**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION SUMMARY**
**ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

**A. 510(k) Number:**
K070174

**B. Purpose for Submission:**
To add the option for automated swab specimen preparation and control preparation using the software accessory "Roche Scripts for AMPLICOR™ CT/NG Test for *Chlamydia trachomatis*" to direct the Tecan Genesis RSP 150 Workstation.

**C. Measurand:**
*Chlamydia trachomatis* DNA

**D. Type of Test:**
Qualitative determination using Polymerase Chain Reaction (PCR) DNA amplification and colorimetric detection

**E. Applicant:**
Roche Diagnostics Corporation

**F. Proprietary and Established Names:**
AMPLICOR™ CT/NG Test for *Chlamydia trachomatis*; Roche Scripts for AMPLICOR CT/NG Test (Roche Scripts Accessory)

**G. Regulatory Information:**

1. Regulation section:
   866.3120 Chlamydia serological reagents

2. Classification:
   Class I

3. Product code:
   MKZ – DNA probe, nucleic acid amplification, Chlamydia

4. Panel:
   83 Microbiology

**H. Intended Use:**

1. Intended use:

The AMPLICOR CT/NG test for *Chlamydia trachomatis* is a qualitative in vitro test for the detection of *C. trachomatis DNA* in urine from symptomatic or asymptomatic males, in

endocervical swab specimens from symptomatic or asymptomatic females, and in urethral swab specimens from symptomatic males as evidence of infection with *C. trachomatis. C. trachomatis DNA* is detected by Polymerase Chain Reaction (PCR) amplification of target DNA and by hybridization capture of amplified target.

Sample and control preparation can either be accomplished manually or automated using the optional Roche Scripts for AMPLICOR CT/NG Test accessory to direct the Tecan Genesis RSP 150 workstation. Urine specimens are not indicated for use with the automated sample preparation option.

The Roche Scripts for AMPLICOR CT/NG Test are intended to provide software scripts to direct the automated Tecan Genesis RSP 150 workstation to process swab samples or control material for analysis using either of the following four 510(k)-cleared assay test systems:

- AMPLICOR ® CT/NG test for *Chlamydia trachomatis*
- AMPLICOR ® CT/NG test for *Neisseria gonorrhoeae*

2. Indication(s) for use:
   Same as intended use

3. Special conditions for use statement(s):
   Urine specimens are not indicated for use with the automated sample preparation option.
   Prescription Use only.

4. Special instrument requirements:
   Not applicable

**I. Device Description:**

The AMPLICOR CT/NG Test for *Chlamydia trachomatis* with optional Roche Scripts accessory is a qualitative in vitro diagnostic test for the detection of *Chlamydia trachomatis* DNA performing automated swab specimen preparation with the Tecan Genesis RSP 150 workstation.

The RSP Genesis 150 Workstation with Gemini software v4.2 is a microprocessor controlled sample diluter and dispenser. The Roche Scripts for AMPLICOR™ CT/NG Test accessory consists of a compact disc (CD) containing scripts to direct the automated Tecan Genesis RSP 150 workstation with Gemini software v4.2 to process swab samples or control material for analysis.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):
   AMPLICOR™ CT/NG test for *Chlamydia trachomatis*
2. Predicate 510(k) number(s):
   K973707

3. Comparison with predicate:

| Similarities | | |
|---|---|---|
| Item | Device<br>AMPLICOR™ CT/NG for *C. trachomatis* with optional Roche Scripts accessory | Predicate<br>AMPLICOR™ CT/NG for *C. trachomatis* |
| Intended Use | The AMPLICOR CT/NG test for *Chlamydia trachomatis* is a qualitative in vitro test for the detection of *C. trachomatis DNA* in urine from symptomatic or asymptomatic males, in endocervical swab specimens from symptomatic or asymptomatic females, and in urethral swab specimens from symptomatic males as evidence of infection with *C. trachomatis. C. trachomatis DNA* is detected by Polymerase Chain Reaction (PCR) amplification of target DNA and by hybridization capture of amplified target. | Same plus:<br>Sample and control preparation can either be accomplished manually or automated using the optional Roche Scripts for AMPLICOR CT/NG Test accessory to direct the Tecan Genesis RSP 150 workstation. Urine specimens are not indicated for use with the automated sample preparation option.<br><br>The Roche Scripts for AMPLICOR CT/NG Test are intended to provide software scripts to direct the automated Tecan Genesis RSP 150 workstation to process swab samples or control material for analysis using either of the following four 510(k)-cleared assay test systems:<br>• AMPLICOR ® CT/NG test for *Chlamydia trachomatis*<br>• AMPLICOR ® CT/NG test for *Neisseria gonorrhoeae* |
| Test Principle | DNA detection via PCR amplification of target DNA followed by hybridization capture, and colorimetric absorbance determination performed on the AMPLICOR ™ Analyzer. | Same |
| Controls provided | Positive control: plasmid DNA from *C. trachomatis*<br>Negative control: DNA from *N. gonorrhoeae*<br>Optional internal control:<br>Plasmid DNA with primer binding regions identical to *C. trachomatis* target sequence | Same |

| Differences | | |
|---|---|---|
| Item | Device | Predicate |
| Analytical sensitivity - Limit of Detection, Expressed as Inclusion Forming Units (IFU) | Revised test performance: 20 IFU/mL for *C. trachomatis* with swab specimens | Labeled test performance: 20 IFU/mL for urine specimens and 80 IFU/mL for *C. trachomatis* with swab specimens |
| Specimen type | Urethral and endocervical swabs only (no urine samples) | Male or female urine specimens; urethral and endocervical swabs |
| Specimen and Control preparation | Manual or automated using the Roche Scripts to direct the Tecan Genesis RSP 150 workstation | Manual |

**K. Standard/Guidance Document Referenced (if applicable):**

CLSI EP5-A2 "Evaluation of Precision Performance of Quantitative Measurement Methods", CLSI EP12-A "User Protocol for Evaluation of Qualitative Test Performance".

**L. Test Principle:**

DNA detection via PCR amplification of target DNA followed by hybridization capture of amplified target using the AMPLICOR ™ Analyzer. The PCR-based test system reagents and testing procedures are unchanged.

**M. Performance Characteristics (if/when applicable):**

The purpose of this submission is to demonstrate equivalency between the automated specimen preparation using Roche Scripts to direct the Tecan Genesis TSP 150, and the manual specimen preparation method.

1. Analytical performance:

*a. Precision/Reproducibility:*

The precision of the AMPLICOR CT/NG Test for *Chlamydia trachomatis* with automated specimen preparation was determined a multi-operator study. Three independent operators using three different Tecan Genesis RSP 150 Instruments tested two panels of culture transport media specimens containing samples described in the table below. The two panels were prepared on separate days but were identical in composition. The first panel was tested by all three sites. The first two sites tested the panel in triplicate, once a day. The third site tested the panel in triplicate on nine separate days over a period of three weeks. The second panel was tested by only one site in triplicate, once a day, over a period of five days. Each run consisted of automated specimen preparation using the Roche Scripts to direct the Tecan Genesis RSP 150 Instrument, followed by amplification and detection according to the standard test protocol.

**Description of Panels used for Precision Testing**

| Panel Sample | Description |
|---|---|
| PS1 | Spiked M4 media at ~500 IFU/mL CT* |
| PS2 | Spiked M4 media at ~300 IFU/mL CT |
| PS3 | Spiked M4 media at ~100 IFU/mL CT |
| PS4 | Spiked M4 media at ~100 IFU/mL CT and ~5000 cfu/mL NG** |
| PS5 | Spiked M4 media at ~300 IFU/mL CT and ~3000 cfu/mL NG |
| PS6 | Spiked M4 media at ~500 IFU/ml CT and ~1000 cfu/mL NG |
| PS7 | Spiked M4 media at ~1000 cfu/mL NG |
| PS8 | Spiked M4 media at ~3000 cfu/mL NG |
| PS9 | Spiked M4 media at ~5000 cfu/mL NG |
| PS10 | Spiked M4 media at ~500 IFU/mL CT and ~5000 cfu/mL NG |
| PS11 | M4 media blank |

*CT = *Chlamydia trachomatis*

**NG = *Neisseria gonorrhoeae*

**Summary of Result for Precision: AMPLICOR™ CT/NG for *C. trachomatis***

| Panel Member | Number of Replicates | Number of Correct Results* | Percentage of Correct Results* | Number of Initially Equivocal Results+ | Percentage of Initially Correct Results |
|---|---|---|---|---|---|
| PS1 | 63 | 63 | 100.0% | 0 | 100.0% |
| PS2 | 63 | 63 | 100.0% | 0 | 100.0% |
| PS3 | 63 | 63 | 100.0% | 0 | 100.0% |
| PS4 | 63 | 63 | 100.0% | 0 | 100.0% |
| PS5 | 63 | 62 | 98.4% | 1 | 98.4% |
| PS6 | 63 | 63 | 100.0% | 0 | 100.0% |
| PS7 | 63 | 63 | 100.0% | 1 | 100.0% |
| PS8 | 63 | 63 | 100.0% | 0 | 100.0% |
| PS9 | 63 | 61 | 96.8% | 1 | 95.2% |
| PS10 | 63 | 63 | 100.0% | 0 | 100.0% |
| PS11 | 63 | 63 | 100.0% | 1 | 100.0% |
| overall | 693 | 690 | 99.6% | 4 | 99.4% |

*Samples with absorbance values of $\geq$ 2.0 were considered positive, and samples with absorbance value <2.0 negative in this set of calculations.
+Initially equivocal samples had an absorbance of $\geq$0.2 and < 2.0

The overall precision of the automated method using a cutoff of 2.0 is 99.6%, compared to the precision of the manual method of 99.4%. When calculations are based on using initially equivocal results, overall precision of the automated method is 96.0%, and manual 98.4%.

*b.* *Linearity/reportable range:*
Not applicable

*c.* *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The recommended Positive, Negative and Internal Control material were tested a sufficient number of times with acceptable results on all testing days.

*d.* *Detection limit:*
The Limit of Detection of the AMPLICOR™ CT/NG for *C. trachomatis* with automated specimen preparation was determined using M4 culture transport media specimens containing 1.44 x 107 IFU/mL of *Chlamydia trachomatis* culture (ATCC strain Vr 885, Serovar D). The panel contained the following dilutions, which bracket the current labeled limit of detection (in bold):

CT: 2, 5, 20, 50, **80**, 100 IFU/mL (0.025, 0.062, 0.250, 0.625. 1.0 and 2.5 IFU/test)

A total of 24 replicates per dilution for each sample processing/testing combination. Equivocal results were not repeated, and samples returning inhibitory results were excluded from data analysis. Absorbance values ≥ 2.0 were considered positive, and < 2.0 negative.

The Limit of Detection (LoD), the lowest dilution at which ≥ 95% of replicates yielded positive results, for COBAS AMPLICOR™ CT/NG for *C. trachomatis* is 20 IFU/mL (0.25 IFU/test) for both the automated and manual specimen preparation. The results of study are presented in the table below.

**This result differs from the current labeled LoD of 80 IFU/mL.**

**Summary of LoD for AMPLICOR™ CT/NG Test for *C. trachomatis* with automated and manual specimen preparation**

| **Sample** | **Dilution** | **N** | **Automated** | | **N** | **Manual** | |
|---|---|---|---|---|---|---|---|
| | | | Positive | % | | Positive | % |
| CT -1 0.025 | 2 | 24 | 16 | 66.7 | 24 | 7 | 29.2 |
| CT – 2 0.062 | 5 | 24 | 22 | 91.7 | 24 | 20 | 83.3 |
| CT – 3 0.25 | 20 | 24 | 24 | 100.0 | 24 | 24 | 100.0 |
| CT-4 0.625 | 50 | 24 | 23 | 95.8 | 24 | 23 | 95.8 |
| CT-5 1 | 80 | 24 | 24 | 100.0 | 24 | 24 | 100.0 |

*e.* *Analytical specificity:*
The analytical specificity was tested using the automated sample preparation directed by the Roche Scripts compared to the manual method by examining spiked samples with varying concentrations of the test analytes. The same

method and concentrations used in the Precision panel testing were utilized for analytical specificity. Data from the different sites were pooled and the percentage of false positives and negatives were calculated. The following table shows the agreement of the specificity results between the automated and the manual methods.

**Specificity results for AMPLICOR CT/NG test for *C. trachomatis***

| | | **AMPLICOR CT/NG test for *C. trachomatis*** | | | | |
|---|---|---|---|---|---|---|
| | N | Automated* | | N | Manual** | |
| Positive Samples | | False negatives | % | | False negatives | % |
| PS1 | 63 | 0 | 0.0 | 63 | 1 | 1.6 |
| PS2 | 63 | 0 | 0.0 | 63 | 0 | 0.0 |
| PS3 | 63 | 0 | 0.0 | 63 | 0 | 0.0 |
| PS4 | 63 | 0 | 0.0 | 63 | 0 | 0.0 |
| PS5 | 63 | 0 | 0.0 | 63 | 0 | 0.0 |
| PS6 | 63 | 0 | 0.0 | 63 | 0 | 0.0 |
| PS10 | 63 | 0 | 0.0 | 63 | 0 | 0.0 |
| **Overall** | **441** | **0** | **0.0** | **441** | **1** | **0.2** |
| Negative Samples | | False positives | | | False positives | |
| PS7 | 63 | 0 | 0.0 | 63 | 2 | 3.2 |
| PS8 | 63 | 0 | 0.0 | 63 | 0 | 0.0 |
| PS9 | 63 | 2 | 3.2 | 63 | 0 | 0.0 |
| PS11 | 63 | 0 | 0.0 | 63 | 1 | 1.6 |
| **Overall** | **252** | **2** | **0.8** | **252** | **3** | **1.1** |

*The automated sample preparation had 4 equivocal results
**The manual sample preparation had 6 equivocal results

The false negative rate and false positive rate for automated sample preparation are within 95% of false negative and false positive rate for manual sample preparation. The analytical specificity automated results are equivalent to the manual method results.

*f. Assay cut-off:*

Not applicable

*g. Cross-Contamination Testing*

This study evaluated the potential for cross contamination / carryover of the AMPLICOR™ CT/NG for *C. trachomatis* assay with automated sample preparation directed by the Roche Scripts; in comparison to manual sample preparation. This study was designed to observe the effects of potential aerosolization / splash over the whole plate. Positive specimens or M4 media blanks were prepared as samples.

Positive samples were prepared from stock cultures of *C. trachomatis* (ATCC

strain Vr 885, Serovar D) at 1.44 x $10^7$ IFU/mL and *Neisseria gonorrhoeae* (ATCC 19242) at 1.0 x $10^8$ IFU/mL diluted in M4 media. High concentration of ~ 10,000 IFU/mL of *C. trachomatis* alone and ~10,000 IFU/mL of both *C. trachomatis* and *N. gonorrhoeae* combined were utilized. Map layouts on the 96-well microtiter plates were constructed to provide adequate opportunity to observe cross-contamination over multiple plate positions, including a 'worst case' scenario of blanks surrounded by positives; and to provide a sufficient number of blanks to calculate a false positive rate. In addition, a checkerboard pattern was used to further challenge the performance of the system. Testing was performed over five days. The overall false positive rate was 0.5% (2/370) with automated specimen preparation and 0.3% (1/370) with manual preparation. The differences in the false positivity rates are not statistically significant at the 95% confidence level, and the results are equivalent.

2. Comparison studies:

*a. Method comparison with predicate device:*

This method comparison study was designed to demonstrate the equivalency between the automated specimen preparation technique directed by the Roche Scripts and the manual specimen preparation option when tested in parallel and compared to culture. A prospective study with 727 samples was performed. The acceptance criteria were based on a concordance of the initial test results; inhibitory or equivocal results were not repeated.

Two swab specimens were collected from each of 727 patients. One swab was sent for *C. trachomatis* culture and the other swab was placed in M4RT culture transport media and used for AMPLICOR testing. An aliquot of each M4RT sample was removed and processed manually, and each sample was also subjected to automated specimen preparation using the Roche Scripts to direct the Tecan Genesis RSP 150 Instrument. The specimens were amplified and detected using the AMPLICOR CT/NG Test for *Chlamydia trachomatis* according to the standard test protocol. An initial qualitative test result (positive, negative, equivocal, or inhibitory) was assigned for each sample according to the method manual. Samples were repeated in the case of failed (invalid) run controls. Samples yielding equivocal or inhibitory results were repeated according to package insert directions (i.e., repeated in duplicate) by sampling from the original sample tube, and the final result was used in the data analysis.

Forty-five samples were excluded from the data analyses: 8 because culture results were not obtained, 2 because cultures were declared indeterminate, 3 because of missing specimen chain of command paperwork, 3 due to improper storage, and 29 after giving repeat results of "equivocal" or "inhibitory"; resulting in 682 valid data sets. Of the 29 repeatedly equivocal or inhibitory specimens; 7 were from females and 22 were from males and all 29 yielded negative culture results. The results from the 682 valid data sets are summarized in the table below. This analysis was also performed separately for males and females with the results summarized below; as an overall comparison to culture of all samples (Table A3), and stratified by specimen type: female endocervical and male

urethral swabs in table A4 and Table A5, respectively.

**Table A.3. Three-way method comparison results for AMPLICOR CT/NG Test for *Chlamydia trachomatis* comparing automated and manual specimen preparation and culture**

| Method Result | | Total Specimens | Culture Result | |
|---|---|---|---|---|
| Test Method* | Comparative Method* | | Positive | Negative |
| Positive | Positive | 148 | 101 | 47 |
| Positive | Negative | 0 | 0 | 0 |
| Negative | Positive | 1 | 0 | 1 |
| Negative | Negative | 533 | 4 | 539 |
| Total | | 682 | 105 | 577 |

*the Test Method was the automated and the Comparative Method was the manual specimen preparation

**Table A.4 Three-way method comparison results for AMPLICOR CT/NG Test for *Chlamydia trachomatis* comparing automated and manual specimen preparation and culture for females.**

| Method Result | | Total Specimens | Culture Result | |
|---|---|---|---|---|
| Test Method | Comparative Method | | Positive | Negative |
| Positive | Positive | 69 | 50 | 19 |
| Positive | Negative | 0 | 0 | 0 |
| Negative | Positive | 1 | 0 | 1 |
| Negative | Negative | 380 | 3 | 377 |
| Total | | 450 | 53 | 397 |

**Table A.5 Three-way method comparison results for AMPLICOR CT/NG Test for *Chlamydia trachomatis* comparing automated and manual specimen preparation and culture for males.**

| Method Result | | Total Specimens | Culture Result | |
|---|---|---|---|---|
| Test Method | Comparative Method | | Positive | Negative |
| Positive | Positive | 79 | 51 | 28 |
| Positive | Negative | 0 | 0 | 0 |
| Negative | Positive | 0 | 0 | 0 |
| Negative | Negative | 153 | 1 | 152 |
| Total | | 232 | 52 | 180 |

A secondary analysis was also conducted. For each of the 711 data sets (682 from the main analysis plus 29 that repeated equivocal or inhibitory), the concordance between results obtained using the automated and manual preparation methods was calculated. Specimens that were repeatedly inhibitory by both methods or

repeatedly equivocal by both methods were deemed concordant in these calculations. The percent positive and percent negative agreements were also calculated. These comparative analyses were also performed on sample sets stratified by gender. The results are shown in Table A.6

**Table A.6. Concordance analysis results for AMPLICOR CT/NG Test for *Chlamydia trachomatis* comparing automated and manual specimen preparation**

| Test | Comparison | | Fraction | Agreement | Confidence Interval |
|---|---|---|---|---|---|
| CT Automated | Overall | Concordance | 700 / 711 | 98.5% | ( 97.2% - 99.2% ) |
| | | %Pos Agreement | 148 / 149 | 99.3% | ( 96.3% - 100.0% ) |
| | | %Neg Agreement | 533 / 536 | 99.4% | ( 98.4% - 99.9% ) |
| | Male | Concordance | 248 / 254 | 97.6% | ( 94.9% - 99.1% ) |
| | | %Pos Agreement | 79 / 79 | 100.0% | ( 95.4% - 100.0% ) |
| | | %Neg Agreement | 153 / 156 | 98.1% | ( 94.5% - 99.6% ) |
| | Female | Concordance | 452 / 457 | 98.9% | ( 97.5% - 99.6% ) |
| | | %Pos Agreement | 69 / 70 | 98.6% | ( 92.3% - 100.0% ) |
| | | %Neg Agreement | 380 / 380 | 100.0% | ( 99.0% - 100.0% ) |

*b. Matrix comparison:*
Not applicable

3. Clinical studies:

*a. Clinical Sensitivity:*
Not applicable

*b. Clinical specificity:*
Not applicable

*c. Other clinical supportive data (when a. and b. are not applicable):*

4. Clinical cut-off:
Same cutoffs for the *C. trachomatis* specimen results and the Internal Control (IC) specimen results as determined in the previous submission for AMPLICOR™ CT/NG for *C. trachomatis* (K973707).

5. Expected values/Reference range:
   Not applicable

**N. Instrument Name:**

Tecan Genesis RSP-RC 150 Workstation Instrument, with Roche Scripts accessory

**O. System Descriptions:**

1. Modes of Operation:
   The Genesis RSP 150 Workstation is a multiple task liquid handling system that combines a system of microprocessor-controlled liquid handling and other components to one instrument. The User controls the system via a personal computer, equipped with the Genesis Instrument Software, Gemini Software v4.2, and the Roche Scripts. Genesis RSP is designed to handle liquid volumes ranging between 0.5 µL and 5 µL depending on the installed configuration.

   The liquid system is the central component of Genesis pipetting function. It transmits the precise movement of the diluter pistons to the tips through the system liquid. The system liquid is delivered to the system in a container and is aspirated and distributed in the whole system via tubes, valves and connectors. The distribution of the system liquid is effected by the movement of the diluter pistons in several strokes. Disposable tips with filter shall be used where no carry over is tolerable.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types in submission K963268:

   Yes __X_ or No _____

3. Specimen Identification:
   The Positive Identification (PosID2) reads Barcodes on Carriers, Racks and Containers on both, the primary, e.g. sample tube, as well as the secondary side, e.g. microplates by means of a scanner. With its gripper, it pulls carriers towards the rear of the instrument for barcode identification on tubes and microplates, and then, pushes the carrier back into operating position. To use the PosID2, all carriers, racks and containers (sample tubes, microplates, reagent bottles, troughs) must be labeled with barcodes. Exact positioning of these labels is required for optimal function.

4. Specimen Sampling and Handling:
   To minimize the potential for well-to-well carryover, the Roche Scripts direct the liquid handling arms of the Tecan RSP 150 to discard the pipet tip immediately after any pipetting step involving contact with a sample- or control containing well. Design features intended to minimize the potential for cross-contamination via aerosolization and splashing are listed below:
   - Use of disposable pipet tips with aerosol barrier filters
   - Low disposable tip eject option: tips are ejected into a narrow slit box to

reduce aerosolization during the ejection step

- Use of a deep well plate for extraction steps

The Gemini 4.2 software and the Tecan Genesis RSP 150 have liquid class settings which control the accuracy of the aspiration and dispense functions of the liquid handling arm. These liquid classes have several sub-options which are configured to the liquid being handled. The sub-options include:

- LAG, STAG, TAG (air gap settings at specific places within the pipet tip)
- Rate / speed of aspiration and dispense settings
- Position of pipet tip in well to optimize liquid aspiration
- Mixing characteristics

5. Calibration:
   Liquid class handling settings are determined for each liquid by performing volumetric test cases. The documentation for the test cases is provided in the submission.

6. Quality Control:
   An on-line interactive Troubleshooting guide normally detects problems and offers immediate advice for corrective actions pertaining to operations, communication, positioning, and identification errors. Daily, weekly, and periodic preventative maintenance schedules are provided in the Tecan User Manual.

**P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:**

The sponsor acknowledges that the analytical studies cited in the risk assessment as controls for the Tecan functions are more properly identified as verification activities. Roche audited Tecan, and confirmed that Tecan validated the Gemini v4.2 software.

The Roche Scripts CD's are standalone software designed to run on Off-the-Shelf (OTS) Tecan equipment. Roche Scripts are installed at the user site in read-only mode, and cannot be altered by the User.

**Q. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**R. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.