA Absent**
Negative sample	CT Not Detected, NG Not Detected (3/3)	Invalid (3/3)
Dual CT/NG Positive	CT Detected, NG Detected (3/3)	CT Detected, NG Detected (3/3)

b. Running a test for an assay with no assay script loaded.

A Cartridge loaded with eNAT buffer was inserted on an *io* Instrument that did not have the CT/NG Assay script loaded. The following prompt was displayed: "The detected cartridge is new to this Instrument. Would you like to install this test software?" The study demonstrated that only Cartridges with a validated software script will be processed by the instrument. This situation is also mitigated by the requirement to run external controls with a new shipment of Cartridges.

c. Running an expired Cartridge

A Cartridge with a passed expiration date was loaded with eNAT buffer and inserted on an *io* Instrument. The following error message was displayed "*The cartridge has passed its expiry date. The test cannot be run*"; and the analysis did not proceed.

d. Reusing a Cartridge that was previously run

The system is designed to destroy the barcode once the Cartridge is inserted and processed, making this cartridge unavailable for re-use, or re-test.

When a previously used Cartridge was loaded with 500 µL eNAT buffer and inserted on an *io* Instrument, the following message was displayed "*The cartridge barcode could not be read. Press 'Eject' to try again or cancel the run*." The only option available to the user is to eject the Cartridge. No options are presented that would allow the Cartridge to run.

e. Loss of power during the run

A CT/NG Cartridge was loaded with 500 µL eNAT buffer and run on an *io* Instrument according to the CT/NG Assay instructions for use. Five minutes into the run, the power cord was pulled out of the rear of the *io* Instrument. After a further one minute, the power cord was replaced and the *io* was powered back on. The following error message was displayed "*Unexpected shutdown recovery. To enable restart to complete, close the instrument drawer. If a cartridge is present, please remove this first*." Following re-start, no results were available for the specimen ID entered, as expected.

f. Running same specimen twice

A CT/NG Cartridge was loaded with 500 µL eNAT buffer and run on the *io* Instrument according to the instructions for use. A specimen ID was selected for use that had been run on the *io* Instrument previously and for which a valid result had been provided. The following error message, stating *"The specimen ID entered has already been used. Please enter a unique specimen ID"* was displayed, demonstrating the failure alert mechanism.

L. Demonstrating "Insignificant Risk of an Erroneous Result" –Accuracy

The accuracy of the binx *io* CT/NG Assay was evaluated in three field studies.

1. Prospective Clinical Study

The clinical performance of the binx *io* CT/NG Assay system was evaluated in a prospective multi-site study which was reviewed and documented under K191352 and K200533.

Briefly, the study was conducted between September 2018 and March 2019 and consisted of prospective enrollment of 2,791 participants (1,634 female and 1,157 male) with signs and symptoms of genitourinary infection or who were at risk of such an infection. The patients were enrolled and tested with the binx *io* CT/NG Assay at 11 individual clinical locations representative of point-of-care (POC) environments and included STD clinics, family planning/OB-GYN clinics, and HIV clinics. The testing was performed by 30 operators who were representative of an intended test user in POC environments, i.e., a member of the healthcare providing team without laboratory training.

The test results from the binx *io* CT/NG Assay were compared to a Composite Infected Status (CIS) comparator derived from an algorithm of results from three FDA-cleared CT/NG nucleic acid amplification tests (NAATs) testing clinician-collected vaginal swab specimens for females and urine specimens for males. All specimens were collected according to the instructions for use in their respective binx collection kits. A total of 1,523 vaginal swab specimens and 922 male urine specimens were included in the calculations of sensitivity and specificity of the binx *io* CT/NG Assay.

The clinical performance of the binx *io* CT/NG Assay for *C. trachomatis* and *N. gonorrhoeae* for each specimen type (female vaginal swabs and male urine) is shown below.

C. trachomatis Clinical Performance of binx health _io_ CT/NG Assay with Vaginal Swab Specimens (Tested by Non-laboratorians)

Symptom Status	N	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)
Asx	706	65	2	634	5	97.0% (89.8; 99.2)	99.2% (98.2; 99.7)
Sx	817	59	3	747	8	95.2% (86.7; 98.3)	98.9% (97.9; 99.5)
Total	**1523**	**124**	**5**	**1381**	**13**	**96.1% (91.3; 98.3)**	**99.1% (98.4; 99.5)**

Asx = Asymptomatic; Sx Symptomatic
TP = True Positive FN = False Negative; TN = True Negative; FP = False Positive
95% Confidence Intervals (CI) by Wilson's Score Method

N. gonorrhoeae Clinical Performance of binx health *io* CT/NG Assay with Vaginal Swab Specimens (Tested by Non-laboratorians)

Symptom Status	N	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)
Asx	706	16	0	689	1	100.0% (80.6; 100.0)	99.9% (99.2; 100.0)
Sx	817	29	0	787	1	100.0% (88.3; 100.0)	99.9% (99.3; 100.0)
Total	**1523**	**45**	**0**	**1476**	**2**	**100.0% (92.1; 100.0)**	**99.9% (99.5; 100.0)**

Asx = Asymptomatic; Sx Symptomatic
TP = True Positive FN = False Negative; TN = True Negative; FP = False Positive
95% Confidence Intervals (CI) by Wilson's Score Method

C. trachomatis Clinical Performance of binx health *io* CT/NG Assay with Male Urine (Tested by Non-laboratorians)

Symptom Status	N	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)
Asx	614	56	4	549	5	93.3% (84.1; 97.4)	99.1% (97.9; 99.6)
Sx	308	55	5	247	1	91.7% (81.9; 96.4)	99.6% (97.8; 99.9)
Total	**922**	**111**	**9**	**796**	**6**	**92.5% (86.4; 96.0)**	**99.3% (98.4; 99.7)**

Asx = Asymptomatic; Sx Symptomatic
TP = True Positive; N = False Negative; TN = True Negative; FP = False Positive
95% Confidence Intervals (CI) by Wilson's Score Method

N. gonorrhoeae Clinical Performance of binx health *io* CT/NG Assay with Male Urine (Tested by Non-laboratorians)

Symptom Status	N	TP	FN	TN	FP	Sensitivity (95% CI)[1]	Specificity (95% CI)[1]
Asx	614	11	1	602	0	91.7% (64.6; 98.5)	100% (99.4; 100)
Sx	308	61	1	246	0	98.4% (91.4; 99.7)	100% (98.5; 100)
Total	**922**	**72**	**2**	**848**	**0**	**97.3% (90.7; 99.3)**	**100% (99.5; 100)**

Asx = Asymptomatic; Sx Symptomatic
TP = True Positive FN = False Negative; TN = True Negative; FP = False Positive
95% Confidence Intervals (CI) by Wilson's Score Method

2. Performance with Samples at Concentrations Near the Cutoff

The binx *io* CT/NG Assay was evaluated in two separate reproducibility studies, conducted at three POC sites with six non-laboratory operators. Because testing female swab and male urine samples follows a different sample handling procedure, it was important to evaluate both workflows when the test is operated by non-laboratorians. Each study included a low-positive and a negative (non-spiked matrix) panel member, in addition to the moderate positive and high positive panel members. The reproducibility of the test system testing female swab samples was conducted between March 26 and April 1, 2019; and with urine samples between February 17 to February 21, 2020; please refer to K191352 and K200533, respectively, for the presentation of data from those two studies.

The data presented in the two tables below show the performance of the binx CT/NG Assay with samples at low organism concentrations, and negative samples, in the hands of non-laboratorian users, as generated in the above studies.

Summary of Results Testing Low Positive Female Vaginal Samples with binx CT/NG Assay by Non-laboratory Users

		Site 1		Site 2		Site 3		All Sites	
Target Conc.		No. Correct/ No. Tested	% Agmt. with Expected Results (95% CI)	No. Correct/ No. Tested	% Agmt. with Expected Results (95% CI)	No. Correct/ No. Tested	% Agmt. with Expected Results (95% CI)	No. Correct/ No. Tested	% Agrmt. with Expected Results (95% CI)
CT Low POS		83/84	98.8% (93.6%-99.8%)	79/84	94.0% (86.8%-97.4%)	83/84	98.8% (93.6%-99.8%)	245/252	97.2% (94.4%-98.7%)
NG Low POS		84/84	100% (95.6%-100%)	83/84	98.8% (93.6%-99.8%)	83/84	98.8% (93.6%-99.8%)	250/252	99.2% (97.2%-99.8%)
Negative	**CT**	26/28	92.9% (77.4%-98.0%)	28/28	100% (87.9%-100%)	26/28	92.9% (77.4%-98.0%)	80/84	95.2% (88.4%-98.1%)
	NG	28/28	100% (87.9%-100%)	28/28	100% (87.9%-100%)	28/28	100% (87.9%-100%)	84/84	100% (95.6%-100%)

Summary of Results Testing Low Positive Male Urine Samples with binx CT/NG Assay by Non-laboratory Users

		Site 1		Site 2		Site 3		All Sites	
Target Conc.		No. Correct/No. Tested	% Agmt. with Expected Results (95% CI)	No. Correct/ No. Tested	% Agmt. with Expected Results (95% CI)	No. Correct/ No. Tested	% Agmt. with Expected Results (95% CI)	No. Correct/ No. Tested	% Agmt. with Expected Results (95% CI)
CT Low POS		84/90	93.3% (86.2%-96.9%)	85/90	94.4% (87.6%-97.6%)	85/90	94.4% (87.6%-97.6%)	254/270	94.1% (90.6%-96.3%)
NG Low POS		84/90	93.3% (86.2%-96.9%)	87/90	96.7% (90.7%-98.9%)	85/90	94.4% (87.6%-97.6%)	256/270	94.8% (91.5%-96.9%)
Negative	**CT**	28/30	93.3% (78.7%-98.2%)	30/30	100% (88.7%-100%)	30/30	100% (88.7%-100%)	88/90	97.8% (92.3%-99.4%)
	NG	30/30	100% (88.7%-100%)	30/30	100% (88.7%-100%)	30/30	100% (88.7%-100%)	90/90	100% (95.9%-100%)

The study results demonstrated that users untrained in laboratory procedures were able to perform the test correctly and the test provided the expected results for samples with organism concentrations near the cut-off.

3. Study with Contrived Samples at CLIA Waived Sites

This study was executed during the COVID-19 pandemic, when conducting prospective clinical studies was exceedingly difficult due to clinics and laboratories operating under reduced capacity with focus on COVID-19-related healthcare. Due to this unprecedented situation, obtaining patient samples for STI testing became extremely challenging. Therefore, this study was designed to augment the already available information about the assay performance in POC environments in the hands of non-laboratorians.

The focus of this study was to demonstrate that untrained operators can perform testing of vaginal swab samples and urine samples using the binx CT/NG Assay accurately and consistently, amidst a busy patient-focused environment. The testing was performed at three outpatient healthcare facilities in three regions of US, performing CLIA waived diagnostic testing while providing healthcare services to the community.

A total of nine untrained Users/Operators were identified (three per site) who performed testing on the binx *io* CT/NG System following only the written instructions provided in the Quick Reference Instructions card over a four-week period.

No training on the use of the binx health *io* Instrument, nor on the use of the chlamydia/gonorrhea (CT/NG) test Cartridges was provided to the operators. Each operator was provided with two *io* Instruments – one to use and a spare in case of any problems. The testing of samples using the binx CT/NG Assay was integrated into the normal daily workflow of the facility, while performing other duties related to patient care. Each of the three operators at each of the sites ran approximately three samples per day. The selected operators included nurses, a physician assistant, and clerical and administrative personnel. The sponsor also provided a full list of the candidate operators who were excluded from the participation in the study. The criteria for operator selection were (a) no previous training or experience with conducting laboratory testing (other than simple CLIA waived tests), and (b) no prior experience using the binx CT/NG Assay.

Because urine samples and vaginal swab samples require a different workflow after sample collection, two sets of samples were used to demonstrate both workflows. All samples were blinded and randomized with respect to the concentration of the target analytes.

1. Female vaginal swab samples were contrived using pooled vaginal swab matrix in eNAT using the binx CT/NG Vaginal Swab Specimen Collection Kit. Three unique pools of negative clinical swab matrix were spiked with the target organisms separately at the target concentrations shown below.

Contrived Vaginal Swab Panels used in the Field Study

	Concentration			
	Low (2x LoD)	**Med (5x LoD)**	**High (20x LoD)**	**Negative (unspiked)**
CT Pos/NG Neg	20	10	5	N/A
CT Neg/NG Pos	20	10	5	N/A
Total	40	20	10	45
TOTAL	70			45

The operators processed the female vaginal swab samples according to the test procedure in the QRI, i.e., (mixing and pipetting the sample into the Cartridge).

2. In contrast to female vaginal swab samples, testing of urine samples requires the user to add the patient's urine into the Male Urine Specimen Collection Kit tube prior to running a test. Therefore, the study was designed to accommodate this step into the workflow to mimic the real-world use of the binx CT/NG Assay. The test samples were contrived by spiking CT and NG organisms directly into eNAT in the Male Urine Specimen Collection Kit tubes. As such, the urine test samples provided to each site for testing on the binx CT/NG Assay consisted of two parts:
    a. Sample tubes containing preservative (either spiked with CT/NG or not), and
    b. Tubes containing pooled male urine, representing the patient's specimen

    Each organism was spiked into the eNAT volume in the Male Collection Kit tubes (444 µL) at a concentration calculated to allow for the addition of 4 mL of a urine sample to achieve the target concentrations, as shown below.

Contrived Male Urine Panels used in the Field Study

	Concentration			
	Low (2x LoD)	Med (5x LoD)	High (20x LoD)	Negative (unspiked)
CT Pos/NG Neg	20	10	5	N/A
CT Neg/NG Pos	20	10	5	N/A
CT Pos/NG Pos	10	5	5	N/A
Total	50	25	15	45
TOTAL	90			45

The study design required the operator to add urine to the sample tube containing preservative, according to the instructions for use in the QRI, using the provided 2 mL pipets supplied in the Male Urine Collection Kit. Once urine was added to the sample collection tubes, users followed the sample preparation instructions in the QRI (mixing by inversion and pipetting into the Cartridge).

The samples were distributed to the three sites, where Site 1 received 83 samples, Site 2 received 84 samples, and Site 3 received 83 samples. All samples were shipped at 2-8 °C and the sites were instructed to keep the panels stored at 2-8 °C up to four weeks. One lot of

Cartridges was used in the study. Each operator performed QC testing once, in accordance with the instructions in the package insert. All QC tests returned expected results.

The testing of female swab samples (total of 115) was conducted between Oct. 26, 2020 and Nov. 11, 2020.

Summary of Results from Testing of Female Vaginal Samples by Untrained Users

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). Based on the analysis of the quantitative electrochemical signal, the FP result was attributed to a very low level of contamination.

Testing of male urine samples (total 135) was conducted between Nov. 4, 2020 and Nov. 17, 2020.

Summary of Results from Testing of Male Urine Samples by Untrained Users

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%)	10/10 (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			

The data showed acceptable results when testing CT/NG positive and negative samples on the binx *io* CT/NG Assay. The summary of results, expressed as positive percent agreement (PPA) and negative percent agreement (NPA), is presented below for each sample type.

Female Vaginal Swabs - *C. trachomatis*
PPA = 100% (35/35), 95% CI (90.1%-100%)
NPA = 98.8% (79/80), 95% CI (93.3%-99.8%)

Female Vaginal Swabs - *N. Gonorrhoeae*
PPA = 100% (35/35), 95% CI (90.1%-100%)
NPA = 100% (80/80), 95% CI (95.4%-100%)

Male Urine - *C. trachomatis*
PPA = 100% (55/55), 95% CI (93.5%-100%)
NPA = 100% (80/80), 95% CI (95.4%-100%)

Male Urine - *N. Gonorrhoeae*
PPA = 100% (55/55), 95% CI (93.5%-100%)
NPA = 100% (80/80), 95% CI (95.4%-100%)

The study demonstrated that untrained non-laboratory users can perform testing using the binx *io* CT/NG Assay accurately amidst performing other patient-related tasks.

There was a total of four samples that initially generated invalid results during the study. Two samples generated a valid result upon one retest. The two invalids obtained by Operator 3 at Site 2 had to be repeated up to 3 times (according to the test procedure) and did not

return a valid result. The operator followed the written procedure, i.e., repeated each sample up to three times and, upon being unsuccessful, contacted the company. The invalid results were investigated, and it was determined that the instrument developed a fault in the detection circuit and required a repair. Those two samples were replaced by the sponsor to complete the data set, i.e., 28 valid results for Operator 3 at site 2. With the exception of one false positive result (mentioned above), there were no erroneous results generated in the study.

4. Operator Questionnaire

Following completion of the study, each operator was asked to complete a short questionnaire about the study on topics covering ease of use, results interpretation, clarity of instructions, etc. All operators found the test easy to use and the instructions easy to follow. With the exception of the single Operator who received invalid results repeatedly on two samples and had to contact the company for further instructions, no other user had the need to contact binx during the study to ask for assistance.

M. Labeling for Waived Devices

The labeling consists of:

1. Assay Instructions for Use (Package insert)
2. Operator Manual for the *io* Instrument
3. Vaginal Swab sample Collection Kit
4. Male Urine sample Collection Kit
5. Quick Reference Instructions (QRI)
6. Quality Controls (Zeptometrix)

The following elements are appropriately present:

- The Quick Reference Instructions (QRI) are written in simple language and contain graphics which visually aid the user in processing samples.
- The labeling identifies the system as CLIA Waived.
- A statement informing the user that the test procedure must be followed as written to maintain the CLIA waived status is present.
- The instrument GUI contains prompts and graphics to ensure that all the procedure steps are performed in the correct sequence.
- Technical support telephone number is prominently displayed.
- All appropriate cautions regarding sample handling and processing are present.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

N. Benefit-Risk Considerations

There has been a significant increase in sexually transmitted infections in recent years, raising a global public health concern. Until now, the testing for *C. trachomatis* and

N. gonorrhoeae has been limited to laboratories that meet the requirements for high or moderate complexity testing, which necessitates sending off specimens and thus delaying the results for at least several days, leading to many patients not returning ("lost to follow up") to obtain treatment. As many patients remain asymptomatic and the infections, if left untreated, can lead to severe reproductive health complications, such as infertility, ectopic pregnancy and congenital infection, as well as an increased risk of acquiring and transmitting HIV, prompt and appropriate treatment is essential in combatting the spread of these STIs.

There have been numerous publications stressing the importance of availability of a POC test for STIs to enable treatment at the time of the patient visit, along with proper counseling and partner management. The binx *io* CT/NG Assay has a small footprint, is easy to use and provides an actionable result in approximately 30 minutes, allowing the clinician to start treatment with appropriate antibiotics immediately. The test was shown to have comparable clinical performance to the laboratory-based tests when used by non-laboratory operators outside of a central laboratory, with sensitivity of and specificity for CT and NG in female vaginal swabs >95%. Although the specificity of the test with male urine was high (>95%) for both CT and NG, the sensitivity for CT in male urine was slightly below the desired 95%; the sensitivity for NG in male urine was >95%. The FDA carefully considered the risk of false negative results for CT in male subjects and, after careful consideration of all the information, including peer-reviewed data, modeled data, and real-world data, FDA concluded that in spite of a slightly lower sensitivity observed for CT in male urine, the use of the device will still result in more cases of infections being treated due to a lowered rate of loss to follow-up. To mitigate the risk of false negative results for CT in male urine, the labeling includes a limiting statement in the intended use: "For a symptomatic male patient with a chlamydia negative test result, further testing with a laboratory-based molecular test is recommended." FDA believes that bringing a POC test for *C. trachomatis* and *N. gonorrhoeae*, the most common sexually transmitted pathogens, into the CLIA waived healthcare settings presents a significant step towards slowing the infection rates for these pathogens. The broadened access of this test to non-traditional healthcare settings, such as doctor's offices, community-based clinics, planned-parenthood clinics, health department clinics and other free-standing counseling testing sites operating under a CLIA Certificate of Waiver, will increase the number of patients screened and treated for these infections. Moreover, by lowering the numbers of untreated cases of STIs, testing with binx CT/NG assay will result in lowered burden of serious sequalae, decreased number of subsequent cases transmitted, and decrease in the administration of empiric antibiotics, which contribute to growing antimicrobial resistance among isolates of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and other bacteria. For those reasons, FDA concluded that the benefits of the device in the CLIA waived healthcare settings outweigh the risks associated with the device.

O. Conclusion:

The submitted information in this CLIA waiver application supports a CLIA waiver approval decision.