State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
Post Office Box 50130
Charleston, WV 25305-0130

Request for Quotation

RFQ NUMBER	PAGE
LBS12044	1

ADDRESS CORRESPONDENCE TO ATTENTION OF:
ROBERTA WAGNER
304-558-0067

VENDOR
RFQ COPY
TYPE NAME/ADDRESS HERE

ORIGINAL

SHIP TO
HEALTH AND HUMAN RESOURCES
BPH - LABORATORY SERVICES

167-ELEVENTH AVENUE
SOUTH CHARLESTON, WV
25303 304-558-3530

DATE PRINTED	TERMS OF SALE	SHIP VIA	F.O.B.	FREIGHT TERMS
11/18/2011				

BID OPENING DATE: 12/22/2011 BID OPENING TIME 01:30PM

LINE	QUANTITY	UOP	CAT. NO.	ITEM NUMBER	UNIT PRICE	AMOUNT
				OPEN-END BLANKET CONTRACT		
0001	1	JB		193-36		

REAGENTS FOR AN AUTOMATED PROCESSING SYSTEM

TO PROVIDE AN FDA CLEARED FULLY AUTOMATED PROCESSING SYSTEM AND REAGENTS TO PERFORM NUCLEIC ACID AMPLIFICATION TESTS (NAAT) FOR THE SIMULTANEOUS DETECTION AND DIFFERENTIATION OF CHLAMYDIA TRACHOMATIS (CT) AND/OR NEISSERIA GONORRHOEAE (GC) ON URINE, CERVICAL, URETHRA OR VAGINAL SWAB SPECIMENS AND TO PERFORM THE TESTING BASED ON THE ATTACHED SPECIFICATIONS.

MUST BE FDA APPROVED

ALL EQUIPMENT, MAINTENANCE AND REPAIR PARTS ON THIS CONTRACT SHALL BE PROVIDED FREE OF CHARGE.

RECEIVED
2011 DEC 20 A 10:32
PURCHASING DIVISION
STATE OF WV

SHIPPING TERMS ARE TO BE F.O.B. DESTINATION UNLESS OTHERWISE STATED IN VENDOR'S QUOTE.

EXHIBIT 3

LIFE OF CONTRACT: THIS CONTRACT BECOMES EFFECTIVE AWARD.....2011 AND EXTENDS FOR A PERIOD OF ONE (1

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE	TELEPHONE	DATE
	800-523-5001	12/19/11

TITLE	FEIN	ADDRESS CHANGES TO BE NOTED ABOVE
VP, AMERICAS & ASIA PAC	33-0767987	

WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

GENERAL TERMS & CONDITIONS
REQUEST FOR QUOTATION (RFQ) AND REQUEST FOR PROPOSAL (RFP)

1. Awards will be made in the best interest of the State of West Virginia.

2. The State may accept or reject in part, or in whole, any bid.

3. Prior to any award, the apparent successful vendor must be properly registered with the Purchasing Division and have paid the required $125 fee.

4. All services performed or goods delivered under State Purchase Order/Contracts are to be continued for the term of the Purchase Order/Contracts, contingent upon funds being appropriated by the Legislature or otherwise being made available. In the event funds are not appropriated or otherwise available for these services or goods this Purchase Order/Contract becomes void and of no effect after June 30.

5. Payment may only be made after the delivery and acceptance of goods or services.

6. Interest may be paid for late payment in accordance with the *West Virginia Code.*

7. Vendor preference will be granted upon written request in accordance with the *West Virginia Code.*

8. The State of West Virginia is exempt from federal and state taxes and will not pay or reimburse such taxes.

9. The Director of Purchasing may cancel any Purchase Order/Contract upon 30 days written notice to the seller.

10. The laws of the State of West Virginia and the *Legislative Rules* of the Purchasing Division shall govern the purchasing process.

11. Any reference to automatic renewal is hereby deleted. The Contract may be renewed only upon mutual written agreement of the parties.

12. BANKRUPTCY: In the event the vendor/contractor files for bankruptcy protection, the State may deem this contract null and void, and terminate such contract without further order.

13. HIPAA BUSINESS ASSOCIATE ADDENDUM: The West Virginia State Government HIPAA Business Associate Addendum (BAA), approved by the Attorney General, is available online at www.state.wv.us/admin/purchase/vrc/hipaa.htm and is hereby made part of the agreement. Provided that the Agency meets the definition of a Cover Entity (45 CFR §160.103) and will be disclosing Protected Health Information (45 CFR §160.103) to the vendor.

14. CONFIDENTIALITY: The vendor agrees that he or she will not disclose to anyone, directly or indirectly, any such personally identifiable information or other confidential information gained from the agency, unless the individual who is the subject of the information consents to the disclosure in writing or the disclosure is made pursuant to the agency's policies, procedures, and rules. Vendor further agrees to comply with the Confidentiality Policies and Information Security Accountability Requirements, set forth in http://www.state.wv.us/admin/purchase/privacy/noticeConfidentiality.pdf.

15. LICENSING: Vendors must be licensed and in good standing in accordance with any and all state and local laws and requirements by any state or local agency of West Virginia, including, but not limited to, the West Virginia Secretary of State's Office, the West Virginia Tax Department, and the West Virginia Insurance Commission. The vendor must provide all necessary releases to obtain information to enable the director or spending unit to verify that the vendor is licensed and in good standing with the above entities.

16. ANTITRUST: In submitting a bid to any agency for the State of West Virginia, the bidder offers and agrees that if the bid is accepted the bidder will convey, sell, assign or transfer to the State of West Virginia all rights, title and interest in and to all causes of action it may now or hereafter acquire under the antitrust laws of the United States and the State of West Virginia for price fixing and/or unreasonable restraints of trade relating to the particular commodities or services purchased or acquired by the State of West Virginia. Such assignment shall be made and become effective at the time the purchasing agency tenders the initial payment to the bidder.

I certify that this bid is made without prior understanding, agreement, or connection with any corporation, firm, limited liability company, partnership, or person or entity submitting a bid for the same material, supplies, equipment or services and is in all respects fair and without collusion or fraud. I further certify that I am authorized to sign the certification on behalf of the bidder or this bid.

INSTRUCTIONS TO BIDDERS

1. Use the quotation forms provided by the Purchasing Division. Complete all sections of the quotation form.

2. Items offered must be in compliance with the specifications. Any deviation from the specifications must be clearly indicated by the bidder. Alternates offered by the bidder as EQUAL to the specifications must be clearly defined. A bidder offering an alternate should attach complete specifications and literature to the bid. The Purchasing Division may waive minor deviations to specifications.

3. Unit prices shall prevail in case of discrepancy. All quotations are considered F.O.B. destination unless alternate shipping terms are clearly identified in the quotation.

4. All quotations must be delivered by the bidder to the office listed below prior to the date and time of the bid opening. Failure of the bidder to deliver the quotations on time will result in bid disqualifications: Department of Administration, Purchasing Division, 2019 Washington Street East, P.O. Box 50130, Charleston, WV 25305-0130

5. Communication during the solicitation, bid, evaluation or award periods, except through the Purchasing Division, is strictly prohibited (W.Va. C.S.R. §148-1-6.6).

State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
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SHIP TO
HEALTH AND HUMAN RESOURCES
BPH - LABORATORY SERVICES

167-ELEVENTH AVENUE
SOUTH CHARLESTON, WV
25303 304-558-3530

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YEAR OR UNTIL SUCH "REASONABLE TIME" THEREAFTER AS IS NECESSARY TO OBTAIN A NEW CONTRACT OR RENEW THE ORIGINAL CONTRACT. THE "REASONABLE TIME" PERIOD SHALL NOT EXCEED TWELVE (12) MONTHS. DURING THIS "REASONABLE TIME" THE VENDOR MAY TERMINATE THIS CONTRACT FOR ANY REASON UPON GIVING THE DIRECTOR OF PURCHASING 30 DAYS WRITTEN NOTICE.

UNLESS SPECIFIC PROVISIONS ARE STIPULATED ELSEWHERE IN THIS CONTRACT DOCUMENT, THE TERMS, CONDITIONS AND PRICING SET HEREIN ARE FIRM FOR THE LIFE OF THE CONTRACT.

RENEWAL: THIS CONTRACT MAY BE RENEWED UPON THE MUTUAL WRITTEN CONSENT OF THE SPENDING UNIT AND VENDOR, SUBMITTED TO THE DIRECTOR OF PURCHASING THIRTY (30) DAYS PRIOR TO THE EXPIRATION DATE. SUCH RENEWAL SHALL BE IN ACCORDANCE WITH THE TERMS AND CONDITIONS OF THE ORIGINAL CONTRACT AND SHALL BE LIMITED TO TWO (2) ONE (1) YEAR PERIOD.

CANCELLATION: THE DIRECTOR OF PURCHASING RESERVES THE RIGHT TO CANCEL THIS CONTRACT IMMEDIATELY UPON WRITTEN NOTICE TO THE VENDOR IF THE COMMODITIES AND/OR SERVICE SUPPLIED ARE OF AN INFERIOR QUALITY OR DO NOT CONFORM TO THE SPECIFICATIONS OF THE BID AND CONTRACT HEREIN.

OPEN MARKET CLAUSE: THE DIRECTOR OF PURCHASING MAY AUTHORIZE A SPENDING UNIT TO PURCHASE ON THE OPEN MARKET, WITHOUT THE FILING OF A REQUISITION OR COST ESTIMATE, ITEMS SPECIFIED ON THIS CONTRACT FOR IMMEDIATE DELIVERY IN EMERGENCIES DUE TO UNFORESEEN CAUSES (INCLUDING BUT NOT LIMITED TO DELAYS IN TRANSPORTATION OR AN UNANTICIPATED INCREASE IN THE VOLUME OF WORK.)

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE [signature] TELEPHONE 800-523-5001 DATE 12/19/11

TITLE VP, AMERICAS & ASIA PACIFIC FEIN 330767987 ADDRESS CHANGES TO BE NOTED ABOVE

WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
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304-558-0067

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QUANTITIES: QUANTITIES LISTED IN THE REQUISITION ARE APPROXIMATIONS ONLY, BASED ON ESTIMATES SUPPLIED BY THE STATE SPENDING UNIT. IT IS UNDERSTOOD AND AGREED THAT THE CONTRACT SHALL COVER THE QUANTITIES ACTUALLY ORDERED FOR DELIVERY DURING THE TERM OF THE CONTRACT, WHETHER MORE OR LESS THAN THE QUANTITIES SHOWN.

ORDERING PROCEDURE: SPENDING UNIT(S) SHALL ISSUE A WRITTEN STATE CONTRACT ORDER (FORM NUMBER WV-39) TO THE VENDOR FOR COMMODITIES COVERED BY THIS CONTRACT. THE ORIGINAL COPY OF THE WV-39 SHALL BE MAILED TO THE VENDOR AS AUTHORIZATION FOR SHIPMENT, A SECOND COPY MAILED TO THE PURCHASING DIVISION, AND A THIRD COPY RETAINED BY THE SPENDING UNIT.

BANKRUPTCY: IN THE EVENT THE VENDOR/CONTRACTOR FILES FOR BANKRUPTCY PROTECTION, THIS CONTRACT IS AUTOMATICALLY NULL AND VOID, AND IS TERMINATED WITHOUT FURTHER ORDER.

THE TERMS AND CONDITIONS CONTAINED IN THIS CONTRACT SHALL SUPERSEDE ANY AND ALL SUBSEQUENT TERMS AND CONDITIONS WHICH MAY APPEAR ON ANY ATTACHED PRINTED DOCUMENTS SUCH AS PRICE LISTS, ORDER FORMS, SALES AGREEMENTS OR MAINTENANCE AGREEMENTS, INCLUDING ANY ELECTRONIC MEDIUM SUCH AS CD-ROM.

REV. 04/11/2001

EXHIBIT 4

BOA

~~LOCAL GOVERNMENT BODIES: UNLESS THE VENDOR INDICATES IN THE BID HIS REFUSAL TO EXTEND THE PRICES, TERMS, AND CONDITIONS OF THE BID TO COUNTY, SCHOOL, MUNICIPAL AND OTHER LOCAL GOVERNMENT BODIES, THE BID SHALL EXTEND TO POLITICAL SUBDIVISIONS OF THE STATE OF WEST~~

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE [signature] TELEPHONE 800-523-5001 DATE 12/19/11

TITLE VP, AMERICAS & ASIA PACIFIC FEIN 33-0767987 ADDRESS CHANGES TO BE NOTED ABOVE

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~~VIRGINIA. IF THE VENDOR DOES NOT WISH TO EXTEND THE PRICES, TERMS, AND CONDITIONS OF THE BID TO ALL POLITICAL SUBDIVISIONS OF THE STATE, THE VENDOR MUST CLEARLY INDICATE SUCH REFUSAL IN HIS BID. SUCH REFUSAL SHALL NOT PREJUDICE THE AWARD OF THIS CONTRACT IN ANY MANNER.~~ Gen-Probe will not extend the prices, terms and conditions to other political entities of West Virginia.

REV. 3/88

INQUIRIES:
WRITTEN QUESTIONS SHALL BE ACCEPTED THROUGH CLOSE OF BUSINESS ON 12/6/2011. QUESTIONS MAY BE SENT VIA USPS, FAX, COURIER OR E-MAIL. IN ORDER TO ASSURE NO VENDOR RECEIVES AN UNFAIR ADVANTAGE, NO SUBSTANTIVE QUESTIONS WILL BE ANSWERED ORALLY. IF POSSIBLE, E-MAIL QUESTIONS ARE PREFERRED. ADDRESS INQUIRIES TO:
ROBERTA WAGNER
DEPARTMENT OF ADMINISTRATION
PURCHASING DIVISION
2019 WASHINGTON STREET, EAST
CHARLESTON, WV 25311

FAX: 304-558-4115
E-MAIL: ROBERTA.A.WAGNER@WV.GOV

THE MODEL/BRAND/SPECIFICATIONS NAMED HEREIN ESTABLISH THE ACCEPTABLE LEVEL OF QUALITY ONLY AND ARE NOT INTENDED TO REFLECT A PREFERENCE OR FAVOR ANY PARTICULAR BRAND OR VENDOR. VENDORS WHO ARE BIDDING ALTERNATES SHOULD SO STATE AND INCLUDE PERTINENT

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE [signature] TELEPHONE 800-523-5001 DATE 12/19/11

TITLE VP Americas & asia Pacific FEIN 33-0767987 ADDRESS CHANGES TO BE NOTED ABOVE

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Request for Quotation

RFQ NUMBER	PAGE
LBS12044	5

ADDRESS CORRESPONDENCE TO ATTENTION OF:
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LITERATURE AND SPECIFICATIONS. FAILURE TO PROVIDE INFORMATION FOR ANY ALTERNATES MAY BE GROUNDS FOR REJECTION OF THE BID. THE STATE RESERVES THE RIGHT TO WAIVE MINOR IRREGULARITIES IN BIDS OR SPECIFICATIONS IN ACCORDANCE WITH SECTION 148-1-4(F) OF THE WEST VIRGINIA LEGISLATIVE RULES AND REGULATIONS.

PURCHASING CARD ACCEPTANCE: THE STATE OF WEST VIRGINIA CURRENTLY UTILIZES A VISA PURCHASING CARD PROGRAM WHICH IS ISSUED THROUGH A BANK. THE SUCCESSFUL VENDOR MUST ACCEPT THE STATE OF WEST VIRGINIA VISA PURCHASING CARD FOR PAYMENT OF ALL ORDERS PLACED BY ANY STATE AGENCY AS A CONDITION OF AWARD.

VENDOR PREFERENCE CERTIFICATE

THIS TEAM EXHIBIT HAS BEEN REPLACED BY THE ONLINE VERSION WHICH IS AVAILABLE HERE:
HTTP://WWW.STATE.WV.US/ADMIN/PURCHASE/VRC/VENPREF.PDF

NOTICE

A SIGNED BID MUST BE SUBMITTED TO:

DEPARTMENT OF ADMINISTRATION
PURCHASING DIVISION
BUILDING 15
2019 WASHINGTON STREET, EAST
CHARLESTON, WV 25305-0130

PLEASE NOTE: A CONVENIENCE COPY WOULD BE APPRECIATED.

THE BID SHOULD CONTAIN THIS INFORMATION ON THE FACE OF THE ENVELOPE OR THE BID MAY NOT BE CONSIDERED:

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE	TELEPHONE	DATE
	800-523-5001	12/19/11

TITLE	FEIN	ADDRESS CHANGES TO BE NOTED ABOVE
VP AMERICAS & ASIA PACIFIC	33-0767987	

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Request for Quotation

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SEALED BID

BUYER:----------ROBERTA WAGNER/FILE 22----------

RFQ. NO.:----------LBS12044----------

BID OPENING DATE:----------12/22/2011----------

BID OPENING TIME:----------1:30 PM----------

PLEASE PROVIDE A FAX NUMBER IN CASE IT IS NECESSARY TO CONTACT YOU REGARDING YOUR BID:

800-523-5001 Phone Fax: 800-288-3141

CONTACT PERSON (PLEASE PRINT CLEARLY):

Allison Horgan

****** THIS IS THE END OF RFQ LBS12044 ****** TOTAL:

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE [signature] TELEPHONE 800-523-5001 DATE 12/19/11

TITLE VP, AMERICAS & ASIA PACIFIC FEIN 33-0767987 ADDRESS CHANGES TO BE NOTED ABOVE

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Glossary of Terms

- ***Assay***: analysis to determine the presence, absence, or quantity of one or more components or organisms; *also*: a test used in this analysis.
- ***Assay Kit:*** items (such as reagents and controls) sold as a unit that would be used to complete an assay.
- ***Reagents:*** substance or compound that is added to a system in order to bring about a chemical reaction or is added to see if a reaction occurs in an assay.
- ***Nucleic Acid Amplification Test (NAAT)***: tests that use molecular biology techniques to detect and identify microorganisms, including viruses and bacterium, on the basis of their nucleic acids. In NAAT testing, a specific sequence of the virus or bacteria's nucleic acid is "amplified" or "copied", which make identification of the organism easier.
- **In vitro Diagnostic (IVD) Tests:** medical devices intended to perform diagnoses from assays in a test tube, or more generally in a controlled environment outside a living organism.
- **Symptomatic:** Indicating or typical signs of a particular illness or disease.
- **Non-symptomatic:** showing no signs or symptoms of an illness or disease
- **F.O.B Destination:** Freight to be paid by Vendor

Summary:

An open ended contract to purchase reagent kits for the simultaneous detection and differentiation of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* using nucleic acid amplification technology (NAAT). Vendor must provide and maintain the fully automated processing system needed to perform the testing at no additional cost to the Office of Laboratory Services (OLS).

Volume:

The estimated average annual volume is 41,500 tests. The number of tests provided is for bidding purpose only and the vendor will be required to provide the quantity of test kits needed, be it more or less.

Test Method and Reagent Kit Requirements:

1. Assay must utilize a form of Nucleic Acid Amplification Testing (NAAT) for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in either symptomatic on non-symptomatic patients; as recommended by the CDC as a "best practice" test type.
2. Assay kit must be approved by the Bureau of Biologics of the Food and Drug Administration (FDA) for *in vitro* diagnostic test use.
   - Proof of FDA approval must be provided.
   - *A copy of the reagent kit package insert stating FDA approval will be considered sufficient proof.*
3. Assay kit or any independent reagents or components needed to complete the test must have at least 180 day expiration date (i.e. "shelf life) remaining at the time they are received in the laboratory.
   - If items have less than 180 day expiration date, the item will be replaced by the vendor at no additional cost to OLS.
4. Assay must be FDA approved for NAAT testing utilizing any of the following specimens types to allow maximum flexibility for the testing program (*a package insert or equivalent documentation from the vendor will be considered sufficient proof of FDA approval*):
   a) endocervical swab specimens
   b) male urethral swab specimens
   c) female or male urine specimens
   d) liquid pap specimens
   e) Vaginal swab (either patient collected and/or physician collected) specimens.
5. All sample types must have a minimum transport time of 30 days at ambient temperature (2° - 30°Celsius).
6. The FDA approved assay must detect and differentiate *Chlamydia trachomatis* and/ or *Neisseria gonorrhoeae* from a single patient sample *(i.e. multiple tests from 1 sample tube or well).*
7. Assays must be adapted to a full automated system, capable of testing at least five hundred samples (patient specimens plus controls) by one technologist in an 8.5 hours period including no more than two hours "hands on" time.

8. Reagents must be supplied in a form requiring minimal preparation, and in a configuration and package size compatible with the projected user volume so as to minimize product waste.
9. Specimen collection kits must be offered by the vendor for use with their NAAT assay.
10. Specimen collection kits must not require refrigeration for storage. Collected specimen transport kit must be acceptable shipped under ambient conditions.
11. The vendor must provide pricing for all the following items:
    - Collection devices for Urine samples.
    - Collection devices for swabs (male, female or unisex).
    - Collection devices for self collected/ and or physician collected vaginal swabs.
    - Collection devices for liquid pap specimens.
    - All reagents needed to run the assay.
    - All controls needed to run the assay (positive, negative, and any other controls or calibrators needed or recommended for the vendor's system).
    - Any diluents or reagents not included with the primary reagents needed to complete the test (examples would include but not limited to specimen diluents, detection reagents, buffers, wash solution or anything of that nature).
    - Tubes needed to perform the testing including but not limited to diluent tubes, sample tubes and or testing tubes.
    - Covers, sealers, caps if needed to perform the test or store the reagents according to the vendor's package insert.
    - Any reagent, diluents or any other item needed to prepare specimens for transport to meet the specifications as listed by the vendors' package insert or operations manual.
    - Any pipette tips, probe covers or other items needed to operate the vendors system as identified in the package insert.
    - Micro- well plates if required by the vendor's system.
    - ***Any other item needed to operate the vendor's system that is not specifically listed above but needed for the proper testing or function of the vendors' system.***

Note: ***Each Vendor must provide a list and item numbers of any item not part of the primary kit that is necessary to run the test. If not provided as part of bid, the vendor will still be expected to provide these items at no extra cost to OLS.***

12. All FDA approved specimen types must be able to be tested without further manipulations such as swab expression, swab removal, or centrifugation.
13. Specimen collection kits must have penetrable caps for use on automated instrumentation for pipetting to eliminate the need for swab removal from the collection tube.
14. Vendor must show research to support that naturally occurring component in patient samples (such as blood, mucous, or bilirubin) and other man-made

components (such as vitamins or gynecological products) should not show interference with the performance of the assay in the detection of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae.*

- *Package insert or vendor/manufacturer documentation should be included as part of the vendor response for this specification.*

15. Vendor must provide research that shows that their assay has no cross-reactions with organisms other than *Chlamydia trachomatis* and *Neisseria gonorrhoeae* noted in testing performance.
    - *Package insert or vendor/manufacturer documentation should be included as part of the vendor response for this specification.*

Instrumentation /Equipment Specifications :

1. Equipment design requirements (i.e. "footprint") must not exceed the area established for the current testing laboratory.
    - Vendor's system must not exceed an area of 12 foot X 9 foot.
    - Vendor's system must not exceed a maximum door clearance of 36 inches.
    - Vendor's system must meet the following power requirements:
        - Not to exceed Electrical input 220 VAC
        - Not to exceed Input Current 30 A
        - Electrical connector L6-30R(USA)
2. Vendor must supply computer, monitor, printer, software, cables, UPS and any other components necessary for operation of the test equipment.
3. Computer operating system must be Windows XP or Windows 7
4. The system software must provide electronic file storage and retrieval capabilities, as well as printed data record.
    - Manufacture Assay procedures must be such that determinations are made and recorded electronically with output hardware capable of interface with a laboratory data processing system (i.e. Laboratory Information System (LIMS).
5. Instrumentation must include at least the following:
    - Must be an integrated platform system.
    - Must have the capability of positive specimen identification.
    - Must have automated reagent and specimen pipetting capabilities.
    - Must have liquid level detection for reagents and patient samples.
    - Must have reagent lot number and expiration date tracking.
    - Automated pipetting instrumentation must have throughput of 450-500 patient specimens and controls per 8 hour shift.
6. If pipette tips are required for the system, they must be provided at no additional charge.
7. The instruments must be controlled by a high performance microprocessor:
    - The instruments must have a safe memory for programs with a file protection scheme.
    - The user must be able to protect programs against unauthorized modifications.

- Software must be menu-driven with a full numeric keypad.
- All output hardware must be designed to interface with a Laboratory network drive.

Equipment Ownership/Maintenance/Technical Assistance Requirements:

1. Vendor must remain the owner and retain the title of the equipment.
2. All instrumentation provided by the vendor must be maintained at the vendor's expense during the term of this contract
3. All Preventative Maintenance service visits to the Office of Laboratory Services must be provided at no additional charge.
4. Vendor technical assistance via telephone must be available within 30 minutes during normal business hours and within four hours during non-business hours for reported problems.
5. On-site technical backup must be guaranteed within twenty-four hours of any reported assay failure for equipment for which in-house or over the phone troubleshooting was unsuccessful.
6. Remote Diagnostic capability is preferred.

Training/ Installation Requirements:

1. Delivery of equipment must be within 90 days of the approved purchase order.
2. Vendor must provide technical training at vendor cost for two technologists per instrument in regards to assay performance, troubleshooting, preventive maintenance, and quality control. Vendor must provide a company representative for installation and training.
3. Vendor must also provide subsequent on-site technical training to pertinent testing personnel in regards to assay performance troubleshooting, preventative maintenance, and quality control as needed by OLS.
4. Successful vendor is required to provide a certificate of Workers' Compensation.
5. Successful vendor shall furnish proof of commercial general liability insurance prior to issuance of contract. Unless otherwise specified in the bid documents, the minimum amount of insurance coverage required is $250,000.

Delivery/shipping Requirements:

1. To be F.O.B Destination, unless vendor states otherwise in submitted quotation.
2. Delivery of assay, additional reagents or supplies must be guaranteed within three working days after receipt of an order.

RFQ Cost Sheet

Item	Description	GenProbe Aptima product Number or Equivalent	Expected # of tests per year	# Tests Per Kit	Kit Price	Estimated Yearly Total Cost
1	Urine Sample collection devices	301040	40,000	50	$62.50	$50,000.00
2	Endocervical swab collection devices	301041	400	50	$62.50	$500.00
3	Male urethral swab collection devices	301041	400	50	$62.50	$500.00
4	Liquid pap collection devices	301154C	50	100	$100.00	$100.00
5	Vaginal swab collection devices	301162	50	50	$62.50	$62.50
6	Aptima Combo2 Assay (1000 test kit)	301130B	41,500	1000	$8,750.00	$363,125.00
7	Aptima System Fluid Preservative	~~302383~~ **302380**	41,500	1000	n/c	n/a
8	Aptima Controls Kits	301110	41,500	5	n/c	n/a
9	Aptima Waste Bag Kits	900907	41,500	30,000	n/c	n/a
10	Aptima waste Deflector	900931	41,500	30,000	n/c	n/a
11	Aptima System Fluid (1000 test)	302380	41,500	1000	n/c	n/a
12	Aptima Wash Solution	LR0356	41,500	1000	n/c	n/a
13	Aptima Combo 2 Auto detect Reagent	301048	41,500	300	n/c	n/a
14	Multi-tube units (MTU)	104772-02	41,500	30,000	n/c	n/a
15	Aptima Waste covers	105523	41,500	30,000	n/c	n/a
16	30mL Reagent Caps	501616	41,500	100	n/c	n/a
17	Small Reagent caps	CL0040	41,500	100	n/c	n/a
18	Reagent Caps	CL0041	41,500	100	n/c	n/a
19	Aptima Buffer Solution	LR0355	41,500	1	n/c	n/a
20	Silicone oil	LR0354	41,500	1	n/c	n/a
21	Endozyme advance clean	901160	41,500	1	n/c	n/a
22	Aptima penetrable caps	105668	41,500	100	n/c	n/a
23	Tips, 1000uL conductive liquid sensing	Gen-Probe 901121	41,500	9600	n/c	n/a
					Annual Grand Total	**$414,287.50**

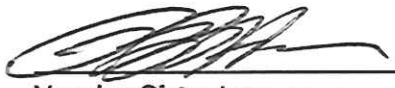
RFQ Cost Sheet

Bidders must provide the cost for the following: PLEASE SEE ATTACHED COST SHEET

Item	Description	GenProbe Aptima product Number or Equivalent	Expected # of tests per year	# Tests Per Kit	Kit Price	Estimated Yearly Total Cost
1	Urine Sample collection devices	301040	40,000			
2	Endocervical swab collection device	301041	400			
3	Male urethral swab collection devices	301041	400			
4	Liquid pap collection devices	301154C	50			
5	Vaginal swab collection devices	301162	50			
6	Aptima Combo2 Assay (1000 test kit)	301130B	41,500			
7	Aptima System Fluid Preservative	302383	41,500			
8	Aptima Control Kits	301110	41,500			
9	Aptima Waste Bag Kits	900907	41,500			
10	Aptima Waste Deflector	900931	41,500			
11	Aptima System Fluid(1000 test)	302380	41,500			
12	Aptima Wash Solution	LR0356	41,500			
13	Aptima Combo 2 Auto detect Reagent	301048	41,500			
14	Multi-tube units (MTU)	104772-02	41,500			
15	Aptima Waste Covers	105523	41,500			
16	30mL Reagent Caps	105523	41,500			
17	Small Reagent caps	CL0040	41,500			
18	Reagent Caps	CL0041	41,500			
19	Aptima Buffer Solution	LR0355	41,500			
20	Silicone Oil	LR0354	41,500			
21	Endozime advance clean	901160	41,500			
22	Aptima Penetrable Caps	105668	41,500			
23	Tip, 1000uL conductive liquid sensing	Tecan 10612513	45,000			

Annual Grand Total $_____

The award will be made to the vendor with the lowest overall total cost which meets all requested specifications and requirements.
Payment of invoices will be made in arrears.



Vendor Signature Brian B. Hansen
VP, Americas & Asia Pacific

12/19/11
Date

ATTACHMENT
P.O.# LB5120144

This agreement constitutes the entire agreement between the parties, and there are no other terms and conditions applicable to the licenses granted hereunder.

Agreed

12/19/11

Signature Date

Title VP Americas & Asia Pacific

Company Name
Gen-Probe Sales & Service, Inc.

Signature Date

Title

Agency/Division

WV-96
Rev. 10/07

AGREEMENT ADDENDUM

In the event of conflict between this addendum and the agreement, this addendum shall control:

1. DISPUTES - Any references in the agreement to arbitration or to the jurisdiction of any court are hereby deleted. Disputes arising out of the agreement shall be presented to the West Virginia Court of Claims.

2. HOLD HARMLESS - Any clause requiring the Agency to indemnify or hold harmless any party is hereby deleted in its entirety.

3. GOVERNING LAW - The agreement shall be governed by the laws of the State of West Virginia. This provision replaces any references to any other State's governing law.

4. TAXES - Provisions in the agreement requiring the Agency to pay taxes are deleted. As a State entity, the Agency is exempt from Federal, State, and local taxes and will not pay taxes for any Vendor including individuals, nor will the Agency file any tax returns or reports on behalf of Vendor or any other party.

5. PAYMENT - Any references to prepayment are deleted. Payment will be in arrears.

6. INTEREST - Should the agreement include a provision for interest on late payments, the Agency agrees to pay the maximum legal rate under West Virginia law. All other references to interest or late charges are deleted.

7. RECOUPMENT - Any language in the agreement waiving the Agency's right to set-off, counterclaim, recoupment, or other defense is hereby deleted.

8. FISCAL YEAR FUNDING - Service performed under the agreement may be continued in succeeding fiscal years for the term of the agreement, contingent upon funds being appropriated by the Legislature or otherwise being available for this service. In the event funds are not appropriated or otherwise available for this service, the agreement shall terminate without penalty on June 30. After that date, the agreement becomes of no effect and is null and void. However, the Agency agrees to use its best efforts to have the amounts contemplated under the agreement included in its budget. Non-appropriation or non-funding shall not be considered an event of default.

9. STATUTE OF LIMITATION - Any clauses limiting the time in which the Agency may bring suit against the Vendor, lessor, individual, or any other party are deleted.

10. SIMILAR SERVICES - Any provisions limiting the Agency's right to obtain similar services or equipment in the event of default or non-funding during the term of the agreement are hereby deleted.

11. ATTORNEY FEES - The Agency recognizes an obligation to pay attorney's fees or costs only when assessed by a court of competent jurisdiction. Any other provision is invalid and considered null and void.

12. ASSIGNMENT - Notwithstanding any clause to the contrary, the Agency reserves the right to assign the agreement to another State of West Virginia agency, board or commission upon thirty (30) days written notice to the Vendor and Vendor shall obtain the written consent of Agency prior to assigning the agreement.

13. LIMITATION OF LIABILITY - The Agency, as a State entity, cannot agree to assume the potential liability of a Vendor. Accordingly, any provision limiting the Vendor's liability for direct damages to a certain dollar amount or to the amount of the agreement is hereby deleted. Limitations on special, incidental or consequential damages are acceptable. In addition, any limitation is null and void to the extent that it precludes any action for injury to persons or for damages to personal property.

14. RIGHT TO TERMINATE - Agency shall have the right to terminate the agreement upon thirty (30) days written notice to Vendor. Agency agrees to pay Vendor for services rendered or goods received prior to the effective date of termination.

15. TERMINATION CHARGES - Any provision requiring the Agency to pay a fixed amount or liquidated damages upon termination of the agreement is hereby deleted. The Agency may only agree to reimburse a Vendor for actual costs incurred or losses sustained during the current fiscal year due to wrongful termination by the Agency prior to the end of any current agreement term.

16. RENEWAL - Any reference to automatic renewal is hereby deleted. The agreement may be renewed only upon mutual written agreement of the parties.

17. INSURANCE - Any provision requiring the Agency to insure equipment or property of any kind and name the Vendor as beneficiary or as an additional insured is hereby deleted.

18. RIGHT TO NOTICE - Any provision for repossession of equipment without notice is hereby deleted. However, the Agency does recognize a right of repossession with notice.

19. ACCELERATION - Any reference to acceleration of payments in the event of default or non-funding is hereby deleted.

20. CONFIDENTIALITY: -Any provision regarding confidentiality of the terms and conditions of the agreement is hereby deleted. State contracts are public records under the West Virginia Freedom of Information Act.

21. AMENDMENTS - All amendments, modifications, alterations or changes to the agreement shall be in writing and signed by both parties. No amendment, modification, alteration or change may be made to this addendum without the express written approval of the Purchasing Division and the Attorney General.

Please see Exhibit A attached

ACCEPTED BY:

STATE OF WEST VIRGINIA

Spending Unit: _____

Signed: _____

Title: _____

Date: _____

VENDOR

Company Name: Gen-Probe Sales & Service, Inc.

Signed: 

Title: Vice President Americas & Asia Pacific

Date: 12/19/11

Rev. 09/08

State of West Virginia
VENDOR PREFERENCE CERTIFICATE N/A

Certification and application* is hereby made for Preference in accordance with *West Virginia Code*, §5A-3-37. (Does not apply to construction contracts). *West Virginia Code*, §5A-3-37, provides an opportunity for qualifying vendors to request (at the time of bid) preference for their residency status. Such preference is an evaluation method only and will be applied only to the cost bid in accordance with the *West Virginia Code*. This certificate for application is to be used to request such preference. The Purchasing Division will make the determination of the Resident Vendor Preference, if applicable. .

1. **Application is made for 2.5% resident vendor preference for the reason checked:**

N/A Bidder is an individual resident vendor and has resided continuously in West Virginia for four (4) years immediately preceding the date of this certification; or,

N/A Bidder is a partnership, association or corporation resident vendor and has maintained its headquarters or principal place of business continuously in West Virginia for four (4) years immediately preceding the date of this certification; or 80% of the ownership interest of Bidder is held by another individual, partnership, association or corporation resident vendor who has maintained its headquarters or principal place of business continuously in West Virginia for four (4) years immediately preceding the date of this certification; or,

N/A Bidder is a nonresident vendor which has an affiliate or subsidiary which employs a minimum of one hundred state residents and which has maintained its headquarters or principal place of business within West Virginia continuously for the four (4) years immediately preceding the date of this certification; or,

2. **Application is made for 2.5% resident vendor preference for the reason checked:**

N/A Bidder is a resident vendor who certifies that, during the life of the contract, on average at least 75% of the employees working on the project being bid are residents of West Virginia who have resided in the state continuously for the two years immediately preceding submission of this bid; or,

3. **Application is made for 2.5% resident vendor preference for the reason checked:**

N/A Bidder is a nonresident vendor employing a minimum of one hundred state residents or is a nonresident vendor with an affiliate or subsidiary which maintains its headquarters or principal place of business within West Virginia employing a minimum of one hundred state residents who certifies that, during the life of the contract, on average at least 75% of the employees or Bidder's affiliate's or subsidiary's employees are residents of West Virginia who have resided in the state continuously for the two years immediately preceding submission of this bid; or,

4. **Application is made for 5% resident vendor preference for the reason checked:**

N/A Bidder meets either the requirement of both subdivisions (1) and (2) or subdivision (1) and (3) as stated above; or,

5. **Application is made for 3.5% resident vendor preference who is a veteran for the reason checked:**

N/A Bidder is an individual resident vendor who is a veteran of the United States armed forces, the reserves or the National Guard and has resided in West Virginia continuously for the four years immediately preceding the date on which the bid is submitted; or,

6. **Application is made for 3.5% resident vendor preference who is a veteran for the reason checked:**

N/A Bidder is a resident vendor who is a veteran of the United States armed forces, the reserves or the National Guard, if, for purposes of producing or distributing the commodities or completing the project which is the subject of the vendor's bid and continuously over the entire term of the project, on average at least seventy-five percent of the vendor's employees are residents of West Virginia who have resided in the state continuously for the two immediately preceding years.

Bidder understands if the Secretary of Revenue determines that a Bidder receiving preference has failed to continue to meet the requirements for such preference, the Secretary may order the Director of Purchasing to: (a) reject the bid; or (b) assess a penalty against such Bidder in an amount not to exceed 5% of the bid amount and that such penalty will be paid to the contracting agency or deducted from any unpaid balance on the contract or purchase order.

By submission of this certificate, Bidder agrees to disclose any reasonably requested information to the Purchasing Division and authorizes the Department of Revenue to disclose to the Director of Purchasing appropriate information verifying that Bidder has paid the required business taxes, provided that such information does not contain the amounts of taxes paid nor any other information deemed by the Tax Commissioner to be confidential.

Under penalty of law for false swearing (West Virginia Code, §61-5-3), Bidder hereby certifies that this certificate is true and accurate in all respects; and that if a contract is issued to Bidder and if anything contained within this certificate changes during the term of the contract, Bidder will notify the Purchasing Division in writing immediately.

Bidder: Gen-Probe Sales & Service, Inc Signed: _____

Date: 12/19/11 Title: VP Americas & Asia Pacific

*Check any combination of preference consideration(s) indicated above, which you are entitled to receive.

EXHIBIT A

TITLE. Gen-Probe shall hold exclusive title to the System and may assign, transfer, pledge or sell Gen-Probe's interest in the System without notice to or approval from Customer. Gen-Probe is Gen-Probe Sales & Service, Inc., a wholly-owned distribution subsidiary of Gen-Probe Incorporated. Customer shall not remove any markings from the System, which identify Gen-Probe as the owner. Customer shall keep the System free from any and all liens, claims and encumbrances and shall not lease, sublease, transfer, sell, or assign the System. Customer does hereby make, constitute and appoint Gen-Probe as Customer's true and lawful attorney-in-fact for the sole purpose of executing and filing, in the name of Customer, a UCC-1 statement in favor of Gen-Probe covering the System.

WARRANTY. Gen-Probe warrants that the Reagents shall meet the required performance specifications to perform the desired tests as described in the package inserts. The extent of Gen-Probe's liability regarding Reagents and Customer's sole and exclusive remedy under this warranty is limited to replacing any defective Reagent, at Gen-Probe's option. Gen-Probe warrants that the System shall conform in all material respects to the performance specifications as described in the operator's manual for a period of twelve (12) months from the date of installation. Gen-Probe does not manufacture the System. The System is fully warranted through its manufacturers and such warranties extend to Gen-Probe's customers. The extent of Gen-Probe's liability regarding the System and at Customer's sole and exclusive remedy under this warranty is limited to the repair or replacement of any defective System, at Gen-Probe's option. The foregoing System warranty shall not apply in the event that: (a) Customer has not used and maintained the System in accordance with the System operator's manual provided to Customer; (b) Customer has used the System with reagents and supplies not expressly authorized by Gen-Probe; (c) the System is repaired or altered by a party other than Gen-Probe without Gen-Probe's prior written consent; (d) Customer has installed commercial or non-System software on the System; or (d) if the System has been subject to misuse, negligence, or accident. THE FOREGOING WARRANTIES ARE IN LIEU OF ALL OTHER WARRANTIES, EXPRESS OR IMPLIED, ARISING BY OPERATION OF LAW OR OTHERWISE, INCLUDING, WITHOUT LIMITATION, THE IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NONINFRINGEMENT AND THOSE ARISING FROM COURSE OF DEALING OR USAGE OR TRADE.

LIMITATION OF LIABILITY. EXCEPT FOR PAYMENTS DUE PURSUANT TO SECTIONS 4 AND 6, IN NO EVENT SHALL EITHER PARTY BE LIABLE FOR INDIRECT, SPECIAL, INCIDENTAL, PUNITIVE, OR CONSEQUENTIAL DAMAGES, INCLUDING, BUT NOT LIMITED TO, LOST PROFITS, EVEN IF ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, OR COSTS INCURRED BY THE OTHER PARTY IN CONNECTION WITH THE USE OF THE SYSTEM OR REAGENTS BY CUSTOMER OR ANY OTHER PERSON UTILIZING THE SYSTEM OR REAGENTS NOR SHALL GEN-PROBE BE LIABLE FOR THE COST OF PROCUREMENT OF SUBSTITUTE REAGENTS OR SYSTEM. Such limitation is intended to apply without regard to whether such damages are claimed, asserted or brought in an action or claim sounding in tort or contract, or on the warranty, or under any other law or form of action.

CONFIDENTIALITY. Customer acknowledges the existence of the trademarks, copyrights, patents, and other intellectual property rights relating to the use or subsisting in or in connection with the System including software, hardware, and other parts thereof in which Gen-Probe or a third party has an interest are, and shall remain, the sole property of Gen-Probe or the respective third party. Customer shall not at any time dispute Gen-Probe's ownership thereof. Customer shall hold in confidence all materials or information disclosed to it by Gen-Probe hereunder ("Confidential Information"). In addition to the foregoing, Gen-Probe Confidential Information includes the operator's manual, the System price and payment terms. Customer agrees to take precautions to prevent the unauthorized disclosure or use of Confidential Information consistent with precautions used to protect its own confidential information, but in no event less than reasonable care. The obligations of Customer hereunder shall not apply to materials or information which (a) is now, or hereafter becomes, through no act or failure to act on the party of Customer, generally known or available; (b) is known by Customer at the time of receiving such information as evidenced by its records; (c) is hereafter furnished to Customer by a third party, as a matter of right and without restriction on disclosure; (d) is independently developed by Customer without any breach of this Agreement; or (e) is the subject of a written permission to disclose provided by Gen-Probe. Notwithstanding any other provision of this Agreement, disclosure of Confidential Information shall not be precluded if such disclosure: (i) is in response to a valid order of a court or other governmental body or is otherwise required by law; provided, however, that Customer shall first have given notice to Gen-Probe and shall have made a reasonable effort to obtain confidential treatment of such Confidential Information; (ii) is otherwise necessary to establish rights or enforce obligations under this Agreement, but only to the extent that any such disclosure is necessary.

MAINTENANCE AND REPAIR. Customer agrees to maintain the System in good operating condition and assumes all risks of loss and damage to the System, except as covered in Section 10. Gen-Probe reserves the right to inspect and service the System at any time.

RFQ No. LB512044

STATE OF WEST VIRGINIA
Purchasing Division

PURCHASING AFFIDAVIT

West Virginia Code §5A-3-10a states: No contract or renewal of any contract may be awarded by the state or any of its political subdivisions to any vendor or prospective vendor when the vendor or prospective vendor or a related party to the vendor or prospective vendor is a debtor and the debt owed is an amount greater than one thousand dollars in the aggregate.

DEFINITIONS:
"Debt" means any assessment, premium, penalty, fine, tax or other amount of money owed to the state or any of its political subdivisions because of a judgment, fine, permit violation, license assessment, defaulted workers' compensation premium, penalty or other assessment presently delinquent or due and required to be paid to the state or any of its political subdivisions, including any interest or additional penalties accrued thereon.

"Debtor" means any individual, corporation, partnership, association, limited liability company or any other form or business association owing a debt to the state or any of its political subdivisions. "Political subdivision" means any county commission; municipality; county board of education; any instrumentality established by a county or municipality; any separate corporation or instrumentality established by one or more counties or municipalities, as permitted by law; or any public body charged by law with the performance of a government function or whose jurisdiction is coextensive with one or more counties or municipalities. "Related party" means a party, whether an individual, corporation, partnership, association, limited liability company or any other form or business association or other entity whatsoever, related to any vendor by blood, marriage, ownership or contract through which the party has a relationship of ownership or other interest with the vendor so that the party will actually or by effect receive or control a portion of the benefit, profit or other consideration from performance of a vendor contract with the party receiving an amount that meets or exceed five percent of the total contract amount.

EXCEPTION: The prohibition of this section does not apply where a vendor has contested any tax administered pursuant to chapter eleven of this code, workers' compensation premium, permit fee or environmental fee or assessment and the matter has not become final or where the vendor has entered into a payment plan or agreement and the vendor is not in default of any of the provisions of such plan or agreement.

Under penalty of law for false swearing (*West Virginia Code* §61-5-3), it is hereby certified that the vendor affirms and acknowledges the information in this affidavit and is in compliance with the requirements as stated.

WITNESS THE FOLLOWING SIGNATURE

Vendor's Name: Gen-Probe Sales & Service, Inc.

Authorized Signature: _____ Date: 12/19/11

Brian B. Hansen

State of CA

County of San Diego, to-wit:

Taken, subscribed, and sworn to before me this 19th day of December, 2011.

My Commission expires July 8th, 2015.

AFFIX SEAL HERE

NOTARY PUBLIC _____

JEFF MAJORIS
Commission # 1940388
Notary Public - California
San Diego County
My Comm. Expires Jul 8, 2015

GEN-PROBE

Dear Valued Customer,

This is in response to your recent question regarding CLIA and CAP Molecular Checklist MOL 34900 inhibition control requirements and the APTIMA COMBO 2® Assay.

On the subject of the need as stated by CLIA for inhibition controls in some amplified assays, the Federal Register Vol. 68, No. 16, p. 3660 states, "...if reaction inhibition is a significant source of false negative results, the laboratory must include a control system to detect such inhibition." Similarly, the CAP Molecular Checklist MOL 34900 asks "In all nucleic acid amplification procedures, are internal controls run to detect a false negative reaction secondary to the presence of inhibitors, when appropriate?"

My interpretation of the CLIA regulations and CAP Molecular Checklist entry is that they apply only to amplification procedures for which the presence of inhibitors or interfering substances may cause false negative results. Furthermore, the CLIA regulations state that it is only when inhibitors are a significant source of false negative results that an inhibition control is required. The conclusions of peer-reviewed studies published in the scientific literature support the interpretation that the CLIA requirements and CAP Molecular Checklist are applicable to first generation amplification assays that have demonstrated sizeable frequencies of specimen inhibition (e.g., LCx, Amplicor, ProbeTec, AMP CT). The studies outlined in a later section of this letter describe the results obtained for APTIMA assays, which show lower frequencies than those seen for first generation assays.

Use of inhibition controls in amplified nucleic acid assays is only one of several solutions to the problem of specimen inhibition. Another, more effective solution is target capture technology. Target capture technology has the advantage of removing potential inhibitors prior to the start of amplification and purifying the target nucleic acid(s) to be amplified, thus allowing amplification and final results reporting for specimens that may have been initially inhibitory. Inhibition controls, when designed appropriately, can be effective in identifying inhibitory specimens. However, results from specimens identified as "inhibitory" cannot be reported since they are considered invalid.

Information on the significance of inhibition in amplified assays is available from many sources – the manufacturers' package inserts, scientific studies presented at major scientific meetings, and peer-reviewed published literature. Discussion of the information from each of these sources and its relevancy to the CLIA regulations and CAP Molecular Checklist is provided below.

The APTIMA COMBO 2 Assay package insert discusses the information available on specimen inhibition in several sections. Note that the term "interference" used in the package insert is inclusive of inhibition since failure to amplify (i.e., inhibition) is the most extreme form of assay interference by specimen materials. In the Introduction section, reference is made to the recent literature articles on the performance of the APTIMA technologies of target capture, transcription-mediated amplification, and hybridization protection assay detection. On the

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(858) 410-8000 · Fax (858) 410-8625

subject of target capture effectiveness to remove specimen inhibitors, the Chong et *al.* paper is referenced; this paper is summarized below. In the Limitations section of the package insert, the first point listed discusses the testing done to determine if blood, gynecological products, vitamins, minerals, and over-the-counter pain relievers interfere with the assay. No interference was observed with any of the materials. The Interfering Substances section of the insert provides more detailed information on the testing described in the Limitations section, including the testing of excessive levels of blood, a substance listed in first generation assay package inserts as a known interferent/inhibitor.

The scientific meeting and peer-reviewed publications cited below include data demonstrating the effectiveness of target capture to remove amplification inhibitors from patient specimens.

1. Chong, S. D. Jang, X. Song, J. Mahony, A. Petrich, P. Barriga, and M. Chernesky. 2003. Specimen Processing and Concentration of *Chlamydia trachomatis* Added Can Influence False-Negative Rates in the LCx Assay but Not in the APTIMA COMBO 2 Assay When Testing for Inhibitors. J. Clinical Microbiology 41(2): 778-782.

In this study, urine specimens were spiked with varying concentrations of *C. trachomatis* elementary bodies and tested for specimen inhibition. Samples that failed to amplify were considered inhibitory. The APTIMA COMBO 2 Assay and the Abbott LCx Assay were compared. Different spiking levels were tested in the two assays, depending on the sensitivities of each assay; lower spiking levels were tested in APTIMA COMBO 2 due to its lower (i.e., more sensitive) limit of detection. At the spiking level used for direct comparison of APTIMA COMBO 2 and LCx, the APTIMA COMBO 2 Assay demonstrated an inhibition frequency of 0.48% (2/415) compared to 13% (44/338) demonstrated by LCx. In the last paragraph of the Conclusions section, the authors state that the prevalence of inhibitors in urine specimens tested in the APTIMA COMBO 2 Assay were very low. As well, the authors concluded that "with this low susceptibility to amplification inhibitors and a high sensitivity level, the APTIMA COMBO 2 Assay has the potential to detect additional cases of *C. trachomatis* in urine specimens."

2. G. S. Hall, M. Katanik, M. Tuohy, and M. Sholtis. 2004. Use of Commercial Amplification Tests in the Clinical Microbiology Laboratory: Test Selection and Quality Assurance. *In* Molecular Microbiology: Diagnostic Principles and Practice, p. 27-36. D. H. Persing et *al.* (Eds), ASM Press, Washington, D.C.

The third paragraph on page 29 of this chapter is a discussion of the various means of detecting specimen inhibitors, including internal controls. In the final sentences of the paragraph, the authors discuss the APTIMA COMBO 2 Assay as an example of a test with a low susceptibility to inhibition that could eliminate the need for inhibition monitoring.

3. D. Ferraro, N. Burgess, D. Schultz, L. Buck, S. Stewart, and L. Traudt. 2002. Head to Head Study Comparing Performance of the Gen-Probe APTIMA COMBO 2, Abbott LCx Probe, Becton Dickinson BDProbeTec ET, and Roche AMPLICOR Nucleic Acid Amplification Assays in Detecting *Chlamydia trachomatis* from First Catch Urine. Abstracts of the Annual Meeting of the American Society for Microbiology.

As part of this study of APTIMA COMBO 2 and first generation amplified assays, the authors observed that two specimens positive by the APTIMA COMBO 2 Assay were inhibitory in the BDProbeTec ET Assay. Although the specimen numbers cited are obviously small, the finding is consistent with those in other studies and supports the effectiveness of target capture to remove specimen inhibitors that cause amplification failure in other amplified assays.

4. B. P. Turner, A. S. Weissfeld, P. H. Vance, E. Trevino, D. Simmons, L. Jones, and A. Hightower. 2001. A Comparison of *Chlamydia trachomatis* Culture and Three Molecular Tests Using Swabs and Urines. Abstracts of the Annual Meeting of the American Society for Microbiology.

The authors of this study state that "sample inhibition is of concern in amplified technology". Based on their study, they concluded that the APTIMA COMBO 2 seems to have solved this problem. Their conclusions are supportive of other similar study findings that been published.

5. A. S. Weissfeld, B. Turner, P. H. Vance, E. Trevino, D. E. Simmons, L. Segura-Jones, and A. Hightower. 2001. A Comparison of *Neisseria gonorrhoeae* Culture to Three Molecular Tests Using Swabs and Urines. Abstracts of the Annual Meeting of the American Society for Microbiology.

As in their study above on *Chlamydia trachomatis*, the authors state that inhibition is a concern for amplified technology. In this study, the APTIMA COMBO 2 assay produced less than 1% false negative results. This is in contrast to a 4% inhibition rate for the AMPLICOR assay. The finding of a lower APTIMA inhibition rate vs. the rates of other amplified assays is consistent with the findings of other studies.

6. J. Shaw, M. Bott, M. Castillo, J. Light, M. Smith, S. Spencer-Covill, T. Bixby, S. Turner, M. Watson, and R. Williams. 2001. APTIMA COMBO 2: Clinical Phase Validation of Analytical Performance. Abstracts of the Annual Meeting of the American Society for Microbiology.

This study describes sensitivity, specificity, target recovery, interference, and inhibition experiments done to verify the analytical performance of the APTIMA COMBO 2 Assay. The study found that none of thirty-three known and potential interfering gynecological-related substances interfered with the APTIMA COMBO 2 Assay. These included substances shown to interfere with first generation amplified assays based on limitations cited in the products' package inserts. As well, in testing of over 400 swab and 400 urine specimens, the authors found 0% inhibition. These findings led the authors to conclude that target capture is effective in removing both endogenous and exogenous specimen inhibitors. These findings are supported by the results of other such studies.

I believe these studies and the testing cited in the APTIMA COMBO 2 Assay package insert provide substantial evidence that inhibition controls are not needed with the APTIMA COMBO 2 Assay.

If you have any questions or concerns regarding this information, please contact Gen-Probe Technical Support at (888) 484-4747.

Martha A. Bott, Ph.D.
Sr. Director, Market Development and Scientific Affairs

L478

GEN-PROBE®

APTIMA®

APTIMA® Specimen Transfer Kit

For Use with PreservCyt Liquid Pap Specimens

For *in vitro* diagnostic use.

Intended Use

The GEN-PROBE APTIMA Specimen Transfer Kit is only for use with GEN-PROBE APTIMA Assays. The GEN-PROBE APTIMA Specimen Transfer Kit allows for APTIMA Assay testing of gynecological specimens collected in Cytyc PreservCyt vials according to the instructions provided.

Reagents

Note: Catalog numbers are listed in parentheses.

Materials Provided

APTIMA® Specimen Transfer Kit (Cat. No. 301154C)

Symbol	Component	Quantity	Description
	APTIMA Specimen Transfer tubes	100 tubes	1 tube x 2.9 mL

Materials Required But Not Provided

One or more kits for the APTIMA Assays

Pipettor, 1000 µL RAININ PR1000 (Cat. No. 901715)

Tips, P1000 Style, special diameter tip only available from Gen-Probe (Cat. No. 105049)

Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution

Test tube rack

Plastic-backed absorbent laboratory bench covers

Fisherbrand BloodBloc Super Absorbency Wipes (available from Fisher Scientific)

Lint-free disposable wipes

Note: See the appropriate package inserts for *Materials Required But Not Provided* for each of the APTIMA Assays.

Optional Materials

Gyn TransCyt Filters (clear) for use with the ThinPrep 2000 Processor

Warnings and Precautions

For handling PreservCyt Solution liquid Pap specimens refer to the *Warnings and Precautions* section in the *Introduction* of the *ThinPrep 2000 Operator's Manual.*

If the Aliquot Removal procedure will be used, refer to the *ThinPrep 2000* or *ThinPrep 3000 Processor Operators Manual-Addendum* for instructions on aliquot removal.

A. Use the APTIMA Specimen Transfer Kit with APTIMA Assays only. Performance has not been established with other products.

B. Do not apply the APTIMA Specimen Transfer medium directly to skin or mucous membranes or take internally.

C. Use only supplied or specified disposable laboratory ware.

D. Use routine laboratory precautions. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye ware, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

E. Specimens may be infectious. Use Universal Precautions when handling specimens. Only laboratory personnel adequately trained in handling infectious materials should be permitted to perform the procedures described in this package insert.

F. Take care to avoid cross-contamination during the specimen handling steps. Specimens may contain high levels of organisms. Change gloves frequently and always change gloves when they come in contact with specimen.

G. Work surfaces, pipettes and other equipment must be regularly decontaminated with 0.5% sodium hypochlorite in deionized (DI) water. If DI water is not used in the 0.5% sodium hypochlorite solution, the effectiveness of the solution may be compromised. Refer to *Procedural Notes*, section C and *Decontamination Instructions*. The effect of the ThinPrep 2000 Processor decontamination procedure was not assessed for its impact on Pap results. Prior to implementing the decontamination procedure, laboratories should validate that the decontamination procedure does not impact Pap results.

H. Only pipette tips with hydrophobic plugs can be used to transfer specimens to the transfer tubes.

I. Do not use this kit after its expiration date.

J. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping and storage conditions other than those recommended has not been evaluated.

K. Dispose of unused PreservCyt solutions and waste in accordance with federal, state, and local regulations.

L. If testing gynecological specimens processed with the ThinPrep 2000 processor, a specific procedure has been validated to mitigate the potential for cross-contamination during Pap processing. Two important steps of the procedure include: (1) bleaching of the PreservCyt filter cap (in 0.5% sodium hypochlorite in DI water) for 1 minute between samples and (2) mandating that the operator change gloves between each sample. Refer to *Procedural Notes* for a detailed protocol. It is important to only dilute bleach with DI water. The pH of tap water varies from lab to lab. Alkaline water can decrease the available chlorine making the bleach less effective for decontaminating equipment.

Storage and Handling Requirements

Gynecological specimens can be stored in the Cytyc PreservCyt Solution vials for 30 days at 2°C to 30°C prior to Pap processing and/or transfer to APTIMA Specimen Transfer tubes. Once the PreservCyt Solution liquid Pap specimen is transferred to the APTIMA Specimen Transfer tube, the specimen must be tested within 30 days when stored at 2°C to 8°C or 14 days at 15°C to 30°C. If longer storage is needed, freeze at -20°C to -70°C for up to 12 months after transfer.

For additional stability requirements, refer to the appropriate APTIMA Assay package insert.

Note: Federal requirements for packaging must be met when specimens are transported by common land and air carriers. Refer to 42 CFR, Part 72. The most current requirements may be obtained from the Centers for Disease Control and Prevention Office of Health and Safety (CDC) in Atlanta, Georgia at 1-800-311-3435.

Procedural Notes

A. Preparation of the Specimen Transfer Area

1. Put on clean gloves.
2. Wipe down work surfaces and pipettors with 0.5% sodium hypochlorite. (Use DI water to dilute bleach. A prepared batch of 0.5% sodium hypochlorite will be effective for 1 week if it is properly stored.)
3. Allow the bleach to contact work surfaces and pipettors for at least 1 minute, then follow with a water rinse. Dry the surfaces with paper towels.
4. Cover the bench with clean, plastic-backed, absorbent laboratory bench covers.
5. In the specimen transfer area, place a test tube rack containing a sufficient number of APTIMA Specimen Transfer tubes corresponding to the number of PreservCyt Solution liquid Pap specimens being tested.
6. Label each APTIMA Specimen Transfer Tube with the accession number or specimen ID number.

Refer to the *ThinPrep 2000 and ThinPrep 3000 Operator's Manual - Addendum* for instructions on aliquot removal. If the ThinPrep aliquot removal procedure will be used, follow the Gen-Probe Specimen Transfer Procedure as defined in section B.

B. Specimen Transfer Procedure for PreservCyt Solution Liquid Pap Specimen Aliquots Removed before Processing with the ThinPrep Processor

1. Put on clean gloves and transfer specimens to be tested to the Specimen Transfer Area.
2. Uncap the APTIMA Specimen Transfer tube, placing the cap on the bench with the threads facing up.
3. Vortex the tube containing the removed aliquot of PreservCyt Solution liquid Pap specimen for 3 to 10 seconds. Uncap the tube, placing the cap on the bench with the threads facing up.
4. Within 1 minute of vortexing, transfer 1 mL of the PreservCyt Solution liquid Pap specimen into the APTIMA Specimen Transfer tube.
5. Dispose of the pipette tip in a container of 0.5% sodium hypochlorite in DI water.
6. Recap the APTIMA Specimen Transfer tube tightly. Gently invert the tube 2 to 3 times to ensure complete mixture of the specimen.
7. Recap the tube containing the removed aliquot of PreservCyt Solution liquid Pap specimen for storage at 2°C to 30°C for 30 days, if desired.
8. Put on clean gloves and repeat steps 1 through 7 above for the transfer of subsequent specimens. To reduce the risk of contaminating other specimens, work with one PreservCyt Solution liquid Pap specimen at a time.
9. Test the PreservCyt Solution liquid Pap specimen from the APTIMA Specimen Transfer tube according to the instructions in the appropriate APTIMA Assay package insert.

If PreservCyt Solution liquid Pap specimens will be transferred into APTIMA Specimen Transfer tubes after processing using the ThinPrep 2000 Processor, perform ThinPrep 2000 processing according to the instructions in section C.

C. Processing PreservCyt Solution Liquid Pap Specimens Using the ThinPrep 2000 Processor

Refer to the *ThinPrep 2000 Operator's Manual* to perform the following procedures:

- Standard Pap processing steps
- Maintenance of the O-rings at the base of the filter cap.

1. Put on clean gloves.
2. Clean 2 filter caps by soaking them in 0.5% sodium hypochlorite in DI water for at least 1 minute, rinse the caps in DI water and dry them thoroughly with a lint-free, disposable wipe. Dispose of the wipe.

Note: Using two filter caps enables the work flow to continue while one filter cap is soaking.

3. Place a clean filter cap on a BloodBloc Super Absorbency Wipe.
4. Place the fixative bath into the ThinPrep 2000 Processor.
5. Create a filter assembly by placing a new Gyn TransCyt Filter in a clean filter cap and insert the filter assembly into the ThinPrep 2000 Processor. (Refer to the *ThinPrep 2000 Processor Operator's Manual* for details on performing this step.)
6. Put a slide in the slide holder. (Refer to the *ThinPrep 2000 Operator's Manual* for details on performing this step.)
7. Uncap the PreservCyt Solution vial, placing the cap on the bench with the threads facing up. Ensure that the bench is clean, with no bleach residue or foreign particles.
8. Load the PreservCyt Solution vial into the ThinPrep 2000 Processor. From the ThinPrep processor main menu, select "4-GYN" by pressing **4** on the keypad.
9. Put on clean gloves.
10. Once the slide preparation is finished, open the door, remove the PreservCyt Solution vial and recap the vial.
11. Remove the fixative bath and place the slide in a 95% ethanol bath.
12. Return the fixative bath to the processor.
13. Remove the filter assembly from the processor using one hand to grasp the filter cap and, using a lint-free, disposable wipe as a barrier, separate the filter from the filter cap. Discard the filter, gloves, and disposable wipe. **Do not discard the filter cap.**
14. Place the filter cap in a container of 0.5% sodium hypochlorite in DI water for at least 1 minute.
15. With clean gloves, rinse the filter cap in DI water, then dry it thoroughly with a lint-free disposable wipe. Dispose of the wipe.
16. Repeat the process for each specimen starting with step 3 of this processing procedure, changing gloves between each specimen, until all of the specimens are processed.

D. Specimen Transfer Procedure for PreservCyt Vials after Processing with the ThinPrep 2000 Processor

1. Put on clean gloves and transfer specimens to be tested to the Specimen Transfer Area.
2. Uncap the APTIMA Specimen Transfer tube, placing the cap on the bench with the threads facing up.
3. Vortex the PreservCyt Solution vial for 3 to 10 seconds. Uncap the vial, placing the cap on the bench with the threads facing up.
4. Within 1 minute of vortexing, transfer 1 mL of the processed PreservCyt Solution liquid Pap specimen into the APTIMA Specimen Transfer tube.
5. Dispose of the pipette tip in a container of 0.5% sodium hypochlorite in DI water.
6. Recap the APTIMA Specimen Transfer tube tightly. Gently invert the tube 2 to 3 times to ensure complete mixture of the specimen. This specimen is referred to as a processed PreservCyt Solution liquid Pap specimen.
7. Recap the PreservCyt Solution vial for storage, if desired.
8. Put on clean gloves and repeat steps 1 through 7 above for the transfer of subsequent specimens. To reduce the risk of contaminating other specimens, work with one processed PreservCyt Solution liquid Pap specimen at a time.

Test Procedure

Test the processed PreservCyt Solution liquid Pap specimen from the APTIMA Specimen Transfer tube according to the instructions in the appropriate APTIMA Assay package insert.

Decontamination Instructions

Note: If PreservCyt Solution liquid Pap specimens are transferred into APTIMA Specimen Transfer tubes after processing using the ThinPrep 2000 Processor, the ThinPrep 2000 Processor must be decontaminated after 8 hours of use.

It is important to clean the processor from the top of the machine to the bottom and to change gloves as instructed in order to prevent recontamination of cleaned surfaces.

Avoid touching the internal instrumentation wiring throughout this process.

Only use 0.5% sodium hypochlorite in DI water to decontaminate the ThinPrep 2000 Processor.

A. Decontamination of the ThinPrep 2000 Processor

1. Put on clean gloves.
2. Wet a lint-free disposable wipe with 0.5% sodium hypochlorite in DI water.
3. Open the sample door, wipe down the slide holder with the disposable wipe, and dispose of the wipe.
4. Close the sample door.
5. Move the internal workings of the processor into the maintenance position by pressing **7** then **2** and **Enter** on the keypad.
6. Open the sample door.
7. Put on clean gloves.
8. Wet a lint-free disposable wipe with 0.5% sodium hypochlorite and wipe down the surfaces from top to bottom. Be sure to thoroughly clean surfaces that are handled during processing such as the slide holder, fixative bath holder, and sample vial holder. Also be sure to clean the cap seal and the inside of the processor's door. Dispose of the wipe.
9. Change gloves. Using a lint-free disposable wipe moistened with 0.5% sodium hypochlorite, clean the exterior of the processor from top to bottom paying close attention to the door handle and the keypad. Dispose of the wipe.
10. Allow the bleach to sit on the equipment for 5 minutes.
11. Return the processor to the working position by closing the sample door and pressing **Enter** on the keypad.
12. Change gloves and wipe down the slide holder with a lint-free, disposable wipe soaked in DI water. Dispose of the wipe.
13. Close the sample door and enter **7** then **2** and **Enter** on the keypad to return the processor to the maintenance position.
14. Open the sample door and, working from top to bottom, wipe the interior with a lint-free, disposable wipe soaked in DI water, being sure to thoroughly remove the bleach from the cap seal. Dispose of the wipe.
15. Repeat steps 1 through 14 to ensure that decontamination is complete.

B. Lab Contamination Monitoring Protocol

There are many laboratory-specific factors that may contribute to contamination, including testing volume, workflow, disease prevalence, and various other laboratory activities. These factors should be taken into consideration when contamination monitoring frequency is being established. Intervals for contamination monitoring should be established based on each laboratory's practices and procedures. Each cytology lab must coordinate with an APTIMA testing site in order to test samples collected for monitoring contamination and receive the sample results.

To monitor for laboratory contamination, the following procedure may be performed using the APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens:

1. Label the swab transport tubes with numbers corresponding to the areas of the lab that will be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the swab transport media and swab the numbered area using a circular motion.
3. Immediately insert the swab into the corresponding transport tube.
4. Carefully break the swab shaft at the score line. Avoid splashing the contents.
5. Re-cap the swab transport tube tightly.
6. Repeat steps 2 to 5 for all areas to be swabbed.
7. Test the swab using the appropriate APTIMA Assay according to the *Test Procedure* section of the appropriate assay package insert.

If the results are positive or equivocal (see the *Test Interpretation* section of the appropriate assay package insert), the surface may be contaminated and should be decontaminated by treating with bleach as recommended in the appropriate Operator's Manual and/or package insert.

PreservCyt Solution Liquid Pap Specimen Contamination Study

To demonstrate that bleaching the filter cap is effective in reducing contamination, 200 negative and 200 high titer (>1x10^6 CFU/mL) GC positive samples were alternately processed first without the bleaching steps and, subsequently, with the bleaching steps. The GC positive samples were generated by spiking the liquid Pap sample with cell equivalents of >5x10^6 fg GC rRNA. Note that the operators changed gloves between handling each sample for both the first and second stages of the study. The same filter cap was used with all 400 samples. After processing liquid Pap in the ThinPrep 2000 instrument, 1 mL of the remaining PreservCyt sample was transferred to an APTIMA Specimen Transfer tube (this is now referred to as the processed liquid Pap sample) then run in the APTIMA COMBO 2 assay. These conditions replicate the processes that are expected to be conducted in a typical clinical setting.

Additionally, an aliquot was removed from each sample prior to processing on the ThinPrep 2000 instrument as a control sample. This aliquot would be tested when a sample produced a false positive result to determine if the contamination occurred prior to sample processing. Further, an additional 20 negative PreservCyt liquid Pap samples were added at the end of the second stage to determine if a build up of cells on the processor (potentially due to the creation of aerosols) could contaminate negative samples.

Without the bleach step there were 24 false positives and 17 equivocal results among the PreservCyt samples for a false result frequency of 20.5%. When the filter cap was bleached between samples the false positive frequency was 1.4% (3 false positives out of 220 negative samples). None of the pre-processed aliquots from the samples producing false results were GC-positive. This is consistent with the notion that the contamination was not introduced prior to processing the sample on the ThinPrep 2000 instrument; rather, contamination was likely introduced during the Pap processing.

These studies demonstrate that incorporation of a contamination mitigation protocol decreases the potential for cross-contamination introduced by the processing steps of the ThinPrep 2000 instrument by > 14 fold.

Limitations

A. Performance of the APTIMA Specimen Transfer kit was not evaluated for testing the same PreservCyt Solution liquid Pap specimen both before and after ThinPrep Pap processing.

B. PreservCyt Solution liquid Pap samples processed with instruments other than the ThinPrep 2000 processor have not been evaluated for use in APTIMA Assays.

C. The APTIMA Specimen Transfer Kit was evaluated using PreservCyt Solution liquid Pap specimens collected with either broom-type or endocervical brush/spatula collection devices. The use of other collection devices was not evaluated for use in APTIMA Assays.

D. The effect of the ThinPrep 2000 Processor decontamination procedure was not assessed for its impact on Pap results. Prior to implementing the decontamination procedure, laboratories should validate that the decontamination procedure does not impact Pap results.

E. If transferring PreservCyt Solution liquid Pap specimens to APTIMA Specimen Transfer tubes following ThinPrep 2000 processing, use of this kit is limited to personnel who have been trained in processing PreservCyt specimens and in decontaminating the ThinPrep 2000 Processor. Failure to follow the instructions to decontaminate the instrument may result in erroneous results.

F. The APTIMA Bleach Enhancer has not been validated for the ThinPrep 2000 Processor decontamination procedure.

G. There is no evidence of degradation of nucleic acids in PreservCyt Solution. If a PreservCyt Solution liquid Pap specimen has small amounts of cellular material, uneven distribution of this material may occur, which may affect the ability to detect target organisms in the collected material. If negative results from the specimen do not fit with the clinical impression, a new specimen may be necessary. When compared to direct sampling with the APTIMA Swab Transport Media for CT and GC, the additional volume of PreservCyt Solution results in greater dilution of the sample material.

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APTIMA®

APTIMA® Vaginal Swab Specimen Collection Kit

For *in vitro* diagnostic use.

Intended Use

The APTIMA Vaginal Swab Specimen Collection Kit is for use with APTIMA assays. The APTIMA Vaginal Swab Specimen Collection Kit is intended to be used for clinician and patient collection of vaginal swab specimens. Patient-collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated. The APTIMA Vaginal Swab Specimen Collection Kit is not for home use.

Materials Provided

50 Vaginal Swab Specimen Collection Kits (Cat. No. 301162)

Each kit contains:

Component	Quantity	Description
Swab	1	*Individually wrapped, sterile swab.*
Transport tube	1	*Tube containing transport medium (2.9 mL).*
Patient collection instructions	1 package	*Pack of patient collection instructions.*

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. Do not apply the transport medium directly to skin or mucous membranes or take internally.

C. Specimens may be infectious. Use Universal Precautions when handling specimens. Only personnel adequately trained in handling infectious materials should be permitted to handle specimens.

D. Take care to avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another, and discard used materials without passing over the containers. If gloves come in contact with specimen, change gloves to avoid cross-contamination.

E. If the contents of the transport tube are spilled at any time during the collection procedure, use a new APTIMA Vaginal Swab Specimen Collection Kit. Failure to use a new kit may invalidate the test results.

F. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

Kit Storage Requirements

Store collection kit at room temperature (15°C to 30°C).

Vaginal Swab Specimen Performance

The assay performance characteristics of the vaginal swab specimen are provided in the appropriate APTIMA assay package insert. The APTIMA assay package inserts may be referenced online at www.gen-probe.com. The performance of the patient-collected vaginal swab specimen has not been established for all APTIMA assays. The table below identifies acceptable specimen types for each of the APTIMA assays.

APTIMA Assay for	Clinician Collected	Patient Collected
Chlamydia trachomatis	Yes	Yes
Chlamydia trachomatis and *Neisseria gonorrhoeae* (APTIMA COMBO 2 Assay)		
Neisseria gonorrhoeae		
Trichomonas vaginalis	Yes	No

Specimen Collection and Handling

Note: Ensure that patients read the Patient Collection Instructions before providing them with a collection kit.

Instructions for vaginal swab specimen collection:

1. Partially peel open the swab package. Remove the swab. Do not touch the soft tip or lay the swab down. If the soft tip is touched, the swab is laid down, or the swab is dropped, use a new APTIMA Vaginal Swab Specimen Collection Kit.
2. Hold the swab, placing your thumb and forefinger in the middle of the swab shaft covering the score line. Do not hold the swab shaft below the score line.
3. Carefully insert the swab into the vagina about 2 inches (5 cm) past the introitus and gently rotate the swab for 10 to 30 seconds. Make sure the swab touches the walls of the vagina so that moisture is absorbed by the swab and then withdraw the swab without touching the skin.
4. While holding the swab in the same hand, unscrew the cap from the tube. Do not spill the contents of the tube. If the contents of the tube are spilled, use a new APTIMA Vaginal Swab Specimen Collection Kit.
5. Immediately place the swab into the transport tube so that the score line is at the top of the tube.
6. Carefully break the swab shaft at the score line against the side of the tube.
7. Immediately discard the top portion of the swab shaft.
8. Tightly screw the cap onto the tube.

Specimen Transport and Storage

Vaginal swab specimens must be transported to the laboratory in the provided swab specimen transport medium and tube. Vaginal swab specimens must be transported to the laboratory at 2°C to 30°C and tested within 60 days of collection. If longer storage is needed, refer to the appropriate APTIMA assay package insert.

Note: Federal requirements for packaging must be met when specimens are transported by common land and air carriers. Refer to 42 CFR, Part 72. The most current requirements may be obtained from the Centers for Disease Control and Prevention Office of Health and Safety in Atlanta, Georgia, USA, at +1 800 467 4922 or the CDC web site.

Limitations

A. Use this collection kit only with the APTIMA assays. Performance has not been established with other products.

B. Vaginal swab sampling is not designed to replace cervical exams and endocervical samples for diagnosis of female urogenital infections. Patients may have cervicitis, urethritis, urinary tract infections, or vaginal infections due to other causes or concurrent infections with other agents.

C. Women who have symptoms suggesting pelvic inflammatory disease (PID) should not use the patient-collected vaginal swab specimen as a replacement for a pelvic exam.

D. The performance of vaginal swab specimen has not been evaluated in pregnant women.

E. The performance of vaginal swab specimen has not been evaluated in teenage women less than 16 years of age.

F. The patient-collected vaginal swab specimen application is limited to health care facilities where support/counseling is available to explain the procedures and precautions.

G. The performance of the patient-collected vaginal swab specimen has not been established for the APTIMA Trichomonas vaginalis assay.

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GEN-PROBE®

APTIMA®

APTIMA® Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens

For *in vitro* diagnostic use.

Intended Use

The APTIMA Unisex Swab Specimen Collection Kit for Female Endocervical and Male Urethral Swab Specimens is for use with APTIMA assays. The APTIMA Unisex Swab Specimen Collection Kit is intended to be used for the collection of female endocervical or male urethral swab specimens.

Materials Provided

50 APTIMA Unisex Swab Specimen Collection Kits for Endocervical and Male Urethral Swab Specimens (Cat. No. 301041)

Each kit contains:

Component	Quantity	Description
Unisex collection swab	1	*Swab for endocervical or male urethral swab specimens.*
Cleaning swab	1	*Female cleaning swab.*
Transport tube	1	*Tube containing APTIMA swab transport medium (2.9 mL).*

Warnings and Precautions

A. Do not apply the transport medium directly to skin or mucous membranes or take internally.

Kit Storage Requirements

Store collection kit at room temperature (15°C to 30°C).

Swab Specimen Performance

The assay performance characteristics of the female endocervical and male urethral swab specimens are provided in the appropriate APTIMA assay package insert. The APTIMA assay package inserts may be referenced online at www.gen-probe.com. The performance of the male urethral swab specimen has not been established for all APTIMA assays. The table below identifies the acceptable specimen types for each of the APTIMA assays.

APTIMA Assay for	Female Swab Specimens	Male Swab Specimens
Chlamydia trachomatis	Yes	Yes
Chlamydia trachomatis and *Neisseria gonorrhoeae* (APTIMA COMBO 2 Assay)		
Neisseria gonorrhoeae		
Trichomonas vaginalis	Yes	No

Specimen Collection and Handling

A. Endocervical swab specimens

1. Remove excess mucus from the cervical os and surrounding mucosa using the cleaning swab (white shaft swab in the package with red printing). Discard this swab.

   Note: To remove excess mucus from the cervical os, a large-tipped swab (not provided) may be used.
2. Insert the specimen collection swab (blue shaft swab in the package with the green printing) into the endocervical canal.
3. Gently rotate the swab clockwise for 10 to 30 seconds in the endocervical canal to ensure adequate sampling.
4. Withdraw the swab carefully; avoid any contact with the vaginal mucosa.
5. Remove the cap from the swab specimen transport tube and immediately place the specimen collection swab into the transport tube.
6. Carefully break the swab shaft against the side of the tube at the scoreline and discard the top portion of the swab shaft; use care to avoid splashing of contents.
7. Re-cap the swab specimen transport tube tightly.

B. Male urethral swab specimens

1. The patient should not have urinated for at least 1 hour prior to sample collection.
2. Insert the specimen collection swab (blue shaft swab in the package with the green printing) 2 to 4 cm into the urethra.
3. Gently rotate the swab clockwise for 2 to 3 seconds in the urethra to ensure adequate sampling.
4. Withdraw the swab carefully.
5. Remove the cap from the swab specimen transport tube and immediately place the specimen collection swab into the transport tube.
6. Carefully break the swab shaft against the side of the tube at the scoreline and discard the top portion of the swab shaft; use care to avoid splashing of contents.
7. Re-cap the swab specimen transport tube tightly.

Specimen Transport and Storage

After collection, transport and store the swab in the swab specimen transport tube at 2°C to 30°C until tested. Specimens must be assayed with the APTIMA assays within 60 days of collection. If longer storage is needed, refer to the appropriate APTIMA assay package insert.

Note: Federal requirements for packaging must be met when specimens are transported by common land and air carriers. Refer to 42 CFR, Part 72. The most current requirements may be obtained from the Centers for Disease Control and Prevention Office of Health and Safety in Atlanta, Georgia, USA, at +1 800 467 4922 or the CDC web site.

Limitations

A. Use this collection kit only with the APTIMA assays. Performance has not been established with other products.

B. The performance of male urethral swab specimens has not been established for the APTIMA Trichomonas vaginalis assay.

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GEN-PROBE®

APTIMA®

APTIMA® System Fluid Preservative Kit (TIGRIS® DTS® System)

For *in vitro* diagnostic use.

Intended Use

The APTIMA System Fluid Preservative Kit is used with the APTIMA Assays on the TIGRIS DTS System. Use the System Fluid Preservative Kit according to the instructions outlined in the appropriate assay package insert and the *TIGRIS DTS System Operator's Manual*.

Reagents

Reagents for the APTIMA System Fluid Preservative Kit are provided below for the TIGRIS DTS System. Reagent identification Symbols are also listed next to the reagent name.

Materials Provided

APTIMA® System Fluid Preservative Kit (1 box) (Cat. No. 302380)

Component	Quantity	Description
APTIMA System Fluid Preservative	1 x 200 mL	*System Fluid Preservative contains 2.5% sodium hypochlorite that inhibits microbial growth in aqueous media.*

Materials Required But Not Provided

Appropriate APTIMA Assay Kit(s)

TIGRIS DTS System

Water for the TIGRIS DTS System. For water specifications for the TIGRIS DTS System, see the *TIGRIS DTS System Operator's Manual*.

Materials Available from Gen-Probe

Appropriate APTIMA® Assay Kit(s)

TIGRIS® DTS® System (Cat. No. 105118)

Warnings and Precautions

A. Undiluted System Fluid Preservative is corrosive.

B. Corrosive

R35 S1/2 S26

S37/39 S45

R35	Causes serious corrosions.
S1/2	Keep in a locked place and out of reach of children.
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S37/39	Wear suitable gloves and eye/face protection.
S45	In case of accident or sickness, seek medical advice immediately and show this container or label.

C. Avoid System Fluid Preservative contact with skin, eyes, and mucous membranes. Wash with water if contact with this fluid occurs. If a spill of this fluid occurs, dilute with water before wiping dry.

D. Use routine laboratory precautions. Do not pipette by mouth, do not eat, drink, or smoke in the laboratory work area. Wash hands thoroughly after handling APTIMA Assay Fluids.

E. Do not top off System Fluid Preservative containers.

Storage and Handling Requirements

A. The APTIMA System Fluid Preservative is stable when stored unopened at 15° to 30°C until the expiration date.

B. Do not use after the expiration date. **Do not freeze.** Once opened, APTIMA System Fluid Preservative is stable for 30 days when stored at room temperature.

C. The APTIMA System Fluid Preservative may be used between 15° and 30°C and 20% to 85% relative humidity.

Procedural Notes

System Fluid

Pour the entire bottle of System Fluid Preservative into an empty TIGRIS DTS System Fluid Container. Fill the System Fluid Container with water for the TIGRIS DTS System until the liquid level is between the two solid lines on the container. For water specifications for the TIGRIS DTS System, see the *TIGRIS DTS System Operator's Manual*. System Fluid is stable for 30 days when stored at room temperature (15° to 30°C) and can be used for up to 14 days after loading onto the TIGRIS DTS System.

Limitations

Assay must be performed, and results interpreted according to the procedures of the appropriate assay kit package insert and the *TIGRIS DTS System Operator's Manual*.

Deviations from these procedures may produce unreliable results. Use of outdated APTIMA System Fluid Preservative may produce erroneous results.

Expected Values

Please reference the appropriate assay package insert for expected results.

CE

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GEN-PROBE®

APTIMA®

APTIMA COMBO 2® Assay

For *in vitro* diagnostic use.

Intended Use

The APTIMA COMBO 2 Assay is a target amplification nucleic acid probe test that utilizes target capture for the *in vitro* qualitative detection and differentiation of ribosomal RNA (rRNA) from *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (GC) to aid in the diagnosis of chlamydial and/or gonococcal urogenital disease using the TIGRIS DTS Automated Analyzer or semi-automated instrumentation as specified. The assay may be used to test the following specimens from symptomatic individuals: clinician-collected endocervical, vaginal and male urethral swab specimens; and female and male urine specimens. The assay may be used to test the following specimens from asymptomatic individuals: clinician-collected endocervical, vaginal and male urethral swab specimens; patient-collected vaginal swab specimens[1]; and female and male urine specimens. The assay is also intended for use with the testing of gynecological specimens, from both symptomatic and asymptomatic patients, collected in the PreservCyt Solution.

[1]Patient-collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated. The vaginal swab specimen collection kit is not for home use.

Summary and Explanation of the Test

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (GC) infections are two of the most common sexually transmitted infections worldwide. In the United States alone, an estimated 1,210,523 new cases of CT and 336,742 new cases of GC infections were reported to the Centers for Disease Control in 2008 (5).

Chlamydiae are nonmotile, gram-negative, obligate intracellular bacteria. The CT species is comprised of fifteen serovars (A, B, Ba, C, D, E, F, G, H, I, J, K, L1, L2 and L3) that can cause disease in humans (32). The serovars D through K are the major cause of genital chlamydial infections in men and women (24). *C. trachomatis* can cause nongonococcal urethritis, epididymitis, proctitis, cervicitis, acute salpingitis, and Pelvic Inflammatory Disease (PID) (3, 15, 26, 27). *C. trachomatis* infections are often asymptomatic in both males and females. Children born to infected mothers are at significantly higher risk for inclusion conjunctivitis and chlamydial pneumonia (1, 11, 25).

Historically, several methods for CT detection have been utilized in the clinical laboratory, including cell culture, direct fluorescent antibody testing, and enzyme immunoassay. More recent methodologies for CT detection include direct DNA probe assays and nucleic acid amplification test (NAAT) DNA probe assays. Cell culture was once considered to be the "gold standard" for detection of CT. Culture is quite specific, but scientific publications have demonstrated that the NAAT DNA probe technologies have a higher clinical sensitivity than culture (2, 9, 17, 28). Due to its lower clinical sensitivity and variable performance between laboratories, culture has been replaced in many laboratories by direct DNA probe and NAATs.

N. gonorrhoeae is the causative agent of gonorrheal disease. *N. gonorrhoeae* are non-motile, gram-negative diplococci. The majority of gonorrheal infections are uncomplicated lower genital tract infections and may be asymptomatic. However, if left untreated in women, infections can ascend and cause PID. PID can manifest as endometritis, salpingitis, pelvic peritonitis, and tubo-ovarian abscesses. A smaller percentage of persons with gonococcal infections may develop Disseminated Gonococcal Infection (DGI) (14, 20).

Conventional diagnosis of GC infection requires isolation of the organism on selective media or the observation of diplococci in Gram stained smears (16). Culture methods can have good clinical sensitivity, but are highly dependent on proper specimen handling. Improper specimen storage and transport can result in the loss of organism viability and yield false negative results. In addition, poor sampling technique, toxic sampling materials, and the inhibition of growth by components of body secretions can also result in false negative results (7, 18). Commonly used non-culture methods for GC detection include direct DNA probe tests and NAATs.

First generation NAATs for CT and GC have technological issues that have limited their performance. These issues include cumbersome specimen processing and specimen inhibition that can yield false negative results (6, 10, 13, 19, 23, 29, 30, 31). The APTIMA COMBO 2 Assay is a second generation NAAT that utilizes target capture, Transcription-Mediated Amplification (TMA), and Dual Kinetic Assay (DKA) technologies to streamline specimen processing, amplify target rRNA, and detect amplicon, respectively. Studies comparing performance and specimen inhibition of various amplification systems have demonstrated the benefits of target capture, TMA, and DKA technologies (8, 12). The APTIMA COMBO 2 Assay qualitatively detects CT and/or GC rRNA in clinician-collected endocervical, vaginal, and male urethral swab specimens, patient-collected vaginal swab specimens, PreservCyt Solution liquid Pap specimens, and in female and male urine specimens from symptomatic and asymptomatic individuals.

Principles of the Procedure

The APTIMA COMBO 2 Assay combines the technologies of target capture, TMA, and DKA.

Specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the rRNA targets and protect them from degradation during storage. When the APTIMA COMBO 2 Assay is performed in the laboratory, the target rRNA molecules are isolated from specimens by use of capture

oligomers via target capture that utilizes magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the target molecules as well as a string of deoxyadenosine residues. A separate capture oligomer is used for each target. During the hybridization step, the sequence specific regions of the capture oligomers bind to specific regions of the target molecules. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured target molecules bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The APTIMA COMBO 2 Assay replicates a specific region of the 23S rRNA from CT and a specific region of the 16S rRNA from GC via DNA intermediates. A unique set of primers is used for each target molecule. Detection of the rRNA amplification product sequences (amplicon) is achieved using nucleic acid hybridization. Single-stranded chemiluminescent DNA probes, which are complementary to a region of each target amplicon, are labeled with different acridinium ester molecules. The labeled DNA probes combine with amplicon to form stable RNA:DNA hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled RNA:DNA hybrids is measured as photon signals in a luminometer, and are reported as Relative Light Units (RLU). In DKA, differences in the kinetic profiles of the CT and GC labeled probes allow for the differentiation of signal; kinetic profiles are derived from measurements of photon output during the detection read time. The chemiluminescent detection reaction for CT signal has very rapid kinetics and has the "flasher" kinetic type. The chemiluminescent detection reaction for GC signal is relatively slower and has the "glower" kinetic type. Assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

DTS Systems Reagents

Reagents for the APTIMA COMBO 2 Assay for CT and GC are listed below for the DTS Systems. Reagent Identification Symbols are also listed next to the reagent name.

Materials Provided

APTIMA COMBO 2 Assay Kit, 100 tests (2 boxes) (Cat. No. 301032)

Refrigerated box (Box 1 of 2)
(store at 2°C to 8°C)

Symbol	Component	Quantity	Description
A	APTIMA COMBO 2 Amplification Reagent	1 vial	*Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*
E	APTIMA COMBO 2 Enzyme Reagent	1 vial	*Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*
P	APTIMA COMBO 2 Probe Reagent	1 vial	*Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*
TCR-B	APTIMA COMBO 2 Target Capture Reagent B	1 x 0.35 mL	*Non-infectious nucleic acid in a buffered solution containing < 5% detergent.*
PCT/ NGC	APTIMA Positive Control, CT / Negative Control, GC	3 x 1.7 mL	*Non-infectious CT nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 1 CT IFU (5 fg/assay*).*
PGC/ NCT	APTIMA Positive Control, GC / Negative Control, CT	3 x 1.7 mL	*Non-infectious GC nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 50 GC cells (250 fg/assay*).*

*The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism.

Also included in the refrigerated box are the following:

Storage Tray
(store at 2°C to 30°C upon receipt)

Symbol	Component	Quantity	Description
AR	APTIMA COMBO 2 Amplification Reconstitution Solution	1 x 9.3 mL	*Aqueous solution containing preservatives.*
ER	APTIMA COMBO 2 Enzyme Reconstitution Solution	1 x 3.3 mL	*HEPES buffered solution containing a surfactant and glycerol.*
PR	APTIMA COMBO 2 Probe Reconstitution Solution	1 x 12.4 mL	*Succinate buffered solution containing < 5% detergent.*

S	APTIMA COMBO 2 Selection Reagent	1 x 31 mL	*600 mM borate buffered solution containing surfactant.*

Also included in the refrigerated box are the following:

	Reconstitution Collars	3 each	
	Sealing Cards	1 package	

Non-Refrigerated Box or APTIMA Assay Fluids (Box 2 of 2) (store at 15°C to 30°Cupon receipt)

Symbol	Component	Quantity	Description
TCR	APTIMA COMBO 2 Target Capture Reagent	1 x 22 mL	*Buffered salt solution containing solid phase and capture oligomers.*
W	APTIMA Wash Solution	1 x 402 mL	*10 mM HEPES buffered solution containing < 2% detergent.*
DF	APTIMA Buffer for Deactivation Fluid	1 x 402 mL	*800 mM bicarbonate buffered solution.*
O	APTIMA Oil Reagent	1 x 24.6 mL	*Silicone Oil*

DTS Systems Materials

Note: Gen-Probe catalog numbers are listed in parentheses.

Materials Required But Available Separately

APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens (Cat. No. 301041)

APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens (Cat. No. 301040)

APTIMA Urine Specimen Transport Tubes for Male and Female Urine Specimens (Cat. No. 105575)

APTIMA Vaginal Swab Specimen Collection Kit(Cat. No. 301162)

APTIMA Specimen Transfer Kit (Cat. No. 301154C)

APTIMA Auto Detect Kit (Cat. No. 301048)

LEADER HC+ Luminometer (Cat. No. 104747-01)

GEN-PROBE Target Capture System (TCS) (Cat. No. 104555)

Incubators and vortexers:

Either:

- 2 Multi-tube vortex mixers (Cat. No. 102160)
- 3 Circulating water baths (62°C ± 1°C, 42°C ± 1°C, 62°C ± 1°C)(Cat. No. 104586)
- 3 Water bath spacers (Cat. No. 104627)

Or:

- 2 SB100 Dry Heat Bath/Vortexers (Cat. No. 105524) (Additional SB100 baths may be required as test volume increases)

2 eppendorf Repeater Plus pipettors (Cat. No. 105725)

2 pipettors, 1000 µL RAININ PR1000 (Cat. No. 901715)

eppendorf pipettor, 20 µL to 200 µL (Cat. No. 105726)

Repeat pipettor tips (2.5 mL, 5.0 mL, 25.0 mL) (Cat. Nos. 21-381-329, 21-381-330, 21-381-115, respectively)

Tips, P1000 Style, special diameter tip only available from Gen-Probe (Cat. No. 105049)

Pipette tips 20 µL to 200 µL, Fisher, 705512

Ten Tube Units (TTU) (Cat. No. TU0022)

Ten Tip Cassettes (TTC) (Cat. No. 104578)

SysCheck calibration standard (Cat. No. 301078)

Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution

Standard urine collection containers, without preservatives

Large-capped plastic container

APTIMA penetrable caps (Cat. No. 105668)

Replacement non-penetrable caps (Cat. No. 103036A)

Optional Materials

PACE Specimen Collection Kit for Male Urethral or Conjunctival Specimens (Cat. No. 103275)

PACE Specimen Collection Kit for Endocervical Specimens (Cat. No. 103300)

APTIMA Adapter Kit (Cat. No. 301087)

APTIMA Controls Kit (Cat. No. 301110)

STD Proficiency Panel (Cat. No. 102325)

TECAN Freedom EVO 100/4 (Cat. No. 900932)

DTS 800 Systems APTIMA COMBO 2 Deck Plate (Cat. No. 105200)

Tips, 1000 µL conductive, liquid sensing, TECAN 10612513

Reagent reservoir (40 mL quarter module) (Cat. No. 104765)

Split reagent reservoir (19 mL x 2 quarter module) (Cat. No. 104763)

GEN-PROBE Bleach Enhancer for Cleaning
for routine cleaning of surfaces and equipment (Cat. No. 302101)

TIGRIS DTS System Reagents

Reagents for the APTIMA COMBO 2 Assay for CT and GC are listed below for the TIGRIS DTS System. Reagent Identification Symbols are also listed next to the reagent name.

Materials Provided

APTIMA COMBO 2 Assay Kit, 250 tests (2 boxes and 1 Controls kit) (Cat. No. 301130)

Refrigerated box (Box 1 of 2) (store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity	Description
A	APTIMA COMBO 2 Amplification Reagent	1 vial	*Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*
E	APTIMA COMBO 2 Enzyme Reagent	1 vial	*Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*
P	APTIMA COMBO 2 Probe Reagent	1 vial	*Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*
TCR-B	APTIMA COMBO 2 Target Capture Reagent B	1 x 0.61 mL	*Non-infectious nucleic acid in a buffered solution containing < 5% detergent.*

Non-Refrigerated Box (Box 2 of 2)
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity	Description
AR	APTIMA COMBO 2 Amplification Reconstitution Solution	1 x 27.7 mL	*Aqueous solution containing preservatives.*
ER	APTIMA COMBO 2 Enzyme Reconstitution Solution	1 x 11.1 mL	*HEPES buffered solution containing a surfactant and glycerol.*
PR	APTIMA COMBO 2 Probe Reconstitution Solution	1 x 35.4 mL	*Succinate buffered solution containing < 5% detergent.*
S	APTIMA COMBO 2 Selection Reagent	1 x 108 mL	*600 mM borate buffered solution containing surfactant.*
TCR	APTIMA COMBO 2 Target Capture Reagent	1 x 54 mL	*Buffered salt solution containing solid phase and capture oligomers.*
	Reconstitution Collars	3	
	Reagent Kit Master Lot Barcode Sheet	1	

APTIMA Controls Kit
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity	Description
PCT/ NGC	APTIMA Positive Control, CT / Negative Control, GC	5 x 1.7 mL	*Non-infectious CT nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 1 CT IFU (5 fg/assay*).*
PGC/ NCT	APTIMA Positive Control, GC / Negative Control, CT	5 x 1.7 mL	*Non-infectious GC nucleic acid in a buffered solution containing < 5% detergent. Each 400 µL sample contains the estimated rRNA equivalent of 50 GC cells (250 fg/assay*).*

*The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism.

TIGRIS DTS System Materials

Materials Required But Available Separately

Note: Gen-Probe catalog numbers are listed in parentheses.

APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens (Cat. No. 301041)

APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens (Cat. No. 301040)

APTIMA Urine Specimen Transport Tubes for Male and Female Urine Specimens (Cat. No. 105575)

APTIMA Vaginal Swab Specimen Collection Kit (Cat. No. 301162)

APTIMA Specimen Transfer Kit (Cat. No. 301154C)

TIGRIS DTS System (Cat. No. 105118)

Tips, 1000 µL conductive, liquid sensing, TECAN 10612513

APTIMA Assay Fluids Kit (Cat. No. 302382)
APTIMA Wash Solution, APTIMA Buffer for Deactivation Fluid, and APTIMA Oil Reagent

APTIMA System Fluid Preservative Kit (Cat. No. 302380)

APTIMA Auto Detect Kit (Cat. No. 301048)

TIGRIS DTS System Run Kit (Cat. No. 301191) or

- Multi-tube Units (MTU) (Cat. No. 104772-02)
- MTU/Tiplet Waste Bag Kit (Cat. No. 900907)
- MTU Waste Deflectors (Cat. No. 900931)
- MTU Waste Covers (Cat. No. 105523)

Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution

Water for the TIGRIS DTS System (consult the *TIGRIS DTS System Operator's Manual* for specifications)

Disposable gloves

SysCheck calibration standard (Cat. No. 301078)

APTIMA penetrable caps (Cat. No. 105668)

Replacement, non-penetrable caps (Cat. No. 103036A)

Replacement Caps for the 250-test kits

- Amplification and Probe reagent reconstitution solutions CL0041 (100 caps)
- Enzyme Reagent reconstitution solution 501616 (100 caps)
- TCR and Selection reagent CL0040 (100 caps)

Optional Materials

APTIMA Controls Kit (Cat. No. 301110)

GEN-PROBE Bleach Enhancer for Cleaning
for routine cleaning of surfaces and equipment (Cat. No. 302101)

Warnings and Precautions

A. For *In vitro* diagnostic use.

B. For additional specific warnings, precautions and procedures to control contamination for the TIGRIS DTS System, consult the *TIGRIS DTS System Operator's Manual.*

Laboratory Related

C. The assay was not evaluated in patient populations with a low prevalence of CT disease; therefore, performance in low prevalence settings has not been determined.

D. Use only supplied or specified disposable laboratory ware.

E. Use routine laboratory precautions. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

F. **Warning: Irritants and Corrosives:** Avoid contact of Auto Detect 1 and Auto Detect 2 with skin, eyes and mucous membranes. If these fluids come into contact with skin or eyes, wash with water. If spills of these fluids occur, dilute with water before wiping dry.

G. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution.

DTS Systems Specific

H. A separate area for DKA is strongly recommended to minimize amplicon contamination in the assay. This dedicated area should be away from the reagent preparation, target capture, and amplification area.

I. To help prevent lab areas from becoming contaminated with amplicon, the laboratory area should be arranged with a unidirectional workflow: from reagent preparation through DKA. Specimens, equipment, and reagents should not be returned to the area where a previous step was performed. Also, personnel should not move back into previous work areas without proper contamination safeguards.

Specimen Related

J. This method has been tested using endocervical and male urethral swab specimens, PreservCyt Solution liquid Pap specimens, vaginal swab specimens, female and male urine specimens only. Performance with specimens other than those collected with the following specimen collection kits have not been evaluated.

- APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
- APTIMA Urine Collection Kit for Male and Female Urine Specimens
- APTIMA Vaginal Swab Specimen Collection Kit
- APTIMA Specimen Transfer Kit

DTS Systems Specific

- PACE Specimen Collection Kit for Male Urethral or Conjunctival Specimens (in conjunction with the APTIMA Adapter Kit)
- PACE Specimen Collection Kit for Endocervical Specimens (in conjunction with the APTIMA Adapter Kit)

The PACE collection kits have been validated for use only with the APTIMA Adapter Kit. Use of the PACE Collection Kit and APTIMA Adapter Kit is currently not qualified for the TIGRIS DTS System. Gynecologic samples collected for preparation using the ThinPrep 2000 System should be collected using broom-type or endocervical brush/plastic spatula combination collection devices. Laboratories may validate other collection devices (21, 22).

K. Expiration dates listed on the collection kits pertain to the collection site and not the testing facility. Samples collected any time prior to the expiration date of the collection kit, and transported and stored in accordance with the package insert, are valid for testing even if the expiration date on the collection tube has passed.

L. The PreservCyt Solution has been validated as an alternative medium for testing with APTIMA COMBO 2 Assay. PreservCyt Solution liquid Pap specimens processed using the ThinPrep 3000 Processor or other instruments have not been evaluated to test for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* using APTIMA COMBO 2 Assay.

M. After urine has been added, the liquid level in the urine transport tube must fall between the two black indicator lines on the tube label. Otherwise, the specimen must be rejected.

N. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

O. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.

P. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another, and discard used materials without passing over open containers. Change gloves if they come in contact with specimen.

Q. If the lab receives a swab specimen transport tube with no swab, two swabs, a cleaning swab, or a swab not supplied by Gen-Probe, the specimen must be rejected. Prior to rejecting a swab transport tube with no swab, verify that it is not an APTIMA Specimen Transfer Tube as this specimen transport tube will not contain a swab.

R. For PreservCyt Solution liquid Pap specimens, collect according to the manufacturer's instructions. Aliquots subsequently removed from the PreservCyt vial for testing by the APTIMA COMBO 2 Assay should be processed using only the APTIMA Specimen Transfer Kit.

S. Upon piercing, liquid can discharge from APTIMA transport tube caps under certain conditions. Follow instructions in the appropriate test procedure to prevent this occurrence.

Assay Related

T. The performance of vaginal swab specimens has not been evaluated in pregnant women.

U. The performance of vaginal swab and PreservCyt Solution liquid Pap specimens has not been evaluated in women less than 16 years of age.

V. Do not use this kit after its expiration date.

W. Do not interchange, mix, or combine assay reagents from kits with different lot numbers. APTIMA controls and assay fluids can be from different lot numbers.

DTS Systems Specific

X. Tips with hydrophobic plugs must be used. A minimum of two repeat pipettors must be dedicated for use with this assay: one for use in the target capture and amplification steps, and one for use in the DKA steps. Two micropipettors must be dedicated for use in this assay: one for use in specimen transfer and one for use in reagent preparation. All pipettors must be cleaned regularly as described in *Procedural Notes.*

Y. When using repeat pipettors for reagent addition, do not touch the tube with the pipette tip to prevent carryover from one tube to another.

Z. Adequate mixing is necessary to achieve accurate assay results. For complete details, see *Procedural Notes*.

AA. Separate water baths must be dedicated for the target capture, amplification, and DKA steps in the assay.

AB. Sealing cards should be disposed of in the waste container immediately after removing them from reaction tubes. Fresh sealing cards should always be used: they should never be re-used from a previous step. Sealing cards should be firmly fixed to the top of all reaction tubes.

Reagent Storage and Handling Requirements

A. The following reagents are stable when stored at 2°C to 8°C (refrigerated):

APTIMA COMBO 2 Amplification Reagent

APTIMA COMBO 2 Enzyme Reagent

APTIMA COMBO 2 Probe Reagent

APTIMA COMBO 2 Target Capture Reagent B

APTIMA Positive Control, CT / Negative Control, GC

APTIMA Positive Control, GC / Negative Control, CT

B. The following reagents are stable when stored at 2°C to 30°C:

APTIMA COMBO 2 Amplification Reconstitution Solution

APTIMA COMBO 2 Enzyme Reconstitution Solution

APTIMA COMBO 2 Probe Reconstitution Solution

APTIMA COMBO 2 Selection Reagent

C. The following reagents are stable when stored at 15°C to 30°C (room temperature):

Target Capture Reagent

APTIMA Wash Solution

APTIMA Buffer for Deactivation Fluid

APTIMA Oil Reagent

D. Working Target Capture Reagent (wTCR) is stable for 30 days when stored at 15°C to 30°C. Do not refrigerate.

E. After reconstitution, the Enzyme Reagent, Amplification Reagent, and Probe Reagent are stable for 30 days when stored at 2°C to 8°C.

F. Discard any unused reconstituted reagents and wTCR after 30 days or after the Master Lot expiration date, whichever comes first.

G. Controls are stable until the date indicated on the vials.

H. Reagents stored on-board the TIGRIS DTS System have 48 hours of on-board stability.

I. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Store the reagents protected from light. The specified reconstituted stability is based on 12 hours exposure of the Reconstituted Probe Reagent to two 60W fluorescent bulbs, at a distance of 17 inches (43 cm), and temperature less than 30°C. Light exposure of the Reconstituted Probe Reagent should be limited accordingly.

J. Upon warming to room temperature, some control tubes may appear cloudy or contain precipitates. Cloudiness or precipitation associated with controls does not affect control performance. The controls may be used whether they are clear or cloudy/precipitated. If clear controls are desired, solubilization may be expedited by incubating them at the upper end of the room temperature range (15°C to 30°C).

K. Do not freeze the reagents.

Specimen Collection and Storage

The APTIMA COMBO 2 Assay is designed to detect the presence of CT and GC in the following specimens: endocervical and male urethral specimens, vaginal swab specimens, PreservCyt Solution liquid Pap specimens, and in female and male urine specimens. Performance with specimens other than those collected with the following specimen collection kits has not been evaluated:

- APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
- APTIMA Urine Collection Kit for Male and Female Urine Specimens
- APTIMA Vaginal Swab Specimen Collection Kit
- APTIMA Specimen Transfer Kit (for use with gynecologic samples collected in PreservCyt Solution)

DTS Systems Specific

- PACE Specimen Collection Kit for Male Urethral or Conjunctival Specimens (in conjunction with the APTIMA Adapter Kit)
- PACE Specimen Collection Kit for Endocervical Specimens (in conjunction with the APTIMA Adapter Kit)

A. Instructions for collection:

Refer to the appropriate specimen collection kit package insert for collection instructions.

B. Specimen transport and storage before testing:

1. Swab specimens:
   a. After collection, transport and store the swab in the swab specimen transport tube at 2°C to 30°C until tested. Specimens must be assayed with the APTIMA COMBO 2 Assay within 60 days of collection. If longer storage is needed, freeze at -20°C to -70°C for up to 12 months after collection (see *Specimen Stability Studies*).
2. Urine specimens:
   a. Urine samples that are still in the primary collection container must be transported to the lab at 2°C to 30°C. Transfer the urine sample into the APTIMA urine specimen transport tube within 24 hours of collection. Store at 2°C to 30°C and test within 30 days of collection.
   b. After collection, transport the processed urine specimens in the GEN-PROBE APTIMA urine specimen transport tube at 2°C to 30°C and store at 2°C to 30°C until tested. Processed urine specimens should be assayed with the APTIMA COMBO 2 Assay within 30 days of collection. If longer storage is needed, freeze at -20°C to -70°C for up to 12 months after collection (see *Specimen Stability Studies*).
3. PreservCyt Solution liquid Pap specimens:
   a. PreservCyt Solution liquid Pap specimens intended for CT and/or GC testing must be processed for cytology and/or transferred to an APTIMA Specimen Transfer tube within 30 days of collection when stored at 2°C to 30°C (see *Specimen Stability Studies*).
   b. If the ThinPrep Aliquot Removal procedure will be used, refer to the *ThinPrep 2000 or ThinPrep 3000 Processor Operator's Manual - Addendum* for instructions on aliquot removal. Transfer 1 mL of the removed aliquot into an APTIMA Specimen Transfer tube according to the instructions in the APTIMA Specimen Transfer Kit package insert.
   c. If testing the specimen after processing using the ThinPrep 2000 Processor, process the PreservCyt Solution liquid Pap specimen in accordance with the *ThinPrep 2000 Processor Operator's Manual* and the APTIMA Specimen Transfer Kit package insert. Transfer 1 mL of the fluid remaining in the PreservCyt Solution vial into an APTIMA Specimen Transfer tube according to the instructions in the APTIMA Specimen Transfer Kit package insert.
   d. Once the PreservCyt Solution liquid Pap specimen is transferred to the APTIMA Specimen Transfer tube, the specimen must be assayed with the APTIMA COMBO 2 Assay within 30 days when stored at 2°C to 8°C or 14 days when stored at 15°C to 30°C. If longer storage is needed, freeze at -20°C to -70°C for up to 12 months after transfer (see *Specimen Stability Studies*).

C. Specimen storage after testing:

1. Specimens that have been assayed must be stored upright in a rack.
2. The specimen transport tubes should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, remove penetrable cap and place new non-penetrable caps on the specimen transport tubes. If specimens need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. **Avoid splashing and cross-contamination.**

Note: Federal requirements for packaging must be met when specimens are transported by common land and air carriers. Refer to 42 CFR, Part 72. The most current requirements may be obtained from the Centers for Disease Control and Prevention Office of Health and Safety in Atlanta, Georgia, or the CDC web site.

DTS Systems Test Procedure

A. Equipment Preparation

1. Adjust one water bath to 62°C ± 1°C (for target capture, and primer annealing), a second water bath to 42°C ± 1°C (for amplification), and a third water bath to 62°C ± 1°C (for DKA). If using the SB100 Dry Heat Bath/Vortexer, refer to the *SB100 Dry Heat Bath/Vortexer Application Sheet (SB100 Application Sheet)*.
2. Prior to starting the assay, wipe down work surfaces and pipettors with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces and pipettors for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the test will be performed with clean, plastic-backed absorbent laboratory bench covers.
3. Place a sufficient number of Ten Tip Cassettes into the Target Capture System (TCS). Ensure that the TCS wash bottle is filled with APTIMA Wash Solution and the aspiration manifold is connected to the vacuum pump. (Refer to the *Target Capture System Operator's Manual.*)

B. Reagent Reconstitution

Reagent Reconstitution *should be performed prior to beginning* specimen transfer.

1. Reconstitute the APTIMA COMBO 2 Enzyme, Amplification, and Probe Reagents:
   a. Pair the appropriate reconstitution solution with the lyophilized reagent. The labels are color coded so that they can be paired correctly.
   b. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1 Step 1).
   c. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
   d. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).
   e. Slowly invert the assembled bottle and vial. Allow the solution to drain from the bottle into the vial (Figure 1, Step 3).
   f. Gently swirl the solution in the vial. Avoid creating foam while swirling the vial (Figure 1, Step 4).
   g. Wait for the lyophilized reagent to go into solution, then invert the assembled bottle and vial again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the bottle.
   h. Remove the reconstitution collar from the bottle (Figure 1, Step 6).
   i. Recap the bottle. Peel and discard the top label. Record operator initials, the reconstitution date, and lyophilized reagent lot number on the remaining label (Figure 1, Step 7).
   j. Discard both the reconstitution collar and vial (Figure 1, Step 8).

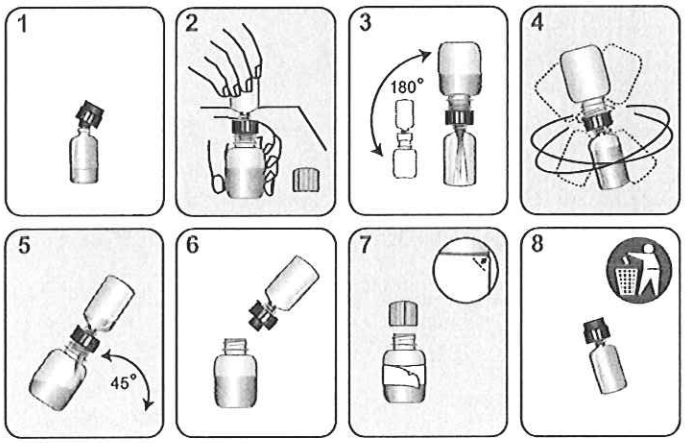

Figure 1. DTS Systems Reconstitution Process

2. Previously reconstituted Probe, Amplification, and Enzyme Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay. If Probe Reagent contains precipitate that does not return to solution at room temperature, heat at 62°C for 1 to 2 minutes. After this heat step, the Probe Reagent may be used even if residual precipitate remains. After resuspension, mix by gentle inversion, being careful not to induce foam.

   Note: This inversion step should be performed any time that the precipitate is being brought into solution, whether by heating at 62°C or by warming at room temperature.

3. Prepare working Target Capture Reagent (wTCR) as follows:
   a. Transfer 20 mL of TCR to an appropriately sized, dedicated, clean, dry container.
   b. Using a micropipettor, add 200 µL of TCR-B into the TCR.
   c. Thoroughly mix the solution by swirling.
   d. Label the container. Record operator initials, preparation date, and both lot numbers.

   Note: For a smaller number of reactions (specimens and controls), use the following to calculate volumes of TCR and TCR-B:

   Volume of TCR (mL) = (number of reactions + 5 extra reactions) x 0.1 mL

   Volume of TCR-B (mL) = Volume of TCR (mL) / 100

C. Target Capture

The repeat pipettor used in target capture and amplification should be dedicated for use in these steps only. See *Warnings and Precautions* for more information.

Rack Setup

1. Allow the controls and specimens to reach room temperature prior to processing.
2. **Do not vortex specimens.**
3. Visually confirm that each specimen tube meets one of the following criteria:
   a. The presence of a single blue APTIMA collection swab in a unisex swab specimen transport tube.
   b. The presence of a single pink APTIMA collection swab in a vaginal swab specimen transport tube.
   c. A final volume of urine between the black fill lines of a urine specimen transport tube.

d. The absence of a swab in the APTIMA specimen transport tube for PreservCyt Solution liquid Pap specimens.

4. Inspect specimen tubes before piercing them:

   a. If a specimen tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.

   b. If a specimen tube has a lower volume than typically observed when collection instructions have been followed, centrifuge the tube for 5 minutes at 420 RCF to ensure that no liquid is in the cap.

   c. If the liquid level in a urine specimen tube is not between the two black indicator lines on the label, the specimen must be rejected. Do not pierce an overfilled tube.

   d. If a urine specimen tube contains precipitate, heat the specimen at 37°C for up to 5 minutes. If the precipitate does not go back into solution, visually ensure that the precipitate does not prevent delivery of the specimen.

   Note: Failure to follow Steps 4a-c may result in liquid discharge from the transport tube cap.

5. If specimens with standard (non-penetrable) caps are to be tested, they must be centrifuged for 5 minutes at 420 RCF (Relative Centrifugal Force) to bring all of the liquid down to the bottom of the tube before uncapping. **Avoid splashing and cross-contamination.**

6. In the Ten Tube Unit (TTU) rack, place enough TTUs to accommodate the controls and specimens.

7. If a worklist is desired, create the worklist at this point. For instructions on creating a worklist, refer to the *APTIMA Assay Software Operator's Manual.*

8. Thoroughly mix the wTCR reagent. Using the repeat pipettor, add 100 µL into each reaction tube.

9. Hold the Positive Control, CT / Negative Control, GC tube in one hand or keep it in a rack. To work properly with the APTIMA Assay software, the Positive Control, CT/Negative Control, GC must be in the first position of the first TTU. This label is pink. The label text is "CONTROL + CT PCT / CONTROL – GC NGC". Using a micropipettor, pierce the cap, taking care not to drive the tip into the bottom of the tube. Add 400 µL of the Positive Control, CT / Negative Control, GC to the first reaction tube. In the same manner and using a new pipette tip, add 400 µL of the Positive Control, GC / Negative Control, CT to the second reaction tube. The label for the second control is blue-green. The label text is "CONTROL + GC PGC / CONTROL – CT NCT".

10. Continue the rack setup procedure by adding 400 µL of each specimen into the remaining reaction tubes. Use a new pipette tip for each specimen and control. The acceptable volume of specimen or control added to a reaction tube is 400 µL ± 100 µL. See *Procedural Notes, Control and Specimen Pipetting* for more information.

Target Capture

Use of the GEN-PROBE Target Capture System is described in the *Target Capture System Operator's Manual.* If using the SB100 Dry Heat Bath/Vortexer, refer to the SB100 Application Sheet.

11. Cover the TTUs with sealing cards and shake the rack gently by hand. **Do not vortex.** Incubate the rack at 62°C ± 1°C in a water bath for 30 ± 5 minutes.

12. Remove the rack from the water bath and blot the bottoms of the tubes dry on absorbent material.

13. Ensure the sealing cards are firmly seated. If necessary, replace them with new sealing cards and seal the TTUs tightly.

14. Vortex the rack for 60 seconds on the multi-tube vortex mixer. See *Procedural Notes, Vortexing* for details. Begin vortexing within 2 minutes of removal of the rack from the water bath.

15. Without removing the sealing cards, incubate the rack at room temperature for 30 ± 5 minutes.

16. Place the rack on the TCS magnetic base for 5 to 10 minutes.

17. Prime the dispense station pump line by pumping APTIMA Wash Solution through the dispense manifold. Pump enough liquid through the system so that there are no air bubbles in the line and that all ten nozzles are delivering a steady stream of liquid.

18. Turn on the vacuum pump and disconnect the aspiration manifold at the first connector between the aspiration manifold and the trap bottle. Ensure that the vacuum gauge meets the leak test specification.[1] It may take 15 seconds to achieve this reading. Reconnect the aspiration manifold, and ensure that the vacuum gauge meets the vacuum level specification. Leave the vacuum pump on until all target capture steps are completed and the aspiration manifold tubing is dry.

19. Firmly attach the aspiration manifold to the first set of tips. Aspirate all liquid by lowering the tips into the first TTU until the tips come into brief contact with the bottoms of the tubes. Do not hold the tips in contact with the bottoms of the tubes.

20. After the aspiration is complete, eject the tips into their original TTC. Repeat the aspiration steps for the remaining TTUs, using a dedicated tip for each specimen.

21. Place the dispense manifold over each TTU and, using the dispense station pump, deliver 1.0 mL of APTIMA Wash Solution into each tube of the TTU.

22. Cover the tubes with a sealing card and remove the rack from the TCS magnetic base. Vortex the rack once on the multi-tube vortex mixer. See *Procedural Notes* for details.

23. Place the rack on the TCS magnetic base for 5 to 10 minutes.

24. Aspirate all liquid as in Steps 19 and 20.

25. After the final aspiration, remove the rack from the TCS magnetic base and visually inspect the tubes to ensure that all liquid has been aspirated, and all tubes contain magnetic particle pellets. If any liquid is visible, place the rack back on the TCS magnetic base for 2 minutes, and repeat the aspiration for that TTU using the same tips used previously for each specimen.

    Note: If a magnetic particle pellet is visible after aspiration is completed, the tube may be accepted. If no pellet is visible, the specimen should be retested. If the same specimen does not contain a magnetic particle pellet at this step in a subsequent run, this may indicate a specimen-specific problem. Re-collection of the specimen is recommended in this situation.

D. Amplification

If using the SB100 Dry Heat Bath/Vortexer, refer to the SB100 Application Sheet.

1. Using the repeat pipettor, add 75 µL of the reconstituted Amplification Reagent to each reaction tube. All reaction mixtures in the rack should now be red.

2. Using the repeat pipettor, add 200 µL of Oil Reagent to each reaction tube.

3. Cover the tubes with a sealing card and vortex them on the multi-tube vortex mixer.

4. Incubate the rack in a water bath at 62°C ± 1°C for 10 ± 5 minutes.

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1 See the Target Capture System Vacuum Specifications Sheet located at the back of the *Target Capture System Operator's Manual* or contact Technical Support.

5. Transfer the rack into a water bath at 42°C ± 1°C and incubate for 5 ± 2 minutes.
6. With the rack in the water bath, carefully remove the sealing card and, using the repeat pipettor, add 25 µL of the reconstituted Enzyme Reagent to each reaction tube. All reaction mixtures should now be orange.
7. Immediately cover the tubes with a fresh sealing card, remove the rack from the water bath, and mix the reaction tubes by gently shaking the rack by hand.
8. Incubate the rack in a water bath at 42°C ± 1°C for 60 ± 15 minutes.

E. Dual Kinetic Assay (DKA)

If using the SB100 Dry Heat Bath/Vortexer, refer to the SB100 Application Sheet.

The repeat pipettor used in the hybridization and selection steps should be dedicated for use in these steps only. See *Warnings and Precautions*.

1. Hybridization
   a. Remove the rack from the water bath and transfer to the DKA area. Using the repeat pipettor, add 100 µL of the reconstituted Probe Reagent to each reaction tube. All reaction mixtures should now be yellow.
   b. Cover the tubes with a sealing card and vortex the rack on the multi-tube vortex mixer.
   c. Incubate the rack in a 62°C ± 1°C water bath for 20 ± 5 minutes.
   d. Remove the rack from the water bath and incubate at room temperature for 5 ± 1 minutes.
2. Selection
   a. Using the repeat pipettor, add 250 µL of Selection Reagent to each reaction tube. All reaction mixtures should now be red.
   b. Cover the tubes with a sealing card, vortex the rack for 10 seconds or until the color is uniform, and incubate the rack in a water bath at 62°C ± 1°C for 10 ± 1 minutes.
   c. Remove the rack from the water bath.
3. Detection

   Detection must be performed at 18°C to 28°C.

   a. Incubate the rack at 18°C to 28°C for 15 ± 3 minutes.

   Note: This temperature range is critical for assay performance.

   b. For use of the LEADER HC+ Luminometer and the APTIMA Assay software, refer to the *LEADER HC+ Luminometer Operator's Manual* and the *APTIMA Assay Software Operator's Manual.*
   c. Ensure there are sufficient volumes of Auto Detect 1 and 2 to complete the tests.
   d. Prepare the LEADER HC+ Luminometer by placing one empty TTU in cassette position number 1 and performing the **Wash** protocol.
   e. Load the TTUs into the luminometer.
   f. Log on to the computer. Click on **New Run,** select the **APTIMA Combo 2** assay protocol, and enter the number of tubes (controls and specimens). Click **Next** to begin the run.

   Note: The run must be completed within 2 hours of the end of the selection step incubation.

   g. Prepare Deactivation Fluid by mixing equal volumes of 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution and APTIMA Buffer for Deactivation Fluid in a large-capped plastic container. Label and write the expiration date on the plastic container. Deactivation Fluid is stable for 4 weeks at room temperature. Discard Deactivation Fluid after 4 weeks or after 100 processed samples have been deactivated (whichever comes first).
   h. After removing the used TTUs from the luminometer, place the TTUs into the container of Deactivation Fluid. Allow the TTUs to sit in the container for 15 minutes before disposal. Proper handling and disposal methods should be established by the laboratory director.

Procedural Notes

A. Controls

To work properly with the APTIMA Assay software, the Positive Control, CT / Negative Control, GC must be in the first position of the first TTU. This control label is pink. The label text is "CONTROL + CT PCT / CONTROL – GC NGC". The Positive Control GC / Negative Control CT must be in the second position of the first TTU. This control label is blue-green. The label text is "CONTROL + GC PGC / CONTROL – CT NCT". Placement in the wrong position will cause the run to fail. Any additional controls must be entered as patient specimens and monitored by the operator for acceptability.

B. Control and Specimen Pipetting

The volume of control or specimen added to the reaction tube should be 400 µL ± 100 µL. Visual inspection of the volume pipetted into the reaction tube is recommended to ensure proper volume transfer. Proper control or specimen volume is needed to provide accurate results. If the proper volume has not been pipetted, re-pipette the wTCR and the control or specimen into a new reaction tube.

C. Reagents

Probe Reconstitution Solution may precipitate during storage. If this occurs, heat the Probe Reconstitution Solution at 62°C for 1 to 2 minutes. After this heat step, the Probe Reconstitution Solution may be used even if residual precipitate remains. After resuspension, mix the vial by gentle inversion, being careful not to induce foam.

D. Temperature

1. The target capture, amplification, hybridization, and selection steps are temperature dependent. Therefore, it is imperative that the water baths be maintained within their specified temperature ranges.
2. Room temperature is defined as 15°C to 30°C.
3. The detection steps in the assay must be carried out at 18°C to 28°C.

E. Time

The target capture, amplification, hybridization, and selection reactions are all time dependent. Adhere to the times specified in the *DTS Systems Test Procedure.*

F. Vortexing

Proper vortexing is important to the successful performance of the APTIMA COMBO 2 Assay. When adequate vortexing motion is achieved, the suspension spins at a rate that raises the solution into the upper half of the tube. This manipulation (vortexing) is maintained for specified periods of time. To vortex reactions, set the multi-tube vortex mixer speed to the lowest setting, secure the rack, and turn on power. Slowly increase speed until the liquid rises halfway up the tube. Vortex for 10 seconds, the indicated amount of time, or until the color is uniform. Then, turn the speed to the lowest setting before turning off the multi-tube vortex mixer and removing the rack. The reaction mixtures should never touch the sealing cards.

G. Water Baths

1. The water level in the water baths must be maintained at 1.5 inches to 2.0 inches (3.8 cm to 5 cm) deep as measured from the supporting metal tray (on the bottom of the water bath) to the surface of the water. This will ensure proper heat transfer.
2. To avoid cross-contamination, water baths should be dedicated to a specific assay step.

H. Decontamination

1. Surfaces and Pipettors

   Laboratory bench surfaces and pipettors must be decontaminated regularly with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute, then follow with a water rinse. **Do not allow the sodium hypochlorite solution to dry.** Chlorine solutions may pit equipment and metal. Thoroughly rinse equipment with water to avoid pitting.

2. TCS Aspiration Manifold

   a. Place a new TTC into the TTC rack. Turn on the vacuum pump. Attach the aspiration manifold to the tips in the TTC. Aspirate all Wash Solution remaining in the priming trough of the Wash Solution dispense station. (Move the dispense manifold out of the way.)

   b. Pour at least 100 mL of 0.5% to 0.7% (0.07 M to 0.1 M), or if preferred 2.5% to 3.5% (0.35 M to 0.5 M), sodium hypochlorite solution into the priming trough. Aspirate all of the solution through the aspiration manifold.

   c. Pour at least 100 mL of deionized water into the priming trough. Aspirate all of the water through the aspiration manifold.

   d. Eject the tips into their original TTC.

   e. Leave the vacuum pump on until the manifold tubing is dry to prevent back flow.

   f. Decontaminate the aspiration manifold surfaces as described in *TCS Unit*.

3. TCS Waste Container

   When the waste bottle is 25% full or weekly, remove the waste bottle from the Target Capture System.

   a. Turn off the vacuum pump and allow the vacuum pressure to equalize.

   b. Release the quick disconnect fittings between the waste bottle and overflow bottle, and the waste bottle and aspiration manifold.

   c. Remove the waste bottle from the vacuum trap enclosure.

   d. Remove the cap and carefully add 400 mL of 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution to the bottle (or 1 L if using a 10 L waste bottle).

      Note: This may be done in a fume hood to avoid the release of fumes into the laboratory.

   e. Cap the waste bottle and gently swirl the contents until fully mixed.

   f. Let the waste bottle sit for 15 minutes and then dispose of the contents (waste).

   g. Rinse the waste bottle with water to remove any remaining waste.

   h. Cap the empty waste bottle and place it in the vacuum trap enclosure. Attach the quick disconnect fitting to the TCS unit. Carefully discard both gloves.

4. TCS Unit

   Wipe the surfaces of the TCS unit, aspiration manifold, and wash buffer ejector tips with paper towels moistened with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Follow the sodium hypochlorite solution step with a water rinse, then dry the surfaces completely with paper towels.

5. Racks

   Submerge the racks in 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution, ensuring that they are covered by the sodium hypochlorite solution. Keep the racks submerged for 10 minutes. Longer exposure could damage the racks. Rinse the racks thoroughly with water, place the racks on a clean absorbent pad, and allow the racks to air-dry thoroughly. To prolong the life of the racks, allow the racks to dry upright, not upside-down.

I. Assay Contamination

1. The introduction of contaminating materials may occur if sufficient care is not taken during the assay protocol.
2. TTUs must be decontaminated in Deactivation Fluid as described under *Detection*. Do not reuse the TTUs.
3. Perform regular decontamination of equipment and work surfaces. See *Procedural Notes, Decontamination* for details.
4. As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

J. Lab Contamination Monitoring Protocol for DTS Systems

There are many laboratory-specific factors that may contribute to contamination, including testing volume, workflow, disease prevalence and various other laboratory activities. These factors should be taken into consideration when contamination monitoring frequency is being established. Intervals for contamination monitoring should be established based on each laboratory's practices and procedures.

To monitor for laboratory contamination, the following procedure may be performed using the APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens:

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the swab transport medium, and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for each area to be swabbed.
7. Test the swab using the APTIMA COMBO 2 Assay according to the *DTS Systems Test Procedure*.

If the results are CT or GC positive or equivocal (see *Test Interpretation - QC/Patient Results*), the surface may be contaminated and should be decontaminated by treating with sodium hypochlorite solution as recommended in *DTS Systems Test Procedure, Equipment Preparation*.

Note: If contamination of the water bath is suspected, the bath water can be tested using the urine specimen test procedure, by adding 2.0 mL of the water to a urine specimen transport tube.

K. Troubleshooting

1. Low positive control values may be caused by incorrect temperatures during various steps in the assay or by allowing the selection time in the selection step to go longer than the recommended time.
2. High backgrounds may occur if the selection time in the selection step is shortened, the selection temperature is not correct, or insufficient mixing occurs after the addition of the Selection Reagent.
3. If the Positive Control, CT / Negative Control, GC is positive or equivocal for GC, or the Positive Control, GC / Negative Control, CT is positive or equivocal for CT, see *Procedural Notes, Assay Contamination* for more information.

TIGRIS DTS System Test Procedure

Note: See *TIGRIS DTS System Operator's Manual* for additional TIGRIS DTS System procedural information.

A. Work Area Preparation

Clean work surfaces where reagents and samples will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reagents and samples will be prepared with clean, plastic-backed absorbent laboratory bench covers.

B. Reagent Reconstitution/Preparation of a New Kit

Reagent Reconstitution *should be performed prior to beginning any work on the TIGRIS DTS System.*

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
    a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and lyophilized reagent have matching label colors before attaching the reconstitution collar.
    b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
    c. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 2, Step 1).
    d. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
    e. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 2, Step 2).
    f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 2, Step 3).
    g. Gently swirl the solution in the vial to mix. Avoid creating foam while swirling the vial (Figure 2, Step 4).
    h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 2, Step 5). Allow all of the liquid to drain back into the plastic bottle.
    i. Remove the reconstitution collar and vial (Figure 2, Step 6).
    j. Recap the bottle. Record operator initials and reconstitution date on the label (Figure 2, Step 7).
    k. Discard the reconstitution collar and glass vial (Figure 2, Step 8).

Warning: Avoid creating foam when reconstituting reagents. Foam compromises the level-sensing in the TIGRIS DTS System.

Note: Thoroughly mix Amplification, Enzyme, Probe, and Selection reagents by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents.

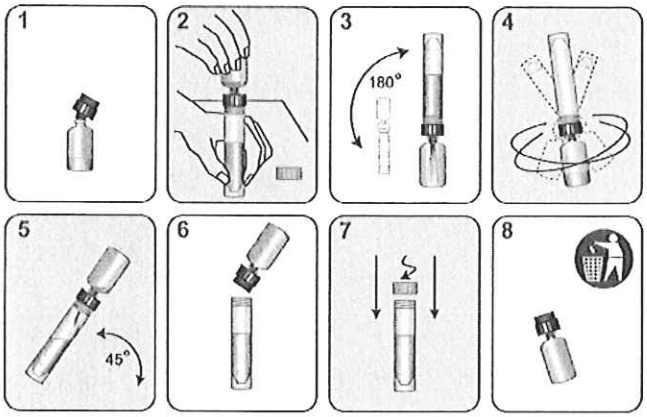

Figure 2. TIGRIS DTS System Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR):
   a. Pair the appropriate bottles of TCR and TCR-B.
   b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
   c. Open the bottle of TCR, and set the cap on a clean, covered work surface.
   d. Open the bottle of TCR-B and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the TCR-B bottle.
   e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.
   f. Record operator initials and the current date on the label. Record the TCR-B lot number.
   g. Discard the TCR-B bottle and cap.
3. Prepare Selection Reagent
   a. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
   b. Record operator initials and the current date on the label.

Note: Thoroughly mix by gently inverting all reagents prior to loading on the system. Avoid creating foam during inversion of reagents.

C. Reagent Preparation for Previously Reconstituted Reagents
1. Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.
2. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. After this heat step, the Probe Reagent may be used even if residual precipitate remains. Mix Probe Reagent by inversion, being careful not to induce foam, prior to loading onto the system.
3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents.
4. Do not top off reagent bottles. The TIGRIS DTS System will recognize and reject bottles that have been topped off.

D. Specimen Handling
1. Allow the controls and specimens to reach room temperature prior to processing.
2. **Do not vortex specimens.**
3. Visually confirm that each specimen tube meets one of the following criteria:
   a. The presence of a single blue APTIMA collection swab in a unisex swab specimen transport tube.
   b. The presence of a single pink APTIMA collection swab in a vaginal swab specimen transport tube.
   c. A final volume of urine between the black fill lines of a urine specimen transport tube.
   d. The absence of a swab in the APTIMA specimen transport tube for PreservCyt Solution liquid Pap specimens.
4. Inspect specimen tubes before loading into rack:
   a. If a specimen tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.
   b. If a specimen tube has a lower volume than typically observed when collection instructions have been followed, centrifuge the tube for 5 minutes at 420 RCF to ensure that no liquid is in the cap.
   c. If the liquid level in a urine transport tube is not between the two black indicator lines on the label, the specimen must be rejected. Do not pierce an overfilled tube.
   d. If a urine specimen tube contains precipitate, heat the specimen at 37°C for up to 5 minutes. If the precipitate does not go back into solution, ensure that the precipitate does not prevent delivery of the specimen.

Note: Failure to follow Steps 4a-c may result in liquid discharge from the transport tube cap.

Note: Up to three separate aliquots can be tested from each specimen. Attempts to pipette more than 3 aliquots from the specimen tube can lead to insufficient volume errors.

E. System Preparation

Set up the system and worklist according to instructions in the *TIGRIS DTS System Operator's Manual* and *Procedural Notes*.

Procedural Notes

A. Controls
1. To work properly with the TIGRIS APTIMA Assay software, front and end controls are required. The Positive Control, CT / Negative Control, GC must be in the first position and second to last position of a worklist. This control label is pink. The label text is "CONTROL + CT PCT / CONTROL – GC NGC". The Positive Control, GC / Negative Control, CT must be in the second position and last position of a worklist. This control label is blue-green. The label text is "CONTROL + GC PGC / CONTROL – CT NCT".
2. Each APTIMA control tube can be tested once. Attempts to pipette more than once from the tube can lead to insufficient volume errors.

B. Temperature

Room temperature is defined as 15°C to 30°C.

C. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

D. Lab Contamination Monitoring Protocol for TIGRIS DTS System

There are many laboratory-specific factors that may contribute to contamination, including testing volume, workflow, disease prevalence and various other laboratory activities. These factors should be taken into consideration when contamination monitoring frequency is being established. Intervals for contamination

monitoring should be established based on each laboratory's practices and procedures.

To monitor for laboratory contamination, the following procedure may be performed using the APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens:

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the swab transport medium, and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for each area to be swabbed.

If the results are CT or GC positive or equivocal, see *Test Interpretation - QC/Patient Results*. For additional TIGRIS DTS System-specific contamination monitoring information, see the *TIGRIS DTS System Operator's Manual*.

Test Interpretation - QC/Patient Results

A. Test Interpretation

Assay test results are automatically interpreted by the APTIMA Assay software, using the APTIMA COMBO 2 protocol, and presented as individual CT and GC test results. A test result may be a negative, equivocal, positive, or invalid as determined by the kinetic type and total RLU in the detection step (see below). A test result may be invalid due to a parameter outside the normal expected ranges. Initial equivocal and invalid test results should be retested.

Kinetic Type	Total RLU (x1000) to give CT Result		
	Negative	Equivocal	Positive
CT only	1 to < 25	25 to < 100	100 to < 4,500
CT and GC	1 to < 85	85 to < 250	250 to < 4,500
CT indeterminate	1 to < 85	85 to < 4,500	N/A

Kinetic Type	Total RLU (x1000) to give GC Result		
	Negative	Equivocal	Positive
GC only	1 to < 60	60 to < 150	150 to < 4,500
GC and CT	1 to < 85	85 to < 250	250 to < 4,500
GC indeterminate	1 to < 85	85 to < 4,500	N/A

B. Quality Control Results and Acceptability

The Positive Control, CT / Negative Control, GC and the Positive Control, GC / Negative Control, CT act as controls for the target capture, amplification, and detection steps of the assay. In accordance with guidelines or requirements of local, state, and/or federal regulations or accrediting organizations, additional controls for cell lysis and RNA stabilization may be included. The Positive Control, CT / Negative Control, GC serves as the negative control for the GC test results. The Positive Control, GC / Negative Control, CT serves as the negative control for the CT test results. If desired, a dual negative control furnished by the user can be added to monitor assay background. Correct preparation of specimens is confirmed visually by the presence of a single APTIMA collection swab in a swab specimen transport tube, a final volume of urine in between the black fill lines of a urine specimen transport tube, or the absence of a swab in an APTIMA specimen transfer tube for liquid Pap specimens.

The Positive Controls must produce the following test results:

Control	Total RLU (x1000)	CT Result	GC Result
Positive Control, CT / Negative Control, GC	≥ 100 and < 3,000	Positive	Negative
Positive Control, GC / Negative Control, CT	≥ 150 and < 3,000	Negative	Positive

1. The APTIMA Assay software automatically evaluates the controls according to the above criteria and will report the Run Status as PASS if the run control criteria are met, and FAIL if the run control criteria are not met.
2. If the Run Status is FAIL, all test results in the same run are invalid and must not be reported.
3. Each laboratory should implement appropriate control procedures to satisfy the requirements of CLIA regulations (section 493.1256).

Note: See *Lab Contamination Monitoring Protocol for DTS Systems* or contact Gen-Probe Technical Support for help with out-of-range controls on the DTS Systems.

4. A TIGRIS DTS System parameter permits each site to specify a "control bracketing" frequency whereby additional sets of controls can be placed at defined intervals within the worklist. If this parameter is specified, the TIGRIS DTS System will require a set of controls to be placed after the defined number of specimens in the control bracket. The TIGRIS DTS System automatically evaluates each control in the worklist according to the above criteria and will invalidate all specimens in the affected control bracket(s) if the control criteria are not met. See the *TIGRIS DTS System Operator's Manual* for additional details.
5. Negative controls may not be effective in monitoring random carryover. See *TIGRIS DTS System Analytical Performance Characteristics* for results from a high-target analytical carryover study that was performed to demonstrate control of carryover on the TIGRIS DTS System.

C. Specimen Preparation Control (Optional)

The Positive Control, CT / Negative Control, GC and the Positive Control, GC / Negative Control, CT provided in the kit act as controls for the target capture, amplification, and detection steps of the assay and must be included in each assay run. If desired, controls for cell lysis and RNA stabilization in appropriate transport media (PreservCyt Solution, STM) can be tested in accordance with the requirements of appropriate accrediting organizations or individual laboratory procedures. Known positive specimens can serve as controls by being prepared and tested in conjunction with unknown specimens. Specimens used as preparation controls must be stored, handled, and tested according to the package insert. Specimen preparation controls should be interpreted in the same manner as described for patient test specimens. See *Test Interpretation - QC/Patient Results*.

D. Patient Test Results

1. If the controls in any run do not yield the expected results, test results on patient specimens in the same run must not be reported.
2. Swab, PreservCyt Solution liquid Pap, and urine specimen results (See below.)

a. Initial results

CT Pos	Positive for CT rRNA.
CT Neg	Presumed negative for CT rRNA.
CT Equiv	Sample should be retested.
GC Pos	Positive for GC rRNA.
GC Neg	Presumed negative for GC rRNA.
GC Equiv	Sample should be retested.
Invalid	Sample should be retested.

b. Retest results

CT Pos	Positive for CT rRNA.
CT Neg	Presumed negative for CT rRNA.
CT Equiv	Indeterminate, a new specimen should be collected.
GC Pos	Positive for GC rRNA.
GC Neg	Presumed negative for GC rRNA.
GC Equiv	Indeterminate, a new specimen should be collected.
Invalid	Indeterminate, a new specimen should be collected

Notes

- Careful consideration of performance data is recommended for interpreting APTIMA COMBO 2 Assay results for asymptomatic individuals or any individuals in low prevalence populations.
- The first valid result for each analyte is the result that should be reported.
- A negative result does not preclude the presence of a CT or GC infection because results are dependent on adequate specimen collection, absence of inhibitors, and sufficient rRNA to be detected. Test results may be affected by improper specimen collection, improper specimen storage, technical error, or specimen mix-up.
- As is true for all non-culture methods, a positive specimen obtained from a patient after therapeutic treatment cannot be interpreted as indicating the presence of viable CT or GC.
- As is true for all urine test methods, a negative urine result for a female patient who is clinically suspected of having a chlamydial or gonococcal infection does not rule out the presence of CT or GC in the urogenital tract. Testing of an endocervical specimen is recommended in such cases. As well, a negative urine result for GC from a female has a lower negative predictive value than does an endocervical swab result.
- Testing of an endocervical specimen is recommended for female patients who are clinically suspected of having a chlamydial or gonococcal infection. If both a Pap and endocervical swab are collected, the PreservCyt Solution liquid Pap specimen must be collected before the endocervical swab specimen.

Limitations

A. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this package insert may result in erroneous results.

B. Swab specimens were evaluated in the APTIMA COMBO 2 Assay on the DTS Systems for interference by blood, gynecological lubricants, and spermicides. Urine specimens were evaluated for interference by blood, commonly used vitamins, minerals, and over-the-counter pain relievers. Blood interference also was evaluated on the TIGRIS DTS System. The data indicated no assay interference by these substances.

C. The effects of tampon use, douching, and specimen collection variables have not been assessed for their impact on the detection of CT or GC.

D. The presence of mucus in endocervical specimens does not interfere with the detection of CT or GC by the APTIMA COMBO 2 Assay. However, to ensure collection of cells infected with CT, columnar epithelial cells lining the endocervix should be sampled. If excess mucus is not removed, sampling of these cells is not ensured.

E. This method has been tested using only the following specimens:

- Clinician-collected endocervical, vaginal, and male urethral swab specimens
- Clinician-collected PreservCyt Solution liquid Pap specimens
- Patient-collected vaginal swab specimens
- Patient-collected female and male urine specimens

Performance with specimens other than those collected with the following specimen collection kits have not been evaluated:

- APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
- APTIMA Urine Collection Kit for Male and Female Urine Specimens
- APTIMA Vaginal Swab Specimen Collection Kit
- APTIMA Specimen Transfer Kit (for use with gynecologic samples collected in PreservCyt Solution)

DTS Systems Specific

- PACE Specimen Collection Kit for Male Urethral or Conjunctival Specimens (in conjunction with the APTIMA Adapter Kit)
- PACE Specimen Collection Kit for Endocervical Specimens (in conjunction with the APTIMA Adapter Kit)

F. Urine, vaginal swab, and PreservCyt Solution liquid Pap specimen sampling is not designed to replace cervical exams and endocervical specimens for diagnosis of female urogenital infections. Patients may have cervicitis, urethritis, urinary tract infections, or vaginal infections due to other causes or concurrent infections with other agents.

G. The APTIMA COMBO 2 Assay is not intended for the evaluation of suspected sexual abuse or for other medico-legal indications. For those patients for whom a false positive result may have adverse psycho-social impact, the CDC recommends retesting (4).

H. Reliable results are dependent on adequate specimen collection. Because the transport system used for this assay does not permit microscopic assessment of specimen adequacy, training of clinicians in proper specimen collection techniques is necessary. Refer to the package insert of the appropriate GEN-PROBE specimen collection kit.

I. Therapeutic failure or success cannot be determined with the APTIMA COMBO 2 Assay since nucleic acid may persist following appropriate antimicrobial therapy.

J. Results from the APTIMA COMBO 2 Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

K. A negative result does not preclude a possible infection because results are dependent on adequate specimen collection. Test results may be affected by improper specimen collection, technical error, specimen mix-up, or target levels below the assay limit of detection.

L. The APTIMA COMBO 2 Assay provides qualitative results. Therefore, a correlation cannot be drawn between the magnitude of a positive assay signal and the number of organisms in a specimen.

M. For the vaginal swab, endocervical swab, male urethral swab and urine specimen clinical studies, performance characteristics for detecting CT and GC are derived from high prevalence populations. Positive results in low prevalence populations should be interpreted carefully with the understanding that the likelihood of a false positive may be higher than a true positive.

N. For the PreservCyt Solution liquid Pap specimen clinical study, the APTIMA COMBO 2 Assay performance for detecting CT and GC is derived primarily from low prevalence populations. Nonetheless, positive results in low prevalence populations should be interpreted carefully with the understanding that the likelihood of a false positive may be higher than a true positive.

O. Performance of the APTIMA Specimen Transfer kit was not evaluated for testing the same PreservCyt Solution liquid Pap specimen both before and after ThinPrep Pap processing.

P. PreservCyt Solution liquid Pap specimens processed with instruments other than the ThinPrep 2000 processor have not been evaluated for use in APTIMA Assays.

Q. Patient-collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated.

R. The patient-collected vaginal swab specimen application is limited to health care facilities where support/counseling is available to explain procedures and precautions.

S. The APTIMA COMBO 2 Assay has not been validated for use with vaginal swab specimens collected by patients at home.

T. The performance of the vaginal swab specimen has not been evaluated in pregnant women.

U. The performance of vaginal swab specimens and PreservCyt Solution liquid Pap specimens have not been evaluated in women less than 16 years of age.

V. The performance of the TIGRIS DTS System has not been determined at altitudes above 7355 feet (2240 m). Additional volumetric verifications and assay specific studies will be performed prior to, or as part of, the installation and acceptance process in laboratories above 7355 foot (2240 m) altitude.

W. There is no evidence of degradation of nucleic acids in PreservCyt Solution. If a PreservCyt Solution liquid Pap specimen has small numbers of CT and GC cellular material, uneven distribution of this cellular material may occur. Also, when compared to direct sampling with the APTIMA Swab Transport Media, the additional volume of PreservCyt Solution results in greater dilution of the sample material. These factors may affect the ability to detect small numbers of organisms in the collected material. If negative results from the specimen do not fit with the clinical impression, a new specimen may be necessary.

X. Customers must independently validate an LIS transfer process.

DTS Systems Expected Values

Prevalence

The prevalence of CT and/or GC disease in patient populations depends on risk factors such as age, gender, the presence of symptoms, the type of clinic, and the test method. A summary of the prevalence of three CT and GC disease outcomes as determined by the APTIMA COMBO 2 Assay is shown in Tables 1a, 1b, and 1c for three multi-center clinical studies by clinical site and overall.

Prevalence of *C. trachomatis* and/or *N. gonorrhoeae* Disease as Determined by the APTIMA COMBO 2 Assay Results by Clinical Site

Table 1a: Endocervical and Male Urethral Swab and Urine Specimens

	Endocervical and Male Urethral Swab % Prevalence (# positive/# tested)						Urine % Prevalence (# positive/# tested)					
Site	CT+/GC+		CT+/GC-		CT-/GC+		CT+/GC+		CT+/GC-		CT-/GC+	
1	10.0	(39/392)	12.8	(50/392)	14.5	(57/392)	8.4	(33/395)	12.9	(51/395)	13.9	(55/395)
2	7.0	(13/186)	12.9	(24/186)	6.5	(12/186)	5.3	(13/245)	13.9	(34/245)	8.6	(21/245)
3	10.4	(48/462)	22.9	(106/462)	14.3	(66/462)	10.3	(48/465)	20.9	(97/465)	12.7	(59/465)
4	3.3	(9/270)	12.2	(33/270)	7.0	(19/270)	3.3	(9/270)	11.5	(31/270)	6.7	(18/270)
5	1.9	(10/533)	8.4	(45/533)	2.3	(12/533)	2.1	(12/567)	9.4	(53/567)	1.8	(10/567)
6	6.3	(43/678)	12.8	(87/678)	16.2	(110/678)	5.9	(40/681)	10.9	(74/681)	13.5	(92/681)
7	4.4	(11/252)	8.7	(22/252)	21.8	(55/252)	4.1	(12/295)	9.2	(27/295)	18.0	(53/295)
All	6.2	(173/2773)	13.2	(367/2773)	11.9	(331/2773)	5.7	(167/2918)	12.6	(367/2918)	10.6	(308/2918)

Table 1b: Patient-Collected Vaginal Swab and Clinician-Collected Vaginal Swab Specimens

	Patient-Collected Vaginal Swab % Prevalence (# positive / # tested)						Clinician-Collected Vaginal Swab % Prevalence (# positive / # tested)					
Site	CT+/GC+		CT+/GC-		CT-/GC+		CT+/GC+		CT+/GC-		CT-/GC+	
1	1.8	(4/220)	16.4	(36/220)	4.1	(9/220)	3	(7/230)	15.7	(36/230)	3.5	(8/230)
2	9.6	(19/198)	18.7	(37/198)	6.6	(13/198)	9.5	(19/199)	18.1	(36/199)	7	(14/199)
3	0.9	(1/111)	9	(10/111)	2.7	(3/111)	0.9	(1/113)	9.7	(11/113)	1.8	(2/113)
4	0.4	(1/266)	9	(24/266)	1.9	(5/266)	0.4	(1/267)	11.2	(30/267)	2.2	(6/267)
5	0.5	(1/199)	7.5	(15/199)	0.5	(1/199)	0.5	(1/199)	7	(14/199)	0.5	(1/199)
6	2.8	(8/290)	10	(29/290)	5.5	(16/290)	2	(6/296)	12.2	(36/296)	5.4	(16/296)
7	0	(0/102)	11.8	(12/102)	0	(0/102)	0	(0/102)	9.8	(10/102)	0	(0/102)
8	0	(0/48)	8.3	(4/48)	2.1	(1/48)	0	(0/51)	7.8	(4/51)	2	(1/51)
All	2.4	(34/1434)	11.6	(167/1434)	3.3	(48/1434)	2.4	(35/1457)	12.1	(177/1457)	3.3	(48/1457)

Table 1c: PreservCyt Solution Liquid Pap Specimen

	PreservCyt liquid Pap % Prevalence (# positive/# tested)		
Site	CT+/GC+	CT+/GC-	CT-/GC+
1	3.0 (3/100)	13.0 (13/100)	2.0 (2/100)
2	0 (0/124)	3.2 (4/124)	0.8 (1/124)
3	0.4 (2/475)	6.1 (29/475)	0.4 (2/475)
4	0.4 (1/287)	4.2 (12/287)	0 (0/287)
5	0 (0/297)	5.1 (15/297)	1.0 (3/297)
6	0 (0/364)	5.5 (20/364)	0.6 (2/364)
ALL	0.4 (6/1647)	5.6 (93/1647)	0.6 (10/1647)

The CT and GC prevalence were calculated using the APTIMA COMBO 2 Assay results of PreservCyt Solution liquid Pap specimen.

Positive and Negative Predictive Values for Hypothetical Prevalence Rates in North America

The estimated positive and negative predictive values (PPV and NPV) for different prevalence rates using the APTIMA COMBO 2 Assay are shown in Tables 2 and 3 below for CT and GC, respectively. These calculations are based on a hypothetical prevalence and the overall sensitivity and specificity calculated from the patient infected status for two multi-center clinical studies. The overall sensitivity and specificity for CT was 96.1% and 98.0%, respectively (Table 2). The overall sensitivity and specificity for GC was 97.8% and 99.2%, respectively (Table 3). The actual PPV and NPV calculated using the clinical trial data are shown in Tables 6a and 10a (swab and urine specimens), Tables 6b and 10b (vaginal swab specimens), and Tables 6c and 10c (PreservCyt Solution liquid Pap specimens).

Table 2: Hypothetical PPV and NPV for CT

Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
1	96.1	98.0	33.1	100.0
2	96.1	98.0	50.0	99.9
5	96.1	98.0	72.0	99.8
10	96.1	98.0	84.5	99.6
15	96.1	98.0	89.6	99.3
20	96.1	98.0	92.4	99.0
25	96.1	98.0	94.2	98.7
30	96.1	98.0	95.4	98.3

Table 3: Hypothetical PPV and NPV for GC

Prevalence Rate (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
1	97.8	99.2	55.3	100.0
2	97.8	99.2	71.4	100.0
5	97.8	99.2	86.6	99.9
10	97.8	99.2	93.2	99.7
15	97.8	99.2	95.6	99.6
20	97.8	99.2	96.8	99.4
25	97.8	99.2	97.6	99.2
30	97.8	99.2	98.1	99.0

DTS Systems Clinical Performance Characteristics

See *TIGRIS DTS System Clinical Specimen Agreement* following the *DTS Systems Analytical Performance Characteristics* section for TIGRIS DTS System-specific clinical performance characteristics.

Clinical Study Results

Performance characteristics for the APTIMA COMBO 2 Assay on DTS Systems were established in three multi-center clinical studies, conducted in North America. The first multi-center clinical study evaluated clinician-collected endocervical and male urethral swabs and male and female urine specimens from 1,363 male and 1,569 female subjects enrolled at seven geographically diverse clinical sites. The second multi-center clinical study evaluated patient-collected and clinician-collected vaginal swab specimens from 1,464 female subjects enrolled at eight geographically diverse clinical sites. The third multi-center clinical study evaluated PreservCyt Solution liquid Pap specimens from 1,647 subjects enrolled at six clinical sites. In performance calculations based on symptom status, subjects were classified as symptomatic if symptoms such as discharge, dysuria, and pelvic pain were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms.

Endocervical Swab, Male Urethral Swab, and Urine Specimen Clinical Study

In the endocervical swab, urethral swab, and urine specimen multi-center clinical study, 2,932 symptomatic and asymptomatic male and female subjects attending STD, OB/GYN and family planning clinics were enrolled in the study. As many as three urethral swabs and a urine specimen were collected from male subjects and four endocervical swabs and a urine specimen were collected from female subjects. For males providing one urethral swab, testing included GC culture only. For males providing three swabs, testing included GC culture, the APTIMA COMBO 2 Assay, and a commercially-available NAAT for CT and GC. Testing on endocervical swabs included the APTIMA COMBO 2 Assay, two commercially-available NAATs for CT, one commercially-available NAAT for GC, and GC culture. The GC culture swab was collected first and the collection order for the remaining swabs was rotated to minimize collection bias. Urine was tested by the APTIMA COMBO 2 Assay, two commercially-available NAATs for CT, and one commercially-available amplified assay for GC. The commercially-available amplification assays were used as reference assays in this APTIMA COMBO 2 Assay clinical study.

All performance calculations were based on the total number of APTIMA COMBO 2 Assay endocervical and male urethral swab and male and female urine specimens compared to a patient infected status algorithm for each gender. In each gender-specific algorithm, the designation of a subject as being infected, not infected, or inconclusive was based on the combined results of the reference NAAT endocervical and male urethral swab and urine results. For CT infected status, any two positive reference NAAT results by any combination of swab and urine designated the subject as infected. If all reference assay results were negative, the subject was designated not infected. If there was one positive result only, the subject was designated inconclusive. For GC infected status, a positive culture, or positive swab and urine results by the amplified reference assay, designated the subject as infected. A negative culture and a single positive result by the amplified reference assay resulted in an inconclusive status. If all reference assay results were negative, the subject was designated not infected. Tables 7a, 7b, 7c, 8, 11a, 11b, 11c, and 12 summarize the frequency of test outcomes for the two reference NAATs and APTIMA COMBO 2 Assay for clinical study subjects.

APTIMA COMBO 2 Assay results from the clinician-collected endocervical and male urethral swab, and male and female urine specimens were compared to the patient infected status algorithm for determination of sensitivity, specificity, and predictive values. A total of 15,661 CT and 14,144 GC test results were used in the data analysis. Sensitivity and specificity for CT by gender, specimen type, and symptom status are presented in Table 5a. Table 6a shows the APTIMA COMBO 2 Assay sensitivity, specificity, and predictive values for CT compared to patient infected status for each clinical site and overall. Sensitivity and specificity for detection of GC by gender, specimen type and symptom status are presented in Table 9a. Table 10a shows the GC sensitivity, specificity, and predictive values for the APTIMA COMBO 2 Assay compared to patient infected status for each clinical site and overall. Samples that were APTIMA COMBO 2 Assay positive and infected patient status negative (i.e., apparent false positives) were tested in GEN-PROBE alternate amplification assays for CT and GC. These assays amplify CT and GC sequences which are different from those amplified in the APTIMA COMBO 2 Assay. Testing was done on a per specimen basis (i.e., not necessarily on paired swab and urine specimens) and the results of the alternate amplification assays were not used to change the original patient categorizations (Tables 5a and 9a).

Endocervical swab specimens were evaluated for the impact of blood on CT and GC assay performance. Of the 2,454 specimens evaluated for CT performance, 234 (9.5%) were bloody. Of the 2,829 specimens evaluated for GC performance, 247 (8.7%) were bloody. Neither the CT nor GC assay performance was statistically different for bloody specimens as compared to non-bloody specimens. Additional data on blood testing can be found in *Interfering Substances*.

Performance of the assay with endocervical swab and urine specimens from pregnant females was assessed in the clinical study. For CT, sensitivity for endocervical swab and urine specimens was 100% (8/8) and 100% (8/8), respectively. Specificity for endocervical swab and urine specimens was 95.8% (23/24) and 100% (24/24), respectively. For GC, sensitivity for endocervical swab and urine specimens was 100% (8/8) and 100% (8/8), respectively. Specificity for endocervical swab and urine specimens was 100% (26/26) and 100% (26/26), respectively.

Of the 11,406 APTIMA COMBO 2 Assay test results from this multi-center clinical study, three CT results and nine GC results were equivocal on repeat testing and were excluded from the analysis. One specimen was invalid for both CT and GC results and was excluded from the study.

Vaginal Swab Specimen Clinical Study

In the vaginal swab multi-center clinical study, 1,464 symptomatic and asymptomatic female subjects attending STD, OB/GYN, teen, and family planning clinics were enrolled into the clinical study. Of the 646 asymptomatic subjects enrolled in the study, two were less than 16 years of age, 158 were between the ages of 16 and 20, 231 were between the ages of 21 and 25, and 255 were more than 25 years of age. Of the 818 symptomatic subjects enrolled in the study, 160 were between the ages of 16 and 20, 324 were between the ages of 21 and 25, and 334 were more than 25 years of age. Five specimens were collected from each eligible subject; one urine specimen, one patient-collected vaginal swab, one clinician-collected vaginal swab, and two randomized endocervical swabs. APTIMA COMBO 2 Assay results were generated from the two vaginal swabs, one of the endocervical swabs, and an aliquot of the urine specimen. The second endocervical swab and a second aliquot of the urine specimen were tested using another commercially-available NAAT for CT and another commercially-available NAAT for GC. Endocervical swab and urine specimens tested in the APTIMA COMBO 2 Assay and the other commercially-available NAATs were used as reference NAATs to determine infected status for each subject in the vaginal swab specimen clinical study. Specimen testing was conducted either at the site of subject enrollment or at an external testing site.

All performance calculations were based on the total number of APTIMA COMBO 2 Assay patient-collected and clinician-collected vaginal swab results compared to a patient infected status algorithm. A total of 2,073 CT and 2,073 GC vaginal swab test results were used in the data analysis. In the algorithm, the designation of a subject as being infected or not infected with CT or GC was based on endocervical swab and urine specimen results from the commercially-available APTIMA COMBO 2 Assay and the other commercially-available NAAT. Subjects were considered infected with CT or GC if two of the four endocervical swab and urine specimens tested positive in the APTIMA COMBO 2 Assay and the other reference NAAT (one specimen testing positive in each NAAT). Subjects were considered non-infected if less than two reference NAAT results were positive. Tables 7b and 11b summarize the number of results from symptomatic and asymptomatic subjects designated as infected or non-infected with CT or GC, respectively, according to the patient infected status algorithm. For this clinical study, two commercially-available NAATs were used to determine GC-infected status. Culture was not used as a reference test since the APTIMA COMBO 2 Assay has already been evaluated against culture for other specimen types (refer to the *Endocervical Swab, Male Urethral Swab, and Urine Specimen Clinical Study* for details).

Sensitivity and specificity for CT by gender, specimen type and symptom status are presented in Table 5b. Table 6b shows the APTIMA COMBO 2 Assay sensitivity, specificity, and predictive values for CT compared to patient infected status for each clinical site and overall. Sensitivity and specificity for detection of GC by gender, specimen type and symptom status are presented in Table 9b. Table 9b shows the GC sensitivity, specificity, and predictive values for the APTIMA COMBO 2 Assay compared to patient infected status for each clinical site and overall. Samples that were APTIMA COMBO 2 Assay positive and infected patient status negative (i.e., apparent false positives) were tested in alternate TMA assays for CT and GC; these alternate TMA assays target sequences which are unique from those targeted in the APTIMA COMBO 2 Assay. The results of the alternate TMA assays were not used to change the original patient categorizations (Tables 5b and 9b).

Of the 1,464 subjects enrolled, there were 13 subjects with unknown CT patient infected status and 14 subjects with unknown GC patient infected status. Subjects were designated with an unknown patient infected status if results were missing that prevented conclusive determination of infected status. These subjects' results were not included in any performance calculations. Of the 5,782 APTIMA COMBO 2 Assay vaginal swab results from the multi-center clinical study, there was a small percentage (28, 0.5%) of vaginal swab specimens that initially tested invalid or equivocal for CT or GC. Upon repeat testing only three CT results and two GC results were equivocal and were excluded from the analysis. No specimens tested invalid on repeat testing.

PreservCyt Solution Liquid Pap Specimen Clinical Study

A prospective multi-center clinical study was conducted to evaluate the use of the PreservCyt Solution (a component of the ThinPrep 2000 System) as an alternative medium for gynecological specimens for the detection of CT and GC. One thousand six hundred forty-seven (1,647) symptomatic and asymptomatic female subjects attending OB/GYN, family planning, public health, women's and STD clinics were evaluated in the clinical study. Of the 1,647 available subjects, 1,288 were asymptomatic subjects and 359 were symptomatic subjects. Subjects were enrolled from sites with CT prevalence that ranged from 3.2% to 14.0% and GC prevalence that ranged from 0% to 5.0%. Two specimens were collected from each eligible subject: one PreservCyt Solution liquid Pap specimen and one endocervical swab. PreservCyt Solution liquid Pap specimens were processed in accordance with the ThinPrep 2000 Processor Operator's Manual and APTIMA Specimen Transfer Kit Package Insert. After processing the PreservCyt Solution liquid Pap specimen with the ThinPrep 2000 Processor, the specimen was transferred into the APTIMA Specimen Transfer Kit for testing with the APTIMA COMBO 2 Assay. The PreservCyt Solution liquid Pap specimens and endocervical swab specimens were tested with the APTIMA COMBO 2 Assay.

Sensitivity and specificity for PreservCyt Solution liquid Pap specimens were calculated by comparing results to a patient infected status algorithm. In the algorithm, the designation of a subject as being infected or non-infected with CT or GC was based on endocervical swab specimen results from two commercially-available NAATs (Table 7c and Table 11c). For CT, the reference NAATs included the APTIMA COMBO 2 Assay and the APTIMA CT Assay. For GC, the reference NAATs included the APTIMA COMBO 2 Assay and the APTIMA GC Assay. Positive results from both reference NAATs were required to establish an *infected* patient. A *non-infected* patient was established if the results from the two reference NAATs disagreed or were negative.

Sensitivity and specificity for CT in PreservCyt Solution liquid Pap specimens tested in the APTIMA COMBO 2 Assay, by symptom status and overall, is presented in Table 5c. For CT, overall sensitivity was 96.7% (87/90). In symptomatic and asymptomatic subjects, sensitivity was 96.7% (29/30) and

96.7% (58/60), respectively. Overall specificity for CT PreservCyt Solution liquid Pap specimens was 99.2% (1545/1557). In symptomatic and asymptomatic subjects, specificity was 98.5% (324/329) and 99.4% (1221/1228), respectively. Table 6c shows the APTIMA COMBO 2 Assay sensitivity and specificity values for CT in PreservCyt Solution liquid Pap specimens by clinical site and overall. For CT, the sensitivity ranged from 92.9% to 100%. The specificity ranged from 97.7% to 100%.

Sensitivity and specificity for GC in PreservCyt Solution liquid Pap specimens tested in the APTIMA COMBO 2 Assay, by symptom status and overall, is presented in Table 9c. For GC, overall sensitivity was 92.3% (12/13). In symptomatic and asymptomatic subjects, sensitivity was 100% (7/7) and 83.3% (5/6), respectively. Overall specificity for GC PreservCyt Solution liquid Pap specimens was 99.8% (1630/1634). In symptomatic and asymptomatic subjects, specificity was 100% (352/352) and 99.7% (1278/1282), respectively. Table 10c shows the APTIMA COMBO 2 Assay sensitivity and specificity values for GC in PreservCyt Solution liquid Pap specimens by clinical site and overall. For GC, the sensitivity ranged from 80.0% to 100%. Specificity ranged from 99.0% to 100%.

The distribution of cervical sampling devices used in this clinical study according to clinical site is summarized in Table 4 below.

Table 4: Summary of Cervical Sampling Devices Used in the PreservCyt Solution Liquid Pap Specimen Study

Cervical sampling device	Clinical Collection Site						Total
	1	2	3	4	5	6	
Spatula/Cytobrush	0	124	475	287	57	364	1307
Broom-type Device	100	0	0	0	240	0	340

Chlamydia trachomatis Performance Tables

C. trachomatis Sensitivity and Specificity

Table 5a: APTIMA COMBO 2 Assay Specimens vs. Patient Infected Status

Specimen		Symptoms Status	N	TP	FP[4]	TN	FN	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Male	Swab	Sympt	676	190	15[a]	464	7	96.4% (92.8–98.6)	96.9% (94.9–98.2)
		Asympt	388	70	5[b]	309	4	94.6% (86.7–98.5)	98.4% (96.3–99.5)
		All[1]	1065	260	20[c]	774	11	95.9% (92.9–98.0)	97.5% (96.1–98.5)
	Urine	Sympt	694	199	8[d]	484	3	98.5% (95.7–99.7)	98.4% (96.8–99.3)
		Asympt	400	77	4[e]	316	3	96.3% (89.4–99.2)	98.8% (96.8–99.7)
		All[1]	1095	276	12[f]	801	6	97.9% (95.4–99.2)	98.5% (97.4–99.2)
Female	Swab	Sympt	819	133	22[g]	653	11	92.4% (86.7–96.1)	96.7% (95.1–97.9)
		Asympt	569	61	6[h]	501	1	98.4% (91.3–100)	98.8% (97.4–99.6)
		All[2]	1389	195	28[i]	1154	12	94.2% (90.1–97.0)	97.6% (96.6–98.4)
	Urine	Sympt	821	136	8[j]	668	9	93.8% (88.5–97.1)	98.8% (97.7–99.5)
		Asympt	569	60	5[k]	502	2	96.8% (88.8–99.6)	99.0% (97.7–99.7)
		All[2]	1391	197	13[l]	1170	11	94.7% (90.7–97.3)	98.9% (98.1–99.4)
Total	Swab	Sympt	1495	323	37[m]	1117	18	94.7% (91.8–96.8)	96.8% (95.6–97.7)
		Asympt	957	131	11[n]	810	5	96.3% (91.6–98.8)	98.7% (97.6–99.3)
		All[3]	2454	455	48[o]	1928	23	95.2% (92.9–96.9)	97.6% (96.8–98.2)
	Urine	Sympt	1515	335	16[p]	1152	12	96.5% (94.0–98.2)	98.6% (97.8–99.2)
		Asympt	969	137	9[q]	818	5	96.5% (92.0–98.8)	98.9% (97.9–99.5)
		All[3]	2486	473	25[r]	1971	17	96.5% (94.5–98.0)	98.7% (98.2–99.2)

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative

[1] Includes 1 male subject for whom symptoms were not reported.

[2] Includes 1 female subject for whom symptoms were not reported.

[3] Includes 1 male and 1 female subject for whom symptoms were not reported.

[4] CT Alternate TMA results represent # positive results/# specimens tested: a: 11/14; b: 3/5; c: 14/19; d: 4/8; e: 0/4; f: 4/12; g: 18/22; h: 4/6; i: 22/28; j: 2/8; k: 1/5; l: 3/13, m: 29/36, n: 7/11, o: 36/47, p: 6/16, q: 1/9, and r: 7/25

Table 5b: APTIMA COMBO 2 Assay Vaginal Swab Specimens vs. Patient Infected Status

Specimen		Symptom Status	N	TP	FP[1]	TN	FN	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Patient-Collected	Vaginal Swab	Asympt	628	60	18[a]	549	1	98.4% (91.2–100)	96.8% (95.0–98.1)
Clinician-Collected	Vaginal Swab	Sympt	809	111	25[b]	669	4	96.5% (91.3–99.0)	96.4% (94.7–97.7)
		Asympt	636	59	16[c]	559	2	96.7% (88.7–99.6)	97.2% (95.5–98.4)
		All	1445	170	41[d]	1228	6	96.6% (92.7–98.7)	96.8% (95.6–97.7)

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.
[1]CT TMA Alternate Amplification results represent # positive results/# specimens tested: a: 15/18, b: 17/25, c: 15/16, and d: 32/41.

Table 5c: APTIMA COMBO 2 Assay PreservCyt Specimens vs. Patient Infected Status

Symptom Status	AC2/CT PreservCyt Result	+/+	+/-	-/+	-/-	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Asympt	Positive	58	1	0	6	96.7% (88.5 - 99.6)	99.4% (98.8 - 99.8)
	Negative	2	1	12	1208		
	Total	60	2	12	1214		
Sympt	Positive	29	0	0	5	96.7% (82.8 - 99.9)	98.5% (96.5 - 99.5)
	Negative	1	3	4	317		
	Total	30	3	4	322		
All	Positive	87	1	0	11	96.7% (90.6 - 99.3)	99.2% (98.7 - 99.6)
	Negative	3	4	16	1525		
	Total	90	5	16	1536		

+/+ = Positive Endocervical Swab Specimen Result in the AC2 Assay / Positive Endocervical Swab Specimen Result in the ACT Assay
+/- = Positive Endocervical Swab Specimen Result in the AC2 Assay / Negative Endocervical Swab Specimen Result in the ACT Assay
-/+ = Negative Endocervical Swab Specimen Result in the AC2 Assay / Positive Endocervical Swab Specimen Result in the ACT Assay
-/- = Negative Endocervical Swab Specimen Result in the AC2 Assay / Negative Endocervical Swab Specimen Result in the ACT Assay

C. trachomatis Performance by Clinical Site

Table 6a: APTIMA COMBO 2 Assay Specimen vs. Patient Infected Status

Specimen		Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (%)	NPV (%)
Male	Swab	1	157	35	6	115	1	22.9	97.2% (85.5–99.9)	95.0% (89.5–98.2)	85.4	99.1
		2	93	19	2	72	0	20.4	100% (82.4–100)	97.3% (90.6–99.7)	90.5	100
		3	248	76	5	165	2	31.5	97.4% (91.0–99.7)	97.1% (93.3–99.0)	93.8	98.8
		4	51	12	1	38	0	23.5	100% (73.5–100)	97.4% (86.5–99.9)	92.3	100
		5	138	24	0	113	1	18.1	96.0% (79.6–99.9)	100% (96.8–100)	100	99.1
		6	353	74	6	268	5	22.4	93.7% (85.8–97.9)	97.8% (95.3–99.2)	92.5	98.2
		7	25	20	0	3	2	88.0*	90.9% (70.8–98.9)	100% (29.2–100)	100	60.0
		ALL	1065	260	20	774	11	25.4	95.9% (92.9–98.0)	97.5% (96.1–98.5)	92.9	98.6
	Urine	1	157	35	6	115	1	22.9	97.2% (85.5–99.9)	95.0% (89.5–98.2)	85.4	99.1
		2	96	22	1	73	0	22.9	100% (84.6–100)	98.6% (92.7–100)	95.7	100
		3	249	78	2	169	0	31.3	100% (95.4–100)	100% (95.8–99.9)	97.5	100
		4	51	12	0	39	0	23.5	100% (73.5–100)	98.8% (91.0–100)	100	100
		5	162	31	2	129	0	19.1	100% (88.8–100)	98.5% (94.6–99.8)	93.9	100
		6	353	74	1	273	5	22.4	93.7% (85.8–97.9)	99.6% (98.0–100)	98.7	98.2
		7	27	24	0	3	0	88.9*	100% (85.8–100)	100% (29.2–100)	100	100
		ALL	1095	276	12	801	6	25.8	97.9% (95.4–99.2)	98.5% (97.4–99.2)	95.8	99.3
Female	Swab	1	150	34	4	110	2	24.0	94.4% (81.3–99.3)	96.5% (91.3–99.0)	89.5	98.2
		2	81	11	1	68	1	14.8	91.7% (61.5–99.8)	98.6% (92.2–100)	91.7	98.6
		3	184	51	13	114	6	31.0	89.5% (78.5–96.0)	89.8% (83.1–94.4)	79.7	95.0
		4	196	27	2	167	0	13.8	100% (87.2–100)	98.8% (95.8–99.9)	93.1	100
		5	370	27	1	341	1	7.6	96.4% (81.7–99.9)	99.7% (98.4–100)	96.4	99.7
		6	274	35	7	230	2	13.5	94.6% (81.8–99.3)	97.0% (94.0–98.8)	83.3	99.1
		7	134	10	0	124	0	7.5	100% (69.2–100)	100% (97.1–100)	100	100
		ALL	1389	195	28	1154	12	14.9	94.2% (90.1–97.0)	97.6% (96.6–98.4)	87.4	99.0
	Urine	1	150	34	4	110	2	24.0	94.4% (81.3–99.3)	96.5% (91.3–99.0)	89.5	98.2
		2	81	12	1	68	0	14.8	100% (73.5–100)	98.6% (92.2–100)	92.3	100
		3	185	54	3	125	3	30.8	94.7% (85.4–98.9)	97.7% (93.3–99.5)	94.7	97.7
		4	196	24	2	167	3	13.8	88.9% (70.8–97.6)	98.8% (95.8–99.9)	92.3	98.2
		5	369	28	2	338	1	7.9	96.6% (82.2–99.9)	99.4% (97.9–99.9)	93.3	99.7
		6	276	35	1	238	2	13.4	94.6% (81.8–99.3)	99.6% (97.7–100)	97.2	99.2
		7	134	10	0	124	0	7.5	100% (69.2–100)	100% (97.1–100)	100	100
		ALL	1391	197	13	1170	11	15.0	94.7% (90.7–97.3)	98.9% (98.1–99.4)	93.8	99.1

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

* Prevalence over-estimated due to initial collection being limited to screening for symptomatic subjects.

Table 6b: APTIMA COMBO 2 Assay Vaginal Swab Specimens vs. Patient Infected Status

Specimen		Site	N	TP	FP	TN	FN	Prev. (%)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (%)	NPV (%)
Patient-Collected	Vaginal Swab	1	70	14	3	53	0	20.0	100% (76.8–100)	94.6% (85.1–98.9)	82.4	100
		2	45	13	3	29	0	28.9	100% (75.3–100)	90.6% (75.0–98.0)	81.3	100
		3	45	4	2	39	0	8.9	100% (39.8–100)	95.1% (83.5–99.4)	66.7	100
		4	152	6	3	142	1	4.6	85.7% (42.1–99.6)	99.7% (94.1–99.6)	66.7	99.3
		5	130	7	3	120	0	5.4	100% (59.0–100)	97.6% (93.0–99.5)	70.0	100
		6	75	8	2	65	0	10.7	100% (63.1–100)	97.0% (89.6–99.6)	80.0	100
		7	68	5	1	62	0	7.4	100% (47.8–100)	98.4% (91.5–100)	83.3	100
		8	43	3	1	39	0	7.0	100% (29.2–100)	97.5% (86.8–99.9)	75.0	100
		ALL	628	60	18	549	1	9.7	98.4% (91.2–100)	96.8% (95.0–98.1)	76.9	99.8
Clinician-Collected	Vaginal Swab	1	227	34	9	182	2	15.9	94.4% (81.3–99.3)	95.3% (91.2–97.8)	79.1	98.9
		2	196	50	5	139	2	26.5	96.2% (86.8–99.5)	96.5% (92.1–98.9)	90.9	98.6
		3	113	9	3	101	0	8.0	100% (66.4–100)	97.1% (91.8–99.4)	75.0	100
		4	262	19	11	231	1	7.6	95.0% (75.1–99.9)	95.5% (92.0–97.7)	63.3	99.6
		5	199	13	2	184	0	6.5	100% (75.3–100)	98.9% (96.2–99.9)	86.7	100
		6	296	33	9	254	0	11.1	100% (89.4–100)	96.6% (93.6–98.4)	78.6	100
		7	102	9	1	91	1	9.8	90.0% (55.5–99.7)	98.9% (94.1–100)	90.0	98.9
		8	50	3	1	46	0	6.0	100% (29.2–100)	97.9% (88.7–99.9)	75.0	100
		ALL	1445	170	41	1228	6	12.2	96.6% (92.7–98.7)	96.8% (95.6–97.7)	80.6	99.5

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

Table 6c: APTIMA COMBO 2 Assay PreservCyt Specimens vs. Patient Infected Status

Site	AC2/CT PreservCyt Result	+/+	+/-	-/+	-/-	Prev (%)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (%)	NPV (%)
1	Positive	14	0	0	2	14.0	100% (76.8 - 100)	97.7% (91.9 - 99.7)	87.5	100
	Negative	0	0	1	83					
	Total	14	0	1	85					
2	Positive	4	0	0	0	3.2	100% (39.8 - 100)	100% (97.0 - 100)	100	100
	Negative	0	0	2	118					
	Total	4	0	2	118					
3	Positive	29	0	0	2	6.5	93.5% (78.6 - 99.2)	99.5% (98.4 - 99.9)	93.5	99.5
	Negative	2	0	2	440					
	Total	31	0	2	442					
4	Positive	8	1	0	4	2.8	100% (63.1 - 100)	98.2% (95.9 - 99.4)	61.5	100
	Negative	0	2	1	271					
	Total	8	3	1	275					
5	Positive	13	0	0	2	4.7	92.9% (66.1 - 99.8)	99.3% (97.5 - 99.9)	86.7	99.6
	Negative	1	1	4	276					
	Total	14	1	4	278					
6	Positive	19	0	0	1	5.2	100% (82.4 - 100)	99.7% (98.4 - 100)	95.0	100
	Negative	0	1	6	337					
	Total	19	1	6	338					
All	Positive	87	1	0	11	5.5	96.7% (90.6 - 99.3)	99.2% (98.7 - 99.6)	87.9	99.8
	Negative	3	4	16	1525					
	Total	90	5	16	1536					

+/+ = Positive Endocervical Swab Specimen Result in the AC2 Assay / Positive Endocervical Swab Specimen Result in the ACT Assay
+/- = Positive Endocervical Swab Specimen Result in the AC2 Assay / Negative Endocervical Swab Specimen Result in the ACT Assay
-/+ = Negative Endocervical Swab Specimen Result in the AC2 Assay / Positive Endocervical Swab Specimen Result in the ACT Assay
-/- = Negative Endocervical Swab Specimen Result in the AC2 Assay / Negative Endocervical Swab Specimen Result in the ACT Assay

***Chlamydia trachomatis* Analysis for Female Patient Infected Status**

Table 7a: Endocervical Swab and Urine Specimen

Patient Infected Status	NAAT 1		NAAT 2		APTIMA COMBO 2 Assay		Symptom Status	
	FU	FS	FU	FS	FU	FS	Sympt	Asympt
Infected	NA	NA	+	+	+	+	1	0
Infected	NA	+	NA	+	+	+	1	0
Infected	NA	+	+	+	-	+	0	1
Infected	-	+	NA	+	-	+	1	0
Infected	-	+	-	+	-	+	4	0
Infected	-	+	-	+	+	+	6	1
Infected	-	+	+	+	-	+	1	0
Infected	-	+	+	+	+	+	7	3
Infected	+	NA	+	+	+	+	1	0
Infected	+	-	NA	+	+	-	1	0
Infected	+	-	+	-	-	-	1	0
Infected	+	-	+	-	+	-	7	1
Infected	+	-	+	-	+	+	2	1
Infected	+	-	+	+	+	-	1	0
Infected	+	-	+	+	+	+	3	3
Infected	+	+	NA	+	+	+	6	2
Infected	+	+	-	NA	+	+	1	0
Infected	+	+	-	+	+	+	7	3
Infected	+	+	+	NA	+	+	1	0
Infected	+	+	+	-	+	+	2	2
Infected	+	+	+	+	-	-	1	0
Infected	+	+	+	+	-	+	1	1
Infected	+	+	+	+	+	NA	1	0
Infected	+	+	+	+	+	+	88	44
Non-infected	-	-	-	-	NA	-	1	1
Non-infected	-	-	-	-	-	NA	2	1
Non-infected	-	-	-	-	-	-	648	497
Non-infected	-	-	-	-	-	+	18	4
Non-infected	-	-	-	-	+	-	4	3
Non-infected	-	-	-	-	+	+	4	2
Total							822	570

FU = Female Urine; **FS** = Female Endocervical swab
"NA" represents specimen not obtained or available for testing.

Table 7b: Patient-Collected and Clinician-Collected Vaginal Swab Specimen

	NAAT 1		NAAT 2 (APTIMA COMBO 2)		APTIMA COMBO 2 Assay		Symptom Status		
Patient Infected Status	FS	FU	FS	FU	PVS	CVS	Symp	Asymp	Total
Infected	+	+	+	+	+	+	79	43	122
Infected	+	+	+	+	+	-	0	1	1
Infected	+	+	+	+	-	+	1	0	1
Infected	+	+	+	+	NA	-	1	0	1
Infected	+	-	+	+	+	+	8	5	13
Infected	+	-	+	+	-	-	1	0	1
Infected	+	-	+	+	NA	+	1	0	1
Infected	+	=	+	+	+	+	1	0	1
Infected	-	+	+	+	+	+	8	3	11
Infected	-	+	+	+	-	-	1	0	1
Infected	-	-	+	+	+	+	1	2	3
Infected	-	NA	+	+	+	+	1	0	1
Infected	+	+	+	-	+	+	5	3	8
Infected	+	-	+	-	+	+	5	0	5
Infected	+	-	+	-	-	+	2	0	2
Infected	+	+	-	+	+	+	0	1	1
Infected	-	+	-	+	+	+	1	4	5
Infected	-	+	-	+	+	-	1	0	1
Infected	-	+	-	+	-	-	0	1	1
Non-infected	-	-	+	-	+	+	0	4	4
Non-infected	-	-	+	-	+	-	2	1	3
Non-infected	-	-	+	-	-	+	2	1	3
Non-infected	-	-	+	-	-	-	6	4	10
Non-infected	-	-	+	-	NA	+	1	0	1
Non-infected	-	-	+	-	NA	-	1	0	1
Non-infected	-	-	-	+	+	+	4	2	6
Non-infected	-	-	-	+	+	-	1	0	1
Non-infected	-	-	-	+	-	-	0	2	2
Non-infected	+	-	-	-	-	-	1	1	2
Non-infected	-	+	-	-	-	-	1	2	3
Non-infected	-	-	-	-	+	+	3	2	5
Non-infected	-	-	-	-	+	-	2	7	9
Non-infected	-	-	-	-	-	+	12	3	15
Non-infected	-	-	-	-	-	-	623	516	1139
Non-infected	-	-	-	-	-	NA	0	2	2
Non-infected	-	-	-	-	-	=	1	0	1
Non-infected	-	-	-	-	NA	+	0	1	1
Non-infected	-	-	-	-	NA	-	11	8	19
Non-infected	-	-	-	-	NA	NA	1	0	1
Non-infected	-	-	-	-	NA	=	0	1	1
Non-infected	-	-	-	-	=	+	0	1	1
Non-infected	-	NA	-	-	-	-	2	2	4
Non-infected	-	NA	-	-	NA	-	0	1	1
Non-infected	-	=	-	-	-	-	12	9	21
Non-infected	-	=	-	-	-	NA	0	1	1
Non-infected	=	-	-	-	-	-	1	1	2
Non-infected	-	-	-	NA	-	-	0	1	1

Table 7b: Patient-Collected and Clinician-Collected Vaginal Swab Specimen (Continued)

Patient Infected Status	NAAT 1		NAAT 2 (APTIMA COMBO 2)		APTIMA COMBO 2 Assay		Symptom Status		
	FS	FU	FS	FU	PVS	CVS	Symp	Asymp	Total
Non-infected	-	-	NA	-	-	-	5	4	9
Non-infected	-	-	=	-	-	+	1	0	1
Non-infected	-	-	=	-	-	-	1	0	1
Total							811	640	1451

FS = Female Endocervical swab; **FU** = Female Urine; **PVS** = Asymptomatic Patient-Collected Vaginal Swab; **CVS** = Clinician-Collected Vaginal Swab. "NA" represents specimen not obtained or available for testing. The equal symbol (=) represents equivocal on repeat testing.

Table 7c: PreservCyt Solution Liquid Pap Specimen Clinical Study Patient Infected Status Results for *C. trachomatis*

Patient Infected Status	Endocervical Swab Result		Symptom Status	
	AC2	ACT	Symp	Asymp
Infected	+	+	30	60
Non-Infected	-	+	4	12
Non-Infected	+	-	3	2
Non-Infected	-	-	322	1214
Total			359	1288

***C. trachomatis* Analysis for Male Patient Infected Status**

Table 8: *C. trachomatis* Urethral Swab and Urine Specimen Analysis for Male Patient Infected Status

Patient Infected Status	NAAT 1		NAAT 2	APTIMA COMBO 2 Assay		Symptom Status	
	MU	MS	MU	MU	MS	Sympt	Asympt
Infected	NA	+	+	+	+	2	0
Infected	-	+	+	+	+	10	4
Infected	+	NA	+	+	NA	4	6
Infected	+	NA	+	+	-	2	0
Infected	+	NA	+	+	+	21	1
Infected	+	-	+	+	-	3	3
Infected	+	-	+	+	+	4	3
Infected	+	+	NA	-	+	1	0
Infected	+	+	NA	+	+	8	2
Infected	+	+	-	+	+	12	4
Infected	+	+	+	-	-	1	0
Infected	+	+	+	-	+	1	3
Infected	+	+	+	+	NA	1	0
Infected	+	+	+	+	-	1	1
Infected	+	+	+	+	+	131	53
Non-infected	-	-	-	NA	-	0	2
Non-infected	-	-	-	-	NA	13	8
Non-infected	-	-	-	-	-	461	303
Non-infected	-	-	-	-	+	10	5
Non-infected	-	-	-	+	-	3	4
Non-infected	-	-	-	+	+	5	0
Total						694	402

MU = Male Urine; **MS** = Male Urethral Swab
"NA" represents specimen not obtained or available for testing.

Neisseria gonorrhoeae Performance Tables

N. gonorrhoeae Sensitivity and Specificity

Table 9a: APTIMA COMBO 2 Assay Specimens vs. Patient Infected Status

Specimen		Symptoms	N	TP	FP[4]	TN	FN	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Male	Swab	Sympt	724	304	5[a]	412	3	99.0% (97.2–99.8)	98.8% (97.2–99.6)
		Asympt	378	15	12[b]	351	0	100% (78.2–100)	96.7% (94.3–98.3)
		All[1]	1103	319	17[c]	764	3	99.1% (97.3–99.8)	97.8% (96.5–98.7)
	Urine	Sympt	750	311	1[d]	433	5	98.4% (96.3–99.5)	99.8% (98.7–100)
		Asympt	383	13	2[e]	368	0	100% (75.3–100)	99.5% (98.1–99.9)
		All[1]	1134	324	3[f]	802	5	98.5% (96.5–99.5)	99.6% (98.9–99.9)
Female	Swab	Sympt	881	94	15[g]	772	0	100% (96.2–100)	98.1% (96.9–98.9)
		Asympt	596	31	2[h]	562	1	96.9% (83.8–99.9)	99.6% (98.7–100)
		All[2]	1479	126	17[i]	1335	1	99.2% (95.7–100)	98.7% (98.0–99.3)
	Urine	Sympt	883	87	7[j]	782	7	92.6% (85.3–97.0)	99.1% (98.2–99.6)
		Asympt	599	28	3[k]	564	4	87.5% (71.0–96.5)	99.5% (98.5–99.9)
		All[2]	1484	116	10[l]	1347	11	91.3% (85.0–95.6)	99.3% (98.6–99.6)
Total	Swab	Sympt	1605	398	20[m]	1184	3	99.3% (97.8–99.8)	98.3% (97.4–99.0)
		Asympt	974	46	14[n]	913	1	97.9% (88.7–99.9)	98.5% (97.5–99.2)
		All[3]	2582	445	34[o]	2099	4	99.1% (97.7–99.8)	98.4% (97.8–98.9)
	Urine	Sympt	1633	398	8[p]	1215	12	97.1% (94.9–98.5)	99.3% (98.7–99.7)
		Asympt	982	41	5[q]	932	4	91.1% (78.8–97.5)	99.5% (98.8–99.8)
		All[3]	2618	440	13[r]	2149	16	96.5% (94.4–98.0)	99.4% (99.0–99.7)

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

[1] Includes 1 male subject for whom symptoms were not reported.

[2] Includes 1 female for whom symptoms were not reported.

[3] Includes 1 male and 1 female for whom symptoms were not reported.

[4] GC Alternate TMA results represents # positive results/# specimens tested: a: 5/5, b: 12/12, c: 17/17, d: 0/1, e: 2/2, f: 2/3, g: 13/15, h: 2/2, i: 15/17, j: 4/7, k: 0/2, l: 4/9, m: 18/20, n: 14/14, o: 32/34, p: 4/8, q: 2/4, and r: 6/12

Table 9b: APTIMA COMBO 2 Assay Vaginal Swab Specimens vs. Patient Infected Status

Specimen		Symptom Status	N	TP	FP[1]	TN	FN	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Patient-Collected	Vaginal Swab	Asympt	629	21	3[a]	605	0	100% (83.9–100)	99.5% (98.6–99.9)
Clinician-Collected	Vaginal Swab	Sympt	807	51	7[b]	747	2	96.2% (87.0–99.5)	99.1% (98.1–99.6)
		Asympt	637	21	4[c]	611	1	95.5% (77.2–99.9)	99.3% (98.3–99.8)
		All	1444	72	11[d]	1358	3	96.0% (88.8–99.2)	99.2% (98.6–99.6)

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

[1]GC TMA Alternate Amplification results represents # positive results/# specimens tested: a: 3/3, b: 6/7, c: 3/4, and d: 9/11.

Table 9c: APTIMA COMBO 2 Assay PreservCyt Specimens vs. Patient Infected Status

Symptom Status	AC2/GC PreservCyt Result	+/+	+/-	-/+	-/-	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Asympt	Positive	5	0	1[1]	3	83.3% (35.9 - 99.6)	99.7% (99.2 - 99.9)
	Negative	1	0	5	1273		
	Total	6	0	6	1276		
Sympt	Positive	7	0	0	0	100% (59.0 - 100)	100% (99.0 - 100)
	Negative	0	0	0	352		
	Total	7	0	0	352		
All	Positive	12	0	1	3	92.3% (64.0 - 99.8)	99.8% (99.4 - 99.9)
	Negative	1	0	5	1625		
	Total	13	0	6	1628		

[1] One specimen had a discordant result: Equivocal endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA GC Assay.

+/+ = Positive Endocervical Swab Specimen Result in the AC2 Assay / Positive Endocervical Swab Specimen Result in the AGC Assay

+/- = Positive Endocervical Swab Specimen Result in the AC2 Assay / Negative Endocervical Swab Specimen Result in the AGC Assay

-/+ = Negative Endocervical Swab Specimen Result in the AC2 Assay / Positive Endocervical Swab Specimen Result in the AGC Assay

-/- = Negative Endocervical Swab Specimen Result in the AC2 Assay / Negative Endocervical Swab Specimen Result in the AGC Assay

Neisseria gonorrhoeae Performance by Clinical Site

Table 10a: APTIMA COMBO 2 Assay Specimens vs. Patient Infected Status

Specimen		Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (%)	NPV (%)
Male	Swab	1	159	56	1	101	1	35.8	98.2% (90.6–100)	99.0% (94.7–100)	98.2	99.0
		2	97	13	0	84	0	13.4	100% (75.3–100)	100% (95.7–100)	100	100
		3	264	71	6	187	0	26.9	100% (94.9–100)	96.9% (93.4–98.9)	92.2	100
		4	53	20	0	33	0	37.7	100% (83.2–100)	100% (89.4–100)	100	100
		5	139	12	0	127	0	8.6	100% (73.5–100)	100% (97.1–100)	100	100
		6	336	94	10	231	1	28.3	98.9% (94.3–100)	95.9% (92.5–98.0)	90.4	99.6
		7	55	53	0	1	1	98.2*	98.1% (90.1–100)	100% (2.5–100)	100	50.0
		ALL	1103	319	17	764	3	29.2	99.1% (97.3–99.8)	97.8% (96.5–98.7)	94.9	99.6
	Urine	1	161	57	0	103	1	36.0	98.3% (90.8–100)	100% (96.5–100)	100	99.0
		2	104	19	0	85	0	18.3	100% (82.4–100)	100% (95.8–100)	100	100
		3	265	71	2	192	0	26.8	100% (94.9–100)	99.0% (96.3–99.9)	97.3	100
		4	53	20	0	33	0	37.7	100% (83.2–100)	100% (89.4–100)	100	100
		5	160	14	0	146	0	8.8	100% (76.8–100)	100% (97.5–100)	100	100
		6	335	89	1	241	4	27.8	95.7% (89.4–98.8)	99.6% (97.7–100)	98.9	98.4
		7	56	54	0	2	0	96.4*	100% (93.4–100)	100% (15.8–100)	100	100
		ALL	1134	324	3	802	5	29.0	98.5% (96.5–99.5)	99.6% (98.9–99.9)	99.1	99.4
Female	Swab	1	196	30	2	164	0	15.3	100%(88.4–100)	98.8% (95.7–99.9)	93.8	100
		2	83	9	1	72	1	12.0	90.0% (55.5–99.7)	98.6% (92.6–100)	90.0	98.6
		3	191	31	2	158	0	16.2	100% (88.8–100)	98.8% (95.6–99.8)	93.9	100
		4	215	7	0	208	0	3.3	100% (59.0–100)	100% (98.2–100)	100	100
		5	382	8	1	373	0	2.1	100% (63.1–100)	99.7% (98.5–100)	88.9	100
		6	278	36	8	234	0	12.9	100% (90.3–100)	96.7% (93.6–98.6)	81.8	100
		7	134	5	3	126	0	3.7	100% (47.8–100)	97.7% (93.4–99.5)	62.5	100
		ALL	1479	126	17	1335	1	8.6	99.2% (95.7–100)	98.7% (98.0–99.3)	88.1	99.9
	Urine	1	196	24	2	164	6	15.3	80.0% (61.4–92.3)	98.8% (95.7–99.9)	92.3	96.5
		2	83	9	1	72	1	12.0	90.0% (55.5–99.7)	98.6% (92.6–100)	90.0	98.6
		3	191	30	2	158	1	16.2	96.8% (83.3–99.9)	98.8% (95.6–99.8)	93.8	99.4
		4	215	5	2	206	2	3.3	71.4% (29.0–96.3)	99.0% (96.6–99.9)	71.4	99.0
		5	383	8	0	375	0	2.1	100% (63.1–100)	100% (99.0–100)	100	100
		6	282	35	2	244	1	12.8	97.2% (85.5–99.9)	99.2% (97.1–99.9)	94.6	99.6
		7	134	5	1	128	0	3.7	100% (47.8–100)	99.2% (95.8–100)	83.3	100
		ALL	1484	116	10	1347	11	8.6	91.3% (85.0–95.6)	99.3% (98.6–99.6)	92.1	99.2

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.
* Prevalence over-estimated due to initial collection being limited to screening for symptomatic subjects

Table 10b: APTIMA COMBO 2 Assay Vaginal Swab Specimens vs. Patient Infected Status

Specimen		Site	N	TP	FP	TN	FN	Prev (%)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (%)	NPV (%)
Patient-Collected	Vaginal Swab	1	70	5	1	65	0	7.1	100% (47.8 - 100)	98.5 (91.7 - 100)	83.3	100
		2	46	7	0	39	0	15.2	100% (59.0 - 100)	100% (91.0 - 100)	100	100
		3	45	2	0	43	0	4.4	100% (15.8 - 100)	100% (91.8 - 100)	100	100
		4	152	1	0	151	0	0.7	100% (2.5 - 100)	100% (97.6 - 100)	100	100
		5	130	1	0	129	0	0.8	100% (2.5 - 100)	100% (97.2 - 100)	100	100
		6	75	5	2	68	0	6.7	100% (47.8 - 100)	97.1 (90.1 - 99.7)	71.4	100
		7	68	0	0	68	0	0.0	N/A	100% (94.7 - 100)	N/A	100
		8	43	0	0	43	0	0.0	N/A	100% (91.8 - 100)	N/A	100
		ALL	629	21	3	605	0	3.3	100% (83.9 - 100)	99.5 (98.6 - 99.9)	87.5	100
Clinician-Collected	Vaginal Swab	1	227	12	3	212	0	5.3	100% (73.5 - 100)	98.6% (96.0 - 99.7)	80.0	100
		2	196	31	2	163	0	15.8	100% (88.8 - 100)	98.8% (95.7 - 99.9)	93.9	100
		3	113	3	0	109	1	3.5	75.0% (19.4 - 99.4)	100% (96.7 - 100)	100	99.1
		4	262	5	2	255	0	1.9	100% (47.8 - 100)	99.2% (97.2 - 99.9)	71.4	100
		5	198	2	0	196	0	1.0	100% (15.8 - 100)	100% (98.1 - 100)	100	100
		6	296	18	4	272	2	6.8	90.0% (68.3 - 98.8)	98.6% (96.3 - 99.6)	81.8	99.3
		7	102	0	0	102	0	0.0	NA	100% (96.4 - 100)	NA	100
		8	50	1	0	49	0	2.0	100% (2.5 - 100)	100% (92.7 - 100)	100	100
		ALL	1444	72	11	1358	3	5.2	96.0% (88.8 - 99.2)	99.2% (98.6 - 99.6)	86.7	99.8

TP = True Positive; **FP** = False Positive; **TN** = True Negative; **FN** = False Negative.

Table 10c: APTIMA COMBO 2 Assay PreservCyt Specimens vs. Patient Infected Status

Site	AC2/GC PreservCyt Result	+/+	+/-	-/+	-/-	Prev (%)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (%)	NPV (%)
1	Positive	5	0	0	0	5.0	100% (47.8 - 100)	100% (96.2 - 100)	100	100
	Negative	0	0	0	95					
	Total	5	0	0	95					
2	Positive	1	0	0	0	0.8	100% (2.5 - 100)	100% (97.0 - 100)	100	100
	Negative	0	0	0	123					
	Total	1	0	0	123					
3	Positive	4	0	0	0	1.1	80.0% (28.4 - 99.5)	100% (99.2 - 100)	100	99.8
	Negative	1	0	0	470					
	Total	5	0	0	470					
4	Positive	1	0	0	0	0.3	100% (2.5 - 100)	100% (98.7 - 100)	100	100
	Negative	0	0	3	283					
	Total	1	0	3	283					
5	Positive	0	0	0	3	0.0	N/A	99.0% (97.1 - 99.8)	0.0	100
	Negative	0	0	0	294					
	Total	0	0	0	297					
6	Positive	1	0	1[1]	0	0.3	100% (2.5 - 100)	99.7% (98.5 - 100)	50.0	100
	Negative	0	0	2	360					
	Total	1	0	3	360					
All	Positive	12	0	1	3	0.8	92.3% (64.0 - 99.8)	99.8% (99.4 - 99.9)	75.0	99.9
	Negative	1	0	5	1625					
	Total	13	0	6	1628					

[1] One specimen had a discordant result: Equivocal endocervical swab specimen result in the APTIMA COMBO 2 Assay/Positive endocervical swab specimen result in the APTIMA GC Assay.

+/+ = Positive Endocervical Swab Specimen Result in the AC2 Assay / Positive Endocervical Swab Specimen Result in the AGC Assay
+/- = Positive Endocervical Swab Specimen Result in the AC2 Assay / Negative Endocervical Swab Specimen Result in the AGC Assay
-/+ = Negative Endocervical Swab Specimen Result in the AC2 Assay / Positive Endocervical Swab Specimen Result in the AGC Assay
-/- = Negative Endocervical Swab Specimen Result in the AC2 Assay / Negative Endocervical Swab Specimen Result in the AGC Assay

***Neisseria gonorrhoeae* Analysis for Female Patient Infected Status**

Table 11a: Endocervical Swab and Urine Specimen

Patient Infected Status	APTIMA COMBO 2 Assay					Symptom Status	
	NAAT		Culture				
	FU	FS	FS	FU	FS	Symp	Asymp
Infected	NA	+	+	+	+	1	1
Infected	-	-	+	-	-	0	1
Infected	-	+	+	-	+	5	2
Infected	-	+	+	+	+	9	2
Infected	+	NA	+	+	+	1	0
Infected	+	-	+	+	+	3	1
Infected	+	+	NA	+	+	0	1
Infected	+	+	-	+	+	11	2
Infected	+	+	+	-	+	2	1
Infected	+	+	+	+	+	62	21
Non-Infected	-	-	-	-	NA	2	3
Non-Infected	-	-	-	-	-	768	559
Non-Infected	-	-	-	-	+	12	2
Non-Infected	-	-	-	+	-	4	3
Non-Infected	-	-	-	+	+	3	0
Total						883	599

FU = Female Urine; **FS** = Female Endocervical Swab
"NA" represents specimen not obtained or available for testing

Table 11b: Patient-Collected and Clinician-Collected Vaginal Swab Specimen Analysis

Patient Infected Status	NAAT 1		NAAT 2		APTIMA COMBO 2 Assay		Symptom Status		Total
	FS	FU	FS	FU	PVS	CVS	Sympt	Asympt	
Infected	+	+	+	+	+	+	44	15	59
Infected	+	+	+	+	+	-	1	0	1
Infected	+	+	+	+	NA	+	0	1	1
Infected	+	-	+	+	+	+	2	2	4
Infected	+	NA	+	+	+	+	1	0	1
Infected	-	+	+	+	+	+	1	1	2
Infected	-	-	+	+	+	+	1	1	2
Infected	+	+	+	-	+	+	1	0	1
Infected	+	-	+	-	+	+	1	1	2
Infected	+	-	+	-	+	-	1	0	1
Infected	+	+	-	+	+	+	1	0	1
Infected	-	+	-	+	+	+	0	1	1
Infected	-	+	-	+	+	-	0	1	1
Infected	+	+	-	-	-	+	1	0	1
Non-infected	-	-	+	-	-	-	5	1	6
Non-infected	-	-	-	+	-	-	1	0	1
Non-infected	+	-	-	-	+	+	1	0	1
Non-infected	+	-	-	-	-	-	5	2	7
Non-infected	-	+	-	-	+	+	0	1	1
Non-infected	-	+	-	-	-	-	2	1	3

FS = Female Endocervical swab; **FU** = Female Urine; **PVS** = Asymptomatic Patient-Collected Vaginal Swab; **CVS** = Clinician-Collected Vaginal Swab; "NA" represents specimen not obtained or available for testing. The equal symbol (=) represents equivocal on repeat testing.

Table 11b: Patient-Collected and Clinician-Collected Vaginal Swab Specimen Analysis (Continued)

Patient Infected Status	NAAT 1		NAAT 2		APTIMA COMBO 2 Assay		Symptom Status		Total
	FS	FU	FS	FU	PVS	CVS	Sympt	Asympt	
Non-infected	-	-	-	-	+	+	2	0	2
Non-infected	-	-	-	-	+	-	1	1	2
Non-infected	-	-	-	-	-	+	2	2	4
Non-infected	-	-	-	-	-	-	698	577	1275
Non-infected	-	-	-	-	-	NA	0	2	2
Non-infected	-	-	-	-	-	=	2	0	2
Non-infected	-	-	-	-	NA	-	15	9	24
Non-infected	-	-	-	-	NA	NA	1	0	1
Non-infected	-	NA	-	-	-	-	2	2	4
Non-infected	-	NA	-	-	NA	-	0	1	1
Non-infected	-	=	-	-	-	-	11	10	21
Non-infected	-	=	-	-	-	NA	0	1	1
Non-infected	=	-	-	-	-	-	1	1	2
Non-infected	-	-	-	NA	-	-	0	1	1
Non-infected	-	-	NA	-	-	-	5	4	9
Non-infected	-	-	=	-	-	-	1	1	2
Total							810	640	1450

FS = Female Endocervical swab; **FU** = Female Urine; **PVS** = Asymptomatic Patient-Collected Vaginal Swab; **CVS** = Clinician-Collected Vaginal Swab; "NA" represents specimen not obtained or available for testing. The equal symbol (=) represents equivocal on repeat testing.

N. gonorrhoeae Analysis for Female Patient Infected Status

Table 11c: PreservCyt Solution Liquid Pap Specimen Clinical Study Patient Infected Status Results for *N. gonorrhoeae*

Patient Infected Status	Endocervical Swab Result		Symptom Status	
	AC2	AGC	Symp	Asymp
Infected	+	+	7	6
Non-Infected	=	+	0	1
Non-Infected	-	+	0	5
Non-Infected	-	-	352	1276
Total			359	1288

***N. gonorrhoeae* Analysis for Male Patient Infected Status**

Table 12: Urethral Swab and Urine Specimen

Patient Infected Status	NAAT 1		Culture	APTIMA COMBO 2 Assay		Symptom Status	
	MU	MS	MS	MU	MS	Symp	Asymp
Infected	NA	+	+	+	+	1	0
Infected	-	NA	+	NA	+	0	1
Infected	-	NA	+	+	+	1	0
Infected	-	-	+	-	-	1	0
Infected	-	+	+	+	+	4	1
Infected	+	NA	+	NA	+	0	1
Infected	+	NA	+	+	NA	8	0
Infected	+	NA	+	+	-	1	0
Infected	+	NA	+	+	+	50	1
Infected	+	-	+	+	+	4	1
Infected	+	+	NA	+	+	1	0
Infected	+	+	-	+	+	11	1
Infected	+	+	+	-	-	1	0
Infected	+	+	+	-	+	3	0
Infected	+	+	+	+	NA	1	0
Infected	+	+	+	+	+	229	9
Non-infected	-	-	-	NA	-	0	1
Non-infected	-	-	-	NA	+	0	1
Non-infected	-	-	-	-	NA	17	9
Non-infected	-	-	-	-	-	411	349
Non-infected	-	-	-	-	+	5	10
Non-infected	-	-	-	+	-	1	1
Non-infected	-	-	-	+	+	0	1
Total						750	387

MU = Male Urine; **MS** = Male Urethral Swab; NA = Specimen not obtained or available for testing.

RLU Distribution of APTIMA Controls

The distribution of the RLUs for the APTIMA Positive Control, GC / Negative Control, CT and the APTIMA Positive Control, CT / Negative Control, GC from all the APTIMA COMBO 2 Assay runs performed during the clinical specimen studies is presented in Table 13 below.

Table 13: Distribution of Total RLU of the APTIMA COMBO 2 Assay Controls

Control	Statistics	Total RLU (x 1000)		
		Endocervical Swab, Male Urethral Swab, and Urine Specimen Clinical Study	Vaginal Swab Specimen Clinical Study	PreservCyt liquid Pap Specimen Clinical Study
Positive Control, CT / Negative Control, GC	Maximum	1572	1996	1747
	75th Percentile	1160	1279	1264
	Median	1063	1135	1165
	25th Percentile	996	933	1024
	Minimum	274	174	494
Positive Control, GC / Negative Control, CT	Maximum	1359	1420	1438
	75th Percentile	1202	1255	1288
	Median	1093	1169	1201
	25th Percentile	989	1084	1099
	Minimum	167	249	166

Precision Study

Precision testing was performed at three sites to obtain measures of repeatability and reproducibility. Precision studies were conducted as part of the Endocervical Swab, Male Urethral Swab, and Urine Specimen Clinical Study and the PreservCyt Solution liquid Pap Specimen Clinical Study. For the former study, each site was provided with three identical panels of 13 samples containing 0 to 500 fg of CT rRNA, 0 to 25,000 fg of GC rRNA, or combinations of both CT and GC rRNA. Testing was performed over three days using a different assay kit lot each day. The overall RLU, within-run, between-run, and between-site descriptive statistics are summarized in Table 14a.

For the latter precision study, reproducibility was established with a 12-member panel generated by spiking PreservCyt Solution with 0 to 2000 fg/assay of CT and 0 to 5,000 fg/assay of GC rRNA and aliquotting 1.0 mL into the APTIMA Specimen Transfer Kit collection tube. Two (2) operators at each of the three sites performed one run per day on each of three days, totaling three valid runs per operator. Testing was performed using one assay kit lot. The results of this precision study are summarized in Table 14b.

For both studies, reproducibility was established by spiking the appropriate transport medium (STM, PreservCyt Solution) with rRNA. Reproducibility when testing swab, urine, or PreservCyt Solution liquid Pap clinical specimens containing target organism has not been determined.

Table 14a: Swab Transport Medium

Panel Member		N	Mean RLU (x1,000)	Within-Run		Between-Run		Between-Site	
				SD (RLU)	CV (%)	SD (RLU)	CV (%)	SD (RLU)	CV (%)
High	CT Swab	54	1,055	76,588	7.3	83,711	7.9	150,332	14.2
	Dual Swab*	54	2,338	93,449	4.0	90,317	3.9	142,898	6.1
	Dual Urine*	54	2,281	91,487	4.0	106,715	4.7	152,747	6.7
	GC Swab	54	1,265	30,561	2.4	55,642	4.4	34,413	2.7
Mid	CT Swab	54	1,001	69,831	7.0	77,701	7.8	159,774	16.0
	Dual Swab*	54	2,241	152,377	6.8	58,353	2.6	139,983	6.2
	GC Swab	54	1,249	35,142	2.8	60,638	4.9	46,364	3.7
Low	CT Swab	54	1,013	61,795	6.1	90,906	9.0	131,207	13.0
	Dual Swab*	54	2,085	286,034	13.7	161,764	7.8	58,837	2.8
	Dual Urine*	54	2,201	95,705	4.3	118,760	5.4	106,802	4.9
	GC Swab	54	1,177	42,478	3.6	69,821	5.9	29,836	2.5
Negative	Swab	54	7	1,301	18.3	2,311	32.5	1,901	26.8
	Urine	54	7	861	12.0	2,299	32.1	1,994	27.9

* Dual positive panel members contained both CT and GC rRNA.

Table 14b: PreservCyt Solution

Concentration (fg/assay)					Within-Run		Between-Run		Between-Site		Between-Operator	
CT	GC	N	Agreement	Mean RLU (x 1,000)	SD (x 1,000)	CV (%)	SD (x 1,000)	CV (%)	SD (x 1,000)	CV (%)	SD (x 1,000)	CV (%)
0	0	162	97.5%	9.7	31.6	N/A	3.4	N/A	6.4	N/A	4.7	N/A
0	5,000	54	96.3%	1296	146	11.3	54.8	4.2	0.0	0.0	0.0	0.0
2,000	0	54	100%	1140	54.1	4.7	79.8	7.0	101	8.9	2.4	0.2
2,000	5,000	54	100%	2345	79.6	3.4	78.0	3.3	94.7	4.0	37.9	1.6
0	250	54	100%	953	114	12.0	0.0	0.0	161	16.9	90.7	9.5
5	0	54	100%	971	58.3	6.0	71.7	7.4	22.8	2.4	85.0	8.8
1,000	2,500	54	100%	2294	114	5.0	88.9	3.9	153	6.7	0.0	0.0
100	250	54	98.1%	1911	139	7.3	130	6.8	348	18.2	39.7	2.1
5	5,000	54	100%	2136	113	5.3	130	6.1	98.8	4.6	166	7.8
2,000	250	54	96.3%	2044	138	6.7	169	8.3	360	17.6	26.9	1.3

RLU - Relative Light Units; **SD** = Standard Deviation; **CV** = Coefficient of Variation; N/A represents specimen not applicable for negative panel members.
Samples with discordant and equivocal results were included in the signal variability analysis.
For CV and SD values equal to 0.0, the variability due to this source is very small relative to other sources of variation.

DTS Systems Analytical Performance Characteristics

See *TIGRIS DTS System Analytical Performance Characteristics* following the *TIGRIS DTS System Clinical Specimen Agreement* section for TIGRIS DTS System-specific analytical performance characteristics.

Analytical Sensitivity

Chlamydia trachomatis analytical sensitivity (limits of detection) was determined by directly comparing dilutions of CT organisms in cell culture and in the assay. The analytical sensitivity claim for the assay is one Inclusion-Forming Unit (IFU) per assay (7.25 IFU/swab, 5.0 IFU/mL urine, 9.75 IFU/mL PreservCyt Solution liquid Pap) for all 15 CT serovars (A, B, Ba, C, D, E, F, G, H, I, J, K, L1, L2 and L3). However, dilutions of less than 1.0 IFU/assay of all serovars tested positive in the APTIMA COMBO 2 Assay.

Neisseria gonorrhoeae analytical sensitivity was determined by directly comparing dilutions of 57 different clinical isolates in culture and in the APTIMA COMBO 2 Assay with swab and urine specimens and 20 clinical isolates with PreservCyt Solution liquid Pap specimens. The analytical sensitivity claim for the assay is 50 cells/assay (362 cells/swab, 250 cells/mL urine, 488 cells/mL PreservCyt Solution liquid Pap). However, all strains tested were positive at less than 50 cells/assay.

Analytical Specificity

A total of 154 culture isolates were evaluated using the APTIMA COMBO 2 Assay. These isolates included 86 organisms that may be isolated from the urogenital tract and 68 additional organisms that represent a phylogenetic cross-section of organisms. The tested organisms included bacteria, fungi, yeast, parasites, and viruses. All organisms except *C. psittaci, C. pneumoniae*, and the viruses were tested at 1.0×10^6 cells/assay in both swab and urine transport medium. The Chlamydia and Neisseria organisms were tested in PreservCyt solution medium. *C. psittaci* and *C. pneumoniae* were tested at 1.0×10^5 IFU/assay. The viruses were tested as follows: (a) herpes simplex viruses I and II: 2.5×10^4 $TCID_{50}$/assay, (b) human papilloma virus 16: 2.9×10^6 DNA copies/assay and (c) cytomegalovirus: 4.8×10^5 infected cell culture cells/assay. Only CT and GC samples produced positive results in the APTIMA COMBO 2 Assay. The list of organisms tested is shown in Table 15 below.

Table 15: Analytical Specificity

Organism	Organism	Organism
Achromobacter xerosis	*Escherichia coli*	*Neisseria mucosa (3)*
Acinetobacter calcoaceticus	*Flavobacterium meningosepticum*	*Neisseria sicca (3)*
Acinetobacter lwoffi	*Fusobacterium nucleatum*	*Neisseria subflava (14)*
Actinomyces israelii	*Gardnerella vaginalis*	*Neisseria perflava*
Actinomyces pyogenes	*Gemella haemolysans*	*Neisseria polysaccharea*
Aerococcus viridans	*Haemophilus ducreyi*	*Paracoccus denitrificans*
Aeromonas hydrophila	*Haemophilus influenzae*	*Peptostreptococcus anaerobius*
Agrobacterium radiobacter	Herpes simplex virus I	*Peptostreptococcus productus*
Alcaligenes faecalis	Herpes simplex virus II	*Plesiomonas shigelloides*
Bacillus subtilis	Human papilloma virus 16	*Propionibacterium acnes*
Bacteriodes fragilis	*Kingella dentrificans*	*Proteus mirabilis*
Bacteriodes ureolyticus	*Kingella kingae*	*Proteus vulgaris*
Bifidobacterium adolescentis	*Klebsiella oxytoca*	*Providencia stuartii*
Bifidobacterium brevi	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
Branhamella catarrhalis	*Lactobacillus acidophilus*	*Pseudomonas fluorescens*
Brevibacterium linens	*Lactobacillus brevis*	*Pseudomonas putida*
Campylobacter jejuni	*Lactobacillus jensonii*	*Rahnella aquatilis*
Candida albicans	*Lactobacillus lactis*	*Rhodospirillum rubrum*
Candida glabrata	*Legionella pneumophila (2)*	*Saccharomyces cerevisiae*
Candida parapsilosis	*Leuconostoc paramensenteroides*	*Salmonella minnesota*
Candida tropicalis	*Listeria monocytogenes*	*Salmonella typhimurium*
Chlamydia pneumoniae	*Micrococcus luteus*	*Serratia marcescens*
Chlamydia psittaci (2)	*Moraxella lacunata*	*Staphylococcus saprophyticus*
Chromobacterium violaceum	*Moraxella osloensis*	*Staphylococcus aureus*
Citrobacter freundii	*Morganella morganii*	*Staphylococcus epidermidis*
Clostridium perfringens	*Mycobacterium smegmatis*	*Streptococcus agalactiae*
Corynebacterium genitalium	*Mycoplasma genitalium*	*Streptococcus bovis*
Corynebacterium xerosis	*Mycoplasma hominis*	*Streptococcus mitis*
Cryptococcus neoformans	*N. meningitidis Serogroup A*	*Streptococcus mutans*
Cytomegalovirus	*N. meningitidis Serogroup B*	*Streptococcus pneumoniae*
Deinococcus radiodurans	*N. meningitidis Serogroup C (4)*	*Streptococcus pyogenes*
Derxia gummosa	*N. meningitidis Serogroup D*	*Streptococcus salivarius*
Eikenella corrodens	*N. meningitidis Serogroup Y*	*Streptococcus sanguis*
Enterobacter aerogenes	*N. meningitidis Serogroup W135*	*Streptomyces griseinus*
Enterobacter cloacae	*Neisseria cinerea (4)*	*Trichomonas vaginalis*
Entercoccus avium	*Neisseria dentrificans*	*Ureaplasma urealyticum*
Entercoccus faecalis	*Neisseria elongata (3)*	*Vibrio parahaemolyticus*
Entercoccus faecium	*Neisseria flava*	*Yersinia enterocolitica*
Erwinia herbicola	*Neisseria flavescens (2)*	
Erysipelothrix rhusiopathiae	*Neisseria lactamica (9)*	

"(n)" represents the number of strains tested.
All organisms tested produced a negative result in the APTIMA COMBO 2 Assay based on kinetic profile type and RLU.

Interfering Substances

The following interfering substances were individually spiked into Swab and PreservCyt Solution liquid Pap specimens: 10% blood, contraceptive jelly, spermicide, moisturizer, hemorrhoidal anesthetic, body oil, powder, anti-fungal cream, vaginal lubricants, feminine spray and leukocytes (1.0×10^6 cells/mL). The following interfering substances were individually spiked into urine specimens: 30% blood, urine analytes, protein, glucose, ketones, bilirubin, nitrate, urobilinogen, pH 4 (acidic), pH 9 (alkaline), leukocytes (1.0×10^6 cells/mL), cellular debris, vitamins, minerals, acetaminophen, aspirin and ibuprofen. All were tested for potential assay interference in the absence and presence of CT and GC at the estimated rRNA equivalent of 1.0 CT IFU/assay (5 fg/assay) and 50 GC cells/assay (250 fg/assay). The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism.

No interference was observed with any of the tested substances. No inhibitors of amplification were observed in the APTIMA COMBO 2 Assay.

Recovery

Escherichia coli and *Gardnerella vaginalis* (2.4×10^5 cells/assay) and *Lactobacillus acidophilus*, *Gardnerella vaginalis*, *Bacteroides ureolyticus* and *Staphylococcus epidermis* (1.0×10^8 cells/assay) were added to samples containing the rRNA equivalent of approximately 1.0 CT IFU (5 fg) and 50 GC cells (250 fg). These additions did not interfere with the amplification and detection of CT or GC rRNA using the APTIMA COMBO 2 Assay.

Specimen Stability Studies

A. Endocervical Swab Specimens

Data to support the recommended shipping and storage conditions for endocervical swab samples were generated with pooled negative swab samples. Five pooled samples were spiked with CT and GC at final concentrations of 10 IFU and 100 CFU per reaction, respectively. The spiked samples were held at -70°C, -20°C, 4°C, and 30°C. Samples were tested in duplicate at days 0, 20, 35, 60, and 90. All test conditions were positive for both CT and GC at all times and temperatures.

B. PreservCyt Solution Liquid Pap Specimens

Data to support the recommended shipping and storage conditions for PreservCyt Solution liquid Pap samples were generated with pooled negative PreservCyt Solution liquid Pap samples. Four pooled samples were spiked with CT and GC at final concentrations of 10 IFU and 100 CFU per reaction, respectively. The PreservCyt Solution liquid Pap samples were placed at 30°C for 7 days, after which 1.0 mL of the sample was added to an APTIMA Transfer Tube. The spiked samples were held at 4°C, 10°C and 30°C. Samples stored at 4°C and 10°C were tested in duplicate at days 0, 6, 13, 26, 30 and 36. Samples stored at 30°C were tested in duplicate at days 0, 5, 8, 14 and 17. Four spiked PreservCyt Solution liquid Pap sample pools were added to APTIMA Transfer Tubes and placed at 30°C for 14 days before being stored at either -20°C or -70°C. The -20°C samples and the -70°C samples were tested in duplicate after 0, 30, 60, 90 and 106 days of storage. All test conditions were positive for both CT and GC at all times and temperatures.

C. Vaginal Swab Specimens

Data to support the recommended shipping and storage conditions for vaginal swab samples were generated with pooled negative swab samples. Fifteen vaginal swab pools were spiked with CT and GC at final concentrations of 1.0 IFU and 50 CFU per reaction, respectively. The spiked samples were held at -70°C, -20°C, 4°C, and 30°C. Samples were tested using one aliquot at days 0, 20, 36, 73, and 114. All test conditions were positive for both CT and GC at all times and temperatures.

D. Urine Specimens

Data to support the recommended shipping and storage conditions for urine samples were generated with ten female and ten male negative urine samples. The urine samples were spiked with CT and GC at final concentrations of 10 IFU and 100 CFU per reaction, respectively. Two sets of the spiked urine samples were held at 4°C and 30°C for 24 hours prior to being added to the Urine Transport Media (UTM). The two sets of UTM samples then were held at 4°C and 30°C, and tested in triplicate at days 0, 1, 5, 20, and 35. All samples were positive for both CT and GC when the urine samples were held at 4°C prior to addition of the UTM. When the urine samples were held at 30°C prior to addition of the UTM, all of the samples were positive for CT and 95% of the samples were positive for GC at Day 35. These same samples were tested after 116 days of storage at -20°C and -70°C. All samples were positive for both CT and GC under both storage conditions.

E. Additional Frozen (at -20°C) Specimen Stability Study

Data to support the recommended storage condition at -20°C for endocervical swab, urethral swab, vaginal swab, female urine, male urine, and PreservCyt Solution liquid Pap specimens were generated using 90 specimens for each type with negative result, where 30 specimens were spiked with CT and GC at 1.0 IFU and 50 CFU per reaction, respectively; 30 specimens were spiked at 0.1 IFU and 5 CFU per reaction, respectively; and 30 specimens were unspiked. The specimens were stored at -20°C and were tested at days 0, 200, and 400 days. All spiked specimens met the acceptance criteria of 95% agreement with expected results.

TIGRIS DTS System Clinical Specimen Agreement

TIGRIS DTS System Agreement

Agreement between APTIMA COMBO 2 Assay results generated on the fully automated TIGRIS DTS System and semi-automated DTS Systems was evaluated by testing endocervical swab, male urethral swab, female and male urine, vaginal swab, and PreservCyt Solution liquid Pap specimens. Each of the clinical specimens was tested individually with the APTIMA COMBO 2 Assay on both the TIGRIS DTS and DTS Systems at Gen-Probe.

Clinical Specimen Agreement Study - Endocervical Swab, Male Urethral Swab, and Female and Male Urine Specimens

Male and female subjects attending STD, urgent care, public health, and family planning clinics were enrolled at seven geographically diverse clinical sites with low to high prevalence for CT and GC. The clinical specimen agreement study evaluated agreement between the two systems using swab and urine specimens from 485 male and 576 female subjects. Of the 1,991 specimens tested, there was a small percentage that initially tested invalid or equivocal for CT or GC on the TIGRIS DTS System (20, 1.0%) and on the DTS Systems (14, 0.7%). Upon repeat testing, there were two (2) clinical specimens with equivocal GC results on the TIGRIS DTS System, which are not included in equivalence calculations. Overall percent agreement and percent positive and negative agreements were calculated. Specimens yielding discordant results between the DTS and TIGRIS DTS System were tested in alternate TMA amplification assays for CT and GC, which are nucleic acid amplification tests (NAATs) that target CT or GC rRNA sequences that differ from those targeted in the APTIMA COMBO 2 Assay. APTIMA COMBO 2 Assay repeat testing on the DTS Systems was also conducted on specimens yielding discordant TIGRIS DTS System and DTS Systems results.

Tables 16 and 17 show the overall percent agreements for all paired test results obtained on the TIGRIS DTS and DTS Systems for swab and urine specimens, respectively. Overall agreements were 98.3% for swab specimens and 99.2% for urine specimens. Refer to Tables 5a and 9a for APTIMA COMBO 2 performance estimates for endocervical swab, male urethral swab, and female and male urine specimens tested on the DTS Systems. Clinical performance estimates for the TIGRIS DTS System with endocervical swab, male urethral swab, and female and male urine specimens would be expected to be similar given the agreement findings.

Clinical Specimen Agreement Study – Vaginal Swab and PreservCyt Solution Liquid Pap Specimens

Female subjects attending STD, public health, and OB/GYN clinics contributed vaginal swab and PreservCyt Solution liquid Pap specimens. The vaginal swab specimens were transferred directly to Gen-Probe for testing while the PreservCyt Solution liquid Pap specimens were processed at 2 cytopathology laboratories before being transferred. At Gen-Probe, vaginal swab and PreservCyt Solution liquid Pap specimens were first screened with the APTIMA COMBO 2 Assay on the DTS Systems. Specimens with final invalid or equivocal DTS Systems results were not selected for further testing on the TIGRIS DTS System. APTIMA COMBO 2 Assay positive specimens and a subset of APTIMA COMBO 2 Assay negative specimens

were selected for comparison testing on the TIGRIS DTS System. One hundred seventy (170) vaginal swab and 170 PreservCyt Solution liquid Pap specimens from 181 female subjects were tested on both systems. The majority of specimens (110 vaginal swab and 107 PreservCyt Solution liquid Pap specimens) selected for comparison testing were from symptomatic women. Seventeen (17) worklists were initiated: 13 (76.5%) were valid and 4 (23.5%) were invalidated because the instrument detected high background at the luminometer. The instrument had loose Detect 1 and 2 fittings that could have allowed air into the lines or incorrect amounts of detect reagents to be injected. These worklists were valid when retested. Of the 340 specimens tested, none had initial invalid or equivocal test results on the TIGRIS DTS System.

Tables 18 and 19 show the overall percent agreements for CT and GC detection for all paired test results obtained on the TIGRIS DTS and DTS Systems for vaginal swab and PreservCyt Solution liquid Pap specimens, respectively. Overall agreements were 98.2% for vaginal swab specimens and 98.2% for PreservCyt Solution liquid Pap specimens. Refer to Tables 5b, 5c, 9b, and 9c for APTIMA COMBO 2 Assay performance estimates for vaginal swab and PreservCyt Solution liquid Pap specimens tested on the DTS Systems. Clinical performance estimates for the TIGRIS DTS System with vaginal swab and PreservCyt Solution liquid Pap specimens would be expected to be similar given the agreement findings.

CT/GC Clinical Panel Agreement Study - Endocervical Swab, Male Urethral Swab, and Female and Male Urine Specimens

The CT/GC clinical panel agreement study evaluated equivalence between the two systems using 13 GEN-PROBE-prepared CT/GC clinical panels containing 0 to 2,500 Inclusion Forming Units (IFU)/mL of CT and/or 0 to 125,000 Colony Forming Units (CFU)/mL of GC. The CT/GC clinical panels were created from swab and urine specimens collected from 222 male and 117 female subjects who were determined to be non-infected based on negative APTIMA COMBO 2 Assay swab and urine specimen results on the DTS Systems. Each of the 13 CT/GC panels consisted of 5 replicates of each specimen type (endocervical swab, male urethral swab, female urine, male urine) for a total of 20 replicates per panel.

Table 20 shows the percent agreements with expected CT and GC results for the TIGRIS DTS System and for the DTS Systems for each of the 13 CT/GC panels. The concentrations ranged from 10 fold below to 1000 fold above the APTIMA COMBO 2 Assay analytical claim limits of 1 IFU/assay for CT and 50 CFU/assay for GC. Also shown in Table 20 is the overall percent agreement (99.3%) between CT/GC panel results from the TIGRIS DTS System and from the DTS Systems. Positive and negative agreements are shown in Tables 21 and 22 for CT and GC panel results, respectively. For swab and urine panels, positive agreements were 100% and 96.2% respectively for CT, and were both 100% for GC. Swab and urine negative agreements were 100% and 98.0%, respectively, for CT, and were both 100% for GC. Three of 5 female urine panel replicates, which were one log below the APTIMA COMBO 2 Assay analytical sensitivity claim of 1 IFU/assay for CT, were CT- on the TIGRIS System. One of 5 female urine panel replicates from a separate panel was CT- on the DTS Systems.

Table 16: Clinical Specimen Agreement Study: Endocervical and Male Urethral Swab Specimen Results[1]

TIGRIS DTS System	DTS Systems				Total
	CT+/GC+	CT+/GC-	CT-/GC+	CT-/GC-	
CT+/GC+	**30**	0	0	0	30
CT+/GC-	0	**108**	0	2[5]	110
CT-/GC+	1[2]	0	**67**	0	68
CT-/GC-	0	12[3]	2[4]	**796**	810
Total	31	120	69	798	1018
Percent Agreement (95% C.I.)	96.8% (83.3-99.9)	90.0% (83.2-94.7)	97.1% (89.9-99.6)	99.7% (99.1-100)	n/a
Overall Percent Agreement (95% C.I.): 98.3% (97.3- 99.0)					

+ denotes Positive,- denotes Negative, n/a = Not Applicable

[1]Data not shown: Two specimens tested CT-/GC equivocal on both the TIGRIS and DTS Systems. One specimen tested CT-/GC- on the TIGRIS DTS System, but CT-/GC equivocal on the DTS Systems. When retested in the APTIMA COMBO 2 Assay on the DTS Systems, this specimen tested CT-/GC-. The specimen also tested GC- in the alternate TMA amplification assay.

[2]1/1 was CT+/GC+ when retested on the DTS Systems and was CT+ in the alternate TMA amplification assay.

[3]11/12 were retested. 11/11 were CT-/GC- when retested in the APTIMA COMBO 2 Assay on the DTS Systems. 9/11 were CT- when tested in the alternate TMA amplification assay and 2/11 were CT+.

[4]2/2 were CT-/GC- when retested in the APTIMA COMBO 2 Assay on the DTS Systems and were GC- in the alternate TMA amplification assay.

[5]2/2 were CT-/GC- when retested in the APTIMA COMBO 2 Assay on the DTS Systems and were CT- in the alternate TMA amplification assay.

Table 17: Clinical Specimen Agreement Study: Female and Male Urine Specimen Results

TIGRIS DTS System	DTS Systems				Total
	CT+/GC+	CT+/GC-	CT-/GC+	CT-/GC-	
CT+/GC+	**32**	0	0	0	32
CT+/GC-	0	**100**	0	1[3]	101
CT-/GC+	0	0	**52**	0	52
CT-/GC-	0	8[1]	1[2]	**776**	785
Total	32	108	53	777	970
Percent Agreement (95% C.I.)	100% (89.1-100)	92.6% (85.9-96.7)	98.1% (89.9-100)	99.9% (99.3-100)	n/a
Overall Percent Agreement (95% C.I.): 99.2% (98.1- 99.5)					

+ denotes Positive,- denotes Negative,n/a = Not Applicable

[1] 7/8 were CT-/GC- when retested in the APTIMA COMBO 2 Assay on the DTS Systems and were CT- in the alternate TMA amplification assay. 1/8 was CT+/GC- when retested in the APTIMA COMBO 2 Assay on the DTS Systems and was CT+ in the alternate TMA amplification assay.
[2] 1/1 was CT-/GC- when retested in the APTIMA COMBO 2 Assay on the DTS Systems and was GC- in the alternate TMA amplification assay.
[3] 1/1 was CT-/GC- when retested in the APTIMA COMBO 2 Assay on the DTS Systems and was CT+ in the alternate TMA amplification assay.

Table 18: Clinical Specimen Agreement Study: Vaginal Swab Specimen Results

TIGRIS DTS System	DTS Systems				Total
	CT+/GC+	CT+/GC-	CT-/GC+	CT-/GC-	
CT+/GC+	**26**	0	0	0	26
CT+/GC-	0	**44**	0	2	46
CT-/GC+	0	0	**24**	0	24
CT-/GC-	0	0	1	**73**	74
Total	26	44	25	75	170
Percent Agreement (95% C.I.)	100% (86.8-100)	100% (92.0-100)	96.0% (79.6-99.9)	97.3% (90.7-99.7)	n/a
Overall Percent Agreement (95% CI): 98.2% (94.9-99.6)					

+ denotes Positive, - denotes Negative, n/a = Not Applicable

Table 19: Clinical Specimen Agreement Study: PreservCyt Solution Liquid Pap Specimen Results

TIGRIS DTS System	DTS Systems				Total
	CT+/GC+	CT+/GC-	CT-/GC+	CT-/GC-	
CT+/GC+	**26**	0	0	0	26
CT+/GC-	0	**44**	0	1	45
CT-/GC+	0	0	**24**	0	24
CT-/GC-	0	1	1	**73**	75
Total	26	45	25	74	170
Percent Agreement (95% C.I.)	100% (86.8-100)	97.8% (88.2-99.9)	96.0% (79.6-99.9)	98.6% (92.7-100)	n/a
Overall Percent Agreement (95% CI): 98.2% (94.9-99.6)					

+ denotes Positive, - denotes Negative, n/a = Not Applicable

Table 20: CT/GC Clinical Panel Agreement Study: Agreement with Expected CT and GC Results for Endocervical Swab, Male Urethral Swab, and Female and Male Urine Panels

Panel Member CT/GC	Panel Member Concentration[1]		Replicates	CT		GC	
	CT IFU/mL	GC CFU/mL		TIGRIS %Agrmt	DTS %Agrmt	TIGRIS %Agrmt	DTS %Agrmt
Low/Low	2.5	125	20	100	100	100	100
Low/High	2.5	125,000	20	100	95[3]	100	100
High/Low	2,500	125	20	100	100	100	100
High/High	2,500	125,000	20	100	100	100	100
Very Low/Neg	0.25[2]	0	20	85[4]	100	100	100
Low/Neg	2.5	0	20	100	100	100	100
Medium/Neg	25	0	20	100	100	100	100
High/Neg	2,500	0	20	100	100	100	100
Neg/Very Low	0	12.5	20	100	100	100	100
Neg/Low	0	125	20	100	100	100	100
Neg/Medium	0	1,250	19	100	100	100	100
Neg/High	0	125,000	20	100	100	100	100
Neg/Neg	0	0	20	100	100	100	100
Overall Percent Agreement between TIGRIS and DTS (95% C.I.): 99.3% (98.3-99.8)							

IFU – Inclusion Forming Units, CFU – Colony Forming Units, TIGRIS %Agrmt = Agreement between TIGRIS with expected results
DTS %Agrmt = Agreement between DTS with expected results
[1]A collection tube contains approximately 2.9 mL of transport medium for swab specimens and 4.0 mL of transport medium/urine mixture for urine specimens.
[2]The CT concentration in this CT/GC clinical panel member is one log below the APTIMA COMBO 2 Assay analytical sensitivity claim of 1 IFU/assay (7.25 IFU/swab, 5 IFU/mL urine).
[3]One of 5 female urine panel replicates was CT- on the DTS Systems.
[4]Three of 5 female urine panel replicates were CT- on the TIGRIS System.

Table 21: CT/GC Clinical Panel Agreement Study: CT Results for the Endocervical and Male Urethral Swab and Female and Male Urine Panels

Specimen	N	DTS+ TIGRIS+ n	DTS+ TIGRIS- n	DTS- TIGRIS+ n	DTS- TIGRIS- n	Positive Agreement (95% C.I.)	Negative Agreement (95% C.I.)
Swab	129	80	0	0	49	100 (95.5-100)	100 (92.7-100)
Urine	130	76	3[1]	1[2]	50	96.2 (89.3-99.2)	98.0 (89.6-100)

+ denotes Positive,- denotes Negative,C.I. = Confidence Interval
[1]Three of 5 female urine panel replicates, which were one log below the APTIMA COMBO 2 Assay analytical sensitivity claim of 1 IFU/assay for CT, were CT- on the TIGRIS System.
[2]One of 5 female urine panel replicates was CT- on the DTS Systems.

Table 22: CT/GC Clinical Panel Agreement Study: GC Results for the Endocervical and Male Urethral Swab and Female and Male Urine Panels

Specimen	N	DTS+ TIGRIS+ n	DTS+ TIGRIS- n	DTS- TIGRIS+ n	DTS- TIGRIS- n	Positive Agreement (95% C.I.)	Negative Agreement (95% C.I.)
Swab	129	79	0	0	50	100 (95.4-100)	100 (92.9-100)
Urine	130	80	0	0	50	100 (95.5-100)	100 (92.9-100)

+ denotes Positive, - denotes Negative, C.I. = Confidence Interval, TIGRIS = TIGRIS DTS

Precision Study

TIGRIS DTS System precision (i.e., reproducibility) was evaluated at one external clinical site and at GEN-PROBE. APTIMA COMBO 2 Assay precision was evaluated across three TIGRIS Systems, two study sites, two APTIMA COMBO 2 Assay kit lots and four operators. Table 23 presents the precision RLU data in terms of Mean, Standard Deviation, Coefficient of Variation (CV), and percent agreement with expected results for calculations of between-site, between-operator, between-lot, between-run, and within-run variability.

At the external site, two operators performed three worklists (i.e., runs) per APTIMA COMBO 2 Assay kit lot on one TIGRIS DTS System, completing a total of 6 worklists each. At GEN-PROBE, two operators performed three worklists per APTIMA COMBO 2 Assay kit lot on each of two TIGRIS DTS System, completing a total of 12 worklists each. Thus, a total of 36 worklists were completed overall. Each worklist was composed of six identical, 12-member precision panels containing 0 to 2,000 fg/assay of CT rRNA and/or 0 to 2,433 fg/assay of GC rRNA. Each worklist was composed of six identical, 12-member precision panels containing 0 to 2,000 fg/assay of CT rRNA and/or 0 to 5,000 fg/assay of GC rRNA. Panel members containing CT and GC were categorized as having low (5 or 100 fg/assay), mid (1000 fg/assay), or high (≥ 2000 fg/assay) concentrations of CT and as having low (≤ 250 fg/assay), mid (approx. 2400 fg/assay), or high (5000 fg/assay) concentrations of GC. Reproducibility was established by spiking swab transport medium with rRNA. Reproducibility when testing swab and urine specimens containing target organism has not been determined. Precision was estimated according to NCCLS Guidelines (NCCLS document EP5-A, 1999).

Table 23. TIGRIS DTS System Precision Data

Conc.					Within-Run		Between-Site		Between-Lot		Between-Operator		Between-Run	
CT	GC	N	Mean RLU (x1000)	% Agrmt	SD (RLU x1000)	CV (%)	SD (RLU x1000)	CV (%)	SD (RLU x1000)	CV (%)	SD (RLU x1000)	CV (%)	SD (RLU x1000)	CV (%)
Neg	Neg	647	4	100	1.25	26.2	0.66	13.9	0.05	1.0	0.08	1.7	0.30	6.4
Neg	High	215	1,216	100	28.5	2.3	61.2	5.0	10.0	0.8	0	0	17.1	1.4
High	Neg	216	1,266	100	38.8	3.0	0	0	93.1	7.3	40.8	3.2	40.4	3.1
High	High	210	2,445	100	54.2	2.2	40.0	1.6	110.3	4.5	28.4	1.1	52.3	2.1
Neg	Low[1]	217	1,132	100	30.3	2.6	61.0	5.3	0	0.0	20.7	1.8	18.5	1.6
Low[1]	Neg	214	1,053	100	72.8	6.9	1.5	0.1	73.8	7.0	28.5	2.7	26.9	2.5
Mid	Mid	214	2,429	100	48.8	2.0	40.0	1.6	101.1	4.1	0	0	52.9	2.1
Low[1]	Low[1]	216	2,112	99.5	112.3	5.3	84.1	3.9	33.2	1.5	34.2	1.6	52.9	2.5
Low[1]	High	216	2,282	100	77.3	3.3	97.8	4.2	59.3	2.6	0	0	41.7	1.8
High	Low[1]	215	2,318	100	61.1	2.6	50.7	2.1	86.2	3.7	4.6	0.2	42.4	1.8

SD = Standard Deviation, %CV = Percent Coefficient of Variation, % Agrmt. = Percent Agreement, Conc. = Concentration

Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, the variability as measured with standard deviation and %CV is set to 0. See NCCLS Approved Guidelines EP5-A, 1999.

[1]Low panel members were spiked at the claimed analytical sensitivities of the assay (5 fg CT rRNA/assay, 250 fg GC rRNA/assay, or both for the dual positive panel member). For CT, the target level tested is the equivalent of approximately 36 fg/swab and 25 fg/mL urine. For GC, the target level tested is the equivalent of approximately 1800 fg/swab and 1250 fg/mL urine. Based on genome size and estimated DNA:RNA ratio/cell of each organism, 5 fg is the equivalent of 1 IFU CT and 250 fg is the equivalent of 50 cells GC.

TIGRIS DTS System Analytical Performance Characteristics

Analytical Sensitivity Equivalence Study

Dilutions of three CT serovars (E, F, G) associated with urogenital disease were tested on three TIGRIS DTS System instruments and in parallel on the DTS Systems. The CT serovars were diluted into swab transport media and a pool of processed urine specimen. Concentrations ranged from 3 Inclusion-Forming Units (IFU) per assay to 0.1 IFU per assay, which is one log below the analytical sensitivity claim for the assay of one IFU per assay (7.25 IFU/swab, 5 IFU/mL urine). Percent positivity between the TIGRIS DTS and DTS Systems was equivalent to 95% confidence for all three serovars down to the analytical claim level. Dilutions below the level also tested positive on both platforms. Overall, comparable sensitivity was demonstrated at a detection level of one IFU per assay between the TIGRIS DTS and DTS Systems.

One sensitivity panel in vaginal specimen pool and one sensitivity panel in post-processed PreservCyt Solution liquid Pap specimen pool were prepared at CT 5 fg rRNA and tested 60 replicates on the TIGRIS DTS System. Percent positivity (95% C.I.) on the TIGRIS DTS System for vaginal swab specimen was 100% (95.1 – 100) and post-processed PreservCyt Solution liquid Pap specimen was 100% (95.1 – 100).

Dilutions of three GC clinical isolates were tested on three TIGRIS DTS System and in parallel on the DTS Systems. The GC isolates were diluted into swab transport media and a pool of processed urine specimen. Concentrations ranged from 150 cells per assay to 5 cells per assay, which is one log below the analytical sensitivity claim for the assay of 50 cells/assay (362 cells/swab, 250 cells/mL urine). Percent positivity between the TIGRIS DTS and DTS Systems was equivalent to 95% confidence for all three isolates down to the analytical claim level. Dilutions below the level also tested positive on both platforms. Overall, comparable sensitivity was demonstrated at a detection level of 50 cells per assay between the TIGRIS DTS and DTS Systems.

One sensitivity panel in vaginal specimen pool and one sensitivity panel in post-processed PreservCyt Solution liquid Pap specimen pool were prepared at GC 250 fg rRNA and tested 60 replicates on the TIGRIS DTS System. Percent positivity (95% C.I.) on the TIGRIS DTS System for vaginal swab specimen was 100% (95.1 – 100) and post-processed PreservCyt Solution liquid Pap specimen was 100% (95.1 – 100).

CT/GC rRNA Spiked Clinical Panel Study – Vaginal Swab and PreservCyt Solution Liquid Pap Specimens

The CT/GC rRNA spiked clinical panel study evaluated agreement between the two systems using two Gen-Probe-prepared CT/GC clinical panels spiked with 0 to 5,000 fg rRNA/assay of CT and/or 0 to 250,000 fg rRNA/assay of GC. The CT/GC clinical panels were created from vaginal swab and PreservCyt Solution liquid Pap specimens collected from 309 female subjects whose specimens had negative APTIMA COMBO 2 Assay results on the DTS Systems when tested at Gen-Probe. Negative specimens were pooled by specimen type, spiked or not spiked with CT and/or GC rRNA, and aliquotted as replicates of each panel member. Replicates of each of 13 panel members with different spiked rRNA levels were combined to create one clinical panel for each specimen type. Each panel contained a total of 132 replicates.

One vaginal swab replicate from the very low CT concentration panel member (0.05 fg rRNA/assay) had an equivocal CT result on the DTS Systems.

Table 24 shows the percent agreements for each level of rRNA in the vaginal swab and PreservCyt Solution liquid Pap panels, respectively, with expected CT and GC results for the TIGRIS DTS System and for the DTS Systems. The concentrations ranged from 1 log below to 3 logs above the 5 fg rRNA/assay for CT and 250 fg rRNA/assay for GC. Also shown in Table 24 are the overall percent agreements (99.2% for the vaginal swab panel and 100% for the PreservCyt Solution liquid Pap panel).

Table 24: CT/GC rRNA Spiked Clinical Panel Agreement Study: Agreement with Expected CT and GC Results for the Vaginal Swab Panel and PreservCyt Solution Liquid Pap Panel

Panel Member CT/GC	Concentration (fg rRNA/assay)		Replicates	Vaginal Swab Panel				PreservCyt Solution Liquid Pap Panel			
				CT		GC		CT		GC	
	CT	GC		TIGRIS %Agrmt	DTS %Agrmt	TIGRIS %Agrmt	DTS %Agrmt	TIGRIS %Agrmt	DTS %Agrmt	TIGRIS %Agrmt	DTS %Agrmt
Low/Low	5	250	10	100	100	100	100	100	100	100	100
Low/High	5	250,000	10	100	100	100	100	100	100	100	100
High/Low	5000	250	10	100	100	100	100	100	100	100	100
High/High	5000	250,000	10	100	100	100	100	100	100	100	100
Very Low/Neg	0.5	0	10	100	88.9[1]	100	100	100	100	100	100
Low/Neg	5	0	10	100	100	100	100	100	100	100	100
Medium/Neg	50	0	10	100	100	100	100	100	100	100	100
High/Neg	5000	0	10	100	100	100	100	100	100	100	100
Neg/Very Low	0	25	10	100	100	100	100	100	100	100	100
Neg/Low	0	250	10	100	100	100	100	100	100	100	100
Neg/Medium	0	2500	10	100	100	100	100	100	100	100	100
Neg/High	0	250,000	10	100	100	100	100	100	100	100	100
Neg/Neg	0	0	12	100	100	100	100	100	100	100	100
				Overall Percent Agreement between TIGRIS and DTS (95% CI): 99.2% (95.8-100)				Overall Percent Agreement between TIGRIS and DTS (95% CI): 100% (97.2-100)			

DTS % Agrmt = Agreement between DTS and expected results, TIGRIS % Agrmt = Agreement between TIGRIS DTS and expected results

[1] 1/10 replicates had equivocal CT results on the DTS Systems and was excluded from this analysis. 8/9 agreed with expected results. 1/9 was CT- on the DTS Systems. The CT concentration of this panel member is 1 log below 5 fg rRNA/assay.

Analytical Specificity Equivalence Study

For a nucleic acid amplification assay, analytical specificity with respect to individual organisms is largely determined by the chemistry of the assay (e.g. oligonucleotide sequences) rather than by the platform. Because the reagents for the APTIMA COMBO 2 Assay are identical between the TIGRIS DTS System and the DTS Systems, analytical specificity experiments on the TIGRIS DTS System were designed to focus on the most challenging culture isolates. These organisms included those known to cross-react in other amplification assays. Twenty-four (24) culture isolates were selected from the panel of organisms in Table 15, including 3 organisms that are most closely related to CT and 17 organisms that are most closely related to GC. All of the organisms tested produced negative results on the TIGRIS DTS System.

Interfering Substances Equivalence Study

Blood commonly found in urogenital specimens may interfere in some amplification assays. Whole blood was used to establish the degree of blood interference on the TIGRIS DTS and equivalence between the TIGRIS DTS System and DTS Systems with respect to this potential interferant. Fresh blood was added to clinical swab, vaginal swab, post-processed PreservCyt Solution liquid Pap, and urine specimen pools, then tested for potential assay interference in the absence and presence of CT and GC target. The estimated rRNA equivalent of one CT IFU/assay (5 fg/assay) and 50 GC cells/assay (250 fg/assay) were used as these represent the analytical sensitivity of the assay. The rRNA equivalents were calculated based on the genome size and estimated DNA:RNA ratio/cell of each organism. Specimens were tested on two TIGRIS DTS Systems. All samples containing target nucleic acid were positive when tested at a level of 10% (vol/vol) blood in swab specimens, vaginal swab specimens, post-processed PreservCyt Solution liquid Pap specimens and 30% (vol/vol) blood in urine specimens. All samples that did not contain target were correctly identified

as negative for both CT and GC. These results are identical to those demonstrated for the DTS Systems when spiked with the same quantities of blood.

Blood added to swab, vaginal swab, post-processed PreservCyt Solution liquid Pap specimens, and urine specimens at levels much higher than could be expected with normal specimen collection, did not interfere with results on the TIGRIS DTS System.

Carryover Studies for the TIGRIS DTS System

To establish that the TIGRIS DTS System minimizes the risk of false positive results arising from carryover contamination, a multi-day analytical study was conducted using spiked panels on three TIGRIS DTS System. The study used 20% high-target GC samples containing 1.0 x 10^9 cells/reaction, which were randomly spaced amongst 80% negative samples containing swab transport media. Over the course of the study, 1,372 high-target samples and 5,516 negative samples were tested across the three TIGRIS DTS System. The overall carryover rate, including both false positive and equivocal results, averaged 0.3% (18/5491). A total of 25 negative samples were reported as invalid and were excluded from the calculation. A separate analysis was conducted on a subset of the study population comprised of the negative samples that immediately followed a high-target positive. The carryover rate for this subset of the population, including both false positive and equivocal results, averaged 1.1% (12/1097). For false positives in this subset, the carryover rate ranged from 0% to 1.1% across the three TIGRIS DTS System. For equivocals in this subset, the carryover rate ranged from 0% to 0.9% across the three TIGRIS DTS System. These results demonstrate that carryover contamination is minimized on the TIGRIS DTS System.

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