Rheonix, Inc
Richard Montagna
Senior Vice President, Scientific & Clinical Affairs
2680 Grand Island Boulevard, Suite 1
Grand Island, New York 14072

December 17, 2021

Re: K193081
Trade/Device Name: Rheonix STI TriPlex Assay, Rheonix EncompassMDx Workstation
Regulation Number: 21 CFR 866.3393
Regulation Name: Device To Detect Nucleic Acids From Non-Viral Microorganism(S) Causing Sexually Transmitted Infections And Associated Resistance Marker(S)
Regulatory Class: Class II
Product Code: QEP, NSU, LSL, OUY, MKZ
Dated: March 31, 2020
Received: April 1, 2020

Dear Richard Montagna:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part

801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K193081

Device Name
Rheonix STI TriPlex Assay
Rheonix Encompass MDx Workstation

Indications for Use *(Describe)*
For In Vitro Diagnostic Use.
The Rheonix STI TriPlex™ Assay, as performed on the Rheonix Encompass MDx® Workstation, is an automated DNA extraction and multiplex PCR amplification test system intended for the direct, qualitative detection of DNA from Chlamydia trachomatis (CT), and/or Neisseria gonorrhoeae (NG), and/or Trichomonas vaginalis (TV) in male urine specimens collected with the Rheonix Urine Specimen Collection Kit. The test is indicated to aid in the diagnosis of chlamydial urogenital disease, gonococcal urogenital disease and trichomoniasis in asymptomatic or symptomatic male individuals.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary
Date of Summary: December 02, 2021

A. APPLICANT

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B. CONTACT PERSON

Richard A. Montagna, PhD, FACB
Senior Vice President, Scientific & Clinical Affairs
Direct line: 716-773-4232, Ext 11.
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C. MEASURAND

Chlamydia trachomatis (CT), *Neisseria gonorrhoeae* (NG), *and Trichomonas vaginalis* (TV) DNA.

D. TYPE OF TEST

Nucleic acid amplification assay (end point polymerase chain reaction)

E. TRADE NAME OF DEVICE(S)

Rheonix STI TriPlex™ Assay on Rheonix Encompass MDx® Workstation

F. IDENTIFICATION OF LEGALLY MARKETED PREDICATE DEVICE

BD MAX CTGCTV2 (assay) and BD MAX™ System (instrument)
510(k) Number: K182692

G. REGULATORY INFORMATION

1. REGULATION SECTION:

21 CFR 866.3393 – Nucleic acid detection system for non-viral microorganism(s) causing sexually transmitted infections

2. CLASSIFICATION:

Class II

3. PRODUCT CODE:

QEP: Nucleic acid detection system for non-viral microorganism(s) causing sexually transmitted infections
OUY: Trichomonas vaginalis Nucleic Acid Amplification Test System
MKZ: DNA Probe, Nucleic Acid Amplification, Chlamydia
LSL: DNA-Reagents, Neisseria
NSU: Instrumentation for Clinical Multiplex Test Systems

4. PANEL:

Microbiology (83)

H. INTENDED USE

1. INTENDED USE(S):

The Rheonix STI TriPlex™ Assay, as performed on the Rheonix Encompass MDx® Workstation, is an automated DNA extraction and multiplex PCR amplification test system intended for the direct, qualitative detection of DNA from *Chlamydia trachomatis* (CT), and/or *Neisseria gonorrhoeae* (NG), and/or *Trichomonas vaginalis* (TV) in male urine specimens collected with the Rheonix Urine Specimen Collection Kit. The test is indicated to aid in the diagnosis of chlamydial urogenital disease, gonococcal urogenital disease and trichomoniasis in asymptomatic or symptomatic male individuals.

2. INDICATION(S) FOR USE:

Same as Intended Use

3. SPECIAL CONDITIONS FOR USE STATEMENT(S):

For prescription use only.

4. SPECIAL INSTRUMENT REQUIREMENTS:

The Rheonix STI TriPlex Assay must be performed on the Rheonix Encompass MDx® Workstation

5. SPECIAL SPECIMEN COLLECTION REQUIREMENTS:

Specimens analyzed using the Rheonix STI TriPlex Assay as performed using the Rheonix Encompass MDx Workstation must be collected in the Rheonix Urine Specimen Collection Kit.

I. DEVICE DESCRIPTION

The Rheonix Encompass MDx® Workstation and the Rheonix STI TriPlex™ Assay are comprised of an instrument with associated hardware and accessories, disposable microfluidic CARD cartridges, master mixes and reagent components used to extract, amplify, and detect DNA using end point PCR. In addition, all male urine specimens in this system must be collected using the Rheonix Urine Specimen Collection Kit. The process is fully automated with the user intervention required only for loading and unloading the samples and disposable assay components. The Rheonix Encompass MDx Workstation's software automatically interprets test results which may be called as POS (positive), NEG (negative), or IND (indeterminate) for each of the assay's three targets. In addition, if the instrument encounters an error during the performance of the assay, it will report an ERR code. If either an IND or ERR code results, the same specimen should be reanalyzed for the presence of the target for which the indeterminate or error code occurred. Each assay has a built-in process control that assures that the individual steps of the entire process occurred properly. The user may also include external positive and/or negative controls to monitor the assay performance.

J. SUBSTANTIAL EQUIVALENCE INFORMATION

1. PREDICATE DEVICE NAME(S):
BD MAX CTGCTV2 (assay) and BD MAX™ System (instrument)

2. PREDICATE 510(K) NUMBER: K182692

3. COMPARISON WITH PREDICATE

Item	Device under investigation	Predicate Device (K182692)
Intended Use	The Rheonix STI TriPlex™ Assay, as performed on the Rheonix Encompass MDx® Workstation, is an automated DNA extraction and multiplex PCR amplification test system intended for the direct, qualitative detection of DNA from *Chlamydia trachomatis* (CT), and/or *Neisseria gonorrhoeae* (NG), and/or *Trichomonas vaginalis* (TV) in male urine specimens collected with the Rheonix Urine Specimen Collection Kit. The test is indicated to aid in the diagnosis of chlamydial urogenital disease, gonococcal urogenital disease and trichomoniasis in asymptomatic or symptomatic male individuals.	The BD MAX CTGCTV2 assay, as performed using the BD MAX System incorporates automated DNA extraction and real-time polymerase chain reaction (PCR) for the direct, qualitative detection of DNA from *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG) and/or *Trichomonas vaginalis* (TV). The assay may be used for detection of CT and/or NG DNA in male urine specimens, and the detection of CT, NG, and/or TV DNA in female urine specimens, clinician-collected female endocervical swab specimens and patient-collected vaginal swab specimens (in a clinical setting). The assay is indicated for use to aid in the diagnosis of chlamydial urogenital disease, gonococcal urogenital disease and/or trichomoniasis in asymptomatic and symptomatic individuals.
Assay Results	Qualitative	Qualitative
Organisms Detected	CT/NG/TV	CT/NG/TV
Instrument	Rheonix Encompass MDx Workstation	BD MAX™ System

Item	Device under investigation	Predicate Device (K182692)
Technology	NAAT using end point PCR with detection via colorimetric detection	NAAT using Real-time multiplex PCR with detection via fluorescence.
Specimen Types	Male urine	Male and female urine, Endocervical swab, and patient-collected vaginal swabs, Liquid-Based Cytology (LBC) specimens

K. STANDARD/GUIDANCE DOCUMENT REFERENCED (IF APPLICABLE)

Establishing the Performance Characteristics of In Vitro Diagnostic Devices for Chlamydia trachomatis and/or Neisseria gonorrhea: Screening and Diagnostic Testing - Draft Guidance for Industry and FDA Staff, May 11, 2011.

Class II Special Controls Guideline: Nucleic Acid Amplification Assays for the detection of *Trichomonas vaginalis* – Guideline for Industry and FDA Staff, August 4, 2015.

EP7-A2, 2005 – Interference Testing in Clinical Chemistry, CLSI Approved Guideline.

EP12-A2, 2008 – User Protocol for Evaluation of Qualitative Test Performance; CLSI Approved Guideline.

EP17-A2, 2012 – Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition

L. TEST PRINCIPLE

The Rheonix STI TriPlex Assay, as performed on the Rheonix Encompass MDx Workstation consists of automated DNA extraction and end-point PCR for the qualitative detection of CT/NG/TV DNA from urogenital specimens. The specimens collected using the Rheonix Urine Specimen Collection Kit are loaded directly onto the Rheonix Encompass MDx Workstation to which the various disposable consumables contained within the Rheonix STI TriPlex Assay have also been loaded by the user. The user interface of the Rheonix Encompass MDx Workstation guides users regarding the proper placement of the various disposable assay consumables and patient specimens. The workstation automates sample preparation, including target organism lysis, DNA extraction and concentration, reagent rehydration and amplification of target DNA using

polymerase chain reaction (PCR). Detection of the various targets and controls is achieved by end point hybridization of the resulting amplicons with DNA probes located on microarray filters contained within the disposable microfluidic CARD cartridges. The Rheonix Encompass MDx Workstation performs results interpretation automatically.

All assay steps are automatically performed within the microfluidic CARD cartridges and include cell lysis, extraction and purification of DNA, multiplex PCR and finally hybridization of the resulting target and control amplicons with their corresponding probes located on the integrated DNA array. The control and target amplicons are each generated using biotinylated primer sets and the hybridization spots are detected by the sequential addition of streptavidinylated horseradish peroxidase and its substrate, 3, 3', 5, 5' tetramethylbenzidine (TMB). The resulting blue colored hybridization spots are detected by the Workstation's CMOS camera and the instrument's imaging software interprets the location and intensity of the various hybridization spots. The results are reported as POS (positive), NEG (negative) or IND (indeterminate), based on how the intensity of the hybridization spots corresponds to the threshold intensities established to differentiate a POS from a NEG result. Specimens that yield signal intensities between the POS and NEG thresholds are reported as IND and should be repeated using the same specimen.

M. PERFORMANCE CHARACTERISTICS (IF/WHEN APPLICABLE)

1. ANALYTICAL PERFORMANCE:

A. PRECISION

Within-laboratory precision was evaluated for the Rheonix STI TriPlex assay at one site. Testing was performed by three operators performing 2 runs/day over 3 nonconsecutive days over a 12-day period with each sample tested in duplicate. The blinded Precision Test Panel (PTP) contained negative male urine specimen matrix individually spiked with CT, NG or TV at the concentrations specified below.:

- True Negative (TN): no targets
- High Negative (HN): 0.25x LoD
- Low Positive (LP): 2x LoD
- Moderate Positive (MP): 5x LoD

Precision study results for the Rheonix STI TriPlex Assay are described in Table 1.

Table 1: Overall Precision Study Results Using One Lot of the Rheonix STI TriPlex Assay Kit

Panel Member	Percent (%) Observed versus Expected		
	C. trachomatis	*N. gonorrhoeae*	*T. vaginalis*
	Urine	Urine	Urine
[a]TN	97.2% (35/36) 85.8% - 99.5%	100% (36/36) 90.4% - 100%	100% (36/36) 90.4% - 100%
[b]HN	33.3% (12/36) 20.2% - 49.7%	85.3% (29/34) 69.9% - 93.6%	72.2% (26/36) 56.0% - 84.2%
LP	91.7% (33/36) 78.2% - 97.1%	100% (36/36) 90.4% - 100%	100% (36/36) 90.4% - 100%
MP	97.2% (35/36) 85.8% - 99.5%	97.2% (35/36) 85.8% - 99.5%	100% (36/36) 90.4% - 100%

[a]*For the True Negative (TN) category, the reported agreement indicates the percent of negative results.*
[b]*For the High Negative (HN) category, the reported agreement indicates the percent of positive results.*

B. REPRODUCIBILITY

Site-to-Site reproducibility of the Rheonix STI TriPlex Assay was evaluated at three sites (two external and one internal). Each site was provided with the same panels as described for the Precision study above. The testing was performed by two operators per site who performed two runs per day (each specimen analyzed in duplicate) over 5 non-consecutive days. The results for Site-to-Site Reproducibility study are shown in Tables 2-5 as below.

Table 2: Rheonix STI TriPlex Assay Site-to-Site Reproducibility Study Results

Panel Member	Percent (%) Observed versus Expected		
	C. trachomatis	*N. gonorrhoeae*	*T. vaginalis*
	Urine	Urine	Urine
[a]TN	100% (120/120) 96.9% - 100%	100% (120/120) 96.9% - 100%	100% (120/120) 96.9% - 100%
[b]HN	44.1% (52/118) 35.4% - 53.1%	79.2% (95/120) 71.1% - 85.5%	78.8% (93/118) 70.6% - 85.2%
LP	100% (120/120) 96.9% - 100%	100% (120/120) 96.9% - 100%	100% (120/120) 96.9% - 100%
MP	100% (120/120) 96.9% - 100%	100% (120/120) 96.9% - 100%	100% (120/120) 96.9% - 100%

[a]*For the True Negative (TN) category, the reported agreement indicates the percent of negative results.*
[b]*For the High Negative (HN) category, the reported agreement indicates the percent of positive results.*

The qualitative reproducibility across sites and by target is presented below in Tables 3 – 5.

Table 3 Percent Agreement with Expected Result by Site for Qualitative CT Results in Urine

Sample ID	Site	Sample Size	Number Positive	Percent Positive Tests	95% CI
TN	1	40	0	0.0%	(0.0%, 8.8%)
	2	40	0	0.0%	(0.0%, 8.8%)
	3	40	0	0.0%	(0.0%, 8.8%)
HN	1	40	18	45.0%	(30.7%, 60.17%)
	2	40	20	50.0%	(35.2%, 64.8%)
	3	38	14	36.8%	(23.4%, 52.7%)
LP	1	40	40	100.0%	(91.2%, 100.0%)
	2	40	40	100.0%	(91.2%, 100.0%)
	3	40	40	100.0%	(91.2%, 100.0%)
MP	1	40	40	100.0%	(91.2%, 100.0%)
	2	40	40	100.0%	(91.2%, 100.0%)
	3	40	40	100.0%	(91.2%, 100.0%)

Table 4 Percent Agreement with Expected Result by Site for Qualitative NG Results in Urine

Sample ID	Site	Sample Size	Number Positive	Percent Positive Tests	95% CI
TN	1	40	0	0.0%	(0.0%, 8.8%)
	2	40	0	0.0%	(0.0%, 8.8%)
	3	40	0	0.0%	(0.0%, 9.0%)
HN	1	40	34	85.0%	(70.9%, 92.9%)
	2	40	31	77.5%	(62.5%, 87.7%)
	3	40	30	75.0%	(59.8%, 85.8%)
LP	1	40	40	100.0%	(91.2%, 100.0%)
	2	40	40	100.0%	(91.2%, 100.0%)
	3	40	40	100.0%	(91.2%, 100.0%)
MP	1	40	40	100.0%	(91.2%, 100.0%)
	2	40	40	100.0%	(91.2%, 100.0%)
	3	40	40	100.0%	(91.2%, 100.0%)

Table 5 Percent Agreement with Expected Result by Site for Qualitative TV Results in Urine

Sample ID	Site	Sample Size	Number Positive	Percent Positive Tests	95% CI
TN	1	40	0	0.0%	(0.0%, 8.8%)
	2	40	0	0.0%	(0.0%, 8.8%)
	3	40	0	0.0%	(0.0%, 9.0%)
HN	1	40	32	80.0%	(65.2%, 89.5%)
	2	40	34	85.0%	(70.9%, 92.9%)
	3	40	27	67.5%	(52.0%, 79.9%)
LP	1	40	40	100.0%	(91.2%, 100.0%)
	2	40	40	100.0%	(91.2%, 100.0%)
	3	40	40	100.0%	(91.2%, 100.0%)
MP	1	40	40	100.0%	(91.2%, 100.0%)
	2	40	40	100.0%	(91.2%, 100.0%)
	3	40	40	100.0%	(91.2%, 100.0%)

For the Lot-to-Lot reproducibility, a single operator completed two separate runs per day on a single instrument for each of three separate lots of Rheonix STI TriPlex Assay kits for 3 non-consecutive days over a 12 day period. The test panel used was the same as described for the Precision study. The results for Lot-to-Lot reproducibility are shown below.

Table 3: Lot-to-Lot Reproducibility

Target	Sample Type	Panel Member ID	Correct	Total	% Correct	95% CI
CT	Urine	TN*	36	36	100	90.4% - 100%
		HN**	14	36	38.9	24.8% - 55.1%
		LP	36	36	100	90.4% - 100%
		MP	35	36	97.2	85.8% - 99.5%
NG		TN*	36	36	100	90.4% - 100%
		HN**	7	35	20.0	10.0% - 35.9%
		LP	36	36	100	90.4% - 100%
		MP	36	36	100	90.4% - 100%
TV		TN*	36	36	100	90.4% - 100%
		HN**	10	36	27.8	15.9% - 44.0%
		LP	36	36	100	90.4% - 100%
		MP	36	36	100	90.4% - 100%

**TN samples do not contain any target analytes. Therefore "% Correct" refers to the percent of negative test results.*
***HN samples, the "% Correct" refers to the percent of positive results.*

C. CARRY OVER/CROSS-CONTAMINATION STUDIES

A study was conducted to investigate within-run carryover and between-run carryover while processing samples with high microbial loads of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* in the Rheonix STI TriPlex Assay. Two different composite samples were tested. One panel member consisted of all three targets at high concentration (*Chlamydia trachomatis,* serovar D at 10^6 IFU/ml, *Neisseria gonorrhoeae,* ATCC strain 49226, at 10^6 CFU/ml and *Trichomonas vaginalis,* ATCC strain 30236 at 10^5 trophozoites/mL) and a second panel member consisted of only the matrix without any spiked targets. The high positive samples were run in a CARD cartridge lane immediately adjacent to lane that contained the negative sample. A total of 144 alternating samples were run across nine days and six runs on a single Rheonix Encompass MDx Workstation. In all cases, the high positive sample yielded positive results for all targets in each run while the negative sample yielded negative results for all targets in each run. Therefore, no carry over or cross contamination was observed.

D. LINEARITY/ASSAY REPORTABLE RANGE

Not applicable.

E. TRACEABILITY, STABILITY, EXPECTED VALUES (CONTROLS, CALIBRATORS, OR METHODS)

Controls:

External controls are not provided by the manufacturer, but commercially available positive and negative controls were used throughout the clinical studies. Contrived positive controls can also be used by spiking *Chlamydia trachomatis* (Serovar H, ATCC VR-879 or Serovar D, ZeptoMetrix Z054), *Neisseria gonorrhoeae* (ATCC 19424 or ATCC 49226) and *Trichomonas vaginalis* (ATCC 30236 or ATCC 50143) into negative matrix. Use of a previously characterized clinical sample known to be positive may also be used. Similarly, a previously characterized clinical sample known to be negative may also be used as a negative control.

An internal process control (a chimeric plasmid containing sequences recognized by each of the three sets of PCR primer pairs) is present in the buffer system used in the Rheonix STI TriPlex assay and therefore can confirm if steps from extraction to signal detection of the assay perform properly.

Specimen Stability Studies:

Male urine specimens must be transferred from the collection cup into the transport buffer immediately after collection and are stable for 120 days when stored at 2-8°C or 75 days when stored at 30°C. (Table 7).

Table 4: Sample Stability

Specimen Type	Transport and/or Storage Temperature	
	2-8 °C	30 °C
Male urine	120 days	75 days

Device Stability Studies

1. **Rheonix STI TriPlex Assay**
   When properly stored under recommended conditions at room temperature (i.e., 15 °C – 30 °C), the Rheonix STI TriPlex Assay kit is stable for 24 months.

2. **Rheonix Urine Specimen Collection Kit**
   When properly stored under recommended conditions at room temperature (i.e., 15 °C – 30 °C), the Rheonix Urine Specimen Collection Kit is stable for 6 months.

F. DETECTION LIMITS

The Limit of Detection (LoD) for the Rheonix STI TriPlex Assay in male urine was determined by testing two representative strains of each target organism detected by the assay. Each target organism was prepared and quantified prior to testing and inoculated into pooled male urine matrix at multiple concentrations. The samples were then loaded into the Rheonix Encompass MDx® Workstation in a manner identical to that used for authentic clinical specimens. Each matrix suspension was tested using at least 20 replicates per LoD concentration. Once the preliminary LoD was determined, the LoD was confirmed by evaluating 44 replicates at the presumed LoD concentration. The LoD was defined as the lowest concentration at which at least 95% of all replicates gave a positive test result. The final LoDs for urine matrix are presented in Table 8.

Table 5: Limit of Detection (LoD) for the Rheonix STI TriPlex Assay

Organism	Strain	Specimen	LoD Concentration (units/ml)*
Chlamydia trachomatis	Serovar D	Male Urine	19 IFU/ml
	Serovar H		26 IFU/ml
Neisseria gonorrhoeae	ATCC 49226		180 CFU/ml
	ATCC 19424		110 CFU/ml
Trichomonas vaginalis	ATCC30236**		4 Trophozoites/ml
	ATCC 50143***		5 Trophozoites/ml

**IFU refers to Inclusion Forming Units and CFU refers to colony forming units.*
*** Metronidazole (MTZ) sensitive*
****MTZ resistant*

Inclusivity

Once the LoD was established, an additional 13 serovars of CT, 30 strains of NG and 6 strains of TV were tested in 20 replicates at the most challenging LoDs noted above for each target in pooled urine matrix. If the initial concentration tested yielded lower than 95% positive results for a particular strain, then additional replicates were tested at higher target concentration.

For CT: of all 13 additional strains tested, 11 were detected at 3xLoD or lower target concentrations, and strains L1 and Ba were detected at higher concentrations with ≥95% positivity, as shown in the table below.

For NG: all 30 strains of NG were detected at 95% positive rate or higher at 110 CFU/mL in urine matrix.

For TV: all six strains of TV were detected at 95% positive rate or higher at 4 trophozoites/mL in urine matrix.

The results of the inclusivity study are shown below.

Table 9. Results for Additional Strains of CT

CT Serovar	IFU/mL	x LoD	%Pos
A	11	0.6	100
B	11	0.6	100
C	33	1.7	100
E, nvCT	22	1.2	100
F	11	0.6	100
I	22	1.2	100
J	55	2.9	100
K	33	1.7	95
G	33	1.7	100
L1	154	8.1	100
L2	11	0.6	100
L3	55	2.9	100
Ba	110	5.8	95

Table 10. Results for Additional Strains of NG

NG Strain	CFU/ml	x LoD	%Pos	NG Strain	CFU/ml	x LoD	%Pos
Z423	110	1	100	**Z438**	110	1	100
Z424	110	1	100	**Z439**	110	1	100
Z425	110	1	100	**Z440**	110	1	100
Z426	110	1	100	**Z441**	110	1	100
Z427	110	1	100	**Z442**	110	1	100
Z428	110	1	95	**Z443**	110	1	100
Z429	110	1	100	**Z444**	110	1	100
Z430	110	1	100	**Z445**	110	1	100
Z431	110	1	100	**Z446**	110	1	100
Z432	110	1	100	**Z448**	110	1	100
Z433	110	1	100	**Z449**	110	1	100
Z434	110	1	100	**Z450**	110	1	100
Z435	110	1	100	**Z451**	110	1	100
Z436	110	1	100	**Z452**	110	1	100
Z437	110	1	100	**Z466**	110	1	100

Table 11. Results for Additional Strains of TV

TV Strain	Trophozoites/ml	x LoD	%Pos
Z070	4	1	100
CDC252*	4	1	100
Z158	4	1	100
Z159	4	1	100
ATCC 30238	4	1	100
ATCC 30001	4	1	100

**MTZ resistant*

G. ANALYTICAL SPECIFICITY

The Rheonix STI TriPlex Assay was performed on samples containing phylogenetically related species and other microorganisms that potentially could be found in urogenital specimens to determine if the other microorganisms might cross-react with any of the three targets of the assay. The bacteria, yeasts, and parasites were tested in the urine matrix at 1 x 10^6 cells/ml and viruses were tested in the same matrix at 1 x 10^5 viral particles/ml. Each individual microorganism was tested in triplicate and considered potentially cross-reactive if one or more test replicates yielded a positive result. Of the 156 non-target organisms tested (Table 12), 154 gave negative results for all three replicates. *Herpes Simplex Virus*, Type I gave one positive result out of three replicates for TV while *Neisseria meningitides* serogroup D gave one positive result out of three replicates for NG. Upon retest in triplicate, however, all replicates for both microorganisms gave negative results.

Table 6: Rheonix STI TriPlex Assay Specificity Results (Bacteria, Yeasts, and Viruses)

Microorganisms Tested	
Achromobacter xerosis	*Chlamydophila (Chlamydia) psittaci*
Acinetobacter calcoaceticus	*Chlamydophila (Chlamydia) psittaci*
Acinetobacter lwoffii	*Chromobacterium violaceum*
Actinomyces israelii	*Citrobacter freundii*
Actinomyces pyogenes (Trueperella pyogenes)	*Clostridium difficile*
Aerococcus viridans	*Clostridium perfringens*
Aeromonas hydrophila	*Corynebacterium genitalium*
Alcaligenes faecalis	*Corynebacterium xerosis*
Atopobium vaginae	*Cryptococcus neoformans*
Bacillus subtilis	*Cytomegalovirus (CMV)*
Bacteroides fragilis	*Deinococcus radiodurans*
Bergeriella (Neisseria) denitrificans	*Derxia gummosa*
Bifidobacterium adolescentis	*Eikenella corrodens*
Bifidobacterium breve	*Elizabethkingia meningoseptica (Flavobacterium melingosepticum)*
Blautia producta (Peptostreptococcus productus)	*Enterobacter aerogenes*
Brevibacterium linens	*Enterobacter cloacae*
Campylobacter jejuni	*Enterococcus avium*
Campylobacter ureolyticus (Bacteroides ureolyticus)	*Enterococcus faecalis*
Candida albicans	*Enterococcus faecium*
Candida glabrata	*Erysipelothrix rhusiopathiae*
Candida parapsilosis	*Escherichia coli*
Candida tropicalis	*Fusobacterium nucleatum*
Chlamydophila pneumoniae	*Gardnerella vaginalis*

Microorganisms Tested	
Gemella haemolysans	*Neisseria cinerea*
Haemophilus ducreyi	*Neisseria cinerea*
Haemophilus influenzae	*Neisseria elongata*
*Herpes Simplex Virus , type I (HSV1)**	*Neisseria elongata*
Herpes Simplex Virus , type II (HSV2)	*Neisseria elongata*
HIV type 1	*Neisseria flavescens*
HPV 16	*Neisseria flavescens*
Weissella paramesenteroides (Leuconostoc paramensenteroides)	*Neisseria lactamica*
Human Papilloma Virus 6	*Neisseria lactamica*
Kingella denitrificans	*Neisseria lactamica*
Kingella kingae	*Neisseria lactamica*
Klebsielia oxytoca	*Neisseria lactamica*
Klebsiella pneumoniae	*Neisseria lactamica*
Lactobacillus acidophilus	*Neisseria lactamica*
Lactobacillus brevis	*Neisseria lactamica*
Lactobacillus crispatus	*Neisseria lactamica*
Lactobacillus jensenii	*Neisseria meningitidis serogroup A*
Lactobacillus debrueckii (lactis)	*Neisseria meningitidis serogroup B*
Lactobacillus vaginalis	*Neisseria meningitidis serogroup C*
Legionella pneumophila	*Neisseria meningitidis serogroup C*
Legionella pneumophila	*Neisseria meningitidis serogroup C*
Listeria monocytogenes	*Neisseria meningitidis serogroup C*
Micrococcus luteus	*Neisseria meningitidis serogroup D**
Mobiluncus curtisii	*Rhodospirillum rubrum*
Moraxella (Branhamella) catarrhalis	*Saccharomyces cerevisiae*
Moraxella lacunata	*Yersinia enterocolitica*
Moraxella osloensis	*Neisseria meningitidis serogroup W135*
Morganella morganii	*Neisseria meningitidis serogroup Y*
Mycobacterium smegmatis	*Neisseria mucosa*
Mycoplasma genitalium	*Neisseria mucosa*
Mycoplasma hominis	*Neisseria mucosa*
Neisseria cinerea	*Neisseria polysaccharea*
Neisseria cinerea	*Neisseria sicca*
Neisseria sicca	*Pseudomonas aeruginosa*
Neisseria sicca	*Pseudomonas fluorescens*
Neisseria subflava biovar flava	*Pseudomonas putida*
Neisseria subflava biovar flava	*Rahnella aquatilis*
Neisseria subflava biovar perflava	*Rhizobium (Agrobacterium) radiobacter*
Neisseria subflava biovar perflava	*Salmonella enterica minnesota*
Neisseria subflava biovar perflava	*Salmonella typhimurium*
Neisseria subflava biovar perflava	*Serratia marcescens*
Neisseria subflava biovar perflava	*Staphylococcus aureus*
Neisseria subflava biovar perflava	*Staphylococcus epidermidis*
Neisseria subflava biovar subflava	*Staphylococcus saprophyticus*
Pantoea agglomerans (Erwinia herbicola)	*Streptococcus agalactiae*
Paracoccus denitrificans	*Streptococcus bovis*
Pentatrichomonas hominis	*Streptococcus mitis*
Peptostreptococcus anaerobius	*Streptococcus mutans*
Peptostreptococcus magnus (Finegoldia magna)	*Streptococcus pneumoniae*
Plesiomonas shigelloides	*Streptococcus pyogenes*
Prevotella bivia	*Streptococcus salivarius*
Propionibacterium acnes	*Streptococcus sanguinis*
Proteus mirabilis	*Trichomonas tenax*
Proteus vulgaris	*Ureaplasma urealyticum*
Providencia stuartii	*Vibrio parahaemolyticus*

INTERFERING SUBSTANCES

A total of 26 potentially interfering substances that could be present in urogenital tract specimens were evaluated at concentrations selected to be medically relevant. The testing was performed by analyzing pooled male urine that contained a mixture of the three targets spiked at 1.5 x their respective LoDs. Since a small percentage of samples analyzed at 1.5 x LoD would be expected to return negative results, even in the absence of any interfering substances, if potential interference was observed at 1.5 x LoD, the potential interfering substance was retested using matrix spiked with 3 x their respective LoDs. None of the substances tested yielded interference at the concentrations noted in the following tables.

Table 13. Results for Interfering Substances Studies

Substances Tested in Urine Matrix	
Interfering substance	**Concentration**
Whole Blood	2% (v/v)
Semen	5% (v/v)
Hormones	0.48 ng/mL 17-α-Ethinylestradiol
AntiProtozoal (Metronidazole)	48 µg/mL
Glucose	0.48 mg/mL
Acetylsalicylic Acid	260.8 µg/mL
Azithromycin	4.8 µg/mL
Phenazopyridine Hydrochloride	80 µg/mL
Norithindrone	8 ng/mL
4-Acetaminophenol	80 µg/mL
Naproxen	200 µg/mL
Ibuprofen	200 µg/mL
Amoxicillin Trihydrate	30.08 µg/mL
Tetracycline Hydrochloride	6 µg/mL
Ceftriaxone	324.4 µg/mL
Sulfamethoxazole	160 µg/mL
Trimethoprim	16 µg/mL L
Erythromycin	24 µg/mL
Human Serum Albumin	0.4 mg/mL
Leukocytes	10^6 cells/mL
Feminine Deodorant Spray	0.68% v/v
Talcum Powder[a]	0.6% w/v
Bilirubin	0.08 mg/mL
Biotin	3500 ng/ml
Urine (high pH)	pH 9
Urine (low pH 4)	pH 4

[a] *May interfere with the Rheonix STI TriPlex assay when at concentrations higher than shown.*

MIXED INFECTION/COMPETITIVE INTERFERENCE

The mixed infection/competitive interference study was designed to evaluate the ability of the Rheonix STI TriPlex Assay to detect low positive results in the presence of the other targets at high concentrations in urine matrix. Two (2) organisms (*Neisseria gonorrhoeae* and *Trichomonas vaginalis*) were individually prepared at 1.5X their respective LoD and *Chlamydia trachomatis* was prepared at 1.7X its respective LoD to serve as a low target in the Rheonix STI Collection Buffer. When added as a high target to the mixture, CT was added at 10^6 IFU/ml, NG was added at 10^6 CFU/ml and TV was added at 10^5 trophozoites/ml. No interference was observed when either CT, NG or TV were tested in the presence of exceedingly high concentrations of the other two targets.

H. ASSAY CUT-OFF

The Encompass MDx workstation's optical system and software evaluates the intensity of the end point PCR hybridization spots on the DNA array contained within the CARD cartridge devices used to perform the assay. Based on predetermined intensity values, the software reports the results as either Positive, Negative, Indeterminate or an Error Code for each of the three targets. Intensity values equal to or less than the lower cut off value generate a negative result, values between the lower and the upper cut offs generate an indeterminate result, and values equal to or greater than the upper cut off generate a positive result. If the workstation encounters any unacceptable assay parameters, appropriate Error code messages appear on the final report.

2. COMPARISON STUDIES

A. METHOD COMPARISON WITH PREDICATE DEVICE:

Not Applicable.

B. MATRIX COMPARISON

Not Applicable.

3. CLINICAL STUDIES

A multi-site and geographically diverse study was conducted to evaluate the Rheonix STI TriPlex Assay as performed on the Rheonix Encompass MDx Workstation using male urine specimens collected in the Rheonix Urine Specimen Collection Kit.

During the clinical study to evaluate the performance of the Rheonix STI TriPlex Assay, specimens from a total of 1627 male subjects (aged 14 years and older) were collected at 8 geographically distinct sites in US. The study enrolled both symptomatic and asymptomatic subjects. One first-catch urine specimen was collected from each subject. The collected specimen from each subject was subsequently aliquoted and transferred into a total of four different manufacturers' transport tubes (one from Rheonix Urine Specimen Collection kit and three others for reference methods). All processed urine specimens were shipped via overnight courier to a central laboratory for Patient

Infection Status (PIS) testing and then distributed to one of three separate testing laboratories for test by Rheonix STI TriPlex assay.

Out of the total of 1627 subjects, 14 were excluded from test for reasons including ineligibility issues (N = 1), Patient Withdrawal (N=3), sample mishandling/shipping issues (N=10). Of the remaining 1613 evaluable subjects, 1606 were evaluated for CT and NG (7 subjects were excluded from performance analysis due to testing noncompliance or unevaluable PIS testing results), while 1586 subjects were evaluated for TV (27 were excluded from performance analysis due to testing noncompliance or unevaluable PIS testing results). Of the 1586 subjects evaluated for TV, results from one subject were not included in the calculation of Sensitivity and Specificity for TV because of invalid test results in Rheonix STI TriPlex Assay. Therefore, results from a total of 1585 subjects were used to calculate the Sensitivity and Specificity of the Rheonix STI TriPlex Assay for the TV target while results from a total of 1606 subjects were used to calculate the Sensitivity and Specificity for the CT and NG targets.

The performance characteristics of the Rheonix STI TriPlex Assay was then compared to the PIS results determined from analysis of the comparator test results. For males the PIS testing for CT, NG and TV consisted of up to three different FDA cleared Nucleic Acid Amplification Tests (NAATs). When the first two NAATs yielded either two positive or two negative results, the PIS was defined as either PIS Positive or PIS Negative, respectively. If the first two PIS tests were not concordant, then a third "tie-breaker" test was used to establish the PIS of the specimen whereby the PIS was considered either Positive or Negative based on the results of 2 out of the 3 results being either positive or negative, respectively.

1) The prevalence of each pathogen at each clinical study site and all sites combined based on the comparator results is shown below.

Table 14 Prevalence of each pathogen at each clinical study site and all sites combined based on the comparator results

Collection Site	Positivity Rate based on PIS		
	CT	NG	TV
1	20.4%	18.6%	7.1%
2	4.6%	7.2%	0.0%
3	9.7%	4.7%	1.7%
4	13.5%	5.0%	1.6%
5	12.2%	2.0%	2.0%
6	9.1%	10.6%	5.1%
7	1.9%	4.8%	0.0%
8	23.1%	23.1%	0.0%
All Sites	10.1%	7.1%	2.2%

2) Clinical Sensitivity and Specificity

Based upon comparison against the PIS of each enrollee, the following performance estimates were calculated for each of the analytes:

Table 15 Clinical Performance for CT Detection

Gender	Specimen	Symptom	CT						
			N	TP	FP	TN	FN	%Sens (95% CI)	%Spec (95% CI)
Male	Urine	A	1202	96	0	1104	2	98% (92.9% - 99.4%)	100.0% (99.7% - 100.0%)
		S	404	62	0	339	3	95.4% (87.3% - 98.4%)	100.0% (98.9% - 100.0%)
		ALL	1606	158	0	1443	5	96.9% (93.0% - 98.7%	100.0% (99.7% - 100.0%)

Table 16 Clinical Performance for NG Detection

Gender	Specimen	Symptom	NG						
			N	TP	FP	TN	FN	%Sens (95% CI)	%Spec (95% CI)
Male	Urine	A	1201	14	0	1187	0	100.0% (78.5% - 100.0%)	100.0% (99.7% - 100.0%)
		S	405	99	0	305	1	99% (94.6% - 99.8%)	100.0% (98.8% - 100.0%)
		ALL	1606	113	0	1492	1	99.1% (95.2% - 99.8%)	100.0% (99.7% - 100.0%)

Table 17 Clinical Performance for TV Detection

Gender	Specimen	Symptom	TV						
			N	TP	FP	TN	FN	%Sens (95% CI)	%Spec (95% CI)
Male	Urine	A	1187	22	1	1163	1	95.7% (79.0% - 99.2%)	99.9% (99.5% - 100.0%)
		S	398	12	0	386	0	100% (75.8% - 100.0%)	100% (99.0% - 100.0%
		ALL	1585*	34	1	1549	1	97.1% (85.5% - 99.5%)	99.9% (99.6% - 100.0%)

*One of the tested 1586 subjects was excluded from the above performance analysis due to the lack of valid result for Rheonix STI TriPlex assay.

3) Rate of non-reportable results

Indeterminate or Error Code Results

The Rheonix Encompass MDx workstation reports results as Positive, Negative or Indeterminate for each of the three target microorganisms. In addition, the workstation also reports a variety of

error codes (ERR) that would require testing. On a target-by-target basis, the combined IND and ERR code rates are reported (Table 18). The values reported as "Initial" represent samples that displayed either an IND or ERR code that required a reanalysis. The "Unresolved" values represent those samples that, upon repeat, did not yield valid results. As noted, only one urine specimen yielded an unresolved final result, also representing 0.06% of the male population evaluated. All other initially failed runs yielded valid, interpretable results upon repeat testing.

Table 18 Rates of Initial and Final Unresolved Results

Subject	Chlamydia trachomatis			Neisseria gonorrhoeae			Trichomonas vaginalis		
	Total N	Initial	Unresolved	Total N	Initial	Unresolved	Total N	Initial	Unresolved
Male	1606	20 (1.3%)	0 (0.0%)	1606	16 (1.0%)	0 (0.0%)	1586	24 (1.5%)	1 (0.1%)
		95% CI			95% CI			95% CI	
		(0.8% - 1.9%)	(0.0% - 0.2%)		(0.6% - 1.6%)	(0.0% - 0.2%)		(1.0% - 2.2%)	(0.0% - 0.04%)

4) <u>Hypothetical Positive and Negative Predictive Values</u>

The hypothetical Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated for urine specimens evaluated from male subjects. The calculations are based on the observed clinical sensitivity and clinical specificity for each specimen type, as compared to the Patient Infection Status (Table 19).

Table 19 Hypothetical Positive and Negative Predictive Values of the Rheonix STI TriPlex Assay

Specimen Type	Hypothetical Prevalence	*Chlamydia trachomatis*				*Neisseria gonorrhoeae*				*Trichomonas vaginalis*			
		% Sen	% Spec	% PPV	% NPV	% Sen	% Spec	% PPV	% NPV	% Sen	% Spec	% PPV	% NPV
Male Urine	1%	96.9%	100%	100%	100%	99.1	100	100 %	100%	97.1	99.9	90.8%	100%
	2%			100%	99.9%			100 %	100%			95.2%	99.9%
	5%			100%	99.8%			100%	100%			98.1%	99.9%
	10%			100%	99.7%			100 %	99.9%			99.1%	99.7%
	15%			100%	99.5%			100 %	99.8%			99.4%	99.5%
	20%			100%	99.2%			100 %	99.8%			99.6%	99.3%
	25%			100%	99.0%			100 %	99.7%			99.7%	99.0%

4. CLINICAL CUT-OFF

Not Applicable

5. EXPECTED VALUES/REFERENCE RANGE

The prevalence of infection with CT and/or NG and or TV in patient populations is dependent upon multiple risk factors including age, gender, type of collection site clinic, and the sensitivity of

the test used to detect the three target microorganisms. The observed positivity rates observed using the Rheonix STI TriPlex Assay in male (Table 20) subjects enrolled in the studies are provided.

Table 20. Rheonix STI TriPlex Observed Positivity Rates in Male Subjects

Collection Site	Positivity Rate as Determined by Rheonix STI TriPlex™ Assay by Clinical Site		
	CT	NG	TV
1	19.5%	17.7%	7.1%
2	4.6%	7.2%	0.0%
3	9.7%	4.7%	1.7%
4	13.2%	5.0%	1.3%
5	10.9%	2.0%	2.0%
6	8.7%	10.6%	5.9%
7	1.9%	4.8%	0.0%
8	23.1%	23.1%	0.0%
All Sites	9.8%	7.0%	2.2%

N. INSTRUMENT NAME

Rheonix Encompass MDx® Workstation.