ResistancePlus® MG

Multiplex real-time PCR assay for the identification of *Mycoplasma genitalium* and detection of mutations associated with resistance to azithromycin

CE 0123 IVD UKCA

Product	Platform	Size (reactions)	Catalogue no.
ResistancePlus® MG FleXible	GeneXpert® (I, II, IV, XVI), and Infinity- (48s, 80) Instrument Systems	10	REF **S2A-2000410**

EC REP

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FOR PROFESSIONAL USE ONLY
Not for sale in the USA

Contents

1 Intended use

The ***Resistance****Plus*® MG FleXible assay is a qualitative multiplexed *in vitro* diagnostic real-time PCR test for the identification of *M. genitalium* and detection of mutations in the 23S rRNA gene (A2058G, A2059G, A2058T, A2058C, *Escherichia coli* numbering), that are associated with resistance to azithromycin (macrolide antibiotic). It is intended to aid in the diagnosis of *M. genitalium* and detects mutations associated with azithromycin resistance in *M. genitalium* and should be used in conjunction with clinical and other laboratory information.

The ***Resistance****Plus*® MG FleXible assay may be used with the following specimen types: male and female urine, vaginal swab, cervical swab, rectal swab, urethral swab, from symptomatic and asymptomatic patients.

Negative results do not preclude *M. genitalium* infections and do not provide confirmation of azithromycin susceptibility as there may be other mechanisms of treatment failure.

The ***Resistance****Plus*® MG FleXible assay is intended to be used in professional settings such as hospitals, or reference or state laboratories. It is not intended for self-testing, home use, or point of care use.

The ***Resistance****Plus*® MG FleXible assay is performed on GeneXpert® Instrument Systems.

2 Summary and explanation of the test

2.1 Pathogen description

M. genitalium is a small bacterium that is found in the human urogenital tract and has been associated with a range of sexually transmitted infections (STIs). In men, it is the second most common cause of non-gonococcal urethritis (NGU), and is responsible for 15-40% of cases[1], and it is also associated with prostatitis, epididymitis, and balanoposthitis, inflammation of the glans penis and prepuce[2]. In women, it is associated with cervicitis, pelvic inflammatory disease (PID), including endometritis (inflammation of the endometrial lining) and salpingitis (inflammation of the fallopian tubes)[2, 3, 4].

Azithromycin is commonly used for the treatment of *M. genitalium* and for the syndromic management of STIs such as NGU and cervicitis. Azithromycin belongs to the macrolide class of antibiotics and acts by binding to the 23S rRNA to inhibit protein synthesis. Point mutations in the 23S rRNA gene of *M. genitalium*, A2058G, A2059G, A2058T, A2058C and A2059C (*E. coli* numbering), have been associated with treatment failure and/or *in vitro* resistance to azithromycin[5, 6]. The most common mutations are A2058G and A2059G[7].

2.2 Principle of the procedure

The ***Resistance****Plus*® MG FleXible assay is an *in vitro* diagnostic real-time PCR test for the identification of *M. genitalium* and detection of mutations in the 23S rRNA gene (A2058G, A2059G, A2058T, A2058C, *Escherichia coli* numbering), that are associated with resistance to azithromycin (macrolide antibiotic). The ***Resistance****Plus*® MG FleXible assay is performed on the Cepheid GeneXpert® Instrument Systems.

Cepheid GeneXpert® Instrument Systems integrate sample purification, nucleic acid amplification, real-time PCR detection, and reporting of results. The system consists of an instrument and personal computer, with pre-loaded software to run assays and view results. Refer to the appropriate GeneXpert® Instrument System Operator Manual for more information.

The ***Resistance****Plus*® MG FleXible assay requires single-use FleXible cartridges, to which the user adds the sample and the PCR Reaction Mix, and the cartridge is loaded onto the instrument.

The ***Resistance****Plus*® MG FleXible assay includes an Internal Control to monitor extraction efficiency and PCR inhibition. The Internal Control Cells contain the internal control DNA template which is added to the sample and is co-extracted and co-amplified in the real-time PCR reaction. The interpretation of results from the ***Resistance****Plus*® MG FleXible assay is automated by the GeneXpert® Dx System or Infinity Xpertise software from measured fluorescent signals and embedded calculation algorithms, to determine the detection of *M. genitalium* and 23S rRNA mutations.

The ***Resistance****Plus*® MG FleXible assay utilises ***Plex****Prime*® primers for sensitive and specific amplification of 23S rRNA mutation targets and ***Plex****Zyme*® enzymes for sensitive and specific multiplexed real-time PCR detection of target sequences.

2.3 Principle of the technology

Real-time PCR (qPCR) can be used to amplify and detect specific target nucleic acids from pathogens. ***Plex****PCR*® is a real-time PCR technology utilising ***Plex****Zyme*® enzymes that detect and report the amplified product through the generation of a fluorescent signal (**Figure 1**). ***Plex****Prime*® primers can be used for specific amplification of mutant sequences which is coupled with mutant specific ***Plex****Zyme*® detection (**Figure 2**).

Plex*Zyme*® enzymes are catalytic DNA complexes composed of two DNA oligos referred to as “Partial Enzymes”. Each Partial Enzyme has a target-specific region, a catalytic core and a universal probe binding region. When the target product is present, the two Partial Enzymes bind adjacently to form the active ***Plex****Zyme*® which has catalytic activity to cleave a labelled probe. Cleavage separates the fluorophore and quencher dyes, producing a fluorescent signal that can be

monitored in real time. ***Plex**Zyme*® enzymes have additional specificity compared to alternate detection technologies, since two Partial Enzymes are required to bind for detection. ***Plex**Zyme*® enzymes are also multiple turnover enzymes, and multiple probes can be cleaved during each PCR cycle, resulting in a strong and sensitive signal. ***Plex**Zyme*® assays are highly sensitive and specific and are ideally suited for the multiplexed detection of pathogens.

***Plex**Prime*® primers have three functional regions. The long 5' region anchors the primer to a particular location, and the short 3' region selectively targets extension from the mutant base. An Insert sequence lies between the 5' and 3' regions and acts as a bridging structure which inserts a target-independent sequence into the resulting amplicon and increases the selective pressure of the 3' region. In multiplex, each ***Plex**Prime*® primer is designed to target a specific mutant base and will incorporate a unique Insert sequence, thus producing distinct mutant amplicon sequences. Unlike other probe-based detection technologies, the ***Plex**Zyme*® enzyme can be overlapped with the ***Plex**Prime*® primer to target the specific mutant amplicon containing the mutant base and incorporated Insert sequence. The unique combination of ***Plex**Prime*® primers coupled to ***Plex**Zyme*® enzymes allows the specific amplification of mutant sequences, and sensitive and specific detection in multiplex.

Figure 1. Schematic representation of *PlexZyme*® detection and universal signalling

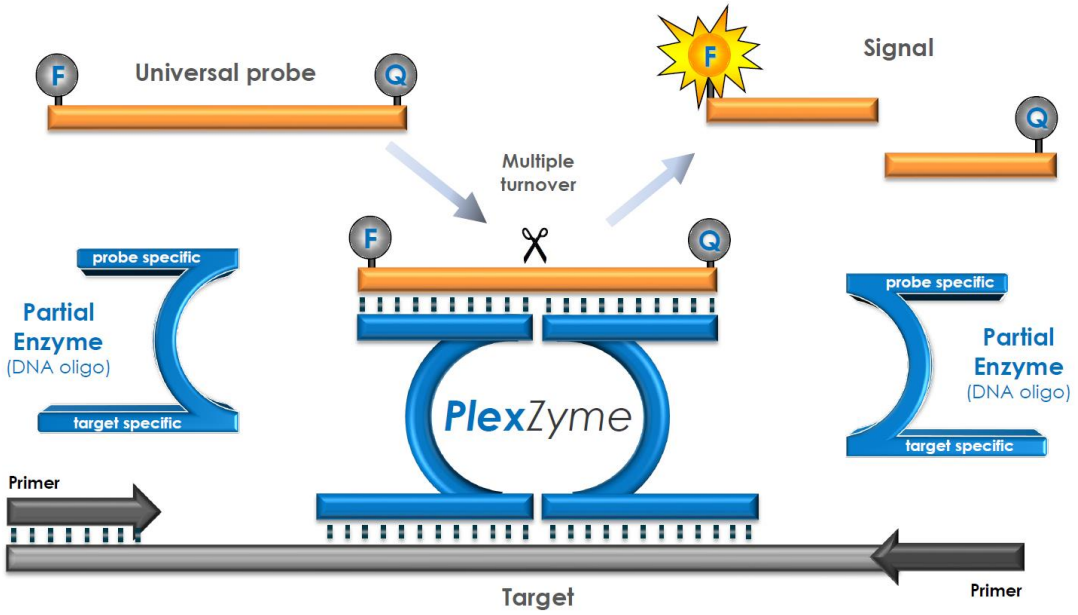
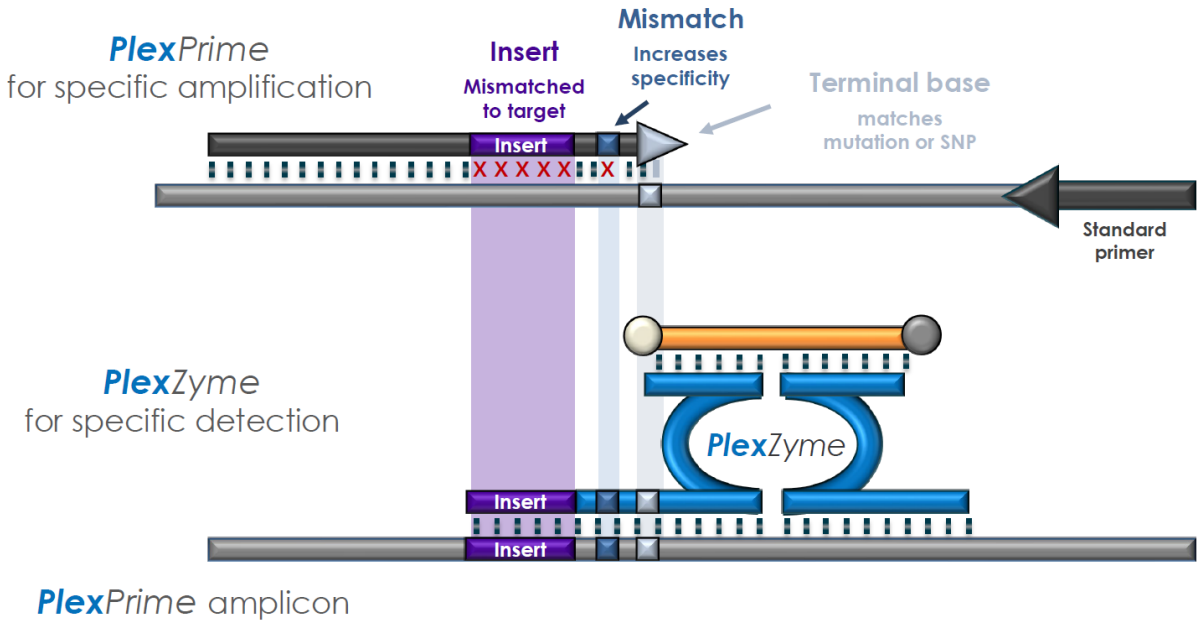


Figure 2. Schematic representation of the *PlexPrime*® primer coupled with *PlexZyme*® detection. The *PlexPrime*® primer specifically amplifies the mutant sequence and *PlexZyme*® enzymes specifically detect the amplicon.



3 Kit contents

Number of tests: 10 reactions

Table 1. Contents for *ResistancePlus*® MG FleXible kit

Box no.	Part no.	Cap colour	Contents	Description	Quantity
1	2000410-R	Blue	***Plex*** Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, DNA polymerase and buffer	1 x 440 µl
		Brown	MG+23S Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of *M. genitalium*, 23S rRNA mutations and internal control	1 x 50 µl
		Red	Internal Control Cells#	Internal control cells containing internal control DNA template to monitor extraction and amplification efficiency	1 x 100 µl
		N/A	***Resistance****Plus*® MG FleXible labels*	Cartridge labels containing Lot-specific barcode, Master Lot number, expiry date and ADF information	10 labels
		N/A	MG FleXible Mix label	Label to identify combined MG FleXible Reaction Mix (optional use)	1 label
2	2000410-CART	N/A	***Resistance****Plus*® MG FleXible Cartridge	Single-use cartridge for sample processing, nucleic acid amplification and detection	10 cartridges

Store template tubes separately from oligo mixes, i.e. template or nucleic acid handling room

^ Oligonucleotides are PCR primer pairs (including ***Plex****Prime*® primers), ***Plex****Zyme*® enzymes and fluorescent probes

* **Do not dispose of cartridge labels**

4 Shipping and storage

- The assay reagents (contained in Box 1) of the ***Resistance****Plus*® MG FleXible kits are shipped on dry ice or ice gel packs. Store assay components at -25°C to -15°C upon receipt. It is recommended that freeze/thaw cycles be limited to less than 8. See **Section 8.2** for storage conditions and freeze/thaw recommendations of the combined MG FleXible reaction mix.
- The ***Resistance****Plus*® MG FleXible cartridges are shipped and stored at 2°C – 28°C.
- When stored under the recommended conditions and handled correctly, activity of the kit is retained until the expiry date stated on the label. Do not use past expiry date.
- Any serious incident shall be reported to SpeeDx by contacting tech@speedx.com.au

5 Warnings and precautions

5.1 General

- For *in vitro* diagnostic use only.
- Carefully read these Instructions for Use prior to use. Closely follow procedures as described to ensure reliability of test results. Any deviation from these procedures may affect test performance.
- Users must be adequately trained in the use of the ***Resistance****Plus*® MG FleXible assay.
- Any serious incident shall be reported to the manufacturer and competent authority of the Member State in which user and/or patient is established

5.2 Laboratory

- Basic precautions for preventing contamination of PCR reactions include the use of sterile filter pipette tips for preparation of PCR Reaction Mix, use of a new pipette tip for every pipetting action, and separation of workflow.
- It is recommended to perform mastermix preparation, sample addition and thermocycling in spatially separated spaces. At a minimum the PCR instrument should ideally be in a separate room to areas where reactions are prepared.

- It is recommended to follow routine laboratory precautions. Wear appropriate personal protective equipment such as gloves, protective eye wear and laboratory coat when handling reagents.
- Pathogenic organisms may be present in clinical specimens as well as used cartridges. Treat all biological specimens and used cartridges as potentially infectious and follow your institution's safety procedures for handling chemicals and biological samples.
- Follow your institution's hazardous waste disposal procedures for proper disposal of used cartridges.

5.3 Specimen handling

- Specimens should be collected, transported and stored using standard laboratory techniques or according to collection kit instructions.

5.4 Assay/Reagent

- Do not open the cartridge lid except when adding the Reaction Mix and sample.
- Do not use a cartridge that appears damaged, has been dropped or shaken, or displays signs of reagent leakage or crystallisation.
- Do not place the sample ID label on the cartridge lid or on the barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Each cartridge is single use only. Do not reuse processed cartridges.
- Do not use reagents, cartridges and labels from different Master Lot numbers.
- Assay reagents contain IDTE Buffer which can cause severe eye irritation. It is recommended to use in a well-ventilated area and wear appropriate personal protective equipment such as gloves, protective eye wear and laboratory coat when handling reagents.

5.5 Safety precautions

⚠ **WARNING** - Lysis reagent (contained in cartridge) contains guanidinium thiocyanate

Hazard class:

- Acute Toxicity Oral 4.
- Skin Mild Irritation 3.
- Eye Mild Irritation 2B.

Hazards statements:

- H302: Harmful if swallowed.
- EUH301: Contact with acids liberates toxic gas.

Precautionary statements:

- P264: Wash thoroughly after handling.
- P270: Do not eat, drink or smoke when using this product.
- P301+P312: IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician if you feel unwell.
- P330: Rinse mouth.
- P501: Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.

Safety Data Sheets (SDS) are available on request. Please contact technical support in **Section 16** for more information.

6 Associated Products and Consumables

Positive Control Material

- ***Resistance****Plus*® MG S2A Positive Control kit (SpeeDx, Cat no. S2A–95004)

General lab consumables

- Gloves and clean lab coats
- Vortex mixer
- Benchtop centrifuge for 1.5 ml tubes
- Micropipettors covering the range 10-100 µl
- Sterile aerosolresistant, DNAse/RNAse free, pipette tips
- Sterile transfer pipettes capable of transferring at least 1 mL volume

For the GeneXpert® Instrument

- GeneXpert® Instrument System: GeneXpert® instrument, computer with GeneXpert® Software Version 4.7b or higher
- Barcode scanner

For the GeneXpert Infinity-48s or Infinity-80:

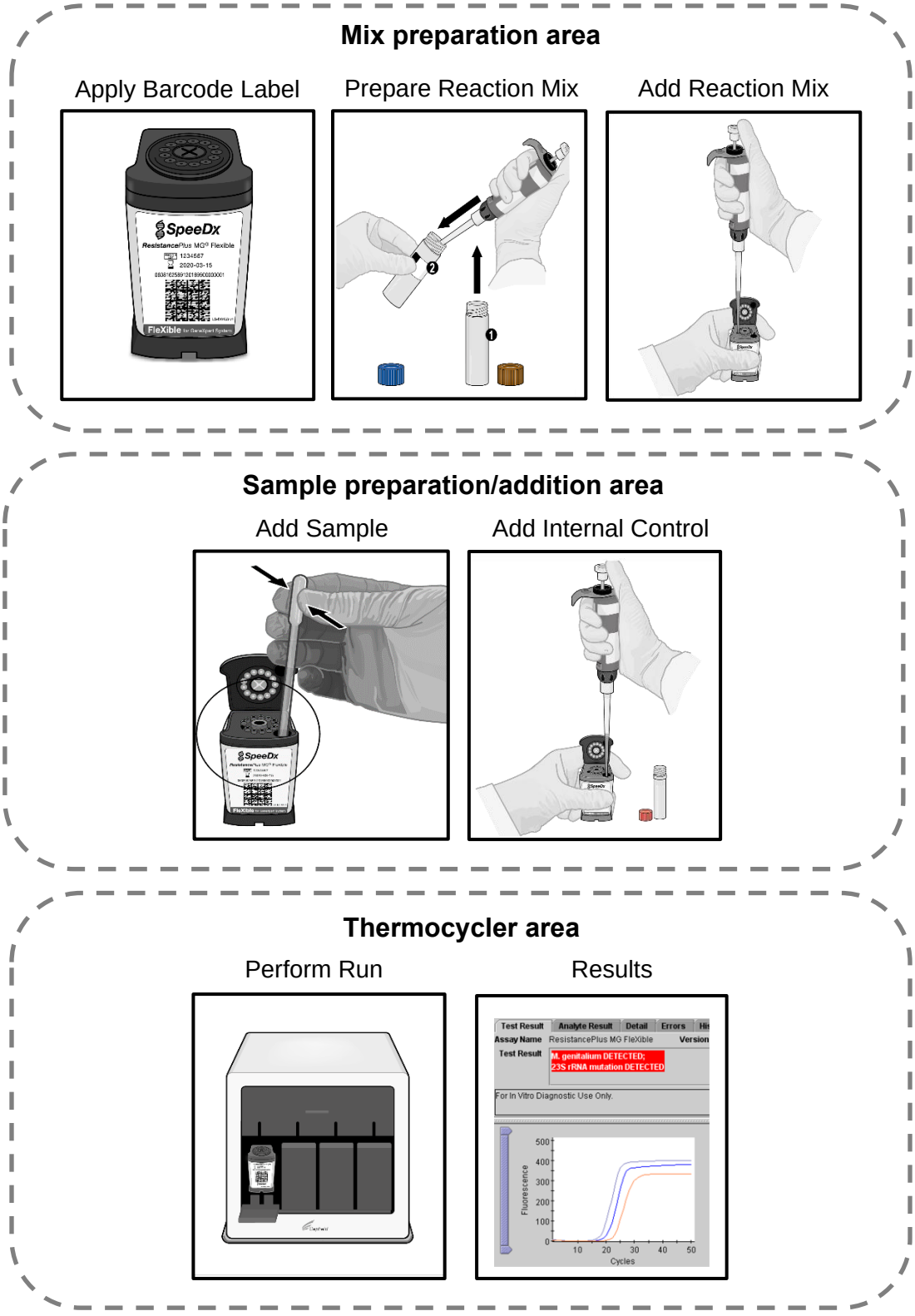
- Xpertise software version 6.4b or higher

Sample Collection Devices

- Xpert® Vaginal/Endocervical Specimen Collection kit (Cepheid, Cat no. SWAB/A-50)
- Xpert® Swab Specimen Collection Kit (Cepheid, Cat no. SWAB/G-50)
- Xpert® Urine Specimen Collection Kit (Cepheid, Cat no. URINE/A-50)
- Sterile urine collection cup
- Regular FLOQSwab™ in 3 ml of UTM™ media (Copan, Cat no. 346C or 306C (USA))
- Cobas® PCR media (Roche, Cat no. 06466281190)
- Dry swab, resuspended in 3 ml of PBS

7 Procedure overview

Figure 3. Procedure overview



8 Detailed procedure

Note: Provided reagents are named in italics and colour of the tube cap follows in brackets.

8.1 Sample collection, transport and storage

Male and female urine, vaginal swab, cervical swab, rectal swab, urethral swab, from symptomatic and asymptomatic patients should be collected, transported and stored using standard laboratory techniques or according to collection kit instructions.

8.1.1 Validated sample collected devices

Inadequate or inappropriate specimen collection, storage and transport are likely to yield false test results. Proper training in specimen collection is highly recommended to ensure specimen quality and stability.

Sample collection devices that have been validated with the ***Resistance****Plus*® MG FleXible kit are included below with short guidance regarding the device manufacturer's instructions for collection, handling and transport. These instructions are not intended to replace or supersede any instructions provided by the manufacturer. Always refer to specimen collection device manufacturer instructions for proper collection methods.

Prior to any collection method, trained staff must ensure proper understanding of the device and methodology. At minimum review the test description for the following: indication of specimen type, sufficient volume, procedure(s), necessary collection materials, patient preparation, and proper handling and storage instructions.

8.1.2 Xpert® Vaginal/Endocervical Specimen Collection kit (Cepheid, Cat no. SWAB/A-50) collection, transport and storage

8.1.2.1 Vaginal swab specimen collection, transport and storage

Directions are summarized below for the collection and transport of female vaginal swab specimens with the Xpert® Vaginal/Endocervical Specimen Collection kit (Cepheid, Cat no. SWAB/A-50)

1. Open the outer peelpack (which contains the two-package kit) and identify the larger cleaning swab and discard.
2. Open the package that contains the pink capped Xpert Swab Transport Reagent tube and individually wrapped collection swab. Set the tube aside before beginning to collect sample.
3. Open the collection swab wrapper by peeling open the top of the wrapper.
4. Remove the swab, taking care not to touch the tip or lay it down. If the soft tip is touched, the swab is laid down, or the swab is dropped, request a new collection kit.
5. Hold the swab in your hand, placing your thumb and forefinger in the middle of the swab shaft.
6. Carefully insert the swab into your vagina about two inches (5 cm) inside the opening of the vagina and gently rotate the swab for 10 to 30 seconds. Ensure the swab touches the walls of the vagina so that moisture is absorbed by the swab.
7. Withdraw the swab carefully.
8. While holding the swab in the same hand, unscrew the cap from the Xpert Swab Transport Reagent tube.
9. Do not spill the contents of the tube. If the contents of the tube are spilled, request a new collection kit.
10. Immediately place the specimen collection swab into the transport reagent tube.
11. Identify the scoreline on the collection swab shaft. 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 contents.
12. Re-cap the swab transport reagent tube and tighten the cap securely.
13. Invert or gently shake the tube 3-4 times to elute material from the swab. Avoid foaming.
14. Label the transport tube with sample identification information, including date of collection, as required.
15. Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with local, state, and country regulations.
16. Transport and store the Xpert Swab Transport Reagent tube at 2ºC to 30ºC for up to 60 days.

8.1.2.2 Endocervical swab specimen collection, transport and storage

Directions are summarized below for the collection and transport of female endocervical swab specimens with the Xpert® Vaginal/Endocervical Specimen Collection kit (Cepheid, Cat no. SWAB/A-50)

1. Open the Xpert CT/NG Vaginal/Endocervical Specimen collection kit.
2. Before collecting the endocervical specimen with the Xpert CT/NG Vaginal/Endocervical Specimen Collection Kit, remove excess mucus from the cervical os and surrounding mucosa using the large individually wrapped cleaning swab. Partially peel open the larger cleaning swab wrapper and remove the swab. Clean the cervical os and surrounding mucosa and then discard the swab.
   Note: If collecting multiple specimens, excess mucus need only be removed once.

3. Open the package that contains the pink capped Xpert Swab Transport Reagent tube and individually wrapped collection swab. Set the tube aside before proceeding.
4. Open the collection swab wrapper by peeling open the top of the wrapper.
5. Remove the swab, taking care not to touch the tip or lay it down. If the soft tip is touched, the swab is laid down, or the swab is dropped, request a new collection kit.
6. Insert the collection swab into the endocervical canal.
7. Gently rotate the swab clockwise for 10 to 30 seconds in the endocervical canal to ensure adequate sampling.
8. Withdraw the swab carefully.
9. While holding the swab in the same hand, unscrew the cap from the Xpert CT/NG Swab Transport Reagent tube.
10. Do not spill the contents of the tube. If the contents of the tube are spilled, request a new collection kit.
11. Identify the scoreline on the collection swab shaft. 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 contents.
12. Re-cap the swab transport reagent tube and tighten the cap securely.
13. Invert or gently shake the tube 3-4 times to elute material from the swab. Avoid foaming.
14. Label the transport tube with sample identification information, including date of collection, as required.
15. Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with local, state, and country regulations.
16. Transport and store the Xpert Swab Transport Reagent tube at 2ºC to 30ºC for up to 60 days.

8.1.3 Xpert® Swab Specimen Collection Kit (Cepheid, Cat no. SWAB/G-50) collection, transport and storage

Directions are summarized below for the collection and transport of male and female rectal swab specimens with the Xpert® Swab Specimen Collection kit (Cepheid, Cat no. SWAB/G-50)

1. Open the Xpert Swab Specimen collection kit.
2. Open the outer peelpack (which contains the two-package kit) and identify the larger cleaning swab and discard it.
3. Open the collection swab wrapper by partially peeling open the top of the wrapper.
4. Remove the swab, taking care not to touch the tip or lay it down. If the soft tip is touched, the swab is laid down, or the swab is dropped, use a new Xpert Swab Specimen Collection Kit.
5. Carefully insert the swab approximately 1 cm beyond the anal sphincter (so that the fiber tips are no longer visible) and rotate gently.
6. While holding the swab in the same hand, unscrew the cap from the Xpert Swab Transport Reagent tube.
7. Do not spill the contents of the tube. If the contents of the tube are spilled, use a new collection kit.
8. Immediately place the swab into the transport reagent tube.
9. Identify the scoreline on the collection swab shaft. 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 the contents. Wash with soap and water if exposed.
10. Re-cap the swab transport reagent tube and tighten the cap securely.
11. Invert or gently shake the tube 3-4 times to elute material from the swab. Avoid foaming.
12. Label the transport tube with the sample identification information, including date of collection, as required.
13. Transport and store the swab samples in the Xpert Swab Transport Reagent tube at 2ºC to 30ºC for up to 60 days.

8.1.4 Xpert® Urine Specimen Collection Kit (Cepheid, Cat no. URINE/A-50) collection, transport and storage

Directions are summarized below for the collection and transport of male and female urine specimens with the Xpert® Urine Specimen Collection kit (Cepheid, Cat no. URINE/A-50)

1. The patient should not have urinated for at least 1 hour prior to specimen collection. Female patients should not cleanse the labial area prior to collecting the specimen. Male patients should not cleanse the tip of the penis prior to collecting specimen.
2. Direct patient to provide first-catch urine (approximately 20 to 50 mL of the initial urine stream) into a urine collection cup free of any preservatives. Collection of larger volumes of urine may esult in specimen dilution that may reduce test sensitivity.
3. Ensure that the urine is well mixed in the urine cup before transferring a sample to the Xpert Urine Transport Reagent tube.
4. Open the packaging of a disposable transfer pipette provided in the kit.
5. Remove the cap from the Xpert Urine Transport Reagent tube and from the urine collection cup.
6. Insert the transfer pipette into the urine cup so that the tip is near the bottom of the cup. Transfer approximately 7 mL of urine into the Xpert Urine Transport Reagent tube using the disposable transfer pipette. The correct volume of urine has been added when the level reaches the black dashed line on the label of the Xpert Urine Transport Reagent tube.
7. Replace the cap on the Xpert Urine Transport Reagent tube and tighten securely.
8. Invert the reagent tube 3-4 times to ensure that the specimen and reagent are well mixed.
9. Recap the urine cup securely.
10. Label the transport tube with sample identification information, including the date of collection, as required. Take care not to obscure the fill line on the Xpert Urine Transport Reagent tube.

11. Transport and store female urine samples in the Xpert Urine Transport Reagent tube at 2ºC to 30ºC for up to 3 days or at 2ºC to 15ºC for up to 45 days.
12. Transport and store male urine samples in the Xpert Urine Transport Reagent tube at 2ºC to 30ºC for up to 45 days.

8.1.5 Sterile urine collection cup collection, transport and storage

A sterile urine collection cup may be used for collection of neat urine specimens. Due to the variability, refer to the manufacturers package insert for appropriate collection methods. Transport and store using standard laboratory techniques.

8.1.6 Regular FLOQSwab™ in 3 ml of UTM™ media (Copan, Cat no. 346C or 306C (USA)) collection, transport and storage

Directions are summarized below for the collection and transport of female vaginal swab specimens with the Regular FLOQSwab™ in 3 ml of UTM™ media (Copan, Cat no. 346C or 306C (USA))

1. Open the UTM kit package and remove the medium test tube and the internal bag containing the sterile swab.
2. Take the sterile swab out of its bag and collect the clinical specimen; to prevent the risk of contamination, make sure that the swab tip comes into contact with the collection site only.
3. After collecting the specimen, unscrew and remove the cap from the test tube taking care not to spill the medium.
4. Insert the swab into the test tube until the breakpoint is level with the test tube opening.
5. Bend and break the swab at the breakpoint holding the test tube away from your face and discard the excess part.
6. Screw the cap back onto the test tube and hermetically seal it.
7. Process the specimen contained in the UTM within 48 hours from collection storing the test tube at 2-25°C.
8. Before processing, vortex for 20 seconds in order to encourage specimen release from the swab and homogenize the medium.

8.1.7 Cobas® PCR media (Roche, Cat no. 06466281190) collection, transport and storage

Directions are summarized below for the collection and transport of male and female urine within cobas® PCR media (Roche, Cat no 06466281190).

1. Mix and transfer the urine into the cobas® PCR Media tube using a disposable pipette (not provided). Note: urine can be stored at 2°C to 30°C for up to 24 hours prior to transferring into the cobas® PCR Media tube
2. The correct volume of urine has been added when the fluid level is between the two black lines on the tube label
3. Tightly re-cap the cobas® PCR Media tube
4. Invert the tube 5 times to mix. The specimen is now ready for transport and testing
5. Transport and store the cobas® PCR Media tube containing the stabilized urine specimen at 2ºC to 30ºC.

8.1.8 Dry swab, resuspended in 3 ml of PBS collection, transport and storage

Dry swabs may be used for various clinician and patient collection specimens. Due to the variability, refer to the manufacturers package insert for appropriate specimen types and collection methods.

8.2 Preparation of MG FleXible Reaction Mix

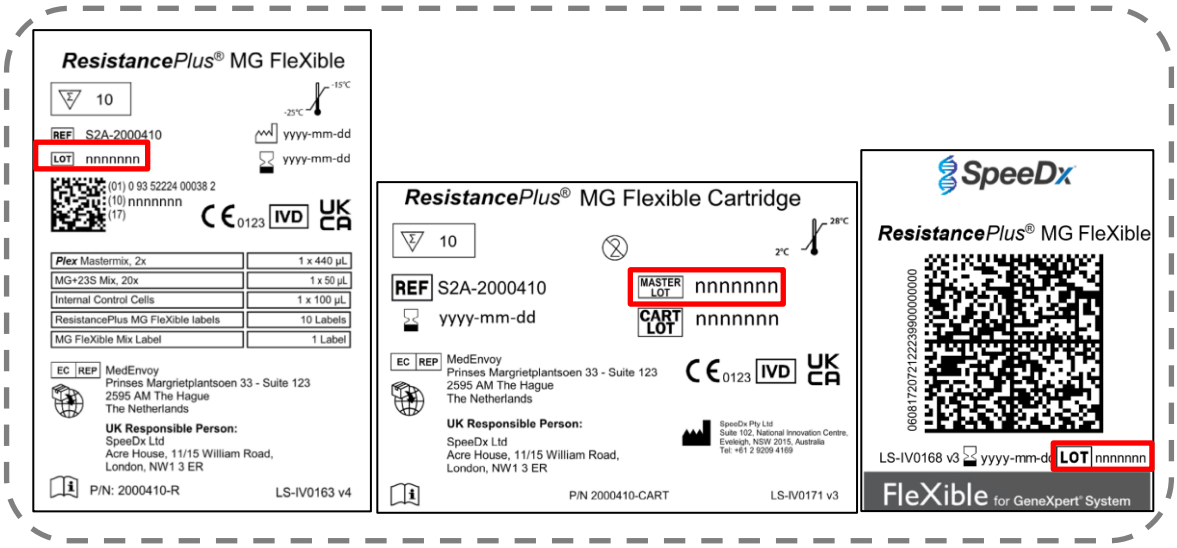
Refer to **Table 1** for description of kit contents.

Note: Assay set up must use the same product Master Lot number for Box 1 (assay reagents), Box 2 (cartridges) and ***Resistance****Plus*® MG FleXible labels (**Figure 4** indicated in red).

Note: Inspect cartridges for integrity and ensure reaction tube is intact.

Note: Check all reagents and cartridges are within the expiration date before use and before labelling the cartridge.

Figure 4. The MASTER LOT number must be the same on Box 1 (left; *ResistancePlus*® MG FleXible assay reagents, P/N:2000410-R), Box 2 (centre; *ResistancePlus*® MG FleXible Cartridges, P/N:2000410-CART) and *ResistancePlus*® MG FleXible labels (right)



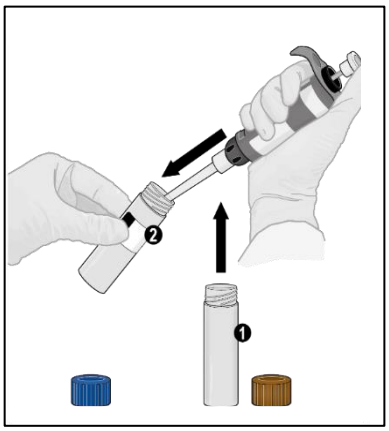
Affix ***ResistancePlus***® MG FleXible label to the front of the cartridge as shown in **Figure 5**.

Figure 5. Apply *ResistancePlus*® MG FleXible label

Note: Before use of reagents, thaw completely, mix thoroughly by briefly vortexing, and spin down

To make the MG FleXible Reaction Mix, pipette 44 µl of *MG+23S Mix* (**BROWN**) into the ***Plex*** *Mastermix* tube (**BLUE**) as shown in **Figure 6**. This Reaction mix is sufficient for 10 reactions.

Figure 6. Make MG FleXible Reaction Mix

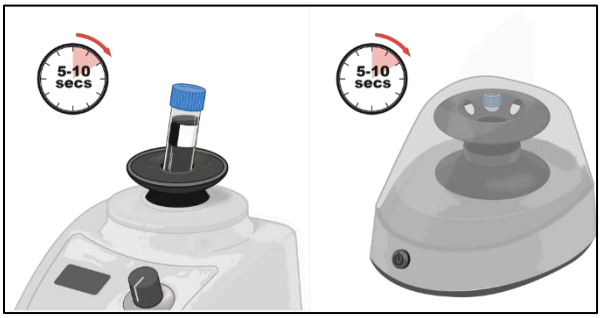


(Optional) To store residual combined MG FleXible Reaction Mix, contents can remain in the ***Plex*** *Mastermix* tube (**BLUE**), and the tube can be relabelled using the *MG FleXible Mix* label. Record the date of preparation in the space provided on the label.

- The combined MG FleXible Reaction Mix can be stored at -25°C to -15°C for up to 8 weeks. It is recommended that freeze/thaw cycles be limited to less than 8.

Return and tighten lid of the ***Plex*** *Mastermix* tube (**BLUE**). Vortex and spin down as shown in **Figure 7**.

Figure 7. Vortex MG FleXible Reaction Mix

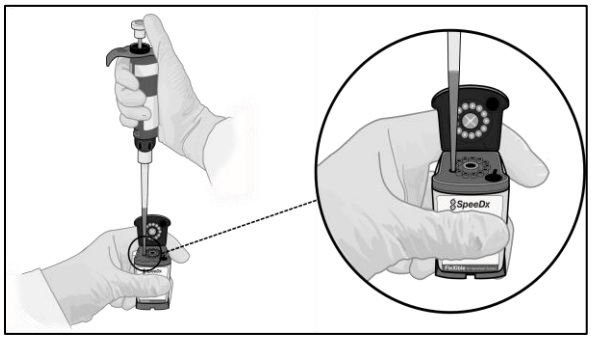


Discard *MG+23S Mix* tube (**BROWN**).

Open cartridge lid.

Pipette 44 µl of the combined MG FleXible Reaction Mix into the Reaction Chamber (small opening on the left of the cartridge as shown in **Figure 8**). Insert tip vertically as far as it will go into chamber before expelling solution. Gently tap the bottom of cart onto the bench to settle solution.

Figure 8. Load MG FleXible Reaction Mix into the Reaction Chamber

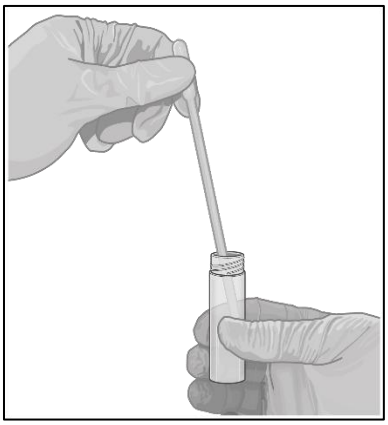


8.3 Addition of sample

Note: Only use transfer pipettes for addition of sample into the cartridge.

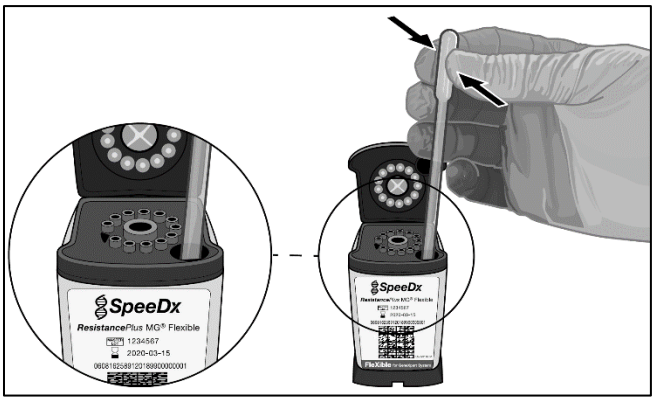
Open the sample tube lid, compress the bulb of the transfer pipette, insert the pipette into the sample tube and slowly release the bulb to fill the transfer pipette to the 1 ml mark on the pipette shaft. The aspirated sample should not contain air bubbles (**Figure 9**).

Figure 9. Aspirate sample into transfer pipette



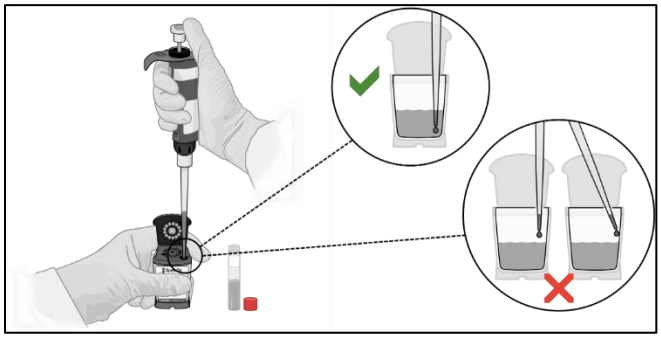
Dispense the sample from the transfer pipette into the Sample Chamber of the cartridge (large opening on the bottom right of the cartridge) shown in **Figure 10**.

Figure 10. Add sample to Sample Chamber



Add 10 µl *Internal Control Cells* (**RED**) into the Sample Chamber as shown in **Figure 11**. Ensure tip is immersed in the sample before expelling *Internal Control Cells*. Do not mix or shake cartridge.

Figure 11. Add Internal Control Cells



Close the cartridge lid as shown in **Figure 12.**

Figure 12. Close lid

Note: The cartridge should be loaded within 30 mins of preparation.

9 Programming the instrument

The *ResistancePlus*® MG FleXible assay must be run on a GeneXpert® Instrument System using GeneXpert® Software Version 4.7b or higher or Infinity Xpertise Software Version 6.4b or higher.

The ResistancePlus MG FleXible Assay Definition File (ADF) must be imported into the software before running the test for the first time.

Note: The steps below are based on GeneXpert® Software Version 4.7b and may differ if the default workflow of the system has been changed by the system administrator

9.1 Importing the ADF into the software

In the main menu of the GeneXpert® Dx software

Select **Define Assays** (**Figure 13**)

Select **Import** (**Figure 14)**

Figure 13. Main menu – Select Define Assays

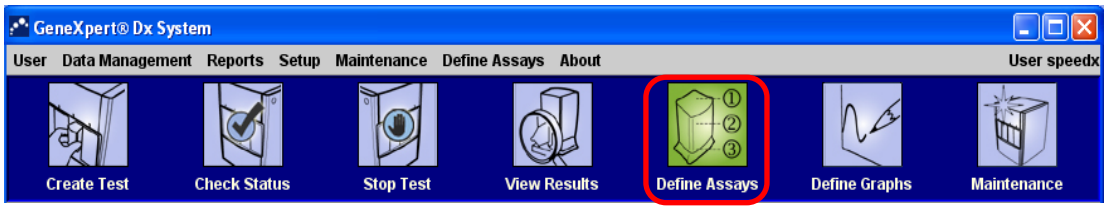
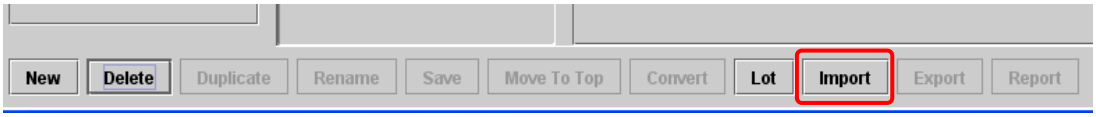
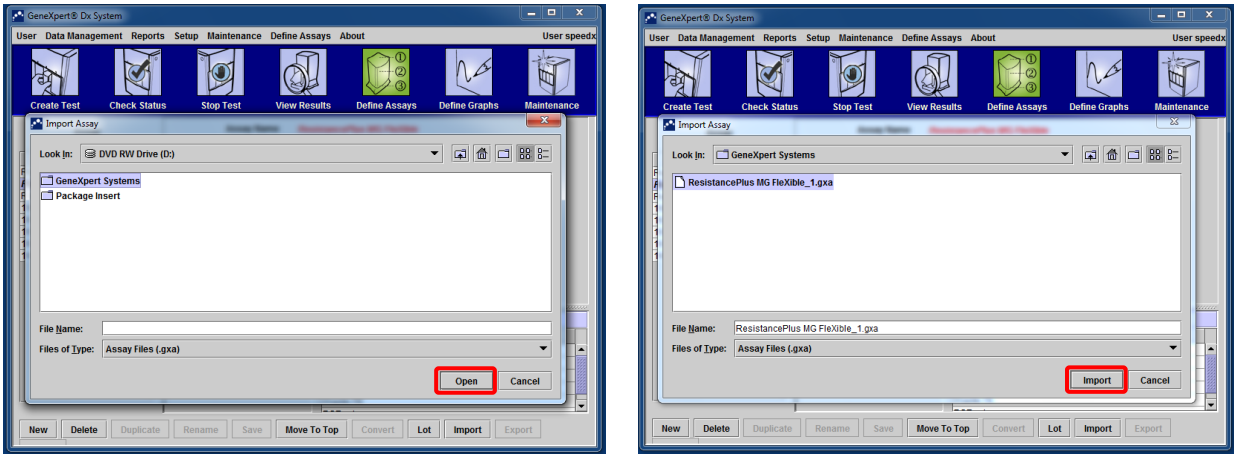


Figure 14. Define assays menu – Select Import



In the **Import Assay** window, browse to the location of the ADF and select **Import** (**Figure 15**)

Figure 15. Browse to the ADF location (left) and Import assay (right)



In the Infinity Xpertise software, select the **Home** icon to display the Xpertise Software Home workspace

Select the **Setup** button

In the Setup menu, select **Manage Assays**

In the Manage Assays workspace, click **Import**. The Import Assay dialogue box will appear.

Browse to the location of the ADF, then click the **Open** button on the Import Assay dialogue box.

9.2 Starting the test

Note: The steps below may differ if the system administrator has changed the default workflow of the system.

In the main menu of the GeneXpert® Dx software

Select **Create Test** (GeneXpert Dx **Figure 16**) or select **Orders** and **Order Test** (Infinity Xpertise)

Figure 16. Main menu – Create Test

In the Create Test window of the GeneXpert® Dx software (**Figure 17**) or the Order Test workspace of the Infinity Xpertise software:

Enter **Patient ID** (optional) by scanning or typing

Enter **Sample ID** by scanning or typing

Select **Scan Cartridge Barcode**

Scan the barcode on the ***Resistance****Plus*® MG FleXible label. Using the barcode information, the software automatically fills the boxes for the following fields:

- Select Assay
- Reagent Lot ID
- Cartridge SN
- Expiration Date

Note: If the barcode on the ***Resistance****Plus*® MG FleXible label does not scan, contact technical support (**Section 16**) for instructions on how to proceed.

For **Select Assay**

Check the **Name** is shown as '**ResistancePlus MG FleXible**'

To start the test on the GeneXpert® Dx Instrument:

Click **Select Module** and choose the required module

Select **Start Test** (enter password, if required)

A flashing green light will indicate the selected module

Load the cartridge with the reaction tube pointing to the back of the module

Close the module door

The green light will stop flashing and remain on, to indicate that the test is running

To start the test on the GeneXpert® Infinity Instrument:

Select **Submit** (enter password, if required)

Place the cartridge on the conveyor belt

The Infinity instrument will automatically load the cartridge and run the test

When the test is finished:

For the GeneXpert® Dx Instrument:

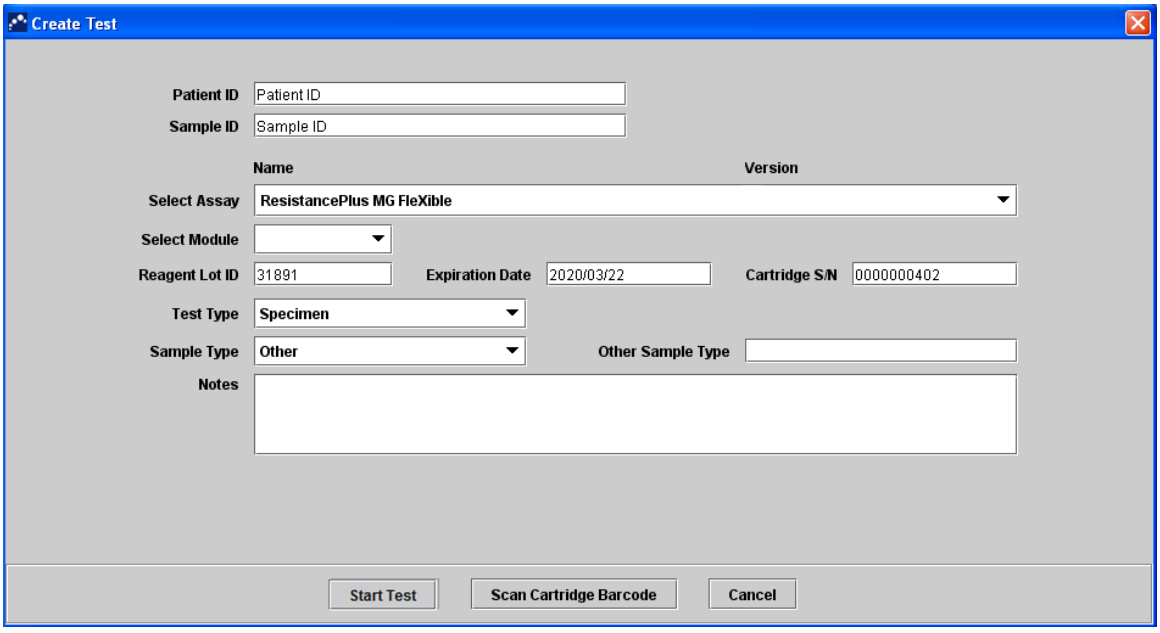
The light will turn off, and the door will open

Cartridges must be manually removed and should be disposed using appropriate hazardous waste disposal procedures

For the GeneXpert Infinity Instrument:

The used cartridge will automatically be placed into the waste container

Figure 17. Create Test window



10 Quality control

Each test includes an Internal control and a Probe Check Control (PCC).

The Internal Control (IC) monitors extraction efficiency and PCR inhibition. The *Internal Control Cells* are *Escherichia coli* cells that contain the internal control DNA template which is added to the sample and is co-extracted and co-amplified in the reaction. The IC is valid if it meets the acceptance criteria. For an analyte negative sample, the IC must be valid for the sample to be determined as a valid negative. For an analyte positive sample, the IC range does not affect the overall test result.

Before the start of the PCR, a Probe Check is performed by the GeneXpert System. The fluorescence signal is measured to monitor mix loading, reaction-tube filling, probe integrity and dye stability. The probe check passes if it meets the validated acceptance criteria.

External Controls (positive and negative controls) should be run in accordance to your institution's protocols. The ***Resistance****Plus*® MG S2A Positive Control kit is recommended as positive control material for nucleic acid amplification. Refer to **Section 11** for instructions to use the ***Resistance****Plus*® MG S2A Positive Controls. A known negative specimen is recommended to be used as a negative control.

11 *ResistancePlus*® MG S2A Positive Control instructions

The ***Resistance****Plus*® MG S2A Positive Control kit contains positive control material for *M. genitalium* 23S rRNA mutants and *M. genitalium* 23S rRNA wild type (**Table 2**).

Table 2. Kit contents for *ResistancePlus*® MG S2A Positive Control kit

Cap colour	Contents	Description	Quantity
White	MG, 23S rRNA wild type	Positive control template for the detection of *M. genitalium*, 23S rRNA wild type	2 x 100 µl
Green	MG, 23S rRNA A2058G	Positive control template for the detection of *M. genitalium*, 23S rRNA A2058G mutation	2 x 100 µl
Orange	MG, 23S rRNA A2059G	Positive control template for the detection of *M. genitalium*, 23S rRNA A2059G mutation	2 x 100 µl
Blue	MG, 23S rRNA A2058T	Positive control template for the detection of *M. genitalium*, 23S rRNA A2058T mutation	2 x 100 µl
Yellow	MG, 23S rRNA A2058C	Positive control template for the detection of *M. genitalium*, 23S rRNA A2058C mutation	2 x 100 µl
Neutral	Dilution Buffer	Diluent	10 x 1 ml

11.1 Instructions for use

Note: Before use of reagents, thaw completely, mix thoroughly by briefly vortexing, and spin down.

Prepare MG FleXible Reaction Mix as described in **Section 8.2**.

11.1.1 Preparation of Positive Control sample with a micropipettor

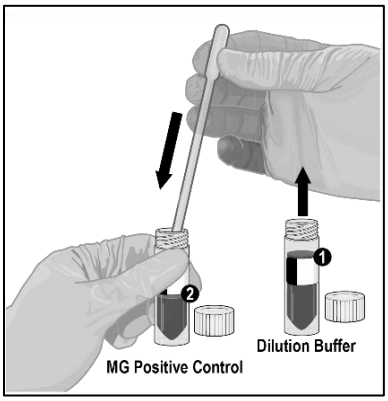
- Pipette 1 ml *Dilution Buffer* (**NEUTRAL**) into a Positive Control tube (e.g. *MG, 23S rRNA wild type* (**WHITE**)).
- Return and tighten lid. Vortex and spin down.
- Add 1 ml of the diluted Positive Control sample to a cartridge as described in **Section 8.3**.
- Start the Positive control test as described in **Section 9.2**.

11.1.2 Preparation of Positive Control sample with a transfer pipette

- Open the *Dilution Buffer* (**NEUTRAL**) tube lid. Compress the bulb of the transfer pipette, slowly insert the tip into the *Dilution Buffer* tube to about a quarter from the bottom. **Gently** release the pressure on the bulb to fill the transfer pipette while slowly moving the tip to the bottom of the tube (**Figure 18**). Ensure the transfer pipette has filled approximately up to the 1 ml mark.
- Insert the transfer pipette into the Positive Control tube (e.g. *MG, 23S rRNA wild type* (**WHITE**)) so that it touches the interior wall, and **gently** release the *Dilution Buffer* from the transfer pipette. Remove the transfer pipette from the tube.
- Compress the bulb of the transfer pipette, slowly insert the tip of the transfer pipette into the diluted Positive Control below the liquid level, and **gently** release the bulb whilst slowly moving the tip to the bottom of the tube. Ensure the transfer pipette has filled approximately up to the 1 ml mark.
- Dispense 1 ml of the diluted Positive Control sample to a cartridge as described in **Section 8.3**.
- Start the Positive control test as described in **Section 9.2**.

Refer to **Section 13** for example results.

Figure 18. Add 1 ml of Dilution Buffer to the MG Positive Control



12 Interpretation of results

The interpretation of results from the *ResistancePlus*® MG FleXible assay is automated by the GeneXpert® System software from measured fluorescent signals and embedded calculation algorithms.

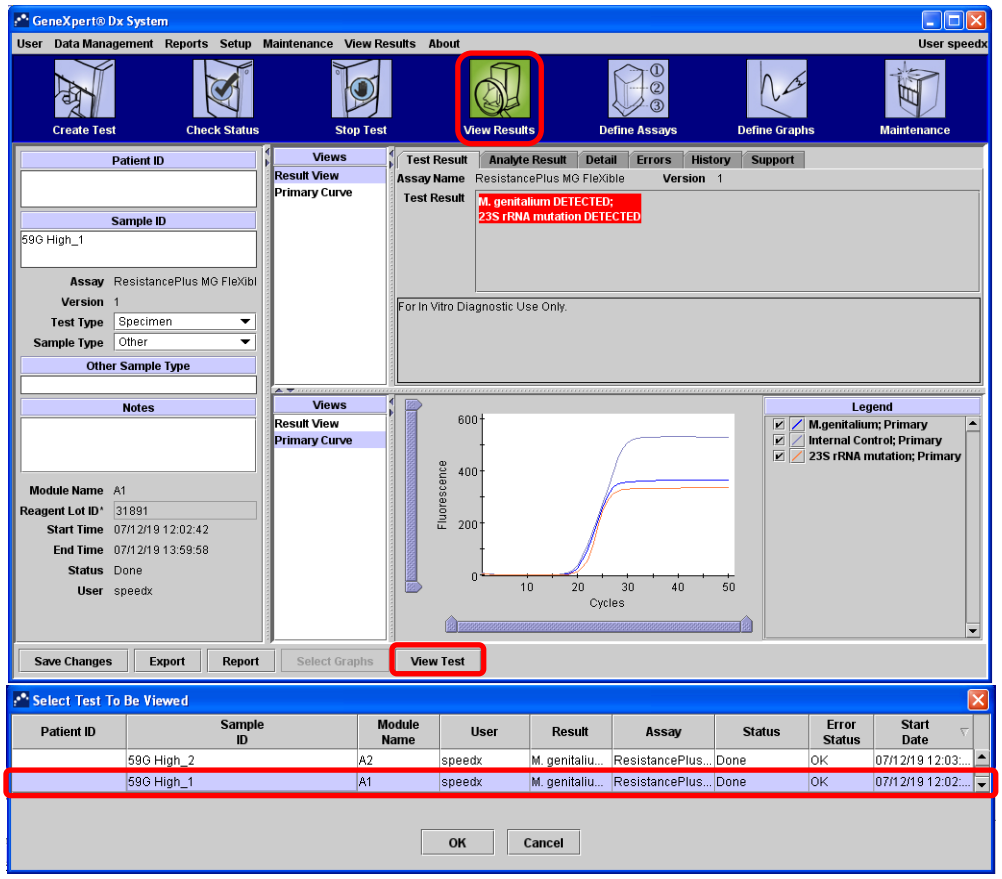
In the main menu of the GeneXpert® Dx software

Select **View Result**s (**Figure 19**) > Select **View Test**

In the **Select Test to Be Viewed** window

Select required test and select **OK**

Figure 19. View Results (display may vary depending on user setup)



In the **View Results** window

In **Views** > Select **Result View**

> Select **Test Result** tab to view overall test result

> Select **Analyte Result** tab to view Ct values for all analytes

In **Views** > Select **Primary curve** to view amplification curve

In the Infinity Xpertise software, select the **Results** button. The Results menu will be displayed.

In the Results menu, select the **View Results** button. The View Results window will be displayed.

In the View Results window

> Select **Test Result** tab to view overall test result

> Select **Analyte Result** tab to view Ct values for all analytes

> Select **Amplification Curve** to view amplification curves

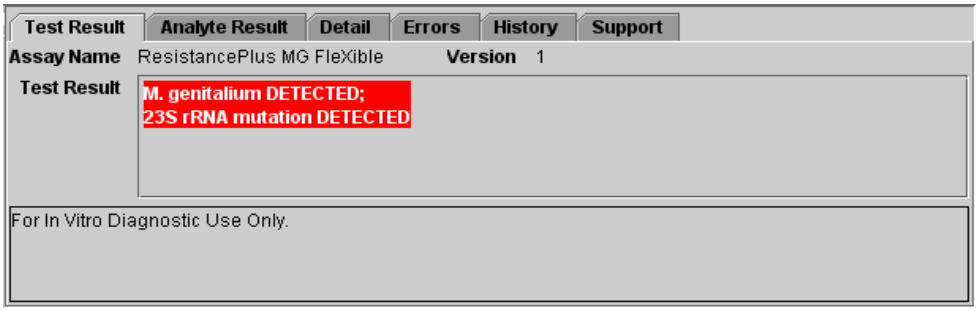
NOTE: It is highly recommended that amplification curves be reviewed for all positive samples.

13 Example results

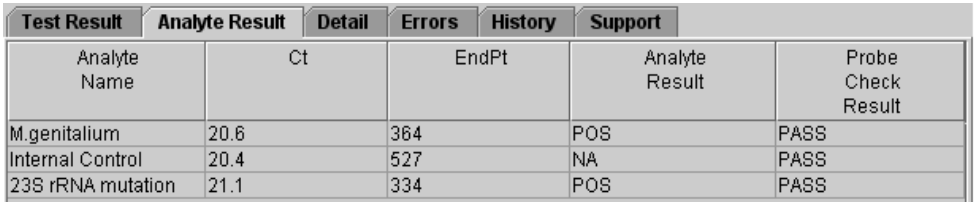
The following examples show the overall Test Result from the **Test Result** tab, amplification curves and Analyte Cts from the **Analyte Result** tab, within the **View Results** window of the GeneXpert® Dx software.

Example 1. *M. genitalium*, 23S rRNA mutant sample

Result view > Test Result tab

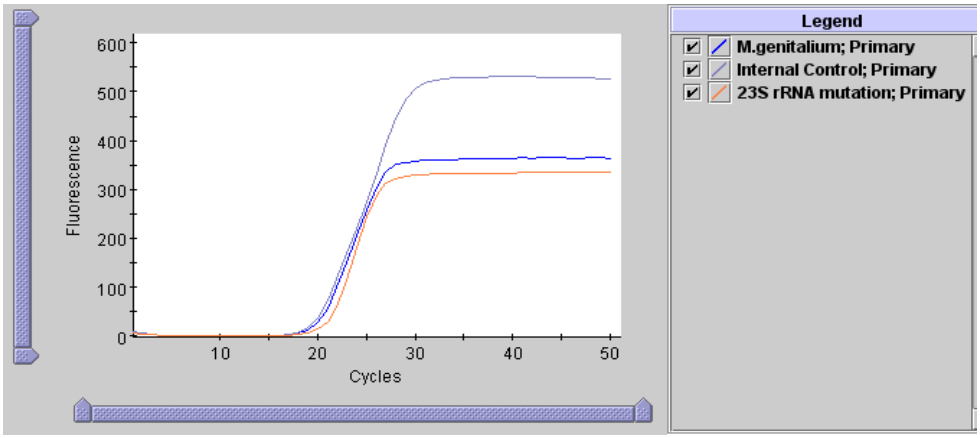


Result view > Analyte Result tab



Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
M.genitalium	20.6	364	POS	PASS
Internal Control	20.4	527	NA	PASS
23S rRNA mutation	21.1	334	POS	PASS

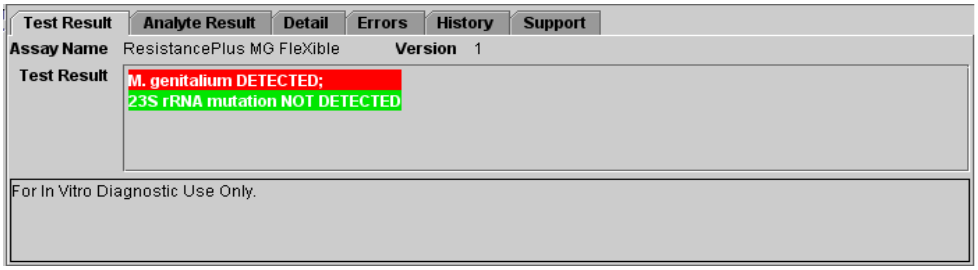
Primary Curve



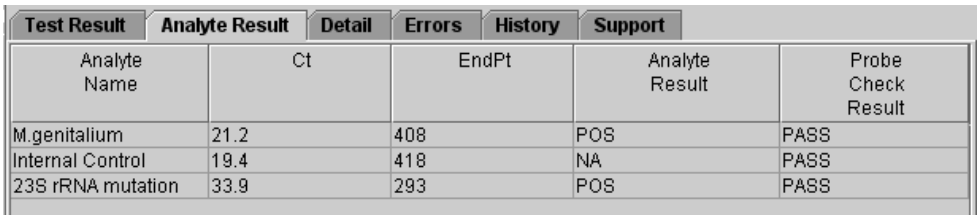
Test Result	Interpretation
M. genitalium DETECTED; **23S rRNA mutation DETECTED**	*M. genitalium* and 23S rRNA mutation target DNA detected • PCR amplification of *M. genitalium* and 23S rRNA mutation targets give Cts within the valid range • Internal control: Not applicable (NA) when *M. genitalium* is detected • Probe check: PASS; All probe check results pass

Example 2. *M. genitalium*, 23S rRNA wild type sample

Result view > Test Result tab

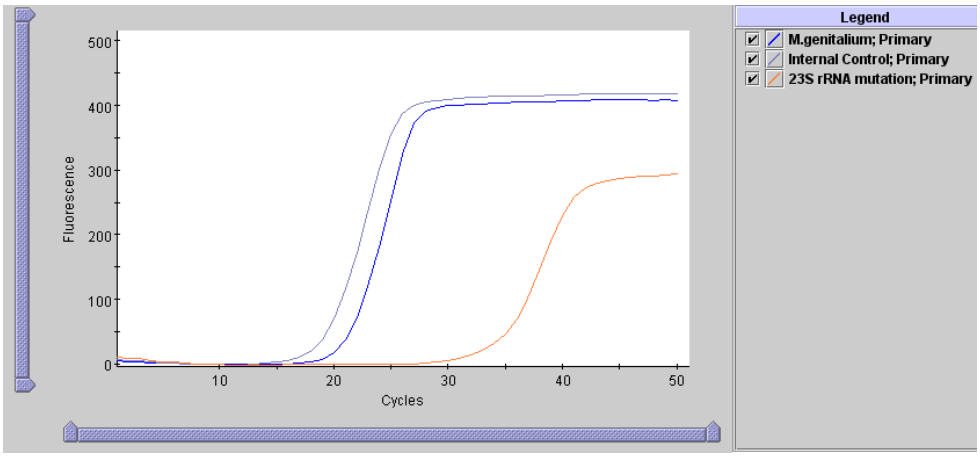


Result view > Analyte Result tab



Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
M.genitalium	21.2	408	POS	PASS
Internal Control	19.4	418	NA	PASS
23S rRNA mutation	33.9	293	POS	PASS

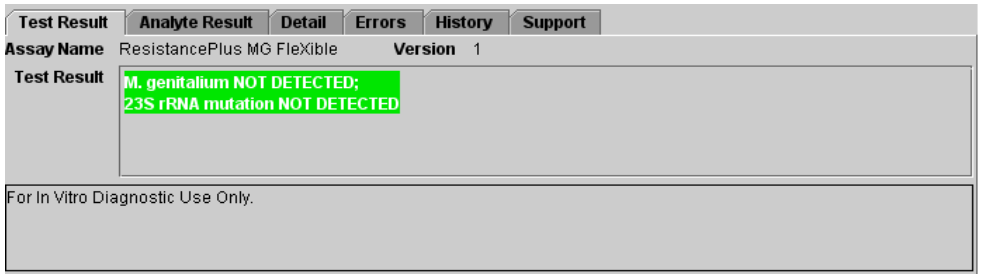
Primary Curve



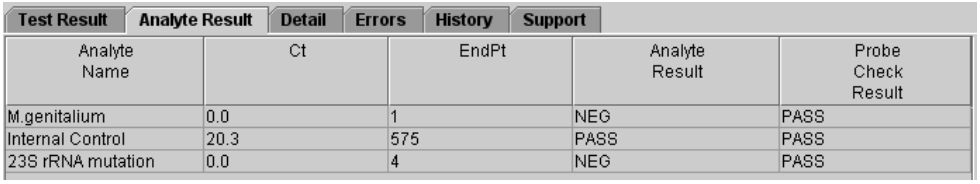
Test Result	Interpretation
M. genitalium DETECTED; **23S rRNA mutation NOT DETECTED**	*M. genitalium* target DNA detected; 23S rRNA mutation target DNA not detected • PCR amplification of *M. genitalium* target gives a Ct within the valid range; 23S rRNA mutation target is absent or not within the valid range • Internal control: Not applicable (NA) when *M. genitalium* is detected • Probe check: PASS; All probe check results pass

Example 3. *M. genitalium* negative sample

Result view > Test Result tab

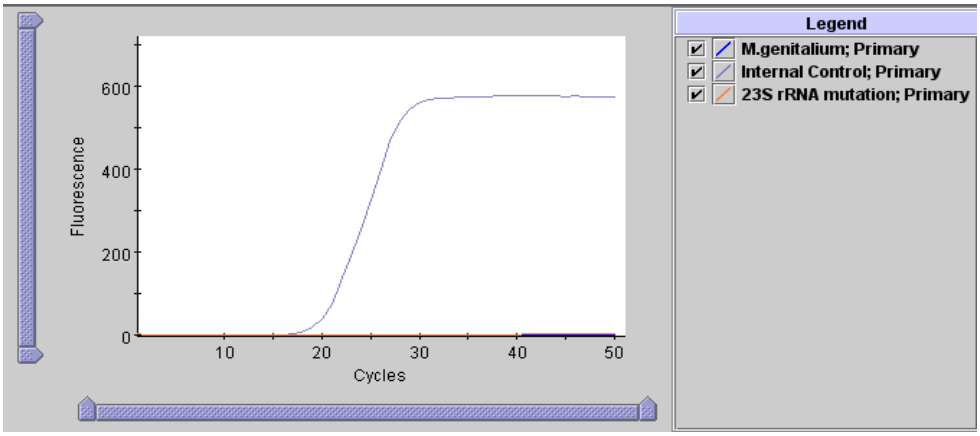


Result view > Analyte Result tab



Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
M.genitalium	0.0	1	NEG	PASS
Internal Control	20.3	575	PASS	PASS
23S rRNA mutation	0.0	4	NEG	PASS

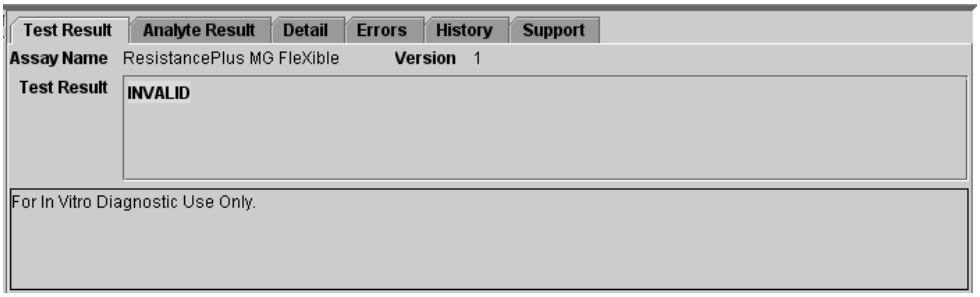
Primary Curve



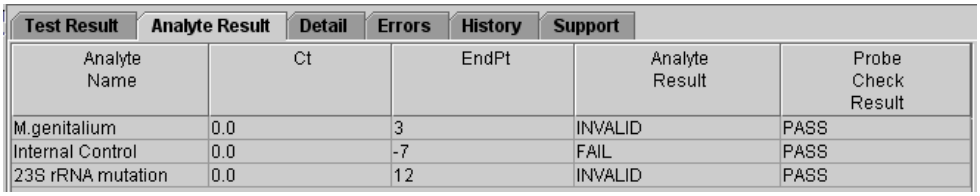
Test Result	Interpretation
M. genitalium NOT DETECTED; **23S rRNA mutation NOT DETECTED**	*M. genitalium* target DNA not detected • *M. genitalium* target absent or outside the valid range • Internal control: PASS; PCR amplification of Internal Control gives a Ct within the valid range • Probe check: PASS; All probe check results pass

Example 4. Invalid sample

Result view > Test Result tab

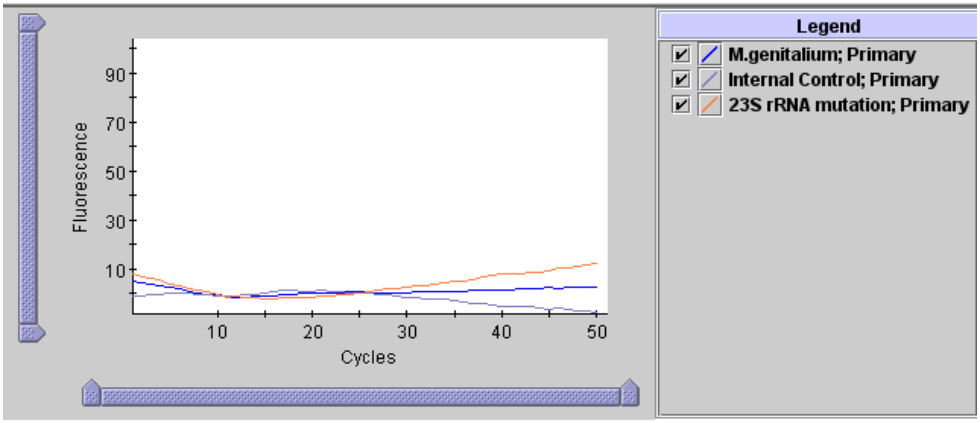


Result view > Analyte Result tab



Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
M.genitalium	0.0	3	INVALID	PASS
Internal Control	0.0	-7	FAIL	PASS
23S rRNA mutation	0.0	12	INVALID	PASS

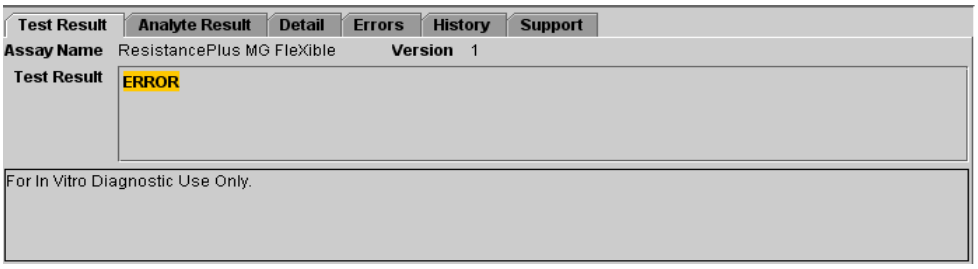
Primary Curve



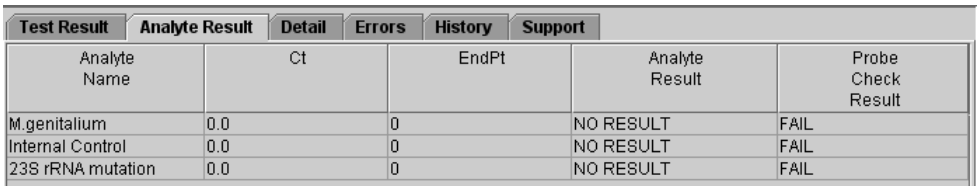
Test Result	Interpretation
INVALID	Presence or absence of *M. genitalium* and 23S rRNA mutation target DNA cannot be determined. **Repeat the test** using the original sample and proceed from **Section 8.2**. If the repeat test does not produce a valid result, collect a new sample to re-test. • Internal control: FAIL; Internal Control result is absent, or Ct is not within the valid range • Probe check: PASS; All probe check results pass

Example 5. Error result

Result view > Test Result tab



Result view > Analyte Result tab



Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
M.genitalium	0.0	0	NO RESULT	FAIL
Internal Control	0.0	0	NO RESULT	FAIL
23S rRNA mutation	0.0	0	NO RESULT	FAIL

Test Result	Interpretation
ERROR	Presence or absence of *M. genitalium* and 23S rRNA mutation target DNA cannot be determined. **Repeat the test** using the original sample and proceed from **Section 8.2.** If the repeat test does not produce a valid result, collect a new sample to re-test. • Internal control: NO RESULT • Probe check: FAIL*; all or one of the probe check results fail. The Probe Check may have failed because the reaction mix was made incorrectly, or the reaction chamber was filled improperly, or a mix integrity problem was detected. * If the probe check passed, the error is caused by system component failure or signal loss detection or other error

Example 6. No result

Result view > Test Result tab

Test Result	Interpretation
NO RESULT	Presence or absence of *M. genitalium* and 23S rRNA mutation target DNA cannot be determined. **Repeat the test** using the original sample and proceed from **Section 8.2.** If the repeat test does not produce a valid result, collect a new sample to re-test. Insufficient data were collected to produce a test result (e.g. Operator stopped a test that was in progress or system component failure occurred).

14 Limitations

- The ***Resistance****Plus*® MG FleXible assay targets the *MgPa* gene for *M. genitalium* and mutations at positions 2058 and 2059 in the 23S rRNA gene (A2058G, A2059G, A2058T, A2058C, *E. coli* numbering) that are associated with resistance to azithromycin (macrolide-based antibiotic).
- The ***Resistance****Plus*® MG FleXible assay has been shown to cross-react with the *M. genitalium*, 23S rRNA A2059C mutant sequences.
- The ***Resistance****Plus*® MG FleXible assay should only be performed by personnel trained in the procedure and should be performed in accordance to these Instructions for Use.
- Reliable results are dependent on adequate specimen collection transport, storage, and processing. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
- The ***Resistance****Plus*® MG FleXible assay is a qualitative assay and does not provide quantitative values or information about organism load.
- With urine specimens, assay interference may be observed in the presence of blood (>0.4% v/v) or bilirubin (>0.18 mg/ml).
- Results from the test must be correlated with the clinical history, epidemiological data, laboratory data and any other data available to the clinician.
- Prevalence of *M. genitalium* and macrolide resistance will affect the positive and negative predictive values for the assay.
- Detection of antibiotic resistance markers may not correlate with phenotypic gene expression.
- Therapeutic failure or success cannot be determined based on the assay results, since nucleic acid may persist following appropriate antimicrobial therapy.
- Negative results do not exclude the possibility of infection due to improper specimen collection, technical error, presence of inhibitors, specimen mix up, or low numbers of organisms in the clinical specimen.
- Negative results for the resistance markers do not indicate susceptibility of detected microorganisms, as resistance markers not measured by the assay or other potential mechanisms of antibiotic resistance may be present.
- False positive results may occur due to cross-contamination by target organisms, their nucleic acids or amplified product.

15 Performance characteristics

15.1 Clinical performance

A prospective-retrospective clinical study was conducted at the STD Laboratory, University of Alabama, Birmingham, Alabama, USA. Samples were collected from September 2018 - March 2019, and based on an in-house *M. genitalium* pdhD real-time PCR (performed at Johns Hopkins Center for the Development of Point-of-Care Sexually Transmitted Diseases, Baltimore, Maryland, USA), 21 *M. genitalium* positive and 54 consecutive *M. genitalium* negative samples were selected for inclusion in the study. The 76 specimens consisted of 38 male urine (collected in Xpert® Urine Specimen Collection Kit) and 38 vaginal swabs (collected in Xpert® Vaginal/Endocervical Specimen Collection kit). To determine performance of the ***Resistance****Plus*® MG FleXible kit, *M. genitalium* detection was compared to the pdhD real-time PCR result, and 23S rRNA mutant detection was compared to Sanger sequencing. The sensitivity and specificity of the ***Resistance****Plus*® MG FleXible kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 3**. One specimen (vaginal swab) was reported as Invalid by ***Resistance****Plus*® MG FleXible and was excluded from analysis. Analysis of 23S rRNA mutation detection only includes samples where the mutant status could be determined. Analysis of results in accordance to specimen type is shown in **Table 4**. The 23S rRNA mutation analysis is shown in **Table 5**.

Table 3. Clinical evaluation of *ResistancePlus*® MG FleXible

		Reference *M. genitalium* detection (pdhD qPCR)			Reference 23S rRNA mutant detection (Sanger Sequencing)	
		MG Positive	MG Negative		Mutant	Wild type
***ResistancePlus* MG FleXible**	**MG Positive**	21	2	**Mutant**	13	0
	MG Negative	0	52	**Mutant not detected**	1	7
	Total	21	54	**Total**	14	7
	Sensitivity	100.0% (95% CI 83.9 – 100.0%)		**Sensitivity**	92.9% (95% CI 66.1 – 99.8%)	
	Specificity	96.3% (95% CI 87.3 – 98.7%)		**Specificity**	100.0% (95% CI 59.0 – 100.0%)	

Table 4. Clinical result analysis in accordance to specimen type[#]

Specimen	Expected MG negative	Expected MG positive, 23S rRNA wild type	Expected MG positive, 23S rRNA mutant
Male urine	33/33	1/1	3/4[1]
Female vaginal swab	22/22	6/6	10/10

[#] Samples were included in this analysis if they had a valid result from both the reference test and ***Resistance****Plus*® MG FleXible

[1] 1 sample was incorrectly called *M. genitalium* detected, 23S rRNA mutant not detected

Table 5. Clinical result analysis according to 23S rRNA mutation

Reference result	*ResistancePlus*® MG FleXible result
Wild type	7/7
A2059G	8/8
A2058G	5/6[1]

[1] 1 sample was incorrectly called *M. genitalium* detected, 23S rRNA mutant not detected

15.2 Analytical performance

15.2.1 Reproducibility

A reproducibility study was performed across testing sites, instruments, lots, operators, runs and days for the ***Resistance****Plus*® MG FleXible kit, using panels prepared from urine and vaginal swab matrix. Testing was performed at two sites. Each panel contained three replicates of a panel member tested at 3x LOD. Each panel contained three negative samples. Panels were tested twice daily over three non-consecutive days by three operators, giving a total of 54 observations per panel member (3 replicates per run x 2 runs x 3 days x 3 operators = 54 observations). At least three lots of the ***Resistance****Plus*® MG FleXible kit were included in the study.

All valid test runs were included in the analyses of the percent agreement for each target of ***Resistance****Plus*® MG FleXible kit for each panel type separately.

Urine Panels Reproducibility results: Percent agreement for all samples was 100% (**Table 6**). Analysis of variance components (**Table 7 – Table 9**) of the Cq values performed on positive panel members yielded overall CV ranges from 0.00% to 4.07%, 0.54% to 4.23%, and 0.25% to 6.04% for the MgPa, IC, and 23S targets respectively.

Vaginal Swab Panels Reproducibility results: Percent agreement for all samples was 100% (**Table 10**). Analysis of variance components (**Table 11 – Table 13**) of the Cq values performed on positive panel members yielded overall CV ranges from 0.10% to 2.69%, 0.02% to 2.57%, and 0.18% to 2.86% for the MgPa, IC, and 23S targets respectively.

Table 6. Urine samples: Percent agreement

M. genitalium **23S rRNA type**	**Site 1 (GeneXpert XVI)**		**Site 2 (GeneXpert Infinity-48s)**		**Total agreement by target**	
	n/N[a]	**Hit rate (%)**	**n/N[a]**	**Hit rate (%)**	**n/N[a]**	**Hit rate (%)**
A2058C	36/36	100.0	18/18	100.0	54/54	100.0
A2058G	36/36	100.0	18/18	100.0	54/54	100.0
A2058T	36/36	100.0	18/18	100.0	54/54	100.0
A2059G,	36/36	100.0	18/18	100.0	54/54	100.0
WT	36/36	100.0	18/18	100.0	54/54	100.0

[a] n/N = number of correctly identified samples/total number of samples tested

Table 7. Urine samples: Summary of reproducibility data for the MgPa target

M. genitalium **23S rRNA type**	**n/N[a]**	**Agreement (%)**	**Mean Cq**	**Between-lot**		**Between-day**		**Between-operator**		**Between-run**		**Between-instrument**	
				SD	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
A2058C	54/54	100	27.93	0.66	2.38	0.23	0.81	0.47	1.66	0.69	2.47	0.57	2.03
A2058G	54/54	100	28.78	1.10	3.88	0.42	1.46	0.62	2.17	0.79	2.78	0.76	2.63
A2058T	54/54	100	31.27	1.12	3.62	0.30	0.98	1.04	3.36	1.21	3.90	1.27	4.07
A2059G	54/54	100	29.42	0.77	2.62	0.59	2.01	0.09	0.32	0.77	2.62	0.00	0.00
Wild type	54/54	100	30.81	0.62	2.01	0.38	1.25	0.21	0.68	0.51	1.65	0.24	0.78
M. genitalium negative	54/54	100	--	--	--	--	--	--	--	--	--	--	--

[a] n/N = number of correctly identified samples/total number of samples tested

Table 8. Urine samples: Summary of reproducibility data for the 23S rRNA mutation target													
***M. genitalium* 23S rRNA type**	**n/N[a]**	**Agreement (%)**	**Mean Cq**	**Between-lot**		**Between-day**		**Between-operator**		**Between-run**		**Between-instrument**	
				SD	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
A2058C	54/54	100	28.47	1.09	3.83	0.61	2.15	0.59	2.07	0.94	3.29	0.85	2.98
A2058G	54/54	100	33.54	0.25	0.74	0.15	0.45	0.26	0.77	0.74	2.21	0.09	0.25
A2058T	54/54	100	32.18	0.73	2.30	0.09	0.28	0.46	1.44	1.21	3.80	0.40	1.23
A2059G	54/54	100	30.02	1.04	3.47	1.20	3.97	1.48	4.88	1.83	6.04	1.24	4.14
Wild type	54/54	100	--	--	--	--	--	--	--	--	--	--	--
M. genitalium negative	54/54	100	--	--	--	--	--	--	--	--	--	--	--

[a] n/N = number of correctly identified samples/total number of samples tested

Table 9. Urine samples: Summary of reproducibility data for the IC target													
***M. genitalium* 23S rRNA type**	**n/N[a]**	**Agreement (%)**	**Mean Cq**	**Between-lot**		**Between-day**		**Between-operator**		**Between-run**		**Between-instrument**	
				SD	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
A2058C	54/54	100	19.46	0.72	3.77	0.34	1.78	0.67	3.49	0.68	3.54	0.82	4.23
A2058G	54/54	100	18.82	0.44	2.37	0.36	1.91	0.11	0.61	0.39	2.09	0.10	0.54
A2058T	54/54	100	19.07	0.29	1.55	0.31	1.62	0.28	1.45	0.57	3.02	0.25	1.31
A2059G	54/54	100	19.17	0.79	4.19	0.17	0.87	0.65	3.45	0.62	3.28	0.80	4.18
Wild type	54/54	100	19.21	0.66	3.44	0.49	2.57	0.54	2.82	0.67	3.54	0.66	3.43
M. genitalium negative	54/54	100	19.72	0.69	3.52	0.42	2.12	0.22	1.13	0.53	2.67	0.27	1.38

[a] n/N = number of correctly identified samples/total number of samples tested

Table 10. Vaginal swab samples: Percent agreement						
***M. genitalium* 23S rRNA type**	**Site 1 (GeneXpert XVI)**		**Site 2 (GeneXpert Infinity-48s)**		**Total agreement by target**	
	n/N[a]	**Hit rate (%)**	**n/N[a]**	**Hit rate (%)**	**n/N[a]**	**Hit rate (%)**
A2058C	36/36	100.0	18/18	100.0	54/54	100.0
A2058G	36/36	100.0	18/18	100.0	54/54	100.0
A2058T	36/36	100.0	18/18	100.0	54/54	100.0
A2059G	36/36	100.0	18/18	100.0	54/54	100.0
WT	36/36	100.0	18/18	100.0	54/54	100.0

[a] n/N = number of correctly identified samples/total number of samples tested

Table 11. Vaginal swab samples: Summary of reproducibility data for the MgPa target													
***M. genitalium* 23S rRNA type**	**n/N[a]**	**Agreement (%)**	**Mean Cq**	**Between-lot**		**Between-day**		**Between-operator**		**Between-run**		**Between-instrument**	
				SD	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
A2058C	54/54	100	26.49	0.09	0.35	0.22	0.85	0.25	0.94	0.41	1.57	0.30	1.12
A2058G	54/54	100	25.69	0.15	0.58	0.17	0.66	0.39	1.53	0.55	2.13	0.19	0.73
A2058T	54/54	100	27.00	0.45	1.68	0.03	0.10	0.64	2.36	0.69	2.55	0.14	0.51
A2059G	54/54	100	27.16	0.15	0.53	0.12	0.45	0.08	0.29	0.37	1.38	0.10	0.36
Wild type	54/54	100	28.38	0.48	1.71	0.32	1.14	0.43	1.52	0.76	2.69	0.44	1.56
M. genitalium negative	54/54	100	--	--	--	--	--	--	--	--	--	--	--

[a] n/N = number of correctly identified samples/total number of samples tested

Table 12. Vaginal swab samples: Summary of reproducibility data for the 23S rRNA mutation target													
***M. genitalium* 23S rRNA type**	**n/N[a]**	**Agreement (%)**	**Mean Cq**	**Between-lot**		**Between-day**		**Between-operator**		**Between-run**		**Between-instrument**	
				SD	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
A2058C	54/54	100	27.13	0.09	0.35	0.20	0.74	0.58	2.15	0.59	2.18	0.66	2.45
A2058G	54/54	100	30.64	0.47	1.54	0.24	0.78	0.18	0.58	0.61	2.00	0.41	1.35
A2058T	54/54	100	28.89	0.46	1.60	0.05	0.18	0.17	0.60	0.53	1.84	0.33	1.13
A2059G	54/54	100	27.71	0.49	1.77	0.19	0.70	0.52	1.88	0.79	2.86	0.23	0.82
Wild type	54/54	100	--	--	--	--	--	--	--	--	--	--	--
M. genitalium negative	54/54	100	--	--	--	--	--	--	--	--	--	--	--

[a] n/N = number of correctly identified samples/total number of samples tested

Table 13. Vaginal swab samples: Summary of reproducibility data for the IC target													
***M. genitalium* 23S rRNA type**	**n/N[a]**	**Agreement (%)**	**Mean Cq**	**Between-lot**		**Between-day**		**Between-operator**		**Between-run**		**Between-instrument**	
				SD	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
A2058C	54/54	100	17.02	0.23	1.36	0.11	0.62	0.24	1.44	0.32	1.89	0.13	0.74
A2058G	54/54	100	17.78	0.33	1.87	0.09	0.51	0.30	1.69	0.42	2.36	0.06	0.32
A2058T	54/54	100	17.06	0.14	0.79	0.10	0.60	0.30	1.76	0.29	1.72	0.36	2.13
A2059G	54/54	100	17.59	0.11	0.65	0.10	0.55	0.09	0.50	0.26	1.47	0.07	0.40
Wild type	54/54	100	17.18	0.25	1.47	0.30	1.72	0.07	0.39	0.31	1.83	0.00	0.02
M. genitalium negative	54/54	100	17.28	0.45	2.57	0.06	0.35	0.41	2.36	0.41	2.37	0.22	1.27

[a] n/N = number of correctly identified samples/total number of samples tested

15.2.2 Analytical sensitivity

Representative *M. genitalium* strains were used to assess analytical sensitivity (Limit of Detection or LOD). Each *M. genitalium* strain was diluted into a matrix of negative urine specimen and negative vaginal swab specimen. The LOD is defined at the lowest concentration (expressed as number of genomes per sample) that can be reproducibly distinguished from negative samples with 95% confidence.

The results for each *M. genitalium* strain are shown in **Table 14**.

Table 14. LOD of each target of the *ResistancePlus*® MG FleXible kit

M. genitalium 23S rRNA type	Strain	Urine	Vaginal swab
		LOD (genomes per sample)	LOD (genomes per sample)
WT	G37	157	157
A2058C	M6302	317	317
A2059G	M6593	147	220
A2058G	M6604	387	387
A2058T	M6926	151	151

15.2.3 Inclusivity

An inclusivity study was conducted to test reactivity of the ***Resistance****Plus*® MG FleXible kit with 8 strains of *M. genitalium*. The *M. genitalium* isolates represented different 23S rRNA mutants from diverse geographical locations (isolates were from the following countries, with number of strains given in brackets: Australia (2), Denmark (3), Norway (1), UK (1), USA (1)). Each strain was diluted into a matrix of negative urine specimen and negative vaginal swab specimen. All isolates were tested at a concentration of 2x LOD in replicates of three using one lot of the ***Resistance****Plus*® MG FleXible kit. All strains were correctly detected.

Results are summarised in **Table 15**.

Table 15. *M. genitalium* strains tested for inclusivity

***M. genitalium* 23S rRNA type**	**Strain**
Wild type	M2300 M2321 M2341 M30 Early
A2058G	M6271
A2059G	M6303 M6320
A2058C	M6848

15.2.4 Cross-reactivity to other 23S rRNA mutations

A synthetic DNA construct containing *M. genitalium* MgPa and A2059C 23S rRNA targets was tested at 5000 copies in a background of 35 ng DNA input per sample. Results demonstrated that the ***Resistance****Plus*® MG FleXible test cross-reacts to the *M. genitalium*, A2059C 23S rRNA target at a > 90% hit rate. Analytical performance of the ***Resistance****Plus*® MG FleXible test in detecting this mutation has not been evaluated.

15.2.5 Analytical specificity

A study was conducted to evaluate cross-reactivity with the ***Resistance****Plus*® MG FleXible kit when non-target organisms are present at high concentrations. A panel of 42 microorganisms consisting of bacteria, viruses, fungi and protozoa representing pathogens or flora commonly present in the urogenital system, or closely related to *M. genitalium*, were evaluated. Each bacterial strain was tested at 1 x 10^6 genomes/mL, unless otherwise stated. Viral strains were tested at 1 x 10^5 genomes/mL, unless otherwise stated. All other organisms were tested at the concentrations stated. All bacterial and viral organisms were quantified using qPCR, except those listed as quantified with Colony Forming Units (CFU) or Plaque Forming Units (PFU) (**Table 16**). All microorganisms were tested in triplicate for cross-reactivity with the ***Resistance****Plus*® MG FleXible kit. All microorganisms tested were diluted into negative clinical matrix (urine).

Results indicated that none of these organisms produced false positive results in the *M. genitalium* negative urine matrix (**Table 16**).

An *in silico* analysis was also performed to evaluate if the oligonucleotides in the ***Resistance****Plus*® MG FleXible kit could amplify and detect nucleic acid sequences from non-target organisms available in BLAST. No significant interactions were detected.

Table 16. Microorganisms tested for analytical specificity

Organism	Concentration (genomes/mL)	Organism	Concentration (genomes/mL)	Organism	Concentration (genomes/mL)
Actinomyces israelii	1 x 10^6	*Fusobacterium nucleatum*	1 x 10^6	*Neisseria gonorrhoeae*	1 x 10^6
Bacteroides fragilis	1 x 10^6	*Haemophilus ducreyi*	1 x 10^6	*Pentatrichomonas hominis*	1 x 10^5^
Bifidobacterium adolescentis	1 x 10^6	Herpes simplex virus I	1 x 10^6	*Peptostreptococcus anaerobius*	1 x 10^6
Campylobacter jejuni	1 x 10^6	Herpes simplex virus II	1 x 10^6	*Prevotella bivia*	1 x 10^6
Candida albicans	1 x 10^5	HPV type 18 (HeLa cells)	1 x 10^5*	*Propionibacterium acnes*	1 x 10^5
Candida glabrata	1 x 10^6	*Klebsiella oxytoca*	1 x 10^6	*Proteus mirabilis*	1 x 10^6
Candida parapsilosis	1 x 10^6	*Lactobacillus acidophilus*	1 x 10^6	*Proteus vulgaris*	1 x 10^6
Candida tropicalis	1 x 10^5	*Lactobacillus crispatus*	1 x 10^6	*Pseudomonas aeruginosa*	1 x 10^6
Chlamydia trachomatis	1 x 10^6	*Lactobacillus jensenii*	1 x 10^6	*Staphylococcus aureus*	1 x 10^6
Clostridium perfringens	1 x 10^6	*Lactobacillus vaginalis*	1 x 10^6	*Staphylococcus saprophyticus*	1 x 10^6
Corynebacterium genitalium	1 x 10^6	*Listeria monocytogenes*	1 x 10^6	*Streptococcus agalactiae*	1 x 10^6
Enterobacter aerogenes	1 x 10^6	*Mycobacterium smegmatis*	1 x 10^5	*Streptococcus pyogenes*	1 x 10^6
Enterobacter cloacae	1 x 10^6	*Mycoplasma hominis*	1 x 10^6	*Trichomonas vaginalis*	1 x 10^5^
Enterococcus faecalis	1 x 10^6	*Mycoplasma pneumoniae*	1 x 10^6	*Ureaplasma urealyticum*	1 x 10^5

* Quantified as PFU/ml

^ Quantified as CFU/ml

15.2.6 Potentially interfering substances

An interfering substances study was carried out to examine if substances or conditions that may be present in clinical specimens could affect the performance of the ***Resistance****Plus*® MG FleXible kit. The panel consisted of endogenous substances such as blood, mucin, leukocytes, and medications (prescription and over-the-counter) that could be used to treat urogenital conditions. All substances were tested in the presence and absence of a representative *M. genitalium* A2058G strain at 3x LOD. All test samples were tested in triplicate. Substances were diluted in negative clinical matrix (either urine or vaginal swab) as appropriate.

Except for bilirubin, whole blood and vagisil intimate powder, results indicated that none of the substances and conditions interfered with detection of the representative *M. genitalium* A2058G strain or produced false positive results in the *M. genitalium* negative matrices at the concentrations stated.

With urine specimens, assay interference may be observed in the presence of:

- Blood at a concentration greater than 0.4% v/v
- Bilirubin at a concentration greater than 0.18 mg/mL

With vaginal swab specimens, assay interference may be observed in the presence of:

- Vagisil intimate powder at a concentration greater than 0.1% w/v

Results are summarised in **Table 17** and **Table 18.**

Table 17. Potentially interfering substances in urine samples

Class/Substance	Product name	Test concentration
Whole blood	-	0.4% v/v^
Semen	-	5.0% v/v
Mucus	Mucin	0.8% w/v
Antibiotic	Azithromycin	1.8 mg/mL
Analgesic	Paracetamol	3.2 mg/mL
Intravaginal hormones	Progesterone; Estradiol	7 mg/mL Progesterone + 0.07 mg/mL Beta Estradiol
Leukocytes	-	10^5 cells/mL
Albumin	Bovine serum albumin	10 mg/ml
Glucose	-	10 mg/ml
Bilirubin	-	0.18 mg/ml*
Acidic urine (pH 4.0)	Urine + N-Acetyl-L-Cysteine	pH 4.0
Alkaline urine (pH 9.0)	Urine + Ammonium Citrate	pH 9.0

*interference may be observed in samples containing greater than 0.18 mg/mL bilirubin

^ interference may be observed in samples containing greater than 0.4% whole blood

Table 18. Potentially interfering substances in vaginal swab samples

Class/Substance	Product name	Test concentration
Over-the-counter vaginal products and contraceptives	Vagisil Anti-Itch Crème (1.0 oz)	0.25% w/v
	K-Y Jelly (4.0 oz)	0.25% w/v
	Options Gynol II Vaginal Contraceptive Gel	0.25% w/v
	Walgreens Clotrimazole Vaginal Cream (1.5 oz)	0.25% w/v
	Vagi-gard douche	0.25% w/v
	Vagisil ProHydrate Natural Feel Internal Moisturizing Gel (0.2 oz x 8 pack)	0.25% w/v
	Vagisil Daily Intimate Deodorant Powder (8.0 oz)	0.10% w/v*
Deodorant & Powders	Summer's Eve Deodorant spray (2.0 oz)	0.25% v/v
Hemorrhoidal cream	Preparation H Hemorrhoidal Cream (0.9 oz)	0.25% w/v
Prescription-only medicines	Estrace® (estradiol vaginal cream, USP 0.01%)	0.25% w/v

*interference may be observed in samples containing greater than 0.1% w/v Vagisil intimate powder

15.2.7 Carry-over contamination study

A study was conducted to demonstrate that single-use GeneXpert cartridges prevent carry-over contamination in negative samples run following very high positive samples in the same GeneXpert module. The study consisted of a negative sample processed in the same GeneXpert module immediately following a sample of high MG concentration (synthetic DNA; 10^6 copies/mL of A2058C target). Samples were tested in two GeneXpert modules for a total of 40 runs, resulting in 20 positives and 20 negatives. No false positives were observed with the negative samples indicating that carry-over contamination does not occur between runs on the GeneXpert.

16 Customer and Technical support

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of instrument
- Error message (if any)
- Software version and, if applicable, Computer Service Tag number

Table 19. Technical assistance contact details

Region	Telephone	Email
Australia and New Zealand	+1800 130 821 +0800 001 028	techsupportANZ@cepheid.com
Austria	+43 720 380 091	support@cepheideurope.com
France	+33 563 825 319	support@cepheideurope.com
Germany	+49 21 513 280 100	support@cepheideurope.com
Italy	+39 800 902 567	support@cepheideurope.com
Spain	+34 919 90 67 62	support@cepheideurope.com
Poland	+48 225 048 523	support@cepheideurope.com
Portugal	+351 800 913 174	support@cepheideurope.com
United Kingdom	+44 3303 332 533	support@cepheideurope.com
Belgium, Netherlands and Luxembourg	+33 563 825 319	support@cepheideurope.com
Other European, Middle East and African countries	+33 563 825 319 +971 4 253 3218	support@cepheideurope.com

Contact information for other Cepheid support offices is available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab. Select the **Contact Us** option.

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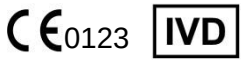
18 Glossary



European Conformity For *In Vitro* Diagnostic Use

Catalogue number

Master lot

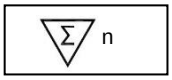
Batch code

Authorised Representative In the European Community

Manufacturer

Date of manufacture

Temperature limitation



Contains sufficent for <n> determinations

Use by Date

Warning

Do Not reuse

Consult instructions for use

Positive control

P/N

Part Number

Importer

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