**Procedure: Coro Molecular Microbiology Lab Aptima Combo 2 Assay (Panther System)**

1. **Principle**
	1. 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 disease using the PANTHER System as specified.
	2. On the PANTHER System, the assay may be used to test the following specimens from symptomatic and asymptomatic individuals: clinician-collected endocervical, vaginal, throat, rectal and male urethral swab specimens: patient-collected vaginal swab specimens and female and male urine specimens. In house validation was performed on the following specimen sources: nasopharyngeal, and eye swabs. In 2019, novel C. trachomatis variants were discovered which contain point mutations affecting detection by the original version of the Aptima Combo 2 assay (18, 23, 29, 30, 41, 42). Variant strains of chlamydia with mutations affecting diagnostic test performance have been reported previously (40) and are a natural product of microbial evolution. The updated version of the Aptima Combo 2 assay provides detection coverage for the variant strains C. trachomatis that emerged in 2019.
	3. The Hologic Aptima Combo 2 Assay combines the technologies of target capture, Hybridization Protection Assay (HPA), Transcription-Mediated Amplification (TMA), and Dual Kinetic Assay (DKA).
	4. 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 the urine and swab samples by the use of capture oligomers in a method called target capture; magnetic microparticles are another key feature of target capture. 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 polydeoxythymidine 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.
	5. 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 Hologic TMA reaction replicates a specific region of the 23S rRNA from *C. trachomatis* and a specific region of the 16S rRNA from *N. gonorrhoeae* 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 updated version of the Aptima Combo 2 assay incorporates a second CT probe, complementary to a unique region of the existing CT amplicon. This tandem probe provides detection coverage for the variant strains of C. trachomatis that emerged in 2019. The labeled probes combine with amplicon to form stable 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 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 *C. trachomatis* and *N. gonorrhoeae* 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 *C. trachomatis* signal has very rapid kinetics and has the "flasher" kinetic type. The chemiluminescent detection reaction for *N. gonorrhoeae* 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.
2. **Availability**
	1. Test is performed daily Monday-Friday. Specimens may be submitted 7 days/week, 24 hours/day.
3. **Test Codes**
	1. Test Codes are source specific:

 For GC: For CT:

EYEG2 EYEC2

 RECG2 RECC2

 THRG1 THRC2

 URIG1 URIC2

 NASG1 NASC2

 CERG1 CERC1

 UREG1 UREC1

 VAGG1 VAGC1

1. **Specimen Collection and Handling**
	1. Instructions for Collection

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

1. Specimens will be rejected if:
2. Wrong swab is submitted in collection tube
3. Swab is absent from collection tube
4. Urine not submitted in collection tube and/or wrong volume of specimen
	1. Specimen Transport and Storage Before Testing
		1. Urogenital Swab specimens:
			1. 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 urogenital specimens in the swab specimen transport tube within 7 days of collection at -20°C to -70°C to allow testing up to 12 months after collection.
		2. Extragenital swab specimens (throat and rectal)
			1. After collection, transport and store the swab in the swab specimen transport tube between 4°C and 30°C, or -20°C and -70°C until tested. Specimens must be assayed with the Aptima Combo 2 assay within 60 days of collection
		3. Urine Specimens
			1. Maintain urine specimen at 2°C to 30°C after collection and transfer to the Aptima urine specimen transport tube within 24 hours of collection. Transport to the lab in the transport tube at 2°C to 30°C. Store at 2°C to 30°C and test the processed urine specimens with the Aptima Combo 2 assay within 30 days of collection.
		4. If longer storage is needed, freeze urine specimens in the Aptima urine specimen transport tube within 7 days of collection at -20°C to -70°C to allow testing up to 12 months after collection
	2. 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. Positive and Negative samples are held for 1 week at room temperature
		4. 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.**
		5. All Child Safe and <14 y/o samples are frozen.
			1. Refer to Appendix D: “Procedure for handling RICHSA, <14 y/o, and alternate site specimens”.

1. **Equipment and Materials**
	1. Equipment
		1. PANTHER System
	2. Reagents and Materials Provided
		1. Aptima Combo 2 Assay Kit
			1. Available in 100 or 250 Tests per Kit (2 boxes and 1 Controls kit)
			2. Box 1 : store at 2° C to 8° C upon receipt
2. Aptima Combo 2 Amplification Reagent
3. Aptima Combo 2 Enzyme Reagent
4. Aptima Combo 2 Probe Reagent
5. Aptima Combo 2 Target Capture Reagent B
	* + 1. Box 2: store at 15°C to 30°C upon receipt
6. Aptima Combo 2 Amplification Reconstitution Solution
7. Aptima Combo 2 Enzyme Reconstitution Solution
8. Aptima Combo 2 Probe Reconstitution Solution
9. Aptima Combo 2 Selection Reagent
10. Aptima Combo 2 Target Capture Reagent
	* 1. Aptima Controls Kit
			1. Store at 2°C to 8°C upon receipt
			2. Aptima Positive Control, CT/Negative Control, GC
			3. Aptima Positive Control, GC/Negative Control , CT
	1. Materials Required but Available Separately – Store at room temperature unless otherwise indicated.
		1. APTIMA Assay Fluids Kit: Store at 15°C to 30°C upon receipt
			1. APTIMA Wash Solution
			2. APTIMA Buffer for Deactivation Fluid
			3. APTIMA Oil Reagent
		2. APTIMA Auto Detect Kit
		3. Multi-tube units (MTUs)
		4. PANTHER Waste Bag Kit
		5. PANTHER Waste Bin Cover

OR

* + 1. PANTHER Run Kit
			1. MTUs
			2. waste bags
			3. waste bin covers
			4. assay fluids
			5. auto detects
		2. Tips, 1000 μL conductive, liquid sensing
		3. APTIMA Multitest Swab Specimen Collection Kit
		4. APTIMA Unisex Swab Specimen Collection Kit
			1. For Endocervical and Male Urethral Swab Specimens
		5. APTIMA Urine Specimen Collection Kit for Male and

 Female Urine Specimens

* + 1. Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution
		2. Disposable gloves
		3. SysCheck calibration standard
		4. APTIMA penetrable caps
		5. Replacement non-penetrable caps
	1. Storage and Handling Requirements
		1. Working Target Capture Reagent (wTCR) is stable for 30 days when stored at 15°C to 30°C. Do not refrigerate.
		2. After reconstitution, the Enzyme Reagent, Amplification Reagent, and Probe Reagent are stable for 30 days when stored at 2°to 8°C.
		3. Discard any unused reconstituted reagents and wTCR after 30 days or after the Master Lot expiration date, whichever comes first.
		4. Controls are stable until the date indicated on the vials.
		5. Reagents stored on-board the PANTHER System have 72 hours of on-board stability.
		6. 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.
		7. 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).
		8. **Do not freeze the reagents**
1. **Warnings and Precautions**
	1. For in vitro diagnostic use.
	2. For additional specific warnings, precautions and procedures to control contamination for the PANTHER System, consult the PANTHER System Operator’s Manual.
	3. The assay was not evaluated in patient populations with a low prevalence of C. trachomatis disease, and therefore, performance in low prevalence settings has not been determined.
	4. Use only supplied or specified disposable laboratory ware.
	5. Use routine laboratory precautions. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
	6. 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.
	7. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. (1:1 dilution of bleach = 1-part bleach, 1-part water).
	8. This assay has been cleared for the following specimens on the PANTHER System:
		1. Clinician-collected endocervical, vaginal, throat, rectal, and male urethral swabs
		2. Female and Male urine specimens
	9. Only specimens collected with the following specimen collection kits have been cleared on the PANTHER System
		1. APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
		2. Aptima Multitest Swab Specimen Collection Kit for Vaginal, Throat, and Rectal Swab Specimens
		3. APTIMA Urine Collection Kit for Male and Female Urine Specimens
	10. 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.
	11. After urine has been added in the urine transport tube, the liquid level must fall between the two black indicator lines on the tube label. Otherwise, the specimen must be rejected.
	12. 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.
	13. 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.
	14. 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 open containers. If gloves come in contact with specimen, change gloves to avoid cross-contamination.
	15. Do not use this kit after its expiration date
	16. Does not interchange, mix, or combine assay reagents from kits with different lot numbers. APTIMA controls and assay fluids (PANTHER System) can be from different lot numbers.
	17. If the lab receives a swab specimen transport tube with no swab, two swabs, or a swab not supplied by Hologic, the specimen must be rejected.
	18. Upon piercing, liquid can discharge from APTIMA transport tube caps under certain conditions. Follow instructions in the PANTHER System Test Procedure to prevent this occurrence
2. **Test Procedure**

**Note:** See **PANTHER System Operator’s Manual** for additional Panther System procedural information.

For quick reference guides refer to Appendix A: Panther System Operation Checklist and Appendix B: Panther Resources Needed located at the end of this procedure.

* 1. Laboratory/ Panther Preparation (Daily)
		1. Prior to starting the assay, wipe down work surfaces with household bleach diluted 1:1 with water (1 part bleach, 1 part water). Allow bleach to contact surfaces for at least 1 minute and then follow with a DI water rinse. Do not allow the bleach to dry. Cover the bench surface on which the test will be performed with clean, plastic-backed absorbent laboratory bench covers.

 \*\*\*CHANGE GLOVES

* + 1. Remove reagent kits from refrigerator and bring to room temp (30 Min). If a new kit needs to be made it should be done at this point. See the REAGENT RECONSTITUTION/PREPERATION section of this procedure.
	1. Reagent Reconstitution/ Preparation

**Note: This step should be performed prior to beginning any work on the PANTHER 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.
			1. 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.
			2. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
			3. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
			4. Open the matching reconstitution solution bottle and set the cap on a clean, covered work surface.
			5. 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).
			6. Slowly invert the assembled bottle and vial. Allow the solution to drain from the bottle into the vial (Figure 1, Step 3).
			7. Gently swirl the solution in the vial to mix. Avoid creating foam while swirling the vial (Figure 1, Step 4).
			8. 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.
			9. Remove the reconstitution collar and glass vial (Figure 1, Step 6).
			10. Recap the bottle. Record operator initials and the reconstitution date. (Figure 1, Step 7).
			11. Discard the reconstitution collar and vial (Figure 1, Step 8).

**Warning:** Avoid creating foam when reconstituting reagents.

 Foam compromises the level-sensing in the PANTHER

 System.

**FIGURE 1**



* + 1. Prepare Working Target Capture Reagent (wTCR)
			1. Pair the appropriate bottles of TCR and TCR-B.
			2. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.
			3. Open the bottle of TCR, and set the cap on a clean, covered work surface.
			4. 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.
			5. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.
			6. Record operator initials and the current date on the label.
			7. Discard the TCR-B bottle and cap.
		2. Prepare Selection Reagent
			1. Check the lot number on the reagent bottle to make sure it matches the lot number on the Master Lot Barcode Sheet.
			2. 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.*

* 1. Reagent Preparation for Previously Reconstituted Reagents
		1. Previously reconstituted Probe, Amplification, and Enzyme Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.
		2. If the reconstituted Probe Reagent contains a 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 PANTHER System will recognize and reject bottles that have been topped off.
	2. Sample and Controls 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:
			1. The presence of a single blue APTIMA collection swab in a unisex swab specimen transport tube.
			2. The presence of a single pink APTIMA collection swab in a vaginal swab specimen transport tube.
			3. A final volume of urine between the black fill lines of a urine specimen transport tube.
		4. Inspect specimen tubes before loading into rack:
			1. 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.
			2. 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.
			3. 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.
			4. 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 these steps may result in liquid discharge from the*

*specimen tube cap.*

**Note**: *Up to 4 separate aliquots can be tested from each specimen*

 *tube. Attempts to pipette more than 4 aliquots from the specimen tube*

 *can lead to processing errors.*

* 1. System Preparation
		1. Set up the system according to the instructions in the PANTHER System Operator’s Manual and Procedural Notes. Make sure that the appropriately sized reagent racks and TCR adapters are used.
		2. Load samples.
1. **Procedural Notes**
	1. Controls
		1. To work properly with the APTIMA Assay software for the PANTHER System, one pair of controls is required. The Positive Control, CT / Negative Control, GC and the Positive Control, GC / Negative Control CT tubes can be loaded in any rack position or in any Sample Bay Lane on the PANTHER System. Patient specimen pipetting will begin when one of the following two conditions has been met:
			1. A pair of controls is currently being processed by the system.
			2. Valid results for the controls are registered on the system.
		2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated kit up to 24 hours unless:
			1. Controls results are invalid.
			2. The associated assay reagent kit is removed from the system.
			3. The associated assay reagent kit has exceeded stability limits.
		3. Each APTIMA control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.
	2. Temperature
		1. Room temperature is defined as 15°C to 30°C.
	3. Glove Powder
		1. As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.
	4. Environmental Testing
		1. 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.
		2. To monitor for laboratory contamination 6 environmental sites per week will be tested. Refer to Environmental Testing QC sheets for specifics.
2. **Quality Control and Acceptability of Results**
	1. Positive and Negative Controls
		1. 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.

* + 1. The Positive Control, CT / Negative Control, GC serves as the negative control for the GC test results.
		2. The Positive Control, GC / Negative Control, CT serves as the negative control for the CT test results.
		3. If desired, a dual negative control furnished by the user can be added to monitor assay background.
		4. Positive and Negative Control Results

**Control Total RLU (x1000) CT Result GC Result**

Positive Control, CT/ ≥ 100 and < 3,000 CT Positive GC Negative

Negative Control, GC

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Positive Control, GC ≥ 150 and < 3,000 CT Negative GC Positive

 Negative Control, CT

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* 1. Acceptability
		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. Notify the Lab Specialist and/or Manager.

 **Note**: See Troubleshooting or contact Hologic Technical

 Support for help with out-of-range controls on the PANTHER Systems.

* + 1. Each kit reagent lot/ new shipment must be QC’d with previously tested Positive and Negative External Controls.
		2. New reagent lots/ new shipments of External Positive and Negative Controls must be tested concurrently with previously tested External Positive and Negative Controls.
1. **Test Interpretation**
	1. Assay test results are automatically interpreted by the APTIMA Combo 2 Assay Software 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 following table). The entire run is considered invalid if either the CT positive control or the GC positive control is not positive. A test result may be invalid due to a parameter outside the normal expected ranges.

|  |  |  |  |
| --- | --- | --- | --- |
| **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 |
| CT and GC | 1 to < 85 | 85 to < 250 | 250 to <4,500 |
| GC indeterminate | 1 to < 85 | 85 to < 4,500 | N/A |

1. **Reporting Patient Results**

**Note:** Refer to Appendix C for Soft Resulting and Appendix D for Handling of Child Safe, <14 y/o, and Alternate site samples.

* 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 and urine specimen results. (See NOTES below.)
		1. Initial Results for CHSA and <14y/o

|  |  |  |
| --- | --- | --- |
| **Instrument Result** | **RLUs** | **Reported Result** |
| CT POS | 900 to <4500 | Positive for Chlamydia trachomatis by amplified technology |
| CT POS (Low) | 100 to <900 | Repeat testing 1 time |
| CT NEG | 1 to <25  | Negative for Chlamydia trachomatis by amplified technology |
| CT EQUIV | 25 to <100 | Equivocal for Chlamydia trachomatis by amplified technology |
| GC POS | 900 to 4500 | Positive for Neisseria gonorrhoeae by amplified technology |
| GC POS (Low) | 150 to <900 | Repeat testing 1 time |
| GC NEG | 1 to <60 | Negative for Neisseria gonorrhoeae by amplified technology |
| GC EQUIV | 60 to <150 | Equivocal for Neisseria gonorrhoeae by amplified technology |
| **Instrument Result** | **RLUs** | **Reported Result** |
| CT and GC POS | 900 to <4500 | Positive for Chlamydia trachomatis and Neisseria gonorrhoeae by amplified technology |
| CT and GC POS (Low) | 250 to <900 | Repeat testing 1 time |
| CT and GC EQUIV | 85 to <250 | Equivocal for Chlamydia trachomatis and Neisseria gonorrhoeae by amplified technology |
| INVALID |  | Repeat testing 1 time |

* + 1. Initial Results for all other patients

|  |  |  |
| --- | --- | --- |
| **Instrument Result** | **RLUs** | **Reported Result** |
| CT POS |  100 to <4500 | Positive for Chlamydia trachomatis by amplified technology |
| CT NEG | 1 to <25 | Negative for Chlamydia trachomatis by amplified technology |
| CT EQUIV | 25 to <100 | Equivocal for Chlamydia trachomatis by amplified technology |
| GC POS | 150 to <4500 | Positive for Neisseria gonorrhoeae by amplified technology |
| GC NEG | 1 to <60 | Negative for Neisseria gonorrhoeae by amplified technology |
| GC EQUIV | 60 to <150 | Equivocal for Neisseria gonorrhoeae by amplified technology |
| **Instrument Result** | **RLUs** | **Reported Result** |
| CT and GC POS | 250 to <4500 | Positive for Chlamydia trachomatis and Neisseria gonorrhoeae by amplified technology |
| CT and GC EQUIV | 85 to <250 | Equivocal for Chlamydia trachomatis and Neisseria gonorrhoeae by amplified technology |
| INVALID |  | Repeat testing 1 time |

* + 1. Retest Results:
			1. Initial Low Positive Results
				1. Result Positive when repeat is Positive
				2. Result Equivocal when repeat is Negative or Equivocal
		2. Invalid Results:
			1. Invalid results that repeat as Invalid due to Volume Verification Failure Sample (VVFS) are resulted as @UNAZ- Unable to analyze due to sampling issues caused by specimen integrity. Please resubmit if clinically warranted.
			2. Invalid results that repeat as Invalid due to any other issue are resulted as Indeterminate with a texted comment stating the reason.
	1. Positivity rate for Chlamydia and GC
		1. The Positivity rate will be recorded on the daily QC sheets for Chlamydia and GC
		2. A positivity rate above the established threshold within a run or after multiple runs

 will prompt an investigation for potential false positive results.

* + 1. Repeat testing of positive and equivocal samples will be necessary when the daily

 positivity rate is 3 X the prevalence rate in a single run.

* + 1. Repeat testing on positive and equivocal samples from the second run is necessary

 when the daily positivity rate is greater than 2 X the prevalence rate for 2 runs in a

 row.

* + 1. If repeat testing confirms the results no further action is required.
		2. The prevalence rate will be monitored and addressed every six months. Statistics will be available on the QC sheets.
		3. Any issues should be brought to the attention of the tech specialist and/or manager.
		4. The positivity rate is determined by dividing the total number of positive and equivocal specimens (excluding controls and repeats) by the total number of specimens in the run (excluding controls).
	1. Notes
		1. Careful consideration of performance data is recommended for interpreting

 APTIMA Combo 2 Assay results for asymptomatic individuals or any individuals in

 low prevalence populations.

* + 1. A negative result does not preclude the presence of a C. trachomatis or N.

 gonorrhoeae infection because results are dependent on adequate specimen

 collection, absence of inhibitors, and sufficient rRNA to be detected. Improper

 specimen collection, improper specimen storage, technical error, or specimen mix-

 up may affect test results.

* + 1. 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 C. trachomatis or N. gonorrhoeae.

* + 1. A vaginal swab is the recommended specimen type for female patients who are clinically suspected of having a chlamydial or gonococcal infection.
		2. 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.

1. **Format for Reporting Results**
	1. Chlamydia trachomatis
		1. Positive for Chlamydia trachomatis

\*\*\* State Health Department Requires Notification of this Positive Result.

**Note:** For a positive result on patients under 14 years of age or eye specimens refer to the Critical Call Policy

* + 1. Negative for Chlamydia trachomatis
		2. Equivocal for Chlamydia trachomatis
		3. Indeterminate for Chlamydia trachomatis
		4. Unable to analyze due to sampling issues caused by specimen integrity.
	1. Neisseria gonorrhoeae
		1. Positive for Neisseria gonorrhoeae

\*\*\* State Health Department Requires Notification of this Positive Result.

**Note:** For a positive result on patients under 14 years of age or eye specimens refer to the Critical Call Policy

* + 1. Negative for Neisseria gonorrhoeae
		2. Equivocal for Neisseria gonorrhoeae
		3. Indeterminate for Neisseria gonorrhoeae
		4. Unable to analyze due to sampling issues caused by specimen integrity
	1. Notes
		1. A positive result on patients under 14 years of age or any positive eye specimens

 refer to the Critical Call Policy for reporting.

* + 1. All positive results must be reported to the State Health Department
		2. A Patient Health Information disclosure form must be filled out for every positive

 result reported to the State Health Department

1. **Limitations of the Procedure**
2. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this procedure may result in erroneous results.
3. The effects of tampon use, douching, and specimen collection variables have not been assessed for their impact on the detection of CT or GC.
4. 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.
5. 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.
6. 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).
7. 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 Hologic specimen collection kit.
8. Therapeutic failure or success cannot be determined with the APTIMA Combo 2 Assay since nucleic acid may persist following appropriate antimicrobial therapy.
9. Results from the APTIMA Combo 2 Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
10. 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.
11. 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.
12. Patient-collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated.
13. The patient-collected vaginal swab specimen application is limited to clinical settings where support/counseling is available to explain procedures and precautions.
14. The Aptima Combo 2 assay has not been validated for use with vaginal swab specimens collected by patients at home.
15. The performance of the Aptima Combo 2 Assay has not been evaluated in adolescents less than 14 years of age.
16. First catch female urine specimens are acceptable but may detect up to 10% fewer CT/GC infections when compared with vaginal and endocervical swab specimens. .
17. **References**
	1. Aptima Combo 2 Assay Package Insert Rev. 004
18. **Technical Support**
	1. Hologic, Inc.

 10210 Genetic Center Drive

 San Diego, CA 92121 USA

* 1. Customer Support
		1. 1 800 442 9892
		2. customersupport@hologic.com
	2. Technical Support
		1. 1 888 484 4747
		2. molecularsupport@hologic.com
	3. Additional contact information
		1. [www.hologic.com](http://www.hologic.com)
1. **Revisions**
	1. July 2018. Addition of female urine as an FDA approved specimen source for assay
	2. Dec. 2018. Updated formatting of procedure to Coro Molecular Microbiology and added Appendixes to procedure.
	3. Jan.2020
		1. Updated resulting information. Deleted the need to repeat low positive results with the exception of CHSA and patients <14 y/o.
		2. Removed footer and added updated signage sheet.
	4. Dec. 2022.
		1. Updated test codes
		2. Updated principle to include the detection of C. trachomatis variants that emerged in 2019.

**Appendix A**

**Panther System Operation Checklist**

1. **Clean and Inspect**
* Clean work areas with 50% bleach (1 min) and rinse with DI H2O

**CHANGE GLOVES**

* Check room humidity (20%-85%)
* Check room temperature (15°-30°C)
* Perform external inspection
* Check for leaks
* Fill DI H2O bucket

**CHANGE GLOVES**

1. **Prepare Reagents**
* Take out reagent kits and bring to room temp (approx. 30 min.)
* Reconstitute reagents, if necessary
* If using previously reconstituted reagents, bring reagents to room temp and gently invert all reagents
* Warm probe reagent, if necessary, and invert.

**CHANGE GLOVES**

1. **Complete Panther System Tasks**
* Load Tips
* Load MTUs

Check for:

* All 5 tiplets present
* No extra MTU tiplets inside tubes
* Barcodes present, properly aligned, undamaged, and intact.
* Distributor feet intact
* Maximum of 25 MTUs loaded
* Load Universal Fluids
* Replace depleted fluids
* Ensure all fittings are secure
* Empty Waste
* Ensure all fittings are secure

**CHANGE GLOVES**

* Perform Maintenance- Tasks
* Complete scheduled maintenance tasks
* Prime, if necessary
* Ensure resources are available
* Load Assay Reagents
* Remove caps, ensure no bubbles and/or precipitate is present
* Ensure bottles are properly seated
* Verify all barcodes are visible
* Activate New Lots of Reagents (if needed)
* Load onto the Panther System
* Ensure wTCR quadrant matches Assay Reagent lane for Amp, Enzyme, Probe, and Selection
* Load Samples
* Ensure sample default settings are accurate or test orders are available via LIS
* Rack calibrators and controls for each Assay Reagent kit loaded
* Confirm correct sample collection and volume
* Place samples into racks
* Verify all barcodes are visible
* Ensure sample retainers are seated
* Load onto the Panther System
* Check racks for any errors
* Manually enter order numbers if necessary
* Manually add test orders when necessary

**CHANGE GLOVES**

1. **Pipetting and Assay Processing**

Sample tube graphic will change from:

* Green- Sample loaded
* Yellow- Pipetting in-process
* Blue- Pipetting complete
* Red- Error
1. **Feed and Monitor**
* Return as needed to load tips, MTUs, additional sample racks, and Assay Reagents

**CHANGE GLOVES**

1. **Review/Manage Results**
* Print Results Report
* Print Exceptions Report
* Verify results
* Send selected results to LIS
1. **Unload Sample Racks**
* Remove sample racks
* Rack samples and cover with parafilm

 **CHANGE GLOVES**

1. **Unload Reagents, if necessary**
* Recap with new caps and store Assay Reagents
* Deactivate any lots when all reagents have been used up.
1. **Check Fluid and Waste**
* Replenish/ Empty as needed

**Appendix B**

**Panther Resources Needed**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|   | **Full Prime** | **Mini-Prime** |  | **Mag Wash Clean Maintenance** |
| **Time to Complete** | 12 Minutes | 10 Minutes |  |   |
| **MTU Count Consumed** | 50/ 10 strips | 15/ 3 strips |  | 10/ 2 strips |
| **Waste Count Consumed** | 50 | 15 |  | 20 |
| **Maximum Fluid Test Count consumed per Kit** | 105 | 8 |  | 15 in Fluids A **OR** B |
| **Mag Wash Cleaning Solution** |   |   |   | 25.5 ml |

**Appendix C**

**Aptima Combo 2 and Trich Panther Procedure Notes and**

**SCC Soft Resulting**

**Test ID:** See Individual Test IDs

**Template:** GC/CT/ Trich –Note: Use GENP (GC/CTs and Trichs)

**Workstation:** RMOLM

1. For Trichomonas
2. Print a “Pending List” and check it against specimens to make sure all samples have been received. Resolve any issues.
3. Create a Tasklist
4. Follow procedure for “Creating a Tasklist” in Soft Manual under TASKLIST

 Template= GENP

1. Delete the Pos and Neg Controls from list.
2. Wand specimens onto the tasklist beginning in position 1.
3. Load Assay Reagents onto Panther
4. Load GC/CT and Trich Controls into Sample Rack- Load onto Panther
5. Load GC/CT and Trich sample tubes into sample Racks- Load onto Panther

Verify that sample barcodes and test orders were read.

1. Results
2. Panther Reports- Report Tab
3. Select “Results by Worklist” Report
4. Choose Worklist ID by Date and Assay
5. Print
6. Select “Exceptions by Worklist” Report

**Note:** List of all Positive and Invalid results

1. Choose Worklist ID by Date and Assay
2. Print
3. Select “Worklist Lot” Report
4. Choose Worklist ID by Date and Assay
5. Print
6. Check reports for any invalid samples, samples that need repeating, and any other result that should not be reported.
7. Check the Positivity rate and repeat all positives if the rate is above the posted limit.
8. Go to Panther Result Tab
9. Filter results by Worklist ID by date and assay
10. “Select All” for Samples to verify.

**Note: “Deselect” any Invalid or any samples results that you do not want to autoverify.**

1. Verify- tab at bottom of screen
2. Send to LIS- tab at bottom of screen

**Note**: Negative Results will Autoverify in Soft

Positive Results will go to the Instrument Menu in Soft for “Posting”

1. From SoftLab, go to “Interfaces”, and “Instrument Menu”.
2. Select “Hologic Panther” (RPANT) for Panther #1 or Hologic Panther 2” (RPAN2) for Panther #2 from Instrument Menu.
3. Select “Loadlist and Today’s Results”, “Not Posted”, “By Sequence”
4. Each order will be highlighted individually. Verify the result against the instrument printout. Click “Post All” for each order to be verified.
5. For any result that is being repeated do not post result.
6. If any Result Comments, ie. Phone reports need to be added:
7. Go to “Lab Result” tab
8. Open “Comment” box and add comment.
9. Save
10. Go back to “Instrument” tab.
11. Post Result
12. Invalid Results that repeat as Invalid due to “volume verification failure sample”.
13. All Invalid results will cross over to Soft as “UNAZ”- “Unable to analyze due to sampling issues caused by specimen integrity. Please resubmit if clinically warranted.
14. “Post” this result when the invalid result is due to error code VVFS- Volume Verification Failure Sample x2.
15. Invalid Results that repeat as Invalid for any other reason.
16. **DO NOT** Post Result (it will say @UNAZ)
17. Go to “Result Entry” and manually enter result.
18. Choose Indeterminate from the keypad and text in an appropriate comment depending on the reason.
19. Cancel any unwanted repeated results from the Instrument Menu by right clicking, choose cancel, Save.
20. Save the Instrument Results printout in the GC/CT, Trich Results Binder. Write the Tasklist number on the first page.

14. Print a “pending worklist” to check for any outstanding orders.

 Resolve all outstanding issues.

**Appendix D**

**Protocols for Handling of Child Safe Clinic, <14 y/o**

**And Alternate Site GC/CT, Trich Specimens**

A. Child Safe Specimens and < 14 y/o

* + 1. Patients are identified as Child Safe by:
			1. Patient location of RXAF noted on slip and/or
			2. Pink slip wrapped around collection tube
			3. Patient location NXAF (NH Child Safe Clinic)
		2. Check age of patient from patient info on Tasklist
		3. Freeze all Negative specimens in the Child Safe Negative Rack labeled Child Safe Neg. Racks #1 and #2. When both racks are full discard the oldest rack.
		4. Positive GC/CT specimens:
			1. Positive GC/CT specimens are resulted according to the criteria for CHSA and <14 y/o patient specimens. Repeat X1 if RLUs are <900.
			2. Result as Positive for CT or GC and call to CHSA or ordering physician.
			3. Place a screen print of the report the front cover of the GC/CT Results Binder.
			4. Place the specimen in the rack labeled Positive Not Confirmed Rack.
			5. Results will be confirmed per physician request. Sample will be sent to Quest for Aptima CT or GC Alternate Probe
			6. If confirmed- Amend original report with the statement Results Confirmed by Secondary Method. Testing performed at Quest Nichols Institute.
			7. There will be no sample left to freeze.
		5. Positive Trichomonas specimens:
			1. Result as Positive for Trichomonas and call to CHSA or ordering physician.
			2. Freeze the specimen in the Child Safe Positive Trichomonas Rack to be held indefinitely.
			3. Place a screen print in the GC/CT Not Confirmed binder.