



## ResistancePlus<sup>®</sup> MG

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



Product	Platform	Size (reactions)	Catalogue no.
ResistancePlus <sup>®</sup> MG	LC480 II z 480	100	<b>REF</b> 20001L-01
ResistancePlus <sup>®</sup> MG	LC480 II z 480	25	<b>REF</b> 2000125
ResistancePlus <sup>®</sup> MG <sub>(550)</sub>	ABI 7500 Fast ABI 7500 Fast Dx	100	<b>REF</b> 2000201
ResistancePlus <sup>®</sup> MG <sub>(550)</sub>	ABI 7500 Fast ABI 7500 Fast Dx	25	<b>REF</b> 2000225
ResistancePlus <sup>®</sup> MG <sub>(675)</sub>	CFX96 <sup>™</sup> Dx CFX96 <sup>™</sup> Touch	100	<b>REF</b> 2000301
ResistancePlus <sup>®</sup> MG <sub>(675)</sub>	CFX96 <sup>™</sup> Dx CFX96 <sup>™</sup> Touch	25	<b>REF</b> 2000325

### Accessory products – Analysis software

ResistancePlus <sup>®</sup> MG (LC480)	<b>REF</b> 99003
ResistancePlus <sup>®</sup> MG (z480)	<b>REF</b> 99018
ResistancePlus <sup>®</sup> MG (7500)	<b>REF</b> 99002
ResistancePlus <sup>®</sup> MG (CFX)	<b>REF</b> 99008
REFLEX ResistancePlus <sup>®</sup> MG (LC480)	<b>REF</b> 99023
REFLEX ResistancePlus <sup>®</sup> MG (z480)	<b>REF</b> 99024
REFLEX ResistancePlus <sup>®</sup> MG (7500)	<b>REF</b> 99026
REFLEX ResistancePlus <sup>®</sup> MG (CFX)	<b>REF</b> 99025



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**FOR PROFESSIONAL USE ONLY**

Not for sale in the USA

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## 1 Product description

The **ResistancePlus**<sup>®</sup> MG kit simultaneously detects *M. genitalium* and 5 mutations at positions 2058 and 2059 in the 23S rRNA gene (*E. coli* numbering) that are associated with resistance to azithromycin (macrolide-based antibiotic). The **ResistancePlus**<sup>®</sup> MG kit is a 1-well real-time PCR multiplex consisting of 3 readouts. Readout 1 indicates the presence or absence of *M. genitalium* through detection of the MgPa gene; Readout 2 indicates the presence of a A2058G, A2059G, A2058T, A2058C or A2059C mutation in the 23S rRNA gene; and Readout 3 is an internal control to monitor extraction efficiency and qPCR inhibition. The **ResistancePlus**<sup>®</sup> MG kit utilises **PlexZyme**<sup>®</sup> and **PlexPrime**<sup>®</sup> for specificity and superior multiplexing capability. The assay is validated on samples extracted using the MagNA Pure 96 System (Roche), MICROLAB STARlet IVD (Hamilton), QIAAsymphony<sup>®</sup> SP (QIAGEN), NUCLISENS<sup>®</sup> easyMAG<sup>®</sup> (Biomérieux) and real-time detection on the Roche LightCycler<sup>®</sup> 480 Instrument II (LC480 II), cobas z 480 analyser (z480), the Applied Biosystems<sup>®</sup> 7500 Fast (7500 Fast), Applied Biosystems<sup>®</sup> 7500 Fast Dx (7500 Fast Dx) and the Bio-Rad CFX96<sup>™</sup> Dx (CFX96 Dx) and CFX96 Touch<sup>™</sup> (CFX96 Touch) Real-time PCR Detection Systems.

## 2 Intended use

The **ResistancePlus**<sup>®</sup> MG kit is a qualitative multiplexed *in vitro* diagnostic real-time PCR test for the identification of *M. genitalium* and detection of 5 mutations in the 23S rRNA gene (A2058G, A2059G, A2058T, A2058C, and A2059C, *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 **ResistancePlus**<sup>®</sup> MG kit may be used with the following specimen types: male and female urine, and anal, rectal, cervical, endocervical, vaginal, urethral, penile, penile meatal and pharyngeal swabs, 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 **ResistancePlus**<sup>®</sup> MG kit 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.

## 3 Pathogen information

*M. genitalium* is small bacterium that is found in the human urogenital tract. *M. genitalium* 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 also associated with prostatitis, epididymitis, and balanoposthitis, inflammation of the glans penis and prepuce<sup>1</sup>. 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)<sup>1,2,3</sup>.

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<sup>4,5</sup>. The most common mutations are A2058G and A2059G, contributing 89% of macrolide resistance mutations in a recent study<sup>6</sup>.

## 4 Kit contents

Cap colour	Contents	Description	Cat no 20001L-01 (100 reactions)	Cat no 2000125 (25 reactions)
Blue	<b>Plex</b> Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, MgCl <sub>2</sub> , DNA polymerase and buffer	1 x 1 ml	1 x 250 µl
Brown	MG+23S Mix, 20x	Mix containing oligonucleotides <sup>^</sup> for amplification and detection of <i>M. genitalium</i> and 23S rRNA mutations	1 x 100 µl	1 x 25 µl
White	Control Mix 1, 20x	Mix containing oligonucleotides <sup>^</sup> for amplification and detection of internal control assay for LC480 II and z 480	1 x 100 µl	1 x 25 µl
Red	Internal Control Cells <sup>#</sup>	Internal control cells containing internal control DNA template to monitor extraction and amplification efficiency	1 x 500 µl	1 x 100 µl
Neutral	Nuclease Free Water	PCR grade water	1 x 1 ml	1 x 1 ml

<sup>#</sup> Store template tubes separately from oligo mixes, i.e. template or nucleic acid handling room

<sup>^</sup> Oligonucleotides are PCR primer pairs (including **PlexPrime**<sup>®</sup> primers), **PlexZyme**<sup>®</sup> enzymes and fluorescent probe

Table 2. Contents for <i>ResistancePlus</i> <sup>®</sup> MG <sub>(550)</sub> kits				
Cap colour	Contents	Description	Cat no 2000201 (100 reactions)	Cat no 2000225 (25 reactions)
Blue	<i>Plex</i> Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, MgCl <sub>2</sub> , DNA polymerase and buffer	1 x 1 ml	1 x 250 µl
Brown	MG+23S Mix, 20x	Mix containing oligonucleotides <sup>^</sup> for amplification and detection of <i>M. genitalium</i> and 23S rRNA mutations	1 x 100 µl	1 x 25 µl
White	Control Mix 2, 20x	Mix containing oligonucleotides <sup>^</sup> for amplification and detection of internal control assay for 7500 Fast and 7500 Fast Dx	1 x 100 µl	1 x 25 µl
Red	Internal Control Cells <sup>#</sup>	Internal control cells containing internal control DNA template to monitor extraction and amplification efficiency	1 x 500 µl	1 x 100 µl
Neutral	Nuclease Free Water	PCR grade water	1 x 1 ml	1 x 1 ml

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

<sup>^</sup> Oligonucleotides are PCR primer pairs (including *PlexPrime*<sup>®</sup> primers), *PlexZyme*<sup>®</sup> enzymes and fluorescent probe

Table 3. Contents for <i>ResistancePlus</i> <sup>®</sup> MG <sub>(675)</sub> kits				
Cap colour	Contents	Description	Cat no 2000301 (100 reactions)	Cat no 2000325 (25 reactions)
Blue	<i>Plex</i> Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, MgCl <sub>2</sub> , DNA polymerase and buffer	1 x 1 ml	1 x 250 µl
Brown	MG+23S Mix, 20x	Mix containing oligonucleotides <sup>^</sup> for amplification and detection of <i>M. genitalium</i> and 23S rRNA mutations	1 x 100 µl	1 x 25 µl
White	Control Mix 3, 20x	Mix containing oligonucleotides <sup>^</sup> for amplification and detection of internal control assay for CFX96 Dx and CFX96 Touch	1 x 100 µl	1 x 25 µl
Red	Internal Control Cells <sup>#</sup>	Internal control cells containing internal control DNA template to monitor extraction and amplification efficiency	1 x 500 µl	1 x 100 µl
Neutral	Nuclease Free Water	PCR grade water	1 x 1 ml	1 x 1 ml

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

<sup>^</sup> Oligonucleotides are PCR primer pairs (including *PlexPrime*<sup>®</sup> primers), *PlexZyme*<sup>®</sup> enzymes and fluorescent probe

## 5 Shipping and storage

- The components of the *ResistancePlus*<sup>®</sup>MG kits are shipped on dry ice or ice gel packs. All components should be stored at -25°C to -15°C upon receipt. It is recommended that freeze/thaw cycles are limited to 15.
- 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](mailto:tech@speedx.com.au)

## 6 Warnings and precautions

### 6.1 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 should be adequately trained in the use of the **ResistancePlus**<sup>®</sup> MG 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.

## 6.2 Laboratory

- It is recommended to perform sample preparation/extraction, 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. Treat all biological specimens 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 specimens, reagents and other potentially contaminated materials.

## 6.3 Specimen handling

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

## 6.4 Assay

- Basic precautions for preventing contamination of PCR reactions include the use of sterile filter pipette tips, use of a new pipette tip for every pipetting action, and separation of workflow.
- PCR tests are prone to contamination from previous PCR products. Never open reaction vessels after the completion of PCR.
- 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.

## 6.5 Safety precautions

- Safety Data Sheets (SDS) are available on request. Please contact [tech@speedx.com.au](mailto:tech@speedx.com.au) for more information.

## 6.6 Assay Plugins: Warnings/Precautions/Limitations

- SpeedX software can only control the analysis of raw data generated from the test kit when used with its respective PCR instrument. It does not control the preparation of samples, reactions, programming of equipment or delivery of treatment.
- Users should be adequately trained in the use of the **ResistancePlus**<sup>®</sup> MG analysis software and the access should be limited to each assigned single user
- It is recommended to implement user authentication access and cybersecurity controls such as anti-virus software or use of a firewall within the IT system and infrastructure which uses the software
- Upon detection of a cybersecurity incident such as unauthorised access and ransomware attacks, please contact [tech@speedx.com.au](mailto:tech@speedx.com.au) for further support.

# 7 Associated Products and Consumables

### *Positive Control Material*

- **ResistancePlus**<sup>®</sup> MG Positive Control kit (SpeedX, Cat no 95001)

### *General lab consumables*

- Gloves and clean lab coats
- Vortex mixer
- Benchtop centrifuge for 0.5 ml and 1.5 ml tubes

- Micropipettors
- Sterile aerosol-resistant pipette tips
- 0.5 ml tubes and 1.5 ml tubes (PCR-grade)
- 2.0 ml tubes (for pre-dilution of internal control cells)

*For MagNA Pure 96 Instrument*

- 1x Phosphate Buffered Saline (PBS)
- MagNA Pure 96 Internal Control Tube (Roche, Cat no 06374905001)
- MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche, Cat no 06543588001)
- MagNA Pure 96 DNA and Viral NA Large Volume Kit (Roche, Cat no 06374891001)
- MagNA Pure 96 System Fluid (external) (Roche, Cat no 06640729001)
- MagNA Pure 96 Processing Cartridge (Roche, Cat no 06241603001)
- MagNA Pure 96 Pure tip 1000uL (Roche, Cat no 6241620001)
- MagNA Pure 96 Output Plate (Roche, Cat no 06241611001)
- MagNA Pure Sealing Foil (Roche, Cat no 06241638001)

*For MICROLAB STARlet Instrument*

- 1x Phosphate Buffered Saline (PBS)
- STARMag 96 X 4 Universal Cartridge kit (384T) kit (Seegene, Cat No 744300.4.UC384)
- 2.0 ml tubes

*For QIASymphony® SP instrument*

- 1x Phosphate Buffered Saline (PBS)
- Sample Prep Cartridges, 8-well (Qiagen, Cat no 997002)
- 8-Rod Covers (Qiagen, Cat no 997004)
- Filter tips, 200 µl and 1500 µl (Qiagen, Cat no 990332 and 997024)
- 2 ml tubes (Sarstedt, Cat no 72.639 or 72.694)
- 14 ml polystyrene tubes (Corning, Cat no 352051)
- DSP Virus/Pathogen Mini Kit (QIAGEN, Cat no 937036)

*For NucliSENS® easyMAG® instrument*

- 1x Phosphate Buffered Saline (PBS)
- NucliSENS® easyMAG® Lysis Buffer 4X1L (Biomerieux, Cat no 280134)
- NucliSENS® easyMAG® Lysis Buffer 2ML 48T (Biomerieux, Cat no 200292)
- NucliSENS® easyMAG® Magnetic Silica (Biomerieux, Cat no 280133)
- NucliSENS® easyMAG® Extraction buffer 1 (Biomerieux, Cat no 280130)
- NucliSENS® easyMAG® Extraction buffer 2 (Biomerieux, Cat no 280131)
- NucliSENS® easyMAG® Extraction buffer 3 (Biomerieux, Cat no 280132)
- NucliSENS® easyMAG® Disposables (Biomerieux, Cat no 280135)

*For LightCycler® 480 Instrument II and cobas z 480 analyser*

- **PlexPCR®** Colour Compensation (CC) kit (SpeedX, Cat no 90001)



- LightCycler® 480 Multiwell Plate 96 (Roche, Cat no 04729692001)
- LightCycler® 480 Sealing Foil (Roche, Cat no 04729757001)

*For Applied Biosystems® 7500 Fast and 7500 Fast Dx*

- MicroAmp® Optical 96-well reaction plates (ThermoFisher Scientific, Cat no 4316813)
- MicroAmp® Optical Adhesive Film (ThermoFisher Scientific, Cat no 4360954)

*For Bio-Rad CFX96™ Dx and CFX96 Touch™ Real-time PCR Detection System*

- Multiplate™ 96-well PCR plates (Bio-Rad, Cat no MLP9601)
- Microseal® 'B' PCR Plate Sealing Film, adhesive, optical (Bio-Rad, Cat no MSB1001)

*Sample Collection Devices*

- Multi-Collect Specimen Collection Kit (Abbott, Cat no 9K12-01)
- Aptima® Urine Collection Kit (Hologic, Cat no 301040)
- Aptima® unisex swab specimen collection kit (Hologic, Cat no 301041)
- DeltaSwab ViCUM® 2 ml + Standard flocked swab (deltalab, Cat no 304278)
- Vacumed® Urine without preservative (FL medical, Cat no 44950)
- Regular FLOQSwab™ in 1 ml of UTM™ media (Copan Cat no 359C)
- cobas® PCR media (Roche, Cat no 06466281190)

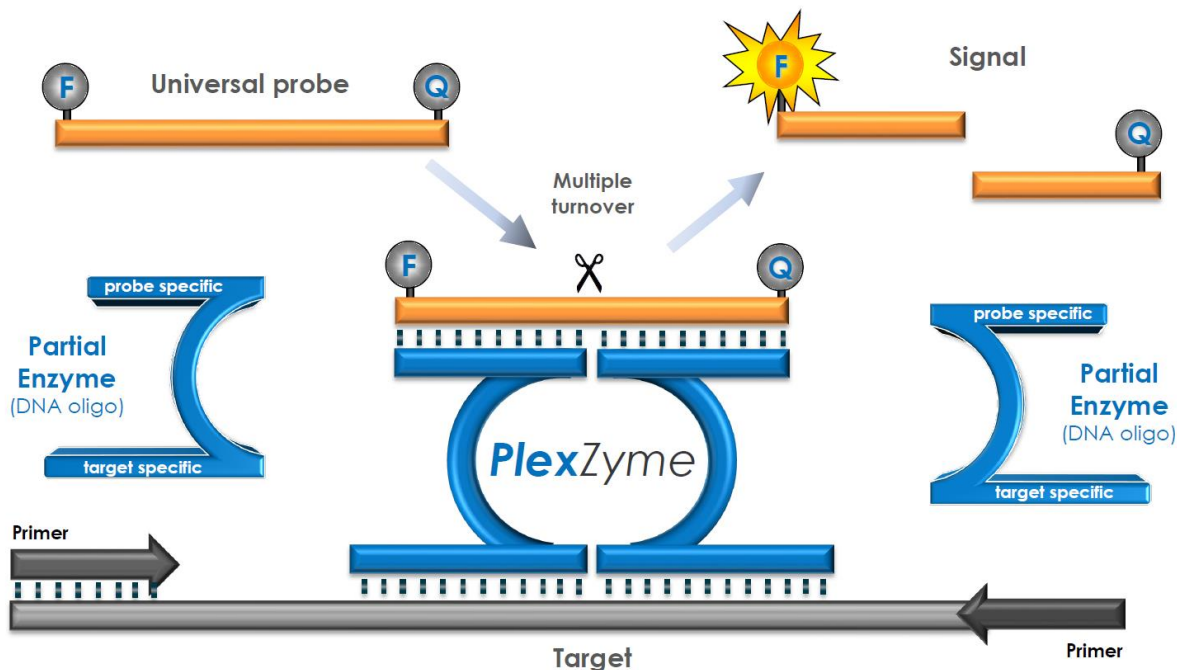
## 8 Principle of the technology

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

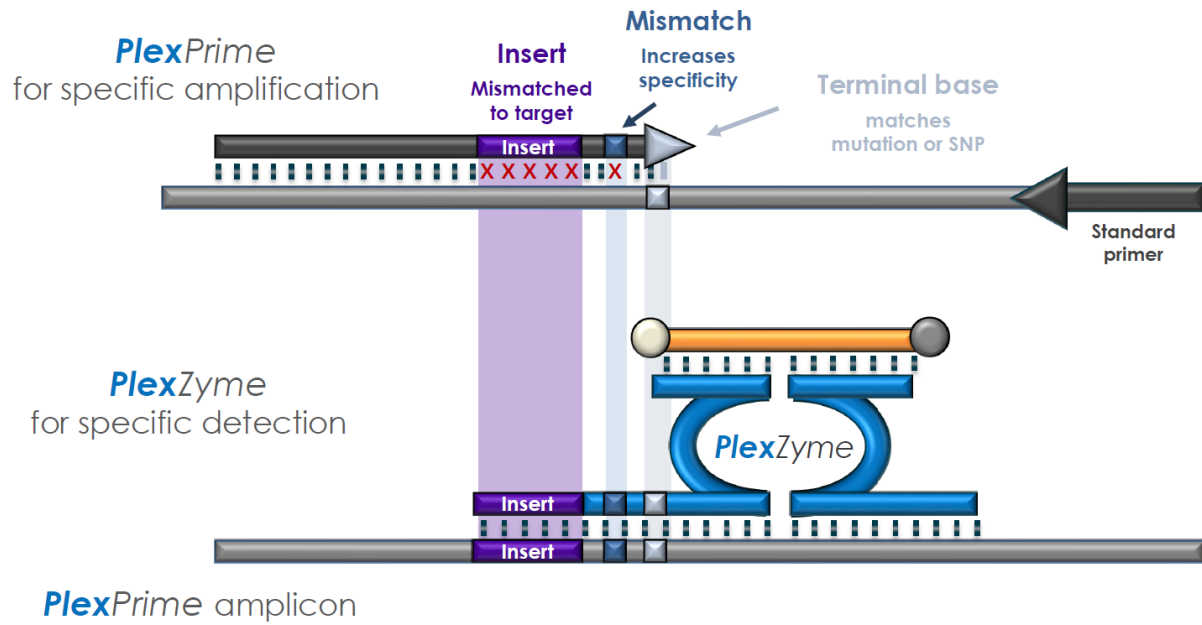
**PlexZyme**<sup>®</sup> enzymes are catalytic DNA complexes composed of two DNA oligos referred to as “Partial Enzymes”. Each Partial Enzyme has a target-specific region, a catalytic core and a universal probe binding region. When the target product is present, the two Partial Enzymes bind adjacently to form the active **PlexZyme**<sup>®</sup> 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. **PlexZyme**<sup>®</sup> enzymes have additional specificity compared to alternate detection technologies, since two Partial Enzymes are required to bind for detection. **PlexZyme**<sup>®</sup> enzymes are also multiple turnover enzymes, and multiple probes can be cleaved during each PCR cycle, resulting in a strong and sensitive signal. **PlexZyme**<sup>®</sup> assays are highly sensitive and specific and are ideally suited for the multiplexed detection of pathogens.

**PlexPrime**<sup>®</sup> 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 **PlexPrime**<sup>®</sup> 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 **PlexZyme**<sup>®</sup> enzyme can be overlapped with the **PlexPrime**<sup>®</sup> primer to target the specific mutant amplicon containing the mutant base and incorporated Insert sequence. The unique combination of **PlexPrime**<sup>®</sup> primers coupled to **PlexZyme**<sup>®</sup> enzymes allows the specific amplification of mutant sequences, and sensitive and specific detection in multiplex.

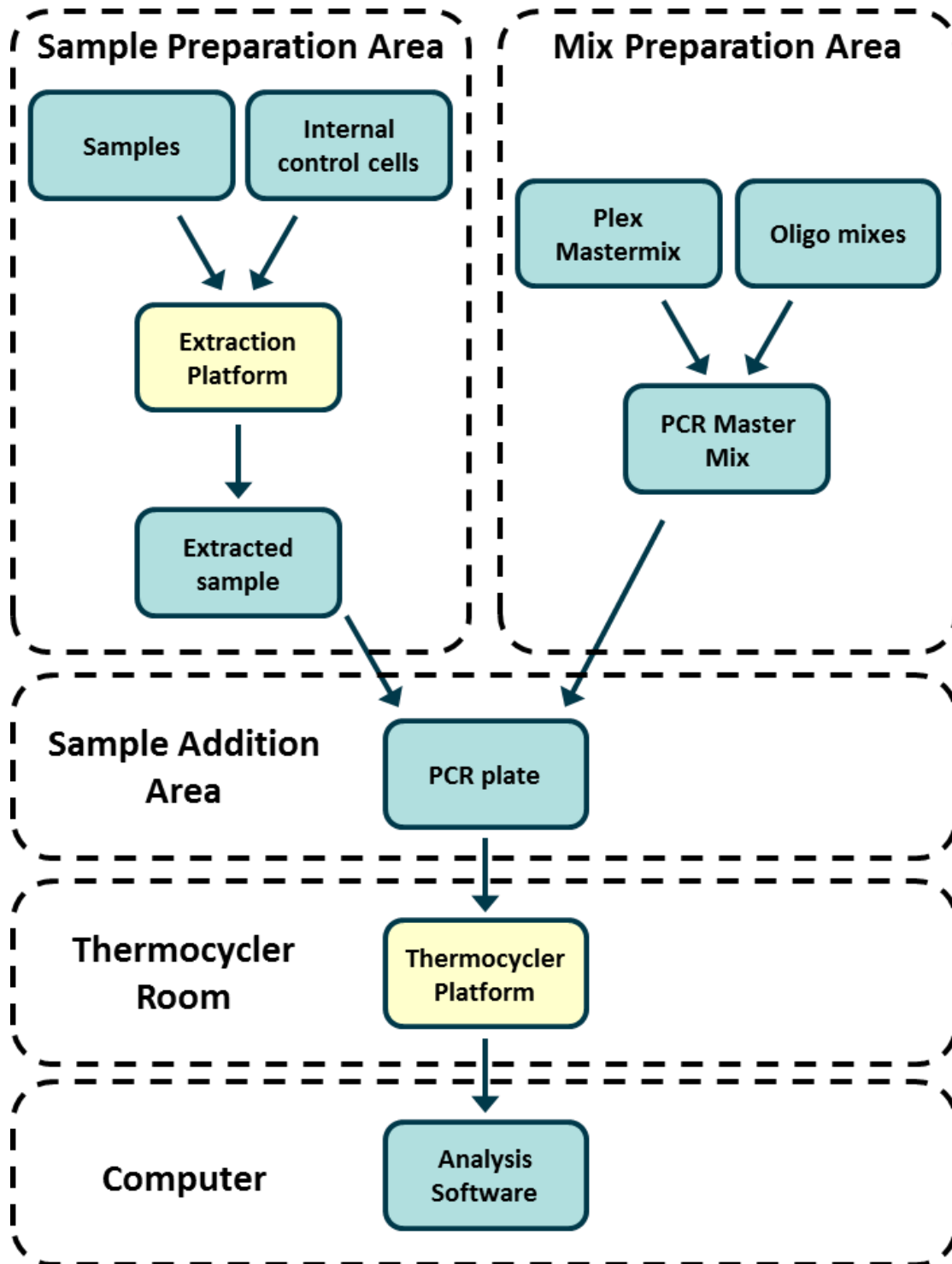
**Figure 1. Schematic representation of *PlexZyme*<sup>®</sup> detection and universal signalling**



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



9 Procedure overview



## 10 Detailed procedure

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

### 10.1 Sample collection, transport and storage

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

#### 10.1.1 Validated sample collection 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 *ResistancePlus*<sup>®</sup>MG 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.

#### 10.1.2 Neat urine collection, transport and storage

1. Use of a clear sterile urine collection cup, free of any preservatives or transport media is recommended for patient self-collection.
2. Patient should collect 20-50ml of first void urine and tightly recap or screw on lid.
3. It is recommended to double bag urine specimen with absorbent pads for transport. Storage temperatures of urine specimen is dependent on the intended processing time.

#### 10.1.3 Dry swab 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.

#### 10.1.4 Multi-Collect Specimen Collection Kit (Abbott, Cat no 9K12-01) collection, transport and storage

Directions are summarized below for the collection and transport of urine, vaginal swabs and male urethral swabs with the multi-Collect Specimen Collection Kit (Abbott, Cat no 9K12-01)

##### 10.1.4.1 Urine specimen collection, transport and storage

1. The patient should not have urinated for at least one hour prior to sample collection.
2. Discard specimen collection swab; it is not required for urine specimen collection.
3. Using a urine specimen collection cup, the patient should collect the first 20 to 30 mL of voided urine (the first part of the stream).
4. Unscrew the transport tube cap, taking care not to spill the transport buffer within.
5. Handle the cap and tube carefully to avoid contamination.
6. Use the plastic transfer pipette to transfer urine from the collection cup into the transport tube until the liquid level in the tube falls within the clear fill window of the transport tube label or else a new specimen should be collected. Do not overfill. Slightly more than one full squeeze of the transfer pipette bulb may be required to transfer the necessary volume of urine specimen.
7. Recap the transport tube carefully. Ensure the cap seals tightly.
8. Label the transport tube with sample identification information, including date of collection using an adhesive label. Take care not to obscure the fill window on the transport tube.
9. After collection, transport and store transport tube at 2°C to 30°C for up to 14 days. If longer storage is needed, store at -10°C or colder for up to 90 days.

##### 10.1.4.2 Vaginal swab specimen collection, transport and storage

1. Discard disposable transfer pipette; it is not required for vaginal swab specimen collection.
2. Remove the sterile swab from the wrapper, taking care not to touch swab tip or lay it down on any surface.

3. Insert the white tip of the specimen collection swab about two inches (5 cm) into the opening of the vagina.
4. Gently rotate the swab for 15 to 30 seconds against the sides of the vagina.
5. Withdraw the swab carefully.
6. Handle the cap and tube carefully to avoid contamination.
7. Unscrew the transport tube cap and immediately place the specimen collection swab into the transport tube so that the white tip is down.
8. Carefully break the swab at the scored line on the shaft; use care to avoid splashing of contents.
9. Recap the transport tube. Ensure the cap seals tightly.
10. Label the transport tube with sample identification information, including date of collection using an adhesive label.
11. After collection, transport and store transport tube at 2°C to 30°C for up to 14 days. If longer storage is needed, store at -10°C or colder for up to 90 days.

#### 10.1.4.3 Male urethral swab specimen collection, transport and storage

1. The patient should not have urinated for at least one hour prior to sample collection.
2. Discard disposable transfer pipette; it is not required for male urethral swab specimen collection.
3. Remove the sterile swab from the wrapper, taking care not to touch swab tip or lay it down on any surface.
4. Insert the white tip of the specimen collection swab 3/4 to 1 1/2 inches (2 to 4 cm) into the urethra.
5. Gently rotate the swab for 2 to 3 seconds to ensure adequate sampling.
6. Withdraw the swab carefully.
7. Handle the cap and tube carefully to avoid contamination.
8. Unscrew the transport tube cap and immediately place the specimen collection swab into the transport tube so that the white tip is down.
9. Carefully break the swab at the scored line on the shaft; use care to avoid splashing of contents.
10. Recap the transport tube. Ensure the cap seals tightly.
11. Label the transport tube with sample identification information, including date of collection using an adhesive label.
12. After collection, transport and store transport tube at 2°C to 30°C for up to 14 days. If longer storage is needed, store at -10°C or colder for up to 90 days.

#### 10.1.5 Aptima® Urine Collection Kit (Hologic, Cat no 301040) collection, transport and storage

Directions are summarized below for the collection and transport of male and female urine specimen with the Aptima® Urine Collection Kit.

1. Use of a clear sterile urine collection cup, free of any preservatives or transport media is recommended for patient self-collection.
2. Patient is directed to provide 20-30 mL of first void urine into provided urine collection cup. Female patients should not cleanse the labial area prior to providing the specimen.
3. Using the pipette and transport tube included in Aptima® Urine Collection Kit, transfer 2mL of urine with the pipette into the uncapped specimen transport tube. Proper urine volume line must fall within the black fill lines on the urine transport tube. Urine must be transferred from the clear sterile urine cup to the Aptima urine specimen tube within 24 hours of collection.
4. Re-cap the urine transport tube tightly.
5. After collection, processed urine specimens in the Aptima urine specimen transport tube should be transported and stored at 2°C to 30°C and store at 2°C to 30°C until tested. Refer to manufacturer's instructions for detailed storage optimization.

#### 10.1.6 Aptima® unisex swab specimen collection kit (Hologic, Cat no 301041) collection, transport and storage

Directions are summarized below for the collection and transport of endocervical swab specimen and male urethral swab with the Aptima® unisex swab specimen collection kit (Hologic, Cat no 301041).

##### 10.1.6.1 Endocervical swab specimen collection, transport and storage

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

#### 10.1.6.2 Male urethral swab specimen collection, transport and storage

1. The patient should not have urinated for at least 1 hour prior to sample collection
2. Insert the specimen collection swab (blue shaft in package with green printing) 2 to 4 cm into the urethra
3. Gently rotate the swab clockwise for 2 to 3 seconds in the urethra to ensure adequate sampling
4. Withdraw the swab carefully
5. Remove the cap from the swab specimen transport tube and immediately place the specimen collection swab into the transport tube
6. Carefully break the swab shaft against the side of the tube at the scoreline and discard the top portion of the swab shaft; use care to avoid splashing of contents
7. Re-cap the swab specimen transport tube tightly. After collection, transport and store the swab in the swab specimen transport tube at 2°C to 30°C until tested.

#### 10.1.7 DeltaSwab ViCUM® 2 ml + Standard flocked swab (deltalab, Cat no 304278) collection, transport and storage

Directions are summarized below for the collection and transport of vaginal, cervical, urethral, pharyngeal and rectal swab specimens with the DeltaSwab ViCUM® 2 ml + Standard flocked swab (deltalab, Cat no 304278).

1. Open peel-pack using both hands pulling opposite sides.
2. Agitate the tube smoothly.
3. Open the flow-pack and collect the sample with the swab.
4. Open the tube with the other hand and place the swab inside so that it is covered with the media.
5. Align the breakpoint of the swab with the top of the tube lightly pressing the swab downwards. Break the swab by the breakpoint by supporting it on the inner edge of the tube.
7. Discard leftover piece of stick, screw cap tightly and shake the sample to elute it into the media.
8. After collection, transport and store the swab in the swab specimen transport tube at 4°C to 25°C until tested.

#### 10.1.8 Vacumed® Urine without preservative (FL medical, Cat no 44950) collection, transport and storage

Directions are summarized below for the collection and transport of male and female urine with the Vacumed® Urine without preservative collection tube (FL medical, Cat no 44950).

1. Open the cap of the urine collection container and lay upside down on a clean surface
2. Do not touch internal surfaces of the container and cap
3. Collect the urine sample. Fill the container up to ¾ of the capacity
4. Replace the cap and turn tightly in a clockwise direction to seal
5. Gently shake the sample
6. Partially raise the protective label (do not remove it completely)
7. Insert the sample tube and apply gentle pressure. Keep the tube connected until it is full (end of flow)
8. Remove the sample tube and fully restick the protective label
9. Store the sample tube at 4°C to 25°C until tested

#### 10.1.9 Regular FLOQSwab™ in 1 ml of UTM™ media (Copan Cat no 359C) collection, transport and storage

Directions are summarized below for the collection and transport of female vaginal swab specimens with the Regular FLOQSwab™ in 1 ml of UTM™ media (Copan Cat no 359C)

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.

#### 10.1.10 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 2°C to 30°C.

#### 10.1.11 Validated sample extracts

Sample extracts validated for use include:

- cobas® x480 (from CT/NG protocol)

See **Section 10.5** for instructions to prepare PCR with Extracted Nucleic-Acids (reflex workflow).

## 10.2 Sample processing

The **ResistancePlus®** MG kit has been validated on the following extraction instruments in **Table 4**.

See **Section 10.3** for instructions to use the Internal Control.

Table 4. Validated extraction protocols				
Instrument	Extraction kit	Sample volume	Protocol	Elution volume
MagNA Pure 96 <sup>a</sup>	MagNA Pure 96 DNA and Viral NA Small Volume Kit	200 µl	Pathogen Universal 200	50 µl or 100 µl
MagNA Pure 96 <sup>a</sup>	MagNA Pure 96 DNA and Viral NA Large Volume Kit	1000 µl <sup>^</sup>	Viral NA Universal LV 1000 3.1	100 µl
MICROLAB STARlet IVD <sup>b</sup>	STARMag 96 x 4 Universal Cartridge kit (Seegene)	300 µl	10 µl diluted Internal Control Cells added per sample Select 'Pause before PCR setup' to perform sample extraction only	100 µl
QIAasymphony SP <sup>c</sup>	DSP Virus/Pathogen Mini Kit	200 µl	Complex200_V6_DSP	110 µl
NucliSENS® easyMAG <sup>d</sup>	NucliSENS® easyMAG® reagents	200 µl swab	Generic 2.01; "On-board" workflow	100 µl
		1000 µl urine	Generic 2.01; "Off-board" workflow	100 µl

<sup>a</sup> See 10.3.1 for how to use the internal control with the MagNA Pure 96

<sup>b</sup> See 10.3.2 for how to use the internal control with the STARlet IVD

<sup>c</sup> See 10.3.3 for how to use the internal control with the QIAasymphony SP

<sup>d</sup> See 10.3.4 for how to use the internal control with the NucliSENS® easyMAG®

<sup>^</sup> Increase sample volume input for specimens collected in media (e.g. Aptima Collection kits)

## 10.3 Internal Control (IC)

The kit includes an internal control to monitor extraction efficiency and qPCR inhibition. The internal control assay is provided as a *Control Mix* (**WHITE**) and *Internal Control Cells* (**RED**). The *Control Mix* is added to the PCR Master Mix ( ). The *Internal Control Cells* contain the internal control DNA template. The *Internal Control Cells* are diluted and processed as below for specific extraction instruments. The internal control DNA template is therefore co-extracted with the sample and co-amplified in the reaction.

### 10.3.1 Internal Control on the MagNA Pure 96

Dilute the *Internal Control Cells* (**RED**) 1 in 200 in 1x PBS (**Table 5**). Adjust volume as required using the same dilution factor (see extraction kit manual for minimum volume for required number of samples). The diluted internal control cells are loaded into the Internal Control Tube on the MagNA Pure 96:

- For the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Pathogen Universal 200 protocol), 20 µl is automatically added to each sample (default).
- For the MagNA Pure 96 DNA and Viral NA Large Volume Kit (Viral NA Universal LV 1000 3.1 protocol), the sample volume is divided and processed in two separate wells of the MagNA Pure 96 Processing Cartridge. A total of 40 µl of diluted internal control cells are automatically added to each sample (20 µl per well of the processing cartridge).

**Note:** Do NOT store diluted Internal Control Cells



**Table 5. Dilution of Internal Control Cells for the MagNA Pure 96 (1 in 200 dilution)**

<i>Internal Control Cells (RED) (μl)</i>	<i>1x PBS (μl)</i>	<i>Total volume (μl)</i>	<i>Volume added to sample (μl)</i>
18	3582	3600	20

### 10.3.2 Internal Control on the MICROLAB STARlet IVD

Dilute the *Internal Control Cells (RED)* 1 in 20 in 1x PBS (**Table 6**). Adjust volume as required using the same dilution factor (see extraction kit manual for minimum volume for required number of samples). The diluted internal control cells are loaded into a 2 ml tube and placed on the reagent support rack, with 10 μl automatically added to each sample.

**Note:** Do NOT store diluted Internal Control Cells

**Table 6. Dilution of Internal Control Cells for the MICROLAB STARlet IVD (1 in 20 dilution)**

<i>Internal Control Cells (RED) (μl)</i>	<i>1x PBS (μl)</i>	<i>Total volume (μl)</i>	<i>Volume added to sample (μl)</i>
50	950	1000	10

### 10.3.3 Internal Control on the QIASymphony® SP

Dilute the *Internal Control Cells (RED)* 1 in 50 in 1x PBS (**Table 7**). Adjust volume as required using the same dilution factor according to the number of samples required.

**Note:** Do NOT store diluted Internal Control Cells

**Table 7. Dilution of Internal Control Cells for the QIASymphony® SP (1 in 50 dilution)**

<i>Internal Control Cells (RED) (μl)</i>	<i>1x PBS (μl)</i>	<i>Total volume (μl)</i>
40	1950	2000

The diluted *Internal Control Cells* are then used to prepare an Internal Control-carrier RNA-Buffer AVE mixture, as shown in **Table 8** below. Adjust volume as required using the same dilution factor for the number of samples required (see extraction kit manual for minimum volume for required number of samples). The Internal Control-carrier RNA-buffer AVE mixture should be prepared immediately before starting the run.

The Internal Control-carrier RNA-buffer AVE mixture is added to a tube, which is placed into a tube carrier and loaded into slot A of the sample drawer in the QIASymphony® SP. 120 μl (default) of the mixture is added to each sample.

**Table 8. Preparation of Internal Control-carrier RNA-buffer AVE mixture for the QIASymphony SP**

<i>Tube type</i>	<i>Number of samples</i>	<i>Volume of diluted IC Cells (μl)</i>	<i>Stock carrier RNA (μl)</i>	<i>Buffer AVE (μl)</i>	<i>Total volume (μl)</i>
-	1	10	3	107	120
2 ml	1 + void volume <sup>^</sup>	40	12	428	480
14 ml	1 + void volume <sup>#</sup>	60	18	642	720

<sup>^</sup> 2 ml tube requires 3 additional samples (360 μl) to account for void volume

<sup>#</sup> 14 ml tube requires 5 additional samples (600 μl) to account for void volume

#### 10.3.4 Internal Control on the easyMAG®

Dilute the *Internal Control Cells* (RED) 1 in 200 in 1x PBS (Table 9). Adjust volume as required using the same dilution factor. Prepare a 'pre-mix' of diluted internal control cells and NucliSENS® easyMAG® Magnetic Silica for the required number of samples (Table 10). 100 µl of pre-mix silica is required per sample.

**Note:** Do NOT store diluted Internal Control Cells

Table 9. Dilution of Internal Control Cells for the NucliSENS® easyMAG® (1 in 200 dilution)			
<i>Internal Control Cells</i> (RED) (µl)	1x PBS (µl)	Total volume (µl)	Dilution factor
10	1990	2000	200

Table 10. Pre-mix of NucliSENS® easyMAG® Magnetic Silica and diluted Internal Control Cells			
Number of samples	Volume of diluted IC Cells (µl)	Volume of Magnetic Silica (µl)	Volume added to sample (µl)
1	50	50	100

"On-board" or "off-board" workflow will be used depending on the specimen type. "Off-board" workflow is used for optimal nucleic acid recovery of urine samples. Refer to the NucliSENS® easyMAG® user manual for more information.

#### "On-board" workflow (swabs)

Transfer specimens into the sample vessel.

Load sample vessels onto the easyMAG.

Program the following Extraction Requests:

Protocol: Generic 2.0.1 (for software version 2.0)

Matrix: Other

Volume (ml): 0.200

Eluate (µl): 100 µl

Type: Primary

After on-board lysis, add 100 µl of pre-mix silica to each sample.

Continue extraction process.

#### "Off-board" workflow (urine)

Briefly spin down NucliSENS Lysis Buffer tube and add 1000 µl urine. Vortex tube.

Let mixture stand at room temperature for 10 mins.

After lysis, transfer lysates to the sample vessels and load onto the easyMAG.

Add 100 µl of pre-mix silica to each sample.

Program the following Extraction Requests:

Protocol: Generic 2.0.1 (for software version 2.0)

Matrix: Other

Volume (ml): 1.000

Eluate (µl): 100 µl

Type: Lysed

Continue extraction process.

#### 10.4 Preparation of real-time PCR

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

Refer to **Table 1 - Table 3** for description of kit contents.

##### 10.4.1 Master Mix preparation

Make up the Master Mix as outlined in .

For a 20 µl reaction volume, 15 µl Master Mix and 5 µl sample is required. Pipette the Master Mix into the PCR plate and then add extracted sample to the reaction.

A no template control (NTC) should be included with each run. For the NTC reaction, add *Nuclease Free Water* (**NEUTRAL**) instead of sample.

Seal plate, centrifuge and transfer to thermocycler.

Table 11. Master Mix		
Reagent	Concentration	Volume per 20 µl reaction (µl)
Nuclease Free Water ( <b>NEUTRAL</b> )	N/A	3.0
<b>Plex</b> Mastermix ( <b>BLUE</b> )	2x	10.0
MG+23S Mix ( <b>BROWN</b> )	20x	1.0
Control Mix* ( <b>WHITE</b> )	20x	1.0
Total volume (µl)		15.0
Add 5 µl sample for a final volume of 20 µl		

\* The Control Mix included in each kit is specific to the PCR instrument used; refer to **Table 1 - Table 3** for correct Control Mix to use

##### 10.4.2 Master Mix stability

The Master Mix can be prepared in bulk and stored at -20°C for up to 4 weeks or stored at 4°C for up to 1 week.

#### 10.5 Preparation of PCR with Extracted Nucleic-Acids (reflex workflow)

Nucleic-acid extracts obtained without the addition of *Internal Control Cells* (**RED**) to samples can be tested using the **ResistancePlus<sup>®</sup>** MG kit.

This procedure should only be followed for extracts that:

Have been tested previously on an alternate assay platform following the manufacturer's instructions for use, and where the previously performed test generated a valid result.

Mastermix should be prepared as per **Section 10.4.1**. In the context of reflex testing, the Internal Control is not present in the sample extract. However, the control mix must be included as described in **Section 10.4.1**.

Refer to **Table 1 - Table 3** for description of kit contents.

Make up the reaction mix as outlined in **Table 11**. For a 20 µl reaction volume, 15 µl Master Mix and 5 µl sample is required. Pipette the Master Mix into the PCR plate and then add extracted sample to the reaction.

A no template control (NTC) should be included with each run. For the NTC reaction, add *Nuclease Free Water* (**NEUTRAL**) instead of sample. Seal plate, centrifuge, and transfer to thermocycler.

## 11 Programming and analysis

Details for programming and analysis are described in the **Section 19 - Section 23**.

The **ResistancePlus**<sup>®</sup> MG kit has three channels for detection of *M. genitalium*, 23S rRNA mutation and Internal Control (**Table 12**).

The **ResistancePlus**<sup>®</sup> MG software is restricted to the analysis of results corresponding to nucleic-acid extracts obtained with the addition of *Internal Control Cells* (**RED**) to samples.

For Nucleic-acid extracts obtained without the addition of *Internal Control Cells* (**RED**) to samples, the REFLEX **ResistancePlus**<sup>®</sup> MG software should be used. The REFLEX **ResistancePlus**<sup>®</sup> MG software has two channels for detection of *M. genitalium* and 23S rRNA mutation (**Table 13**).

*This procedure should only be followed for extracts that:*

*Have been tested previously on an alternate assay platform following the manufacturer's instructions for use, and where the previously performed test generated a valid result.*

Table 12. Channels for <b>ResistancePlus</b> <sup>®</sup> MG targets			
Instrument	Channel A	Channel B	Channel C
	<i>M. genitalium</i> detection (MgPa)	23S rRNA mutation	Internal Control
LC480 II	465-510	533-580	533-640
z 480	465-510	540-580	540-645
7500 Fast and 7500 Fast Dx	FAM	JOE	TAMRA
CFX96 Dx and CFX Touch	FAM	HEX	Quasar 705

Table 13. Channels for <b>ResistancePlus</b> <sup>®</sup> MG targets for reflex workflow		
Instrument	Channel A	Channel B
	<i>M. genitalium</i> detection (MgPa)	23S rRNA mutation
LC480 II	465-510	533-580
z 480	465-510	540-580
7500 Fast and 7500 Fast Dx	FAM	JOE
CFX96 Dx and CFX Touch	FAM	HEX

## 12 Interpretation of results

Data interpretation requires the **ResistancePlus**<sup>®</sup> MG analysis software. While **PlexPrime**<sup>®</sup> primers offer greater specificity than other allele-specific primers, some non-specific amplification from the 23S rRNA mutant assay may be seen in samples that contain high concentrations of *M. genitalium* wild type 23S rRNA. The **ResistancePlus**<sup>®</sup> MG analysis software automates the data interpretation of amplification results and streamlines workflow. Instructions for how to use the analysis software are described in **Section 24**.

See **Table 14** for the appropriate analysis software for each real-time PCR instrument. The analysis software can be supplied on request. Please contact [tech@speedx.com.au](mailto:tech@speedx.com.au) for more information.

Table 14. <b>ResistancePlus</b> <sup>®</sup> MG analysis software		
Cat no	Analysis software*	Real-time PCR instrument
99003	<b>ResistancePlus</b> <sup>®</sup> MG (LC480)	LC480 II
99018	<b>ResistancePlus</b> <sup>®</sup> MG (z 480)	z 480
99002	<b>ResistancePlus</b> <sup>®</sup> MG (7500)	7500 Fast and 7500 Fast Dx
99008	<b>ResistancePlus</b> <sup>®</sup> MG (CFX)	CFX96 Dx and CFX96 Touch

99023	REFLEX <i>ResistancePlus</i> <sup>®</sup> MG (LC480)	LC480 II
99024	REFLEX <i>ResistancePlus</i> <sup>®</sup> MG (z480)	z 480
99026	REFLEX <i>ResistancePlus</i> <sup>®</sup> MG (7500)	7500 Fast and 7500 Fast Dx
99025	REFLEX <i>ResistancePlus</i> <sup>®</sup> MG (CFX)	CFX96 Dx and CFX96 Touch

\* Refer to the website <https://plexpcr.com/products/sexually-transmitted-infections/resistanceplus-mg/#resources> to ensure you are using the most current version of analysis software

### 13 Limitations

- The *ResistancePlus*<sup>®</sup> MG assay targets the *MgPa* gene for *M. genitalium* and mutations at positions 2058 and 2059 in the 23S rRNA gene (A2058G, A2059G, A2058T, A2058C, A2059C, *E. coli* numbering) that are associated with resistance to azithromycin (macrolide-based antibiotic).
- The *ResistancePlus*<sup>®</sup> MG 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 *ResistancePlus*<sup>®</sup> MG assay is a qualitative assay and does not provide quantitative values or information about organism load.
- 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.

## 14 Quality control

The **ResistancePlus**<sup>®</sup> MG kit includes an internal control to monitor extraction efficiency and qPCR inhibition (**Section 10.3**).

When performing reflex testing, the **ResistancePlus**<sup>®</sup> MG kit internal control cells have not been added in the extraction process. Reflex testing can only be performed on samples previously found valid with another system, ensuring extraction efficiency and qPCR inhibition had been monitored.

The **ResistancePlus**<sup>®</sup> MG Positive Control kit (Cat no 95001) is recommended as positive control material for nucleic acid amplification. Refer to **Section 15** for instructions to use the **ResistancePlus**<sup>®</sup> MG Positive Controls. A known negative specimen is recommended to be used as a negative control.

## 15 ResistancePlus<sup>®</sup> MG Positive Control instructions

The **ResistancePlus**<sup>®</sup> MG Positive Control kit contains positive control material for *M. genitalium* 23S rRNA mutants and a *M. genitalium* wild type 23S rRNA (**Table 15**).

Table 15. Contents for <b>ResistancePlus</b> <sup>®</sup> MG Positive Control kit (Cat no 95001)			
Cap colour	Contents	Description	Quantity (10 reactions)
Neutral	MG, 23S rRNA wild type	Positive control template for the detection of <i>M. genitalium</i> , 23S rRNA wild type	1 x 50 µl
Green	MG, 23S rRNA A2058G	Positive control template for the detection of <i>M. genitalium</i> , 23S rRNA A2058G mutation	1 x 50 µl
Red	MG, 23S rRNA A2059G	Positive control template for the detection of <i>M. genitalium</i> , 23S rRNA A2059G mutation	1 x 50 µl
Blue	MG, 23S rRNA A2058T	Positive control template for the detection of <i>M. genitalium</i> , 23S rRNA A2058T mutation	1 x 50 µl
Yellow	MG, 23S rRNA A2058C	Positive control template for the detection of <i>M. genitalium</i> , 23S rRNA A2058C mutation	1 x 50 µl
Purple	MG, 23S rRNA A2059C	Positive control template for the detection of <i>M. genitalium</i> , 23S rRNA A2059C mutation	1 x 50 µl

### 15.1 Instructions for use

Prepare qPCR reactions as described in **Section 10.4** using positive control as sample.

Data interpretation requires the **ResistancePlus**<sup>®</sup> MG analysis software, refer to **Section 24.11** for example results.

## 16 Performance characteristics

### 16.1 Clinical performance

#### 16.1.1 Clinical Study 1

A prospective-retrospective clinical study was conducted at Royal Women's Hospital (RWH), Melbourne, Australia. Samples were collected from May 2016-June 2016 and based on the clinical laboratory results, 111 *M. genitalium* positive and 100 consecutive *M. genitalium* negative samples were selected for inclusion in the study. The 211 samples consisted of 84 urine, 7 anal swabs, 1 urogenital swab (no site specified (nss)), 1 rectal swab, and 1 urethral swab from men, and 33 urine, 33 cervical swabs, 16 endocervical swabs, 14 vaginal swabs, 13 high vaginal swabs, and 8 urogenital swabs (nss) from women. To determine performance of the **ResistancePlus**<sup>®</sup> MG kit, *M. genitalium* detection was compared to the clinical laboratory results from a well-established 16S rRNA qPCR used for routine diagnostics at RWH<sup>7</sup>, and 23S rRNA mutant detection was compared to Sanger sequencing<sup>8</sup>. The **ResistancePlus**<sup>®</sup> MG kit was performed on the LC480 II, after sample extraction on the MagNA Pure 96 Instrument using the MagNA Pure 96 DNA and Viral NA Small Volume Kit using the Universal Pathogen 200 protocol. For *M. genitalium* detection, a composite reference was used for discordant samples using a third qPCR reaction targeting the MgPa gene<sup>9</sup>. For 23S rRNA mutant detection, Sanger sequencing was taken as the true result. Resolved results and sensitivity and specificity of the **ResistancePlus**<sup>®</sup> MG kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 16**. Two specimens were excluded as the Internal Control result was invalid (1 female urine and 1 male urine). 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 17**. The 23S rRNA mutation analysis is shown in **Table 18**.

		<i>M. genitalium</i> detection 16S rRNA qPCR		23S rRNA mutant detection Sequencing		
		Positive	Negative	Mutant	Wild type	
<i>ResistancePlus</i> <sup>®</sup> MG	Positive	106	0	Mutant detected	68	2
	Negative	4	99 <sup>^</sup>	Mutant not detected	2	31
<b>Sensitivity</b>		96.4% (95% CI 91.0-99.0%)		<b>Sensitivity</b>		97.1% (95% CI 90.1-99.7%)
<b>Specificity</b>		100.0% (95% CI 96.3-100.0%)		<b>Specificity</b>		93.8% (95% CI 79.2-99.2%)

95% CI – 95% confidence interval; Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, and A2059C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

<sup>^</sup> The **ResistancePlus**<sup>®</sup> MG kit detected 1 true *M. genitalium* negative using composite reference, table represents resolved results

Table 17. Clinical result analysis in accordance to specimen <sup>a</sup> (Clinical Study 1)			
Specimen	Expected <i>M. genitalium</i> negative	Expected <i>M. genitalium</i> 23S rRNA wild type	Expected <i>M. genitalium</i> 23S rRNA mutant
Male urine	28/28	8/10 <sup>1</sup>	41/42 <sup>1</sup>
Female urine	12/13	11/11	4/6 <sup>2</sup>
Cervical swab	21/21	5/5	7/7 <sup>3</sup>
Endocervical swab	10/10	3/3	3/3 <sup>4</sup>
Vaginal swab	8/8	1/1	2/2 <sup>5</sup>
High vaginal swab	9/9	1/1	4/4 <sup>6</sup>
Male anal swab	3/3	0/0	5/5 <sup>7</sup>
Female swab (nss)	5/5	2/2	1/1 <sup>8</sup>
Male swab (nss)	0/0	0/0	1/1 <sup>9</sup>
Male rectal swab	1/1	0/0	0/0
Male urethral swab	1/1	0/0	0/0

Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, and A2059C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

<sup>a</sup> 2 female urine, 3 male urine, 1 vaginal swab excluded as sequencing failed and mutant status could not be determined

<sup>1</sup> Male urine: 2 *M. genitalium* wildtype miscalled as *M. genitalium* mutant detected, 18 A2058G, 20 A2059G, 3 A2058T correctly detected; 1 A2058G miscalled as *M. genitalium* not detected

<sup>2</sup> Female urine: 1 A2058G, 3 A2059G correctly detected; 2 A2059G miscalled as *M. genitalium* detected, mutant not detected

<sup>3</sup> Cervical swab: 1 A2058G, 6 A2059G correctly detected

<sup>4</sup> Endocervical swab: 2 A2059G, 1 A2058T correctly detected

<sup>5</sup> Vaginal swab: 3 A2058G, 1 A2059G correctly detected

<sup>6</sup> High vaginal swab: 2 A2059G correctly detected

<sup>7</sup> Male anal swab: 1 A2058G, 3 A2059G, 1 A2058T correctly detected.

<sup>8</sup> Female swab (no site specified (nss)): 1 A2059G correctly detected

<sup>9</sup> Male swab (nss): 1 A2059G correctly detected

Table 18. <i>M. genitalium</i> 23S rRNA mutation analysis (Clinical Study 1)	
Reference result <sup>a</sup>	ResistancePlus <sup>®</sup> MG result
Wild type	31/33 <sup>1</sup>
A2058G	24/25 <sup>2</sup>
A2059G	39/41 <sup>3</sup>
A2058T	5/5

<sup>a</sup> For *M. genitalium* positive samples only

<sup>1</sup> Wild type: 2 Male urine miscalled as *M. genitalium* mutant detected

<sup>2</sup> A2058G: 1 Male urine miscalled as *M. genitalium* not detected

<sup>3</sup> A2059G: 2 Female urine miscalled as *M. genitalium* mutant not detected



## 16.1.2 Clinical Study 2

A subset of the extracted specimens from study 1 were run on the 7500 Fast. Results were compared to the clinical result from the 16S rRNA qPCR (Twin 2011) and Sanger sequencing (Twin 2012). Discordant samples for *M. genitalium* detection were re-tested with the 16S rRNA qPCR (Twin 2011) due to suspected sample degradation. Resolved results and sensitivity and specificity of the **ResistancePlus<sup>®</sup> MG<sub>(550)</sub>** kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 19**. Analysis of 23S rRNA mutation detection only includes samples where the mutant status could be determined.

Table 19. Clinical evaluation of the <b>ResistancePlus<sup>®</sup> MG<sub>(550)</sub></b> kit (Clinical Study 2)						
		<i>M. genitalium</i> detection 16S rRNA qPCR		23S rRNA mutant detection Sequencing		
		Positive	Negative	Mutant	Wild type	
<b>ResistancePlus<sup>®</sup> MG</b>	Positive	99	0 <sup>^</sup>	Mutant detected	62	0
	Negative	2	81 <sup>#</sup>	Mutant not detected	5	30
Sensitivity		98.0% (95% CI 93.0-99.8%)		Sensitivity		92.5% (95% CI 83.4-97.5%)
Specificity		100.0% (95% CI 95.6-100.0%)		Specificity		100.0% (95% CI 88.4-100.0%)

95% CI – 95% confidence interval; Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, and A2059C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

<sup>^</sup> The **ResistancePlus<sup>®</sup> MG<sub>(550)</sub>** kit detected 1 true *M. genitalium* positive using reference test, table represents resolved results

<sup>#</sup> The **ResistancePlus<sup>®</sup> MG<sub>(550)</sub>** kit detected 10 samples true *M. genitalium* negatives using reference test, table represents resolved results

## 16.1.3 Clinical Study 3

A retrospective clinical study was conducted at Canterbury Health Laboratories (CHL), Christchurch, New Zealand on characterised, archived samples from 2010-2016, consisting of 103 *M. genitalium* positive and 61 *M. genitalium* negative samples, collected with the multi-Collect Specimen Collection Kit (Abbott). The 164 samples consisted of 110 urine and 4 rectal swabs from men, and 11 urine, 17 cervical swabs, 15 vaginal swabs, 1 urethral swab, 1 urethral/vaginal swab, 1 vaginal/cervical swab and 4 samples from unknown sites from women. To determine performance of the **ResistancePlus<sup>®</sup> MG** kit, *M. genitalium* detection was compared to the clinical laboratory result from a well-established MgPa qPCR, which is also used for routine diagnostics at CHL (Jensen 2004), and 23S rRNA mutant detection was compared to Sanger sequencing (Jensen 2008). The **ResistancePlus<sup>®</sup> MG** kit was performed on the LC480 II, after sample extraction on the MagNA Pure 96 Instrument using the MagNA Pure 96 DNA and Viral NA Small Volume Kit using the Universal Pathogen 200 protocol. For *M. genitalium* detection, the routine MgPa test was repeated for discordant samples. For 23S rRNA mutant detection, Sanger sequencing was taken as the true result. The sensitivity and specificity of the **ResistancePlus<sup>®</sup> MG** kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 20**. Five samples were excluded as the Internal Control result was invalid. 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 21**. The 23S rRNA mutation analysis is shown in **Table 22**.

Table 20. Clinical evaluation of the <b>ResistancePlus<sup>®</sup> MG</b> kit (Clinical Study 3)						
		<i>M. genitalium</i> detection 16S rRNA qPCR		23S rRNA mutant detection Sequencing		
		Positive	Negative	Mutant	Wild type	
<b>ResistancePlus<sup>®</sup> MG</b>	Positive	90	0	Mutant detected	61	1
	Negative	7	67 <sup>^</sup>	Mutant not detected	6	22
Sensitivity		92.8% (95% CI 85.7-97.1%)		Sensitivity		91.0% (95% CI 81.5-96.6%)
Specificity		100.0% (95% CI 94.6-100.0%)		Specificity		95.6% (95% CI 79.7-99.9%)

95% CI – 95% confidence interval; Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, and A2059C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

<sup>^</sup> The **ResistancePlus<sup>®</sup> MG** kit detected 7 true *M. genitalium* negatives, table represents resolved results

**Table 21. Clinical result analysis in accordance to specimen (Clinical Study 3)**

Specimen	Expected <i>M. genitalium</i> negative	Expected <i>M. genitalium</i> wild type	Expected <i>M. genitalium</i> 23S rRNA mutant
Male urine	45/45	17/18 <sup>1</sup>	38/47 <sup>1</sup>
Female urine	4/4	1/1	6/6 <sup>2</sup>
Cervical swab	5/5	3/3	8/9 <sup>3</sup>
Vaginal swab	6/6	1/1	8/8 <sup>4</sup>
Male rectal swab	3/3	0/0	0/1 <sup>5</sup>
Female (unknown site)	1/1	1/1	1/2 <sup>6</sup>
Female urethral swab	1/1	0/0	0/0
Urethral/vaginal swab	1/1	0/0	0/0
Vaginal/cervical swab	1/1	0/0	0/0

Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, and A2059C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

<sup>1</sup> Male urine: 1 *M. genitalium* wild type miscalled as *M. genitalium* mutant detected, 4 A2058G, 32 A2059G, 1 A2058T, 1 A2058C, 1 A2059C, correctly detected; 1 A2058G, 1 A2059G and 1 A2059C miscalled as *M. genitalium* not detected, 3 A2058G and 2 A2059G miscalled as *M. genitalium* mutant not detected

<sup>2</sup> Female urine: 2 A2058G, 4 A2059G correctly detected

<sup>3</sup> Cervical swab: 3 A2058G, 4 A2059G, 1 A2058C correctly detected; 1 A2059G miscalled as *M. genitalium* not detected

<sup>4</sup> Vaginal swab: 1 A2058G, 7 A2059G correctly detected

<sup>5</sup> Male rectal swab: 1 A2059G miscalled as *M. genitalium* not detected

<sup>6</sup> Female (unknown site): 1 A2059G correctly detected; 1 A2059G miscalled as *M. genitalium* mutant not detected

**Table 22. *M. genitalium* 23S rRNA mutation analysis (Clinical Study 3)**

Reference result <sup>^</sup>	ResistancePlus <sup>®</sup> MG result
Wild type	22/23 <sup>1</sup>
A2058G	10/13 <sup>2</sup>
A2059G	47/50 <sup>3</sup>
A2058T	1/1
A2058C	2/2
A2059C	1/1

<sup>^</sup> For *M. genitalium* positive samples only

<sup>1</sup> Wild type: 1 Male urine miscalled as *M. genitalium* mutant detected

<sup>2</sup> A2058G: 3 Male urine miscalled as *M. genitalium* mutant not detected

<sup>3</sup> A2059G: 2 Male urine miscalled as *M. genitalium* mutant not detected, 1 Female sample (unknown site) miscalled as *M. genitalium* mutant not detected

## 16.1.4 Clinical Study 4

A retrospective clinical study was performed at Vall d'Hebron University Hospital (HUVH), Barcelona, Spain, to evaluate the performance of the **ResistancePlus<sup>®</sup> MG<sub>(675)</sub>** kit for the detection of *M. genitalium* and azithromycin resistance-associated mutations in retrospective samples collected between December 2017-April 2018. Samples included 92 *M. genitalium* positive and 108 consecutive *M. genitalium* negative specimens, collected using the DeltaSwab ViCUM<sup>®</sup> (Deltalab, Spain) for swabs or Vacumed<sup>®</sup> Urine (FL medical, Italy) for male and female urine. The 200 samples consisted of 46 urine, 30 vaginal swabs, 30 urethral swabs, 40 cervical swabs, 8 pharyngeal swabs and 46 rectal swabs. Samples were extracted with the STARlet IVD (Hamilton) and run on the CFX96 Dx (Bio-Rad) instrument. To assess the performance, *M. genitalium* detection was compared to Allplex<sup>™</sup> STI Essential (Seegene) as well as to the **ResistancePlus<sup>®</sup> MG kit (SpeedX)** on the LC480 II for both *M. genitalium* detection and 23S rRNA status. The sensitivity and specificity of the **ResistancePlus<sup>®</sup> MG<sub>(675)</sub>** kit for *M. genitalium* detection compared to Allplex<sup>™</sup> STI Essential (Seegene) is shown in **Table 23**. The sensitivity and specificity of the **ResistancePlus<sup>®</sup> MG<sub>(675)</sub>** kit for *M. genitalium* detection was 100.0% (95% CI 95.9-100.0%) and 97.4% (95% CI 92.4-99.5%), respectively and for 23S rRNA mutant detection was as shown in **Table 24**. Analysis of results in accordance to specimen type is shown in **Table 25**.

Table 23. Comparison of <b>ResistancePlus<sup>®</sup> MG<sub>(675)</sub></b> kit with Allplex <sup>™</sup> STI essential (Clinical Study 4)			
		<i>M. genitalium</i> detection Allplex <sup>™</sup> STI Essential	
		Positive	Negative
<b>ResistancePlus<sup>®</sup> MG<sub>(675)</sub></b>	Positive	89	1
	Negative	3	107
Sensitivity		96.7% (95% CI 90.8-99.3%)	
Specificity		99.1% (95% CI 94.95-100.0%)	

Table 24. Clinical evaluation of the <b>ResistancePlus<sup>®</sup> MG<sub>(675)</sub></b> kit (Clinical Study 4)						
		<i>M. genitalium</i> detection <b>ResistancePlus<sup>®</sup> MG (LC480 II)</b>		23S rRNA mutant detection <sup>#</sup> <b>ResistancePlus<sup>®</sup> MG (LC480 II)</b>		
		Positive	Negative	Mutant detected	Mutant not detected	
<b>ResistancePlus<sup>®</sup> MG<sub>(675)</sub></b>	Positive	87	3	Mutant detected	42 <sup>^</sup>	0
	Negative	0	110	Mutant not detected	2	42 <sup>*</sup>
Sensitivity		100.0% (95% CI 95.9-100.0%)		Sensitivity		95.5% (95% CI 84.5-99.4%)
Specificity		97.4% (95% CI 92.4-99.5%)		Specificity		100.0% (95% CI 91.6-100.0%)

95% CI – 95% confidence interval; Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, and A2059C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

<sup>^</sup> The **ResistancePlus<sup>®</sup> MG<sub>(675)</sub>** kit detected 1 true *M. genitalium* mutant using reference test, table represents resolved results

<sup>\*</sup> The **ResistancePlus<sup>®</sup> MG<sub>(675)</sub>** kit detected 1 true *M. genitalium* negative using reference test, table represents resolved results

<sup>#</sup> 1 sample excluded from analysis as this was sequenced as mixed wild-type and mutant

Table 25. Clinical result analysis in accordance to specimen (Clinical Study 4)

Specimen	Expected <i>M. genitalium</i> negative	Expected <i>M. genitalium</i> 23S rRNA wild type	Expected <i>M. genitalium</i> 23S rRNA mutant
Male urine	26/26	5/5	15/15
Male urethral swab	15/15	3/3	11/12 <sup>1</sup>
Female cervical swab	16/16	11/11	2/3 <sup>3</sup>
Female vaginal swab	20/20	15/15	5/5
Male rectal swab	19/22 <sup>1</sup>	5/5	8/8
Female rectal swab	7/7	3/3	0/0
Male pharyngeal swab	5/5	0/0	1/1
Female pharyngeal swab	2/2	0/0	0/0

<sup>1</sup> Male rectal swab: 3 *M. genitalium* negative miscalled as *M. genitalium* positive

<sup>2</sup> Male urethral swab: 1 *M. genitalium* 23S rRNA mutation positive miscalled as *M. genitalium* 23S rRNA mutation negative

<sup>3</sup> Female cervical swab: 1 *M. genitalium* 23S rRNA mutation positive miscalled as *M. genitalium* 23S rRNA mutation negative

#### 16.1.5 Clinical Study 5

A retrospective clinical study was conducted at Royal Women's Hospital (RWH), Melbourne, Australia using Aptima<sup>®</sup> collected urine and swabs from June 2017-November 2017. Matched patient specimens consisted of 98 *M. genitalium* positive and 87 consecutive *M. genitalium* negative, collected as neat urine (routine sample) or with the Aptima<sup>®</sup> Urine Specimen Collection kit (Hologic), or as dry swab (routine sample) or with the Aptima<sup>®</sup> Unisex Swab Specimen Collection kit (Hologic). The 185 samples consisted of 122 urine, 18 rectal swabs, 15 cervical swabs, and 25 vaginal swabs. To determine the performance of Aptima<sup>®</sup> collected samples with the **ResistancePlus<sup>®</sup>** MG kit, *M. genitalium* and 23S rRNA mutant detection was compared to the clinical diagnostic results obtained from the **ResistancePlus<sup>®</sup>** MG kit (SpeedX) using the routine sample. Testing of Aptima<sup>®</sup> collected samples was performed on the LC480 II, after sample extraction on the MagNA Pure 96 Instrument using the MagNA Pure 96 DNA and Viral NA Small Volume Kit using the Viral NA Universal LV 1000 protocol. Clinical diagnostic results from RWH, obtained from a matched diagnostic sample tested with the **ResistancePlus<sup>®</sup>** MG kit (SpeedX), was taken as the true result for *M. genitalium*. For the 23S rRNA mutant detection, the result was compared to the diagnostic result and Sanger sequencing.

The sensitivity and specificity of the **ResistancePlus<sup>®</sup>** MG kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 26**. 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 27**.

Table 26. Clinical evaluation of the **ResistancePlus<sup>®</sup>** MG kit (Clinical Study 5)

		<i>M. genitalium</i> detection <b>ResistancePlus<sup>®</sup></b> MG (routine sample)		23S rRNA mutant detection <b>ResistancePlus<sup>®</sup></b> MG (routine sample)		
		Positive	Negative	Mutant	Wild type	
<b>ResistancePlus<sup>®</sup></b> MG (with 1ml Aptima sample)	Positive	94	3	Mutant detected	65	0
	Negative	4	84	Mutant not detected	1*	28
<b>Sensitivity</b>		95.9% (95% CI 89.9-98.9%)		<b>Sensitivity</b>		98.5% (95% CI 91.8-100.0%)
<b>Specificity</b>		96.6% (95% CI 90.3-99.3%)		<b>Specificity</b>		100.0% (95% CI 87.7-100.0%)

\* Sample could not be sequenced

Table 27. Clinical result analysis in accordance to specimen type (Clinical Study 5)			
Specimen	Expected <i>M. genitalium</i> negative	Expected <i>M. genitalium</i> wild type	Expected <i>M. genitalium</i> 23S rRNA mutant
Urine	50/52 <sup>1</sup>	21/22 <sup>1</sup>	45/48 <sup>1</sup>
Cervical swab	11/11	1/1	3/3
Vaginal swab	14/15 <sup>2</sup>	3/4 <sup>2</sup>	6/6
Rectal swab	9/9	3/3	5/6 <sup>3</sup>
Anal swab	0/0	0/0	5/5

Mutant – 23S rRNA mutation in A2058G, A2059G, A2058T, A2058C, A2059C positions (*E.coli* numbering); Wild type – absence of mutation in these positions

<sup>1</sup> Urine: 2 *M. genitalium* negatives miscalled as *M. genitalium* wild type and mutant respectively; 1 *M. genitalium* wild type miscalled as *M. genitalium* negative; 2 *M. genitalium* mutants miscalled as *M. genitalium* wild type, 1 *M. genitalium* mutant miscalled as *M. genitalium* negative

<sup>2</sup> Vaginal swab: 1 *M. genitalium* negative miscalled as *M. genitalium* wild type; 1 *M. genitalium* wild type miscalled as *M. genitalium* negative

<sup>3</sup> Rectal swab: 1 *M. genitalium* mutant miscalled as *M. genitalium* negative

#### 16.1.6 Clinical Study 6

A retrospective clinical study was conducted at University of Queensland Centre for Clinical Research (UQCCR), Australia, using cobas<sup>®</sup> x480 extracts from urine and swab samples collected from February 2017-February 2019. Specimens consisted of 85 *M. genitalium* positive and 84 *M. genitalium* negative extracts, originally collected as neat urine or with the cobas<sup>®</sup> PCR media collection kit (Roche) and extracted on the cobas<sup>®</sup> x480 (cobas<sup>®</sup> 4800, Roche) instrument using the “Full Workflow” and “CT/NG” protocol, without addition of SpeedX Internal Control Cells. The 169 extracts consisted of 28 rectal swabs, 13 vaginal swabs, 5 high vaginal swabs, 15 cervical swabs, 1 ectocervical swab, 5 urethral swabs, 5 pharyngeal swabs, 1 penile swab, 1 penile meatal, 1 mouth swab, as well as 83 male and 11 female urine specimens.

To determine the performance of cobas<sup>®</sup> extracts with the **ResistancePlus<sup>®</sup> MG<sub>(550)</sub>** kit, *M. genitalium* detection was compared to the routine diagnostic result (MgPa PCR assay (Trembizki *et al.*, 2017)) and 23S rRNA mutant detection was compared to Sanger sequencing. The **ResistancePlus<sup>®</sup> MG<sub>(550)</sub>** kit was performed on the ABI 7500 Fast Dx. The sensitivity and specificity of the **ResistancePlus<sup>®</sup> MG<sub>(550)</sub>** kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 28**. 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 29**. The 23S rRNA mutation analysis is shown in **Table 30**.

Table 28. Clinical evaluation of the <b>ResistancePlus<sup>®</sup> MG<sub>(550)</sub></b> kit (Clinical Study 6)						
		<i>M. genitalium</i> detection MgPa qPCR		23S rRNA mutant detection Sanger Sequencing		
		Positive	Negative	Mutant	Wild type	
<b>ResistancePlus<sup>®</sup> MG<sub>(550)</sub></b>	Positive	80	0	Mutant detected	49 <sup>^</sup>	0
	Negative	5	84	Mutant not detected	0	25
<b>Sensitivity</b>		94.1% (95% CI 86.8-98.1%)		<b>Sensitivity</b>		100.0% (95% CI 92.8-100.0%)
<b>Specificity</b>		100.0% (95% CI 95.7-100.0%)		<b>Specificity</b>		100.0% (95% CI 86.3-100.0%)

<sup>^</sup> 1 vaginal sample returned a mixed wild-type/A2059G sequencing result which was correctly identified as mutant by the **ResistancePlus<sup>®</sup> MG<sub>(550)</sub>** assay

Table 29. Clinical result analysis in accordance to specimen (Clinical Study 6) <sup>*</sup>			
Specimen	Expected <i>M. genitalium</i> negative	Expected <i>M. genitalium</i> 23S rRNA wild type	Expected <i>M. genitalium</i> 23S rRNA mutant
Male urine	42/42	13/13	26/27 <sup>1</sup>
Female urine	6/6	1/1	3/3 <sup>2</sup>
Cervical swab	5/5	6/6	2/2 <sup>3</sup>
Ectocervical swab	1/1	-	-
Vaginal swab	1/1	1/2	7/7 <sup>4</sup> <sup>^</sup>
High vaginal swab	2/2	2/2	1/1 <sup>5</sup>
Male rectal swab	17/17	1/1	7/8 <sup>6</sup>
Female rectal swab	1/1	-	-
Male urethral swab	3/3	-	2/2 <sup>7</sup>
Male pharyngeal	5/5	-	-
Penile swab	-	1/1	-
Penile meatal swab	-	-	1/1 <sup>8</sup>
Male mouth swab	1/1	-	-

<sup>\*</sup> 6 samples were excluded as sequencing failed and true 23S status could not be determined, including: 2 cervical, 2 urine, 1 vaginal and 1 rectal sample

<sup>1</sup> Male urine: 8 A2058G, 3 A2058T and 15 A2059G correctly identified; 1 A2058T was incorrectly identified as *M. genitalium* not detected

<sup>2</sup> Female urine: 2 A2058G and 1 A2059G correctly identified

<sup>3</sup> Cervical swab: 2 A2058G correctly identified

<sup>4</sup> Vaginal swab: 3 A2058G, 2 A2058T and 1 A2059G correctly identified; <sup>^</sup> 1 vaginal swab was identified as a mixture WT/A2059G

<sup>5</sup> High vaginal swab: 1 A2059G correctly identified

<sup>6</sup> Male rectal swab: 5 A2059G, 1 A2058T and 1 A2058G correctly identified; 1 A2058G was incorrectly identified as *M. genitalium* not detected

<sup>7</sup> Male urethral swab: 2 A2059G correctly identified

<sup>8</sup> Penile meatal swab: 1 A2059G correctly identified

**Table 30. *M. genitalium* 23S rRNA mutation analysis (Clinical Study 6)**

Reference result <sup>^</sup>	<i>ResistancePlus</i> <sup>®</sup> MG result
Wild type	25/26 <sup>1</sup>
A2058G	16/17 <sup>2</sup>
A2059G	27/27 <sup>3</sup>
A2058T	6/7 <sup>4</sup>
A2058C	-
A2059C	-

<sup>^</sup> For *M. genitalium* positive samples only

<sup>1</sup> Wild type: 1 vaginal swab miscalled as *M. genitalium* not detected

<sup>2</sup> A2058G: 1 rectal swab miscalled as *M. genitalium* not detected

<sup>3</sup> A2059G: 1 vaginal swab mixed wild-type/A2059G correctly identified as *M. genitalium*, 23S mutation detected

<sup>4</sup> A2058T: 1 male urine swab miscalled as *M. genitalium* not detected

#### 16.1.7 Clinical Study 7

A retrospective clinical study was conducted at Microbiological Diagnostic Unit Public Health Unit (MDU), Victoria, Australia, using dry swabs and neat urine collected from October 2018-January 2019. Specimens consisted of 59 *M. genitalium* positive and 31 *M. genitalium* negative samples, including 15 anal swabs, 19 vaginal swabs, 2 high vaginal, 8 cervical, 1 urethral swab, as well as 45 male urine specimens.

The *ResistancePlus*<sup>®</sup> MG kit was performed on the LC480 II, after sample extraction on the QIAasymphony SP (QIAGEN) instrument using the DSP Virus/Pathogen Mini kit and the Complex200\_V6\_DSP protocol. Results were compared to the routine diagnostic results obtained from the *ResistancePlus*<sup>®</sup> MG kit (SpeedX) using samples extracted on the MagNA Pure 96 Instrument (MP96). For discordant results, a 16S rRNA qPCR (Twin 2011) test was performed for *M. genitalium* detection, and Sanger sequencing (Twin 2012) was performed for 23S rRNA mutant detection. The sensitivity and specificity of the *ResistancePlus*<sup>®</sup> MG kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 31**. 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 32**.

**Table 31. Clinical evaluation of the *ResistancePlus*<sup>®</sup> MG kit (Clinical Study 7)**

		<i>M. genitalium</i> detection <i>ResistancePlus</i> <sup>®</sup> MG (MP96)		23S rRNA mutant detection <i>ResistancePlus</i> <sup>®</sup> MG (MP96)		
		Positive	Negative	Mutant	Wild type	
<i>ResistancePlus</i> <sup>®</sup> MG (QIAasymphony SP)	Positive	54	0 <sup>%</sup>	Mutant detected	28	1 <sup>#</sup>
	Negative	1 <sup>*</sup>	34	Mutant not detected	1 <sup>#</sup>	22
<b>Sensitivity</b>		98.2% (95% CI 90.3-100.0%)		<b>Sensitivity</b>		96.6% (95% CI 82.2-99.9%)
<b>Specificity</b>		100.0% (95% CI 89.7-100.0%)		<b>Specificity</b>		95.7% (95% CI 78.1-99.9%)

<sup>\*</sup> The *ResistancePlus*<sup>®</sup> MG kit detected 6 true *M. genitalium* negative samples which were positive with reference test, table represents resolved results

<sup>%</sup> The *ResistancePlus*<sup>®</sup> MG kit detected 2 true *M. genitalium* positive samples which were negative with reference test, table represents resolved results

<sup>#</sup> 2 discordant urine samples could not be resolved as sequencing failed

Table 32. Clinical result analysis in accordance to specimen (Clinical Study 7) *			
Specimen	Expected <i>M. genitalium</i> negative	Expected <i>M. genitalium</i> 23S rRNA wild type	Expected <i>M. genitalium</i> 23S rRNA mutant
Male Urine	17/17	9/9	12/14 <sup>1</sup>
Female Urine	1/1	1/2 <sup>2</sup>	1/1
Cervical swab	3/3	2/2	3/3
Vaginal swab	8/8*	7/7	3/3
High vaginal swab	1/1	1/1	-
Male Anal swab	4/4	2/2	8/8
Male urethral swab	-	-	1/1

\* 1 vaginal swab was excluded as it produced an invalid result with the **ResistancePlus**® MG kit

<sup>1</sup> Male urine: 1 *M. genitalium* 23S rRNA wild type was incorrectly identified as *M. genitalium* not detected; 1 *M. genitalium* 23S rRNA mutant was incorrectly identified as *M. genitalium* detected, 23S mutation not detected

<sup>2</sup> Female urine: 1 incorrectly identified as *M. genitalium* detected, 23S rRNA mutation detected

## 16.2 Analytical performance

### 16.2.1 Reproducibility and repeatability

The reproducibility and repeatability of the **ResistancePlus**® MG kit on the LC480 II was assessed using quantified synthetic template for *M. genitalium* MgPa and 23S rRNA targets (A2058G, A2059G, A2058T, A2058C and A2059C) at 10,000 and 3x LOD copies per reaction using 6 replicates (unless otherwise specified). Experiments were performed on the LC480 II.

To determine lot-to-lot variability, two lots were tested, run on one machine performed by one operator (**Table 33**). The two lots showed good reproducibility with coefficient of variation (%CV) between 0.35-2.37% for all targets.

Table 33. Lot-to-lot variability				
	Average Cq	STDEV	%CV	# samples
<b>MgPa 10,000 copies</b>	16.9	0.15	0.89	12/12
<b>MgPa 30 copies</b>	25.5	0.52	2.05	12/12
<b>A2058G 10,000 copies</b>	20.4	0.48	2.37	12/12
<b>A2058G 36 copies</b>	27.8	0.43	1.54	12/12
<b>A2059G 10,000 copies</b>	18.0	0.06	0.35	12/12
<b>A2059G 30 copies</b>	25.6	0.50	1.94	12/12
<b>A2058T 10,000 copies</b>	18.7	0.09	0.46	12/12
<b>A2058T 30 copies</b>	26.2	0.30	1.14	12/12
<b>A2058C 10,000 copies</b>	17.7	0.13	0.75	12/12
<b>A2058C 30 copies</b>	25.4	0.29	1.15	12/12
<b>A2059C 10,000 copies</b>	19.2	0.08	0.42	12/12
<b>A2059C 45 copies</b>	25.0	0.26	1.03	12/12

To determine day-to-day variability, testing was performed over three days by one operator on the same machine (**Table 34**). The three runs showed good reproducibility between different days with coefficient of variation between 0.44-2.31% for all targets.



Table 34. Day-to-day variability				
	Average Cq	STDEV	%CV	# samples
MgPa 10,000 copies	17.0	0.18	1.09	18/18
MgPa 30 copies	25.6	0.59	2.31	18/18
A2058G 10,000 copies	20.2	0.37	1.83	18/18
A2058G 36 copies	27.9	0.51	1.84	18/18
A2059G 10,000 copies	18.1	0.24	1.34	18/18
A2059G 30 copies	25.7	0.32	1.23	18/18
A2058T 10,000 copies	18.7	0.23	1.22	18/18
A2058T 30 copies	26.3	0.31	1.17	18/18
A2058C 10,000 copies	17.8	0.16	0.88	18/18
A2058C 30 copies	25.5	0.31	1.22	18/18
A2059C 10,000 copies	19.2	0.08	0.44	18/18
A2059C 45 copies	25.0	0.46	1.82	18/18

To determine run-to-run variability, three qPCR runs were compared, run on the same day by the same operator (**Table 35**). The three runs showed good reproducibility with coefficient of variation between 0.40-3.20% for all targets.

Table 35. Run-to-run variability				
	Average Cq	STDEV	%CV	# samples
MgPa 10,000 copies	17.0	0.07	0.40	18/18
MgPa 30 copies	25.7	0.47	1.83	18/18
A2058G 10,000 copies	19.8	0.63	3.20	18/18
A2058G 36 copies	27.5	0.51	1.85	18/18
A2059G 10,000 copies	18.4	0.11	0.61	18/18
A2059G 30 copies	25.7	0.39	1.52	18/18
A2058T 10,000 copies	18.7	0.22	1.18	18/18
A2058T 30 copies	26.4	0.42	1.59	18/18
A2058C 10,000 copies	17.8	0.08	0.46	18/18
A2058C 30 copies	25.5	0.31	1.22	18/18
A2059C 10,000 copies	19.2	0.15	0.76	18/18
A2059C 45 copies	25.2	0.40	1.57	18/18

To determine operator variability, two runs were compared from two operators (**Table 36**). The two runs from different operators showed good reproducibility with coefficient of variation between 0.54-1.86% for all targets.

Table 36. Operator variability				
	Average Cq	STDEV	%CV	# samples
MgPa 10,000 copies	16.8	0.12	0.73	12/12
MgPa 30 copies	25.3	0.41	1.61	12/12
A2058G 10,000 copies	20.2	0.24	1.21	12/12
A2058G 36 copies	27.9	0.45	1.62	12/12
A2059G 10,000 copies	17.9	0.10	0.58	12/12
A2059G 30 copies	25.5	0.39	1.53	12/12
A2058T 10,000 copies	18.6	0.10	0.54	12/12
A2058T 30 copies	26.1	0.31	1.20	12/12
A2058C 10,000 copies	17.7	0.13	0.71	12/12
A2058C 30 copies	25.2	0.27	1.06	12/12
A2059C 10,000 copies	19.1	0.16	0.83	12/12
A2059C 45 copies	24.9	0.46	1.86	12/12

To determine instrument variability, two runs from two machines were compared, performed by the same operator (**Table 37**). The runs from different instruments showed good reproducibility with coefficient of variation between 0.21-2.62% for all targets.

Table 37. Instrument variability				
	Average Cq	STDEV	%CV	# samples
MgPa 10,000 copies	16.7	0.10	0.60	12/12
MgPa 30 copies	25.4	0.67	2.62	12/12
A2058G 10,000 copies	20.0	0.07	0.33	12/12
A2058G 36 copies	27.8	0.51	1.82	12/12
A2059G 10,000 copies	17.8	0.05	0.30	12/12
A2059G 30 copies	25.3	0.36	1.41	12/12
A2058T 10,000 copies	18.5	0.09	0.50	12/12
A2058T 30 copies	25.9	0.30	1.16	12/12
A2058C 10,000 copies	17.6	0.13	0.75	12/12
A2058C 30 copies	25.3	0.36	1.44	12/12
A2059C 10,000 copies	18.9	0.04	0.21	12/12
A2059C 45 copies	24.8	0.46	1.85	12/12

To determine within-run variability, three experiments were compared, set up separately by the same operator running each target on the same plate (**Table 38**). The three experiments showed good reproducibility with coefficient of variation between 0.57-3.12% for all targets.

Table 38. Within-run variability				
	Average Cq	STDEV	%CV	# samples
MgPa 10,000 copies	17.3	0.36	2.09	18/18
MgPa 30 copies	25.9	0.81	3.12	18/18
A2058G 10,000 copies	20.2	0.11	0.57	18/18
A2058G 36 copies	28.0	0.65	2.31	18/18
A2059G 10,000 copies	17.9	0.15	0.83	18/18
A2059G 30 copies	25.8	0.38	1.46	18/18
A2058T 10,000 copies	18.8	0.12	0.66	18/18
A2058T 30 copies	26.8	0.38	1.41	18/18
A2058C 10,000 copies	17.8	0.15	0.83	18/18
A2058C 30 copies	25.5	0.36	1.41	18/18
A2059C 10,000 copies	19.0	0.14	0.76	18/18
A2059C 45 copies	25.0	0.42	1.66	18/18

#### 16.2.2 Analytical sensitivity

The analytical sensitivity of the **ResistancePlus**<sup>®</sup> MG kit on the LC480 II was determined by running limited dilution series<sup>1</sup>, using quantified synthetic template for *M. genitalium* MgPa and 23S rRNA targets (A2058G, A2059G, A2058T, A2058C and A2059C). The sensitivity for each target was determined as the number of copies per reaction with  $\geq 95\%$  detection shown in **Table 39**.

Table 39. Analytical sensitivity	
	Analytical Sensitivity (copies/reaction)
MgPa	10
A2058G	12
A2059G	10
A2058T	10
A2058C	10
A2059C	15

#### 16.2.3 Analytical specificity

This study was conducted to evaluate the **ResistancePlus**<sup>®</sup> MG kit when non-target organisms are present at high concentrations. A panel of 65 microorganisms (4 viruses, 2 protozoans, 4 fungi and 55 bacteria) representing pathogens or flora commonly present in the urogenital system, or closely related to *M. genitalium*, were evaluated. Each bacterial strain was tested at  $1 \times 10^6$  genomes/ml, unless otherwise stated. Viral strains were tested at  $1 \times 10^5$  genomes/ml, unless otherwise stated. All other organisms were tested at the concentrations stated. All organisms were quantified using qPCR, except those quantified as Colony Forming Units (CFU) or Plaque Forming Units (PFU) ( ). All microorganisms were tested in triplicate. All microorganisms tested were diluted into negative clinical matrix (either urine or vaginal swab).

Results indicated that none of these organisms produced false positive results in the *M. genitalium* negative matrices (**Table 40**).

An *in silico* analysis was also performed to evaluate if the oligonucleotides in the **ResistancePlus**<sup>®</sup> MG assay could amplify and detect nucleic acid sequences from non-target organisms available in BLAST. No significant interactions were detected.

Table 40. Microorganisms tested for analytical specificity

Organism	Concentration (genomes/ml)	Organism	Concentration (genomes/ml)	Organism	Concentration (genomes/ml)
<i>Actinomyces israelii</i>	1 x 10 <sup>6</sup>	HIV-1 <sup>^</sup>	1 x 10 <sup>3</sup>	<i>Mycoplasma pirum</i> (2) <sup>*</sup>	1 x 10 <sup>6</sup>
<i>Atopobium vaginae</i>	1 x 10 <sup>6</sup>	HPV type 18 (HeLa cells) <sup>^</sup>	1 x 10 <sup>5</sup>	<i>Mycoplasma pneumoniae</i> (6) <sup>*</sup>	1 x 10 <sup>6</sup>
<i>Bacterioides fragilis</i>	1 x 10 <sup>6</sup>	<i>Klebsiella oxytoca</i>	1 x 10 <sup>6</sup>	<i>Mycoplasma primatum</i>	1 x 10 <sup>6</sup>
<i>Bifidobacterium adolescentis</i>	1 x 10 <sup>6</sup>	<i>Lactobacillus acidophilus</i>	1 x 10 <sup>6</sup>	<i>Mycoplasma salivarium</i>	1 x 10 <sup>6</sup>
<i>Campylobacter jejuni</i>	1 x 10 <sup>6</sup>	<i>Lactobacillus crispatus</i>	1 x 10 <sup>6</sup>	<i>Neisseria gonorrhoeae</i>	1 x 10 <sup>6</sup>
<i>Candida albicans</i>	1 x 10 <sup>5</sup>	<i>Lactobacillus jensenii</i>	1 x 10 <sup>6</sup>	<i>Pentatrichomonas hominis</i> <sup>#</sup>	1 x 10 <sup>5</sup>
<i>Candida glabrata</i>	1 x 10 <sup>6</sup>	<i>Lactobacillus vaginalis</i>	1 x 10 <sup>6</sup>	<i>Peptostreptococcus anaerobius</i>	1 x 10 <sup>6</sup>
<i>Candida parapsilosis</i>	1 x 10 <sup>6</sup>	<i>Listeria monocytogenes</i>	1 x 10 <sup>6</sup>	<i>Prevotella bivia</i>	1 x 10 <sup>6</sup>
<i>Candida tropicalis</i>	1 x 10 <sup>5</sup>	<i>Mobiluncus curtisii</i>	1 x 10 <sup>6</sup>	<i>Propionibacterium acnes</i>	1 x 10 <sup>5</sup>
<i>Chlamydia trachomatis</i>	1 x 10 <sup>6</sup>	<i>Mycobacterium smegmatis</i>	1 x 10 <sup>5</sup>	<i>Proteus mirabilis</i>	1 x 10 <sup>6</sup>
<i>Clostridium perfringens</i>	1 x 10 <sup>6</sup>	<i>Mycoplasma alvi</i>	1 x 10 <sup>6</sup>	<i>Proteus vulgaris</i>	1 x 10 <sup>6</sup>
<i>Corynebacterium genitalium</i>	1 x 10 <sup>6</sup>	<i>Mycoplasma amphoriforme</i> (2) <sup>*</sup>	1 x 10 <sup>6</sup>	<i>Pseudomonas aeruginosa</i>	1 x 10 <sup>6</sup>
<i>Enterobacter aerogenes</i>	1 x 10 <sup>6</sup>	<i>Mycoplasma arginini</i>	1 x 10 <sup>6</sup>	<i>Staphylococcus aureus</i>	1 x 10 <sup>6</sup>
<i>Enterobacter cloacae</i>	1 x 10 <sup>6</sup>	<i>Mycoplasma buccale</i>	1 x 10 <sup>6</sup>	<i>Staphylococcus saprophyticus</i>	1 x 10 <sup>6</sup>
<i>Enterococcus faecalis</i>	1 x 10 <sup>6</sup>	<i>Mycoplasma fermentans</i>	1 x 10 <sup>6</sup>	<i>Streptococcus agalactiae</i>	1 x 10 <sup>6</sup>
<i>Fusobacterium nucleatum</i>	1 x 10 <sup>6</sup>	<i>Mycoplasma gallisepticum</i>	1 x 10 <sup>4</sup>	<i>Streptococcus pyogenes</i>	1 x 10 <sup>6</sup>
<i>Gardnerella vaginalis</i>	1 x 10 <sup>6</sup>	<i>Mycoplasma hominis</i>	1 x 10 <sup>6</sup>	<i>Trichomonas vaginalis</i> <sup>#</sup>	1 x 10 <sup>5</sup>
<i>Haemophilus ducreyi</i>	1 x 10 <sup>6</sup>	<i>Mycoplasma lipophilum</i>	1 x 10 <sup>4</sup>	<i>Ureaplasma urealyticum</i>	1 x 10 <sup>5</sup>
Herpes simplex virus I	1 x 10 <sup>6</sup>	<i>Mycoplasma orale</i>	1 x 10 <sup>6</sup>		
Herpes simplex virus II	1 x 10 <sup>6</sup>	<i>Mycoplasma penetrans</i>	1 x 10 <sup>6</sup>		

\* number in brackets denotes the number of strains tested

<sup>^</sup> quantified as PFU/ml

<sup>#</sup> quantified as CFU/ml

#### 16.2.4 Potentially interfering substances

An interfering substances study was carried out to examine if substances or conditions that may be present in urine or vaginal swab specimens could affect the performance of the **ResistancePlus**<sup>®</sup> MG assay. 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 evaluated through the performance of the Internal Control, which monitors extraction and qPCR inhibition. All test samples were tested in triplicate. Substances were diluted in negative clinical matrix (either urine or vaginal swab) as appropriate.

Results indicated that none of the substances and conditions interfered with detection of the Internal Control or produced false positive results.

Results are summarised in **Table 41** and **Table 42**.

Table 41. Potentially interfering substances in urine samples

Class/Substance	Product name	Test concentration
Whole blood	--	1% v/v
Semen	--	5.0% v/v
Mucus	Mucin	0.8% w/v
Antibiotics	Azithromycin	1.8 mg/ml
	Doxycycline	3.6 mg/ml
Analgesics	Aspirin	40 mg/ml
	Paracetamol	3.2 mg/ml
Intravaginal hormones	--	7 mg/ml Progesterone + 0.07 mg/ml Beta Estradiol
Leukocytes	--	10 <sup>5</sup> cells/ml
Albumin	Bovine serum albumin	10 mg/ml
Glucose	--	10 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
Bilirubin	--	1 mg/ml

**Table 42. Potentially interfering substances in vaginal swab samples**

Class/Substance	Product name	Test concentration
Blood	--	60% v/v
Seminal fluid	--	5.0% v/v
Mucus	Mucin	0.8% w/v
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
	Vagisil Sensitive Skin Formula Maximum Strength Anti-Itch Creme with Oatmeal (1.0 oz)	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)	0.25% w/v
	Summer's Eve Medicated Douche	0.25% v/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	Metronidazole Vaginal Gel, 0.75%	0.25% w/v
	Estrace® (estradiol vaginal cream, USP 0.01%)	0.25% w/v
Leukocytes	--	10 <sup>5</sup> cells/ml
Intravaginal hormones	--	7 mg/ml Progesterone + 0.07 mg/ml Beta Estradiol

## 17 Customer and technical support

Please contact Technical Support for questions on reaction setup, cycling conditions and other enquiries.

Tel: +61 2 9209 4169, Email: [tech@speedx.com.au](mailto:tech@speedx.com.au)

## 18 References

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## 19 Appendix 1: LightCycler® 480 instrument II

The following information is based on LightCycler® 480 Software (version 1.5).

The **ResistancePlus**® MG kit contains dyes for the LightCycler® 480 Instrument II. The **PlexPCR**® Colour Compensation kit (Cat no 90001) must be run and applied for LC480 II analysis (see **Section 19.2**). This kit can be supplied on request.

### 19.1 Programming the LightCycler® 480 Instrument II (LC480 II)

#### Detection Format

Create a custom **Detection Format**

**Open Tools > Detection Formats**

Create a New Detection Format, and name '**SpeedX PlexPCR**' (may be created during the generation of SpeedX Colour Compensation file) (See **Figure 3**).

For **Filter Combination Selection** select the following (Excitation-Emission):

Table 43. Filter combinations <sup>^</sup>						
<b>LC480 II</b>	440-488	465-510	533-580	533-610	533-640	618-660

<sup>^</sup> These Filter Combinations are the default names for the channels

Set the **Selected Filter Combination List** for all channels as:

Melt Factor: 1

Quant Factor: 10

Max Integration Time (sec): 1

**Figure 3. Custom SpeedX Detection Format**

**Filter Combination Selection**

		Emission					
		488	510	580	610	640	660
Excitation	440	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	465	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	498	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	533	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	618	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
	<input type="button" value="Clear"/>						

---

**Selected Filter Combination List**

Excitation Filter	Emission Filter	Name	Melt Factor	Quant Factor	Max Integration Time (Sec)
440	488	440-488	1	10	1
465	510	465-510	1	10	1
533	580	533-580	1	10	1
533	610	533-610	1	10	1
533	640	533-640	1	10	1
618	660	618-660	1	10	1

#### Instrument Settings

Create a custom **Detection Format**

**Open Tools > Instruments**

For **Instrument Settings >** select **Barcode Enabled**



**Experiment setup**

Select **New Experiment**

In the **Run Protocol** tab

For **Detection Format** select the custom '**SpeedX PlexPCR**' (Figure 4)

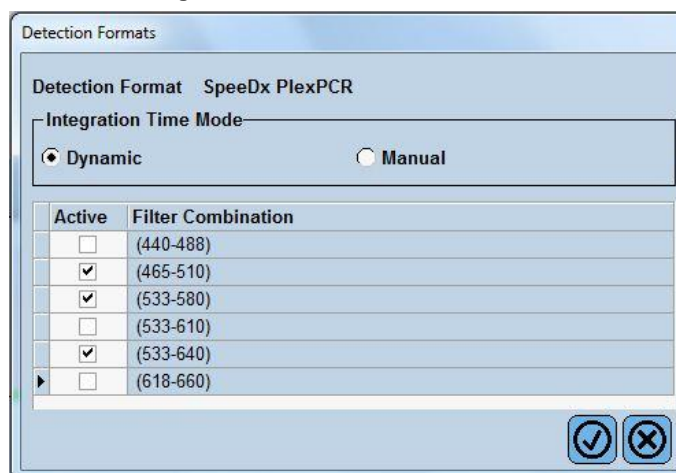
Select **Customize >**

Select **Integration Time Mode > Dynamic**

Select the following Active **Filter Combinations** shown in **Table 44**

Table 44. Channels for <i>ResistancePlus</i> <sup>®</sup> MG targets		
<i>M. genitalium</i> detection (MgPa)	23S rRNA mutation	Internal Control
465-510	533-580	533-640

**Figure 4. Customize Detection Format**



To enable automated sample detection in the analysis software, assign nametags to the wells on the plate

Open the **Sample Editor** module

Select well

Edit **Sample Name** to match nametag defined in Assays module of the analysis software (see **Section 24.4**)

Samples are labelled as *Prefix\_Suffix* (as shown in **Table 45** and **Figure 5**) e.g. Pa\_MG

**NOTE:** Sample nametags are case sensitive. The nametag must match exactly to those assigned in the run file.

Table 45. Sample nametags for analysis software			
Sample type	Prefix (in analysis software)	_Suffix (in analysis software)	Sample name (in LC480)
Regular sample	S	_MG	S_MG
Negative control	N	_MG	N_MG
Positive control (MG, 23S rRNA mutant type) (Pa)	Pa	_MG	Pa_MG
Positive control (MG, 23S rRNA wild type) (Pb)	Pb	_MG	Pb_MG

Figure 5. Sample Editor – Assigning nametags to wells

Pos	Filter combination	Color	Repl Of	Sample Name
A12	465-510 (465-510)	Blue		S_MG
A12	533-580 (533-580)	Blue		S_MG
A12	533-640 (533-640)	Blue		S_MG
B12	465-510 (465-510)	Red		Pa_MG
B12	533-580 (533-580)	Red		Pa_MG
B12	533-640 (533-640)	Red		Pa_MG
C12	465-510 (465-510)	Green		Pb_MG
C12	533-580 (533-580)	Green		Pb_MG
C12	533-640 (533-640)	Green		Pb_MG
G8	465-510 (465-510)	Orange		N_MG
G8	533-580 (533-580)	Orange		N_MG
G8	533-640 (533-640)	Orange		N_MG

Set Reaction Volume > 20 µl

Create the following Program (shown in more detail in Figure 6 – Figure 9):

Table 46. Thermocycling Program				
Program name	Cycles	Target °C	Hold	Ramp rate (°C/s)*
Polymerase activation	1	95°C	2 min	4.4
Touch down cycling <sup>♠</sup> : Step down -0.5°C/cycle	10	95°C	5 s	4.4
		61°C – 56.5°C <sup>♠</sup>	30 s	2.2
Quantification cycling <sup>♠</sup> : Acquisition/Detection	40	95°C	5 s	4.4
		52°C <sup>♠</sup>	40 s	2.2
Cooling	1	40°C	30 s	2.2

\* Default ramp rate (96 well plate)

♠ Step size: -0.5°C/Cycle, Sec Target: 56°C

♠ Analysis mode: Quantification, Acquisition mode: Single

Figure 6. Thermocycling Program – Polymerase activation

The screenshot shows the 'Sample Editor' window in the LightCycler 480 software. The 'Programs' table is as follows:

Program Name	Cycles	Analysis Mode
Polymerase activation	1	None
Touchdown cycling	10	None
Quantification cycling	40	Quantification
Cooling	1	None

Below this, the 'Polymerase activation Temperature Targets' table is shown:

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:02:00	4.4	0	0	0	0

Figure 7. Thermocycling Program – Touchdown cycling

The screenshot shows the 'Programs' table with the following data:

Program Name	Cycles	Analysis Mode
Polymerase activation	1	None
Touchdown cycling	10	None
Quantification cycling	40	Quantification
Cooling	1	None

The 'Touchdown cycling Temperature Targets' table is also visible:

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:00:05	4.4	0	0	0	0
61	None	00:00:30	2.2	56	0.5	0	0

Figure 8. Thermocycling Program – Quantification cycling

The screenshot shows the 'Programs' table with the following data:

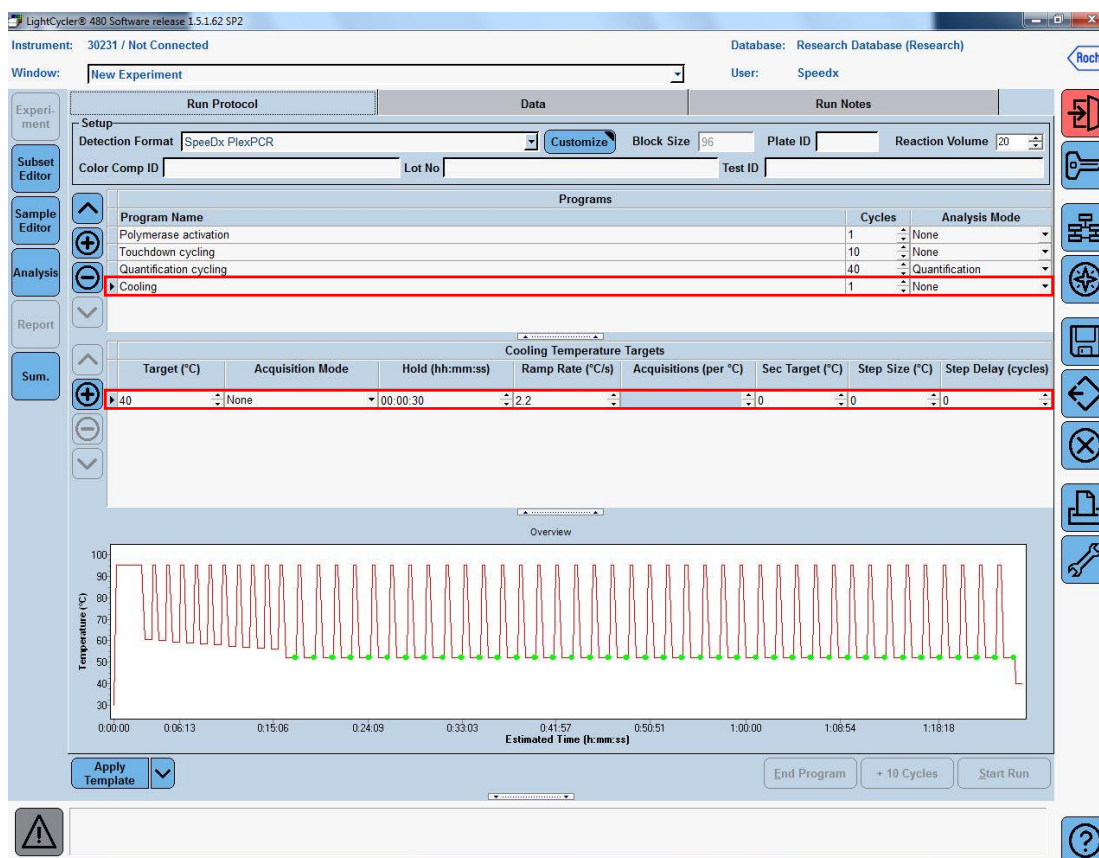
Program Name	Cycles	Analysis Mode
Polymerase activation	1	None
Touchdown cycling	10	None
Quantification cycling	40	Quantification
Cooling	1	None

The 'Quantification cycling Temperature Targets' table is also visible:

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:00:05	4.4	0	0	0	0
52	Single	00:00:40	2.2	0	0	0	0

Figure 9. Thermocycling Program – Cooling



### > Start Run

When the cycling program has finished, export a .ixo file for analysis in the **ResistancePlus®** MG (LC480) analysis software.

#### Select Export

Save in an easily identifiable location

## 19.2 Colour Compensation for LightCycler® 480 Instrument II

**NOTE:** The **PlexPCR®** Colour Compensation (Cat no 90001) kit must be run and applied for LC480 II analysis. This kit can be supplied on request.

For analysis using the software, the Sample Name of the colour compensation reactions must be labelled as shown in **Table 47**.

When the cycling program has finished, export a .ixo file for analysis in the **ResistancePlus®** MG (LC480) analysis software.

#### Select Export

Save in an easily identifiable location and name as “**SpeedX PlexPCR**”

**Table 47. Sample name for colour compensation reactions for the analysis software**

Reactions							
	BLANK	488 mix	510 mix	580 mix	610 mix	640 mix	660 mix
<b>Dominant Channel</b>	Water	440-488	465-510	533-580	533-610	533-640	618-660
<b>Sample Name</b>	BLANK	440-488	465-510	533-580	533-610	533-640	618-660

### 19.3 Interpretation of results

Data interpretation requires the **ResistancePlus**<sup>®</sup> MG (LC480) analysis software. The analysis software can be supplied on request. Please contact [tech@speedx.com.au](mailto:tech@speedx.com.au) for more information.

Refer to **Section 24** for instructions for using the **ResistancePlus**<sup>®</sup> MG (LC480) analysis software.

**20 Appendix 2: cobas z 480 analyser**

The following information is based on the cobas z 480 analyser Software (LightCycler 480 SW UDF 2.1.0). Contact your Roche representative for support in accessing the UDF software on your cobas z 480 analyser.

The **ResistancePlus**® MG kit contains dyes for the cobas z 480 analyser. The **PlexPCR**® Colour Compensation kit (Cat no 90001) must be run and applied for z 480 analysis (see **Section 20.2**). This kit can be supplied on request.

**20.1 Programming the cobas z 480 analyser**

**Detection Format**

Create a custom **Detection Format**

**Open Tools > Detection Formats**

Create a New Detection Format, and name '**SpeedX PlexPCR**' (may be created during the generation of SpeedX Colour Compensation file) (See **Figure 10**).

For **Filter Combination Selection** select the following (Excitation-Emission):

Table 48. Filter combinations <sup>^</sup>					
<b>z 480</b>	465-510	540-580	540-610	540-645	610-670

<sup>^</sup> These Filter Combinations are the default names for the channels

Set the **Selected Filter Combination List** for all channels as:

Melt Factor: 1

Quant Factor: 10

Max Integration Time (sec): 1

**Figure 10. Custom SpeedX Detection Format**

The screenshot shows a software window titled 'Filter Combination Selection'. It features a grid for selecting excitation and emission filters. The 'Excitation' column has options 465, 498, 540, 610, and 680. The 'Emission' columns have options 510, 580, 610, 645, 670, and 700. Checkmarks are placed in the following cells: (465, 510), (540, 580), (540, 610), (540, 645), and (610, 670). A 'Clear' button is located at the bottom right of the grid.

Below the grid is a 'Selected Filter Combination List' table:

Excitation Filter	Emission Filter	Name	Melt Factor	Quant Factor	Max Integration Time (Sec)
465	510	465-510	1	10	1
540	580	540-580	1	10	1
540	610	540-610	1	10	1
540	645	540-645	1	10	1
610	670	610-670	1	10	1

### **Instrument Settings**

Create a custom **Detection Format**

**Open Tools > Instruments**

For **Instrument Settings** > select **Barcode Enabled**

### **Experiment setup**

Select **New Experiment**

In the **Run Protocol** tab

For **Detection Format** select the custom '**SpeedX PlexPCR**' (Figure 11)

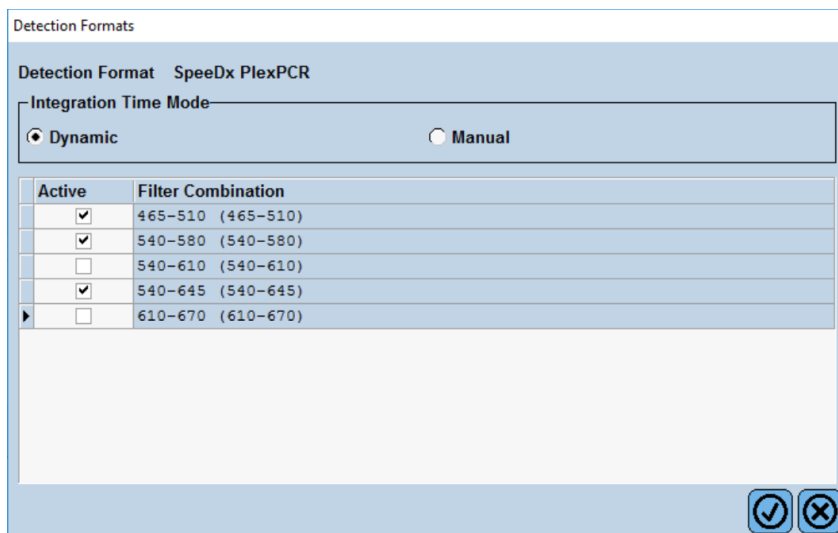
Select **Customize** >

Select **Integration Time Mode** > **Dynamic**

Select the following Active **Filter Combinations** shown in **Table 49**

Table 49. Channels for <i>ResistancePlus</i> <sup>®</sup> MG targets		
<i>M. genitalium</i> detection (MgPa)	23S rRNA mutation	Internal Control
465-510	540-580	540-645

**Figure 11. Customize Detection Format**



Detection Formats

Detection Format SpeedX PlexPCR

Integration Time Mode

Dynamic  Manual

Active	Filter Combination
<input checked="" type="checkbox"/>	465-510 (465-510)
<input checked="" type="checkbox"/>	540-580 (540-580)
<input type="checkbox"/>	540-610 (540-610)
<input checked="" type="checkbox"/>	540-645 (540-645)
<input type="checkbox"/>	610-670 (610-670)

OK Cancel

To enable automated sample detection in the analysis software, assign nametags to the wells on the plate

Open the **Sample Editor** module

Select well













Edit **Sample Name** to match nametag defined in Assays module of the analysis software (see **Section 24.4**)

Samples are labelled as *Prefix\_Suffix* (as shown in **Table 50** and **Figure 12**) e.g. Pa\_MG

**NOTE:** Sample nametags are case sensitive. The nametag must match exactly to those assigned in the run file.

Table 50. Sample nametags for analysis software			
Sample type	Prefix (in analysis software)	_Suffix (in analysis software)	Sample name (in z 480)
Regular sample	S	_MG	S_MG
Negative control	N	_MG	N_MG
Positive control (MG, 23S rRNA mutant type) (Pa)	Pa	_MG	Pa_MG
Positive control (MG, 23S rRNA wild type) (Pb)	Pb	_MG	Pb_MG

Figure 12. Sample Editor – Assigning nametags to wells

Pos	Filter combination	Color	Repl Of	Sample Name	Quantification Sample Type
A12	465–510 (465)			S_MG	Unknown
A12	540–580 (540)			S_MG	Unknown
A12	540–645 (540)			S_MG	Unknown
B12	465–510 (465)			Pa_MG	Unknown
B12	540–580 (540)			Pa_MG	Unknown
B12	540–645 (540)			Pa_MG	Unknown
C12	465–510 (465)			Pb_MG	Unknown
C12	540–580 (540)			Pb_MG	Unknown
C12	540–645 (540)			Pb_MG	Unknown
D12	465–510 (465)			N_MG	Unknown
D12	540–580 (540)			N_MG	Unknown
D12	540–645 (540)			N_MG	Unknown

Set **Reaction Volume** > 20 µl

Create the following Program (shown in more detail in **Figure 13 - Figure 16**):

Table 51. Thermocycling Program				
Program name	Cycles	Target °C	Hold	Ramp rate (°C/s) <sup>‡</sup>
Polymerase activation	1	95°C	2 min	4.4
Touch down cycling <sup>§</sup> : Step down -0.5°C/cycle	10	95°C	5 s	4.4
		61°C – 56.5°C <sup>§</sup>	30 s	2.2
Quantification cycling <sup>‡</sup> : Acquisition/Detection	40	95°C	5 s	4.4
		52°C <sup>‡</sup>	40 s	2.2
Cooling	1	40°C	30 s	2.2

<sup>‡</sup> Default ramp rate (96 well plate)

<sup>§</sup> **Step size:** -0.5°C/Cycle, **Sec Target:** 56°C

<sup>‡</sup> **Analysis mode:** Quantification, **Acquisition mode:** Single



Figure 13. Thermocycling Program – Polymerase activation

LightCycler® 480 SW - User Defined Workflow for cobas z 480  
 Instrument: 54735 / Not Connected Database: June2020 (Research)  
 Window: New Experiment User: Speedx

Program Name	Cycles	Analysis Mode
Polymerase activation	1	None
Touchdown cycling	10	None
Quantification cycling	40	Quantification
Cooling	1	None

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:02:00	4.4	0	0	0	0

Figure 14. Thermocycling Program – Touchdown cycling

LightCycler® 480 SW - User Defined Workflow for cobas z 480  
 Instrument: 54735 / Not Connected Database: June2020 (Research)  
 Window: New Experiment User: Speedx

Program Name	Cycles	Analysis Mode
Polymerase activation	1	None
Touchdown cycling	10	None
Quantification cycling	40	Quantification
Cooling	1	None

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:00:05	4.4	0	0	0	0
61	None	00:00:30	2.2	56	0.5	0	0

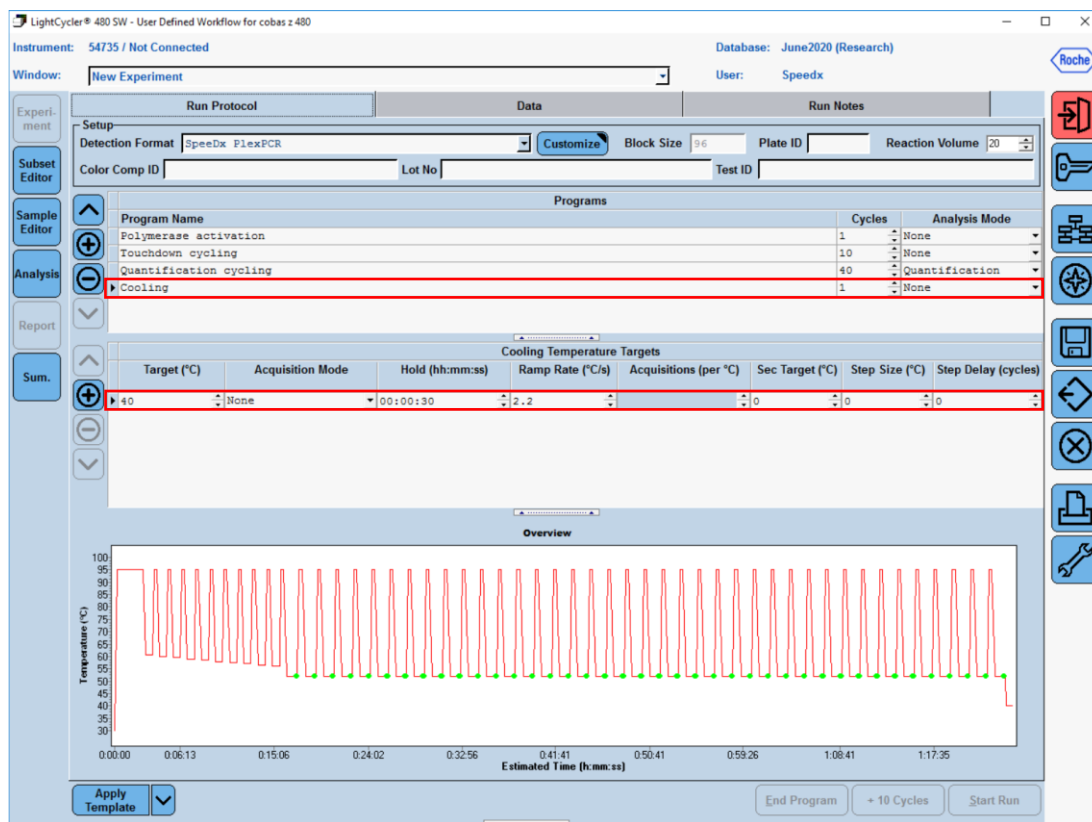
Figure 15. Thermocycling Program – Quantification cycling

LightCycler® 480 SW - User Defined Workflow for cobas z 480  
 Instrument: 54735 / Not Connected Database: June2020 (Research)  
 Window: New Experiment User: Speedx

Program Name	Cycles	Analysis Mode
Polymerase activation	1	None
Touchdown cycling	10	None
Quantification cycling	40	Quantification
Cooling	1	None

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:00:05	4.4	0	0	0	0
52	Single	00:00:40	2.2	0	0	0	0

Figure 16. Thermocycling Program – Cooling



### > Start Run

When the cycling program has finished, export a .ixo file for analysis in the **ResistancePlus®** MG (z480) analysis software.

#### Select **Export**

Save in an easily identifiable location

## 20.2 Colour Compensation for cobas z 480 analyser

**NOTE:** The **PlexPCR®** Colour Compensation (Cat no 90001) kit must be run and applied for z480 analysis. This kit can be supplied on request.

For analysis using the software, the Sample Name of the colour compensation reactions must be labelled as shown in **Table 52**.

When the cycling program has finished, export a .ixo file for analysis in the **ResistancePlus®** MG (z480) analysis software.

#### Select **Export**

Save in an easily identifiable location and name as “**SpeedX PlexPCR**”

Table 52. Sample name for colour compensation reactions for the analysis software						
Reactions						
	BLANK	510 mix	580 mix	610 mix	640 mix	660 mix
<b>Dominant Channel</b>	Water	465-510	540-580	540-610	540-645	610-670
<b>Sample Name</b>	BLANK	465-510	540-580	540-610	540-645	610-670

### 20.3 Interpretation of results

Data interpretation requires the **ResistancePlus**<sup>®</sup> MG (z480) analysis software. The analysis software can be supplied on request. Please contact [tech@speedx.com.au](mailto:tech@speedx.com.au) for more information.

Refer to **Section 24** for instructions for using the **ResistancePlus**<sup>®</sup> MG (z480) analysis software.

## 21 Appendix 3: Applied Biosystems® 7500 Fast

The following information is based on 7500 Software v2.3.

The **ResistancePlus**® MG<sub>(550)</sub> kit contains dyes for the Applied Biosystems® (ABI) 7500 Fast. Default dye calibrations are used for all channels. Custom calibration is not required.

### 21.1 Programming the Applied Biosystems® 7500 Fast

Select **Advanced Setup**

In **Setup** > open **Experiment Properties** and select the following

Name the experiment

**Instrument** > 7500 Fast (96 Wells)

**Type of experiment** > Quantitation – Standard Curve

**Reagents** > Other

**Ramp Speed** > Standard

In **Setup** > open **Plate Setup**

In **Define Targets and Samples** tab >

**Define Targets** as shown below (define colours as required)

Target name	Reporter	Quencher
MgPa	FAM	None
23S rRNA mutation	JOE	None
IC	TAMRA	None

To enable automated sample detection in the analysis software assign nametags to the wells on the plate

In **Setup** > open **Plate Setup**

In **Define Targets and Samples** tab >

**Define Samples**

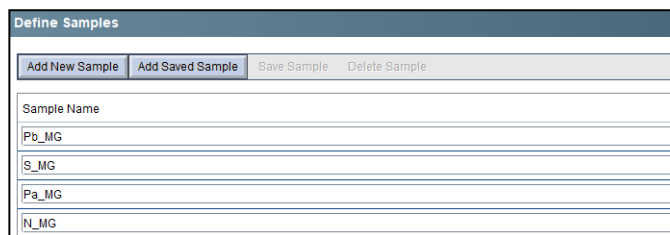
Edit **Sample Name** to match nametags defined in Assays module of the analysis software (see **Section 24.4**)

Samples are labelled as *Prefix\_Suffix* (as shown in **Table 54** and **Figure 17**) e.g. Pa\_MG

**NOTE:** Sample nametags are case sensitive. The nametag must match exactly to those assigned in the run file.

Sample type	Prefix (in analysis software)	_Suffix (in analysis software)	Sample name (in 7500 Fast)
Regular sample	S	_MG	S_MG
Negative control	N	_MG	N_MG
Positive control (MG, 23S rRNA mutant type) (Pa)	Pa	_MG	Pa_MG
Positive control (MG, 23S rRNA wild type) (Pb)	Pb	_MG	Pb_MG

**Figure 17. Sample Editor – Assigning nametags to wells**



In **Assign Targets and Samples** tab >

Select wells and assign targets and samples to the selected wells

Select **Passive reference** > None

In **Setup** > open **Run Method**

Set **Reaction Volume Per Well** > 20 µl

Create the following program (shown in more detail in Graphical View (**Figure 18** and **Figure 19**) and Tabular View (**Figure 20**):

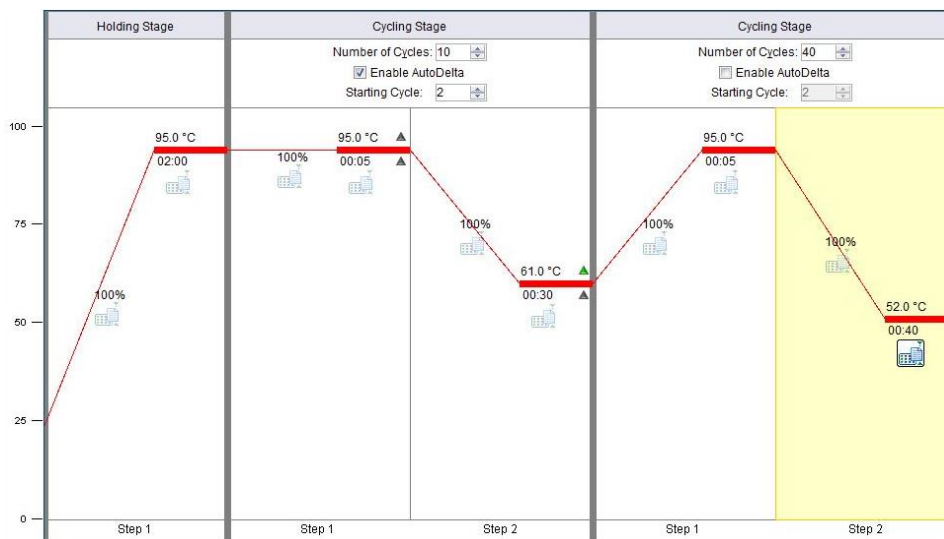
Table 55. Thermocycling Program				
Program name	Cycles	Target °C	Hold	Ramp*
Polymerase activation	1	95°C	2 min	100%
Touch down cycling: Step down -0.5°C/cycle <sup>Ⓞ</sup>	10	95°C	5 s	100%
		61°C – 56.5°C <sup>Ⓞ</sup>	30 s	100%
Quantification cycling*: Acquisition/Detection	40	95°C	5 s	100%
		52°C <sup>+</sup>	40 s	100%

\* Default ramp rate

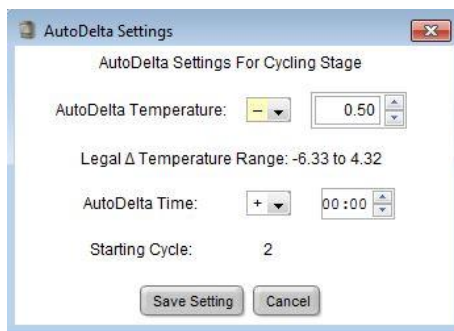
Ⓞ Enable AutoDelta: -0.5°C/cycle

+ Collect data on hold

**Figure 18. Run method – Graphical view**



**Figure 19. Run method – Graphical view – Enable AutoDelta**



**Figure 20. Run method – Tabular view**

	Holding Stage	Cycling Stage		Cycling Stage	
		Number of Cycles: 10 <input checked="" type="checkbox"/> Enable AutoDelta Starting Cycle: 2		Number of Cycles: 40 <input type="checkbox"/> Enable AutoDelta Starting Cycle: 2	
Ramp Rate (%)	100.0	100.0	100.0	100.0	100.0
Temperature (°C)	95.0	95.0	61.0	95.0	52.0
Time	02:00	00:05	00:30	00:05	00:40
AutoDelta Temp.		+ 0.00	- 0.50		
AutoDelta Time		+ 00:00	+ 00:00		
Collect Data on Ramp					
Collect Data on Hold					
	Step 1	Step 1	Step 2	Step 1	Step 2

In **Setup** > open **Run Method**

Select **Start Run**

**21.2 Interpretation of results**

Data interpretation requires the **ResistancePlus® MG (7500)** analysis software. The analysis software can be supplied on request. Please contact [tech@speedx.com.au](mailto:tech@speedx.com.au) for more information.

Refer to **Section 24** for instructions for using the **ResistancePlus® MG (7500)** analysis software.

**22 Appendix 4: Applied Biosystems 7500 Fast Dx**

The following information is based on SDS Software v1.4.1 for the 7500 Fast Dx.

The **ResistancePlus**<sup>®</sup> MG<sub>(550)</sub> kit contains dyes for the Applied Biosystems<sup>®</sup> (ABI) 7500 Fast Dx. Default dye calibrations are used for all channels. Custom calibration is not required.

**22.1 Programming the Applied Biosystems<sup>®</sup> 7500 Fast Dx**

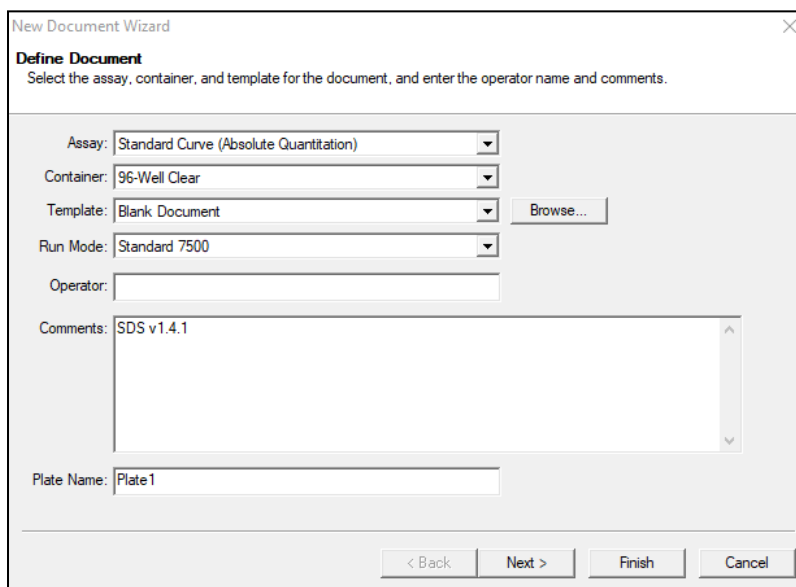
Select Create New Document

In **New Document Wizard** select the following (**Figure 21**):

- Assay** > Standard Curve (Absolute Quantification)
- Container** > 96-Well Clear
- Template** > Blank document
- Run mode** > Standard 7500
- Operator** > Enter Operator's name
- Comments** > Enter any comments or additional notes for the run file
- Plate Name** > Assign a unique name to the run file

Select **Next**

**Figure 21. New Document Wizard window**



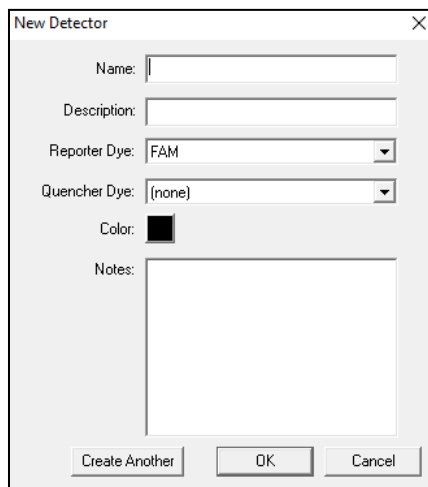
In **Select Detectors** > select **New Detector**

Define detectors as shown below (define colours as required) (**Table 56** and **Figure 22**)

Table 56. Define detectors			
Detectors	Detector name	Reporter dye	Quencher
Detector 1	MgPa	FAM	None
Detector 2	23S rRNA mutation	JOE	None
Detector 3	IC	TAMRA	None

Select **OK**

**Figure 22. New Detector window**

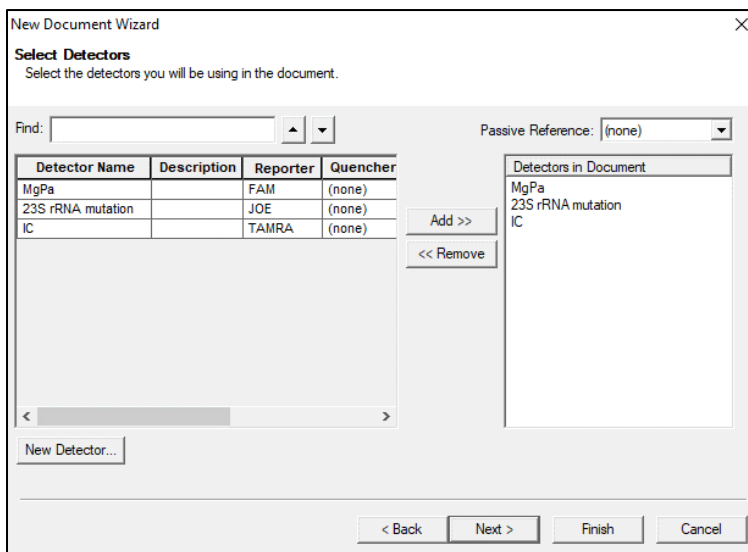


**Select Detectors (Figure 23)**

Select detectors and **Add** to Document

Select **Passive reference** > **None**

**Figure 23. Select Detectors window**



In **Set Up** sample plate >

Select wells and assign 3 detectors to the selected wells

- MgPa
- 23S rRNA mutation
- IC

Select **Next**

To enable automated sample detection in the analysis software, assign nametags to the wells on the plate

In **Setup** > **Plate** tab



Right click on well and select **Well Inspector** > Enter **Sample Name**

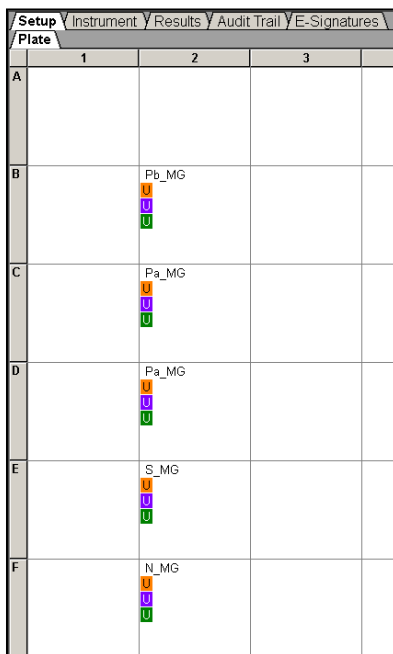
Edit **Sample Name** to match nametag defined in Assays module of the analysis software (see **Section 24.4**)

Samples are labelled as *Prefix\_Suffix* (as shown in **Table 57** and **Figure 24**) e.g. Pb\_MG

**NOTE:** Sample nametags are case sensitive. The nametag must match exactly to those assigned in the run file.

Table 57. Sample nametags for analysis software			
Sample type	Prefix_ (in analysis software)	_Suffix (in analysis software)	Sample name (in 7500 Fast Dx)
Regular sample	S	_MG	S_MG
Negative control	N	_MG	N_MG
Positive control (MG, 23S rRNA mutant type) (Pa)	Pa	_MG	Pa_MG
Positive control (MG, 23S rRNA wild type) (Pb)	Pb	_MG	Pb_MG

**Figure 24. Setup plate view – Assigning nametags to wells**



Select **Next**

In **Instrument** tab

In **Settings** box

For **Sample Volume (µl)**: Enter 20 µl

Create the following thermal cycler protocol (**Table 58** and **Figure 25** and **Figure 26**)

Table 58. Thermal Cycler Protocol				
Program name	Cycles	Target °C	Hold	Ramp*
Polymerase activation	1	95°C	2 min	100%
Touch down cycling: Step down -0.5°C/cycle <sup>Ⓞ</sup>	10	95°C	5 s	100%
		61°C – 56.5°C <sup>Ⓞ</sup>	30 s	100%
Quantification cycling*: Acquisition/Detection	40	95°C	5 s	100%
		52°C <sup>+</sup>	40 s	100%

\* Default ramp rate

Ⓞ Enable AutoDelta: -0.5°C/cycle

+ Collect data on hold

Figure 25. Thermal Cycler Protocol – Thermal profile

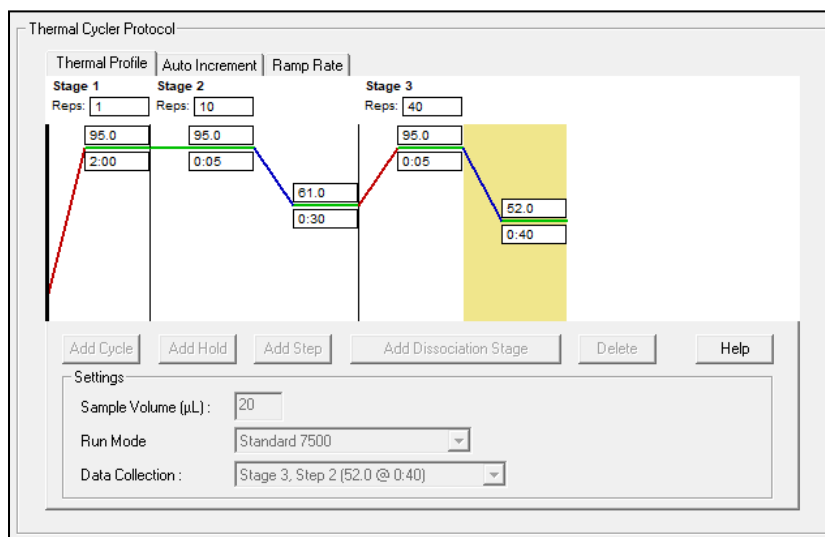
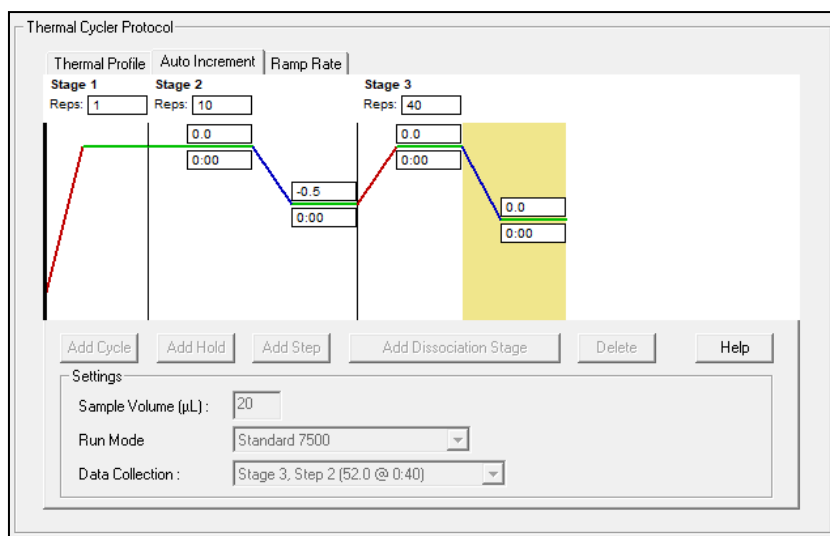


Figure 26. Thermal Cycler Protocol – Auto increment



## 22.2 Interpretation of results

Data interpretation requires the **ResistancePlus**<sup>®</sup> MG (7500) analysis software. The analysis software can be supplied on request. Please contact [tech@speedx.com.au](mailto:tech@speedx.com.au) for more information.

Refer to **Section 24** for instructions for using the **ResistancePlus**<sup>®</sup> MG (7500) analysis software.

**23 Appendix 5: Bio-Rad CFX96™ Dx and CFX96 Touch™ Real-Time PCR System**

The following information is based on Bio-Rad CFX Manager v3.1

The **ResistancePlus®** MG<sub>(675)</sub> kit contains dyes for the CFX96 Real-Time PCR System. Default dye calibrations are used for all channels. Custom calibration is not required.

**23.1 Programming the CFX96™ Dx and CFX96 Touch™ Real-time PCR System**

Select **View > Open Run Setup**

In **Run Setup > Protocol** tab > Select **Create New**

In the **Protocol Editor** (see **Figure 27**):

Set **Sample Volume** > 20 µl

Create the following thermocycling program and save as '**SpeedX PCR**'. This protocol can be selected for future runs.

For Touch down cycling, select Step 3 and select **Step options > Increment: -0.5°C/cycle** (shown in more detail in **Figure 28**).

Table 59. Thermocycling Program			
Program name	Cycles	Target °C	Hold
Polymerase activation	1	95°C	2 min
Touch down cycling <sup>δ</sup> :	10	95°C	5 s
Step down -0.5°C/cycle		61°C – 56.5°C <sup>δ</sup>	30 s
Quantification cycling <sup>+</sup> : Acquisition/Detection	40	95°C	5 s
		52°C <sup>+</sup>	40 s

<sup>δ</sup> **Step options > Increment: -0.5°C/cycle**

<sup>+</sup> **Add Plate Read to Step**

**Figure 27. Thermocycling Protocol – Graphical view**

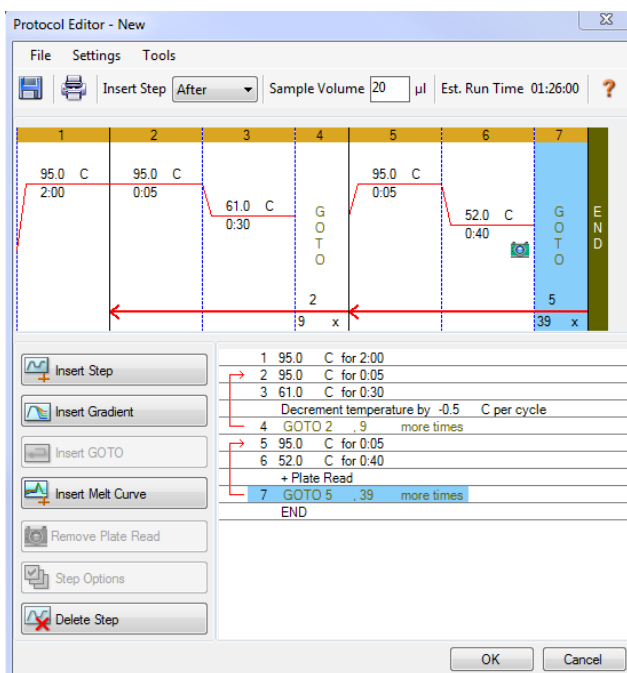
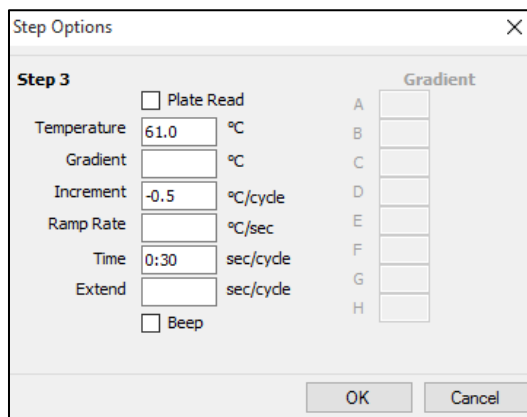


Figure 28. Step options



In **Run Setup** > **Plate** tab

Select **Create New**

Select **Settings** > **Plate Type** > Select **BR Clear**

Set **Scan mode** > All channels

**Select Fluorophores** > FAM, HEX, Quasar 705 (see **Table 60**)

Select wells containing samples and assign **Sample Type** and check **Load** for fluorophores (FAM, HEX, Quasar 705)

Save plate

Table 60. Channels for <i>ResistancePlus</i> <sup>®</sup> MG <sub>(675)</sub> targets		
<i>M. genitalium</i> detection (MgPa)	23S rRNA mutation	Internal control
FAM	HEX	Quasar 705

In **Run Setup** > **Start Run** tab

Select Block

**Start Run**

To enable automated sample detection in the analysis software, assign nametags to the wells on the plate

Open the **Plate Setup** module

Select well

Edit **Sample Name** to match nametag defined in Assays module of the analysis software (see **Section 24.4**)

Samples are labelled as *Prefix\_Suffix* (as shown in **Table 61** and **Figure 29**) e.g. Pb\_MG

**NOTE:** Sample nametags are case sensitive. The nametag must match exactly to those assigned in the run file.

Table 61. Sample nametags for analysis software			
Sample type	Prefix_ (in analysis software)	Suffix (in analysis software)	Sample name (in CFX96)
Regular sample	S	_MG	S_MG
Negative control	N	_MG	N_MG
Positive control (MG, 23S rRNA mutant type) (Pa)	Pa	_MG	Pa_MG
Positive control (MG, 23S rRNA wild type) (Pb)	Pb	_MG	Pb_MG

Figure 29. Sample editor – Assigning nametags to wells

	1	2	3
A	Unk FAM HEX Quasar 705 S_MG		
B	Unk FAM HEX Quasar 705 Pa_MG		
C	Unk FAM HEX Quasar 705 Pb_MG		
D	Unk FAM HEX Quasar 705 N_MG		

**23.2 Interpretation of results**

Data interpretation requires the **ResistancePlus®** MG (CFX) analysis software. The analysis software can be supplied on request. Please contact [tech@speedx.com.au](mailto:tech@speedx.com.au) for more information.

Refer to **Section 24** for instructions for using the **ResistancePlus®** MG (CFX) analysis software.

## 24 Appendix A: Result interpretation

Data interpretation requires the **ResistancePlus**<sup>®</sup> MG analysis software. While **PlexPrime**<sup>®</sup> primers offer greater specificity than other allele-specific primers, some non-specific amplification from the 23S rRNA mutant assay may be seen in samples that contain high concentrations of *M. genitalium* wild type 23S rRNA. The **ResistancePlus**<sup>®</sup> MG analysis software automates the data interpretation of amplification results and streamlines workflow.

See **Table 62** for the appropriate analysis software for each real-time PCR instrument. The analysis software can be supplied on request. Please contact [tech@speedx.com.au](mailto:tech@speedx.com.au) for more information.

Cat no	Analysis software*	Real-time PCR instrument
99003	<b>ResistancePlus</b> <sup>®</sup> MG (LC480)	LC480 II
99018	<b>ResistancePlus</b> <sup>®</sup> MG (z480)	z 480
99002	<b>ResistancePlus</b> <sup>®</sup> MG (7500)	7500 Fast and 7500 Fast Dx
99008	<b>ResistancePlus</b> <sup>®</sup> MG (CFX)	CFX96 Dx and CFX96 Touch
99023	<b>REFLEX ResistancePlus</b> <sup>®</sup> MG (LC480)	LC480 II
99024	<b>REFLEX ResistancePlus</b> <sup>®</sup> MG (z480)	z 480
99026	<b>REFLEX ResistancePlus</b> <sup>®</sup> MG (7500)	7500 Fast and 7500 Fast Dx
99025	<b>REFLEX ResistancePlus</b> <sup>®</sup> MG (CFX)	CFX96 Dx and CFX96 Touch

\* Refer to the website <https://plexpcr.com/products/sexually-transmitted-infections/resistanceplus-mg/#resources> to ensure you are using the most current version of analysis software.

**NOTE:** Follow standard laboratory practices for transfer, reporting and storage of results to prevent loss of sample information.

### 24.1 FastFinder platform – Minimum IT requirements

The analysis software is available within the FastFinder platform (<https://www.ugentec.com/fastfinder/analysis>). The minimum IT requirements for installation of the FastFinder platform are listed below.

#### Hardware requirements

PC (Mac computers are not supported)

Processor: 2 GHz, 2 GB RAM

Disk space: 10Gb

Internet Connection Cable or DSL, proxy not supported

Min. screen resolution: 1366x768 pixels

#### Supported client operating system

Operating system Supported editions

Windows 10 32-bit and 64-bit

Windows 8.1 32-bit, 64-bit, and ARM

Windows 8 32-bit, 64-bit, and ARM

Windows 7 SP1 32-bit and 64-bit

Windows Vista SP2 32-bit and 64-bit

## Supported browsers

FastFinder Administrator account users require one of the following:

- Internet Explorer 11 or newer
- Microsoft Edge 25 or newer
- Firefox 45 or newer
- Google Chrome 47 or newer.

It may run on older versions, but these are not officially supported.

## Software requirements

To use the FastFinder software, at least .NET 4.6.1 is necessary. For more information about the .NET framework, please visit the Microsoft Windows help pages.

## Antivirus settings

Your antivirus software might put the FastFinder installer (UgenTec.FastFinder.Installer.exe) in quarantine. Please add this file to the antivirus whitelist. Example: Symantec (Risk: WS.Reputation.1)

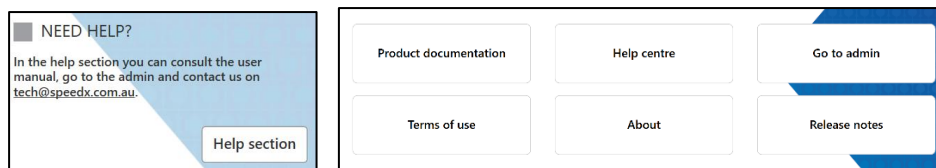
## Firewall requirements

https Connections should be allowed to \*.fastfinderplatform.com:443

For further detailed instructions on the **FastFinder** platform, refer to the **FastFinder Instructions For Use** accessible from the **Help** menu.

To access the Help menu

- Open the start menu 
- Select  or **Help section** and then select **Product Documentation** followed by **Instructions For Use**



## 24.2 Device set up (new user or device)

Refer to the **FastFinder Instructions For Use** for detailed instructions to set up device, accessible from the **Help** menu

Open **FastFinder**


- Select **Devices** from the workflow bar
  - > Select **Add**
  - > Select a file (run file) for the new device
- To change the Current directory
  - > Select **Browse** and select the folder containing relevant files
  - > Select **Next**
- Add device information
  - > Select **Save**



### 24.2.1 Colour Compensation



**NOTE:** See **Section 19.2** and **Section 20.2** for more information on Colour Compensation

For **LC480 II** and **z 480** devices, a colour compensation file must be added to the device

- Select the LC480 II or z 480 device
  - > In the **Colour Compensation** section, select 
  - > Select the colour compensation file for the device from the directory
- To change the Current directory
  - > Select **Browse** and select the folder containing relevant files
- Select **Next**
- Select **ResistancePlus MG (LC480)**, **ResistancePlus MG (z480)**, **REFLEX ResistancePlus MG (LC480)**, or **REFLEX ResistancePlus MG (z480)** from the list to link to this assay
- Select **Save**

New or additional colour compensation files can be added to a device or deactivated as needed.

In the device colour compensation section

- Next to the file name, select 
- Select  to activate or deactivate a colour compensation file for an assay
- Select **Save**


### 24.3 **Assay plug-in (new user)**


Refer to the **FastFinder Instructions For Use** for detailed instructions to set up assays, accessible from the **Help** menu

Open **FastFinder**

- Select **Assays** from the workflow bar
- Select **Add**
  - > For LC480 II > Select **ResistancePlus MG (LC480)** from the list
  - > For z 480 > Select **ResistancePlus MG (z480)** from the list
  - > For 7500 Fast and 7500 Fast Dx > Select **ResistancePlus MG (7500)** from the list
  - > For CFX96 Dx and CFX96 Touch > Select **ResistancePlus MG (CFX)** from the list
  - > For analysis of samples extracted without IC on the LC480 (reflex workflow) > Select **REFLEX ResistancePlus® MG (LC480)** from the list
  - > For analysis of samples extracted without IC on the z 480 (reflex workflow) > Select **REFLEX ResistancePlus® MG (z480)** from the list
  - > For analysis of samples extracted without IC on the 7500 Fast and 7500 Fast Dx (reflex workflow) > Select **REFLEX ResistancePlus® MG (7500)** from the list
  - > For analysis of samples extracted without IC on the CFX96 Dx and CFX96 Touch (reflex workflow) > Select **REFLEX ResistancePlus® MG (CFX)** from the list
- Select **Add**

To activate or de-activate versions of the assay plug-in





- In General assay information
  - > Select  Versions

- > Select  to activate or deactivate the version of the assay
- > Select **Save**

#### 24.4 Sample naming

Sample nametags can be assigned to an assay plug-in to automate detection of wells and sample types for analysis.



Select **Assays** from the workflow bar

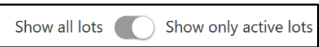
- In the Sample type nametags (prefix), select 
  - > Select  to add a nametag to define sample type nametags (Negative control, Positive control/s and Regular sample)
  - > Add desired word, acronym, or letter to text box
  - > Select **Save**
- In Mix definition nametags (suffix), select 
  - > Select  to add a nametag to define the mix name
  - > Add desired word, acronym, or letter to text box
  - > Select **Save**
- In the instrument software (before or after run is completed) assign the same nametag to appropriate wells
  - > For **LC480 II** see **Section 19** for instructions on programming sample nametags in the run file
  - > For **z 480** see **Section 20** for instructions on programming sample nametags in the run file
  - > For **7500 Fast** see **Section 21** for instructions on programming sample nametags in the run file
  - > For **7500 Fast Dx** see **Section 22** for instructions on programming sample nametags in the run file
  - > For **CFX96 Dx** and **CFX96 Touch** see **Section 23** for instructions on programming sample nametags in the run file

**NOTE:** Sample nametags are case sensitive. The nametag must match exactly to those assigned in the run file.

#### 24.5 Adding mix lot numbers

Mix lot numbers can be assigned to the assay to enable traceability of reagents

- Select **Assays** from the workflow bar
  - > In the **Assay Lot:** Select  to add a new lot or select  to edit an existing lot
  - > Once added, lot numbers will become available in the analysis module

Select  to show all lot numbers or only active lot numbers

#### 24.6 Analysis

Select **Analyses** from the workflow bar to start a new analysis

##### 1 Select datafile

Search for the file to be uploaded for analysis from a specified directory

- To change the **Current directory**
  - > Select **Browse** and select the folder containing relevant files
- Select run (data) file from the list
  - > Select **Next step**

## 2 Assign assay(s)

Assign the assay information to the plate manually if sample naming has not been set up in Assays module

- For **LC480 II** > Select **ResistancePlus MG (LC480)**
- For **z 480** > Select **ResistancePlus MG (z480)**
- For **7500 Fast** and **7500 Fast Dx** > Select **ResistancePlus MG (7500)**
- For **CFX96 Dx** and **CFX96 Touch** > Select **ResistancePlus MG (CFX)**
- For analysis of samples extracted without IC on the LC480 > Select **REFLEX ResistancePlus® MG (LC480)**
- For analysis of samples extracted without IC on the z 480 > Select **REFLEX ResistancePlus® MG (z480)**
- For analysis of samples extracted without IC on the 7500 Fast and 7500 Fast Dx > Select **REFLEX ResistancePlus® MG (7500)**
- For analysis of samples extracted without IC on the CFX96 Dx and CFX96 Touch > Select **REFLEX ResistancePlus® MG (CFX)**
- Select wells and assign as:
  - > Regular sample (S)
  - > Negative control (N)
  - > Positive control (MG, 23S rRNA mutant type) (Pa)
  - > Positive control (MG, 23S rRNA wild type) (Pb)
- Select **Next step**

To save the plate layout as a template for future use

- Select wells and assign types of samples




- > Select  to save template

- Specify template name for future use

- > Select **Save**

To load a previously saved plate template



- Select  to load plate template
  - > Select template from drop down menu
  - > Check the box to load sample types specified within the plate template
  - > Select **Load**

## 3 Configure assay(s)

- For **LC480 II** > Select **ResistancePlus MG (LC480)**
  - > Select the appropriate colour compensation file from the drop down menu
  - > Select **Assay Lot** from the drop down menu
  - > Select **Analyse**
- For **z 480** > Select **ResistancePlus MG (z480)**
  - > Select the appropriate colour compensation file from the drop down menu
  - > Select **Assay Lot** from the drop down menu
  - > Select **Analyse**

- For **7500 Fast** and **7500 Fast Dx** > Select **ResistancePlus MG (7500)**
  - > Select **Assay Lot** from the drop down menu
  - > Select **Analyse**
  
- For **CFX96 Dx** and **CFX96 Touch** > Select **ResistancePlus MG (CFX)**
  - > Select **Assay Lot** from the drop down menu
  - > Select **Analyse**
  
- For samples extracted without IC (reflex workflow) on **LC480 II** > Select **REFLEX ResistancePlus MG (LC480)**
  - > Select the appropriate colour compensation file from the drop down menu
  - > Select **Assay Lot** from the drop down menu
  - > Select **Analyse**
  
- For samples extracted without IC (reflex workflow) on **z 480** > Select **REFLEX ResistancePlus MG (z480)**
  - > Select the appropriate colour compensation file from the drop down menu
  - > Select **Assay Lot** from the drop down menu
  - > Select **Analyse**
  
- For samples extracted without IC (reflex workflow) on **7500 Fast** and **7500 Fast Dx** > Select **REFLEX ResistancePlus MG (7500)**
  - > Select **Assay Lot** from the drop down menu
  - > Select **Analyse**
  
- For samples extracted without IC (reflex workflow) on **CFX96 Dx** and **CFX96 Touch** > Select **REFLEX ResistancePlus MG (CFX)**
  - > Select **Assay Lot** from the drop down menu
  - > Select **Analyse**

## 24.7 Results

See **Table 63** for a summary of possible reported sample results.

**NOTE:** It is highly recommended that amplification curves should be confirmed for all positive samples.

To resolve any uncertain results 

- Select **Resolve** tab
- Select sample to resolve
- Inspect amplification curves for uncertain results
  - > Select  to plot a reference curve on the graph
  - > Select  to plot a positive control on the graph
  - > Select  to plot a negative control on the graph
  - > Select  to confirm suggested result or select  to select a different option
- Confirm as **Negative** or **Inconclusive** and add comments


**NOTE:** For inconclusive samples, re-extract and re-test the samples once. If sample result remains Inconclusive, collect a new sample to re-test.

To finalise analysis and prevent further user edits

- > Select **Authorise Analysis**
- > Select **Yes** to confirm
- To reject analysis or restart the analysis
  - > Select **Restart Analysis** or **Reject Analysis**
  - > Select option to confirm

#### 24.8 Reference curve

A reference curve can be saved and used to compare to samples on the same or across different plates

- Select the sample of interest in either the **Well Details** or **Target Details** menu
- From the amplification graph menu > Select 
  - > Select the check box for the channel of interest and add a label
  - > Select **Save** to add signal as reference curve

This reference curve will now appear linked to the assay in the Assays menu and can be inactivated at any time.

## 24.9 Overview of results

Table 63. Results interpretation <i>ResistancePlus</i> <sup>®</sup> MG analysis software (Results Overview tab)						
	Well	Name	Assay	Result	Cq values <sup>^</sup>	Overall results
	A1	Sample 1	ResistancePlus MG	Negative	CHANNEL C: 25.31	<b>Sample 1 - Negative</b> M. genitalium not detected, IC valid
	A2	Sample 2	ResistancePlus MG	Positive	CHANNEL A: 13.35 CHANNEL B: 24.22 CHANNEL C: 24.36	<b>Sample 2 - Positive</b> M. genitalium detected, 23S rRNA mutation not detected
	A3	Sample 3	ResistancePlus MG	Positive	CHANNEL A: 23.32 CHANNEL B: 31.64	<b>Sample 3 - Positive</b> M. genitalium detected, 23S rRNA mutation not detected
	A4	Sample 4	ResistancePlus MG	Positive	CHANNEL A: 21.32 CHANNEL B: 23.22 CHANNEL C: 24.30	<b>Sample 4 - Positive</b> M. genitalium, 23S rRNA mutation detected
	A5	Sample 5	ResistancePlus MG	Positive	CHANNEL A: 23.16 CHANNEL B: 24.31	<b>Sample 5 - Positive</b> M. genitalium, 23S rRNA mutation detected
	A6	Sample 6	ResistancePlus MG	Invalid	CHANNEL C: 35.02	<b>Sample 6 - Invalid</b> IC invalid, repeat test <sup>1</sup>
ⓘ	A7	Sample 7 (Flagged to resolve)	ResistancePlus MG	Positive	CHANNEL A: 26.27 CHANNEL B: 28.11 <sup>2</sup> CHANNEL C: 28.92	<b>Sample 7 - Positive</b> <sup>2</sup> M. genitalium, 23S rRNA mutation detected
⚠	A7	Sample 7 (Resolve to Inconclusive)	ResistancePlus MG	Invalid	CHANNEL A: 26.27 CHANNEL C: 28.92	<b>Sample 7 - Invalid</b> <sup>3</sup> Inconclusive result, repeat test <sup>1</sup>
	B2	Pa (Mutant type Positive control)	ResistancePlus MG	Positive	CHANNEL A: 25.01 CHANNEL B: 24.23	<b>Pa - Positive</b> Positive Control valid
	B3	Pb (Wild type Positive control)	ResistancePlus MG	Positive	CHANNEL A: 25.90	<b>Pb - Positive</b> Positive Control valid
	B4	N (Negative control)	ResistancePlus MG	Negative	CHANNEL C: 26.25	<b>N - Negative</b> Negative Control valid

<sup>^</sup> Refer to **Table 12** for channel names for different instruments

<sup>1</sup> For IC invalid and inconclusive samples, re-extract and re-test

<sup>2</sup> A sample with an uncertain Cq will be flagged for resolution with ⓘ

<sup>3</sup> A sample resolved to be Inconclusive is flagged with ⚠

## 24.10 Exporting results

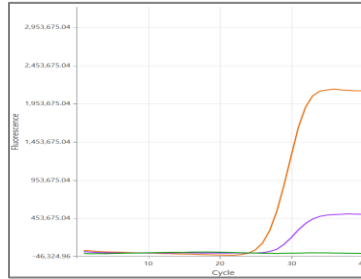
- To export results
  - > Select **Exports** in the workflow bar
  - > Export one or more of the following report types: **Cq values list (CSV)**, **Results (CSV)**, **Generic Amplification CSV** or the appropriate LIS-integration file.
  - > Select **Exports**
- To download exports
  - > Select **Reports** in the workflow bar
  - > Select files and save
- Alternatively export a customised report
  - > Export **Amplification Curve Analysis (PDF)**
  - > Select desired included information (graphs, audit trail, results overview)

- > Select desired report settings to customise sample order
- Select **Exports**
  - > Open in **Report Viewer** to view, save and print

**24.11 Control Example Graphs**

The following examples show the amplification curves (baseline-corrected amplification curves) and the Results overview from the **ResistancePlus MG (7500)** analysis software for control sample types.

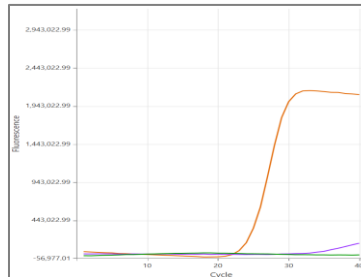
24.11.1 *M. genitalium*, 23S rRNA mutant control (Pa)



CHANNEL A CHANNEL B CHANNEL C

Well	Name	Assay	Result	Cq values	Overall results
B1	Pa	ResistancePlus MG	Positive	CHANNEL A: 26.36 CHANNEL B: 27.38	<b>Pa - Positive</b> Positive Control valid

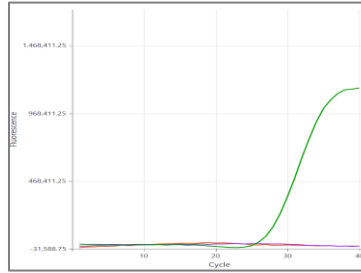
24.11.2 *M. genitalium*, 23S rRNA wild type control (Pb)



CHANNEL A CHANNEL B CHANNEL C

Well	Name	Assay	Result	Cq values	Overall results
D12	Pb	ResistancePlus MG	Positive	CHANNEL A: 24.30 CHANNEL B: 34.29	<b>Pb - Positive</b> Positive Control valid

24.11.3 *M. genitalium* negative control (N) (negative specimen)



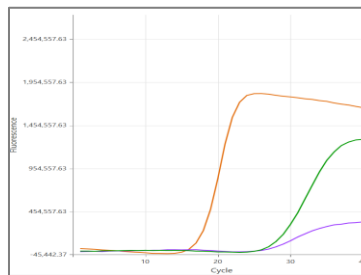
CHANNEL A CHANNEL B CHANNEL C

Well	Name	Assay	Result	Cq values	Overall results
D12	N	ResistancePlus MG	Negative	CHANNEL C: 27.65	N - Negative Negative Control valid

24.12 Examples

The following examples show the amplification curves (baseline-corrected amplification curves) and the Results overview from the **ResistancePlus MG (7500)** analysis software for different samples.

24.12.1 Example 1. High copy *M. genitalium*, 23S rRNA wild type sample

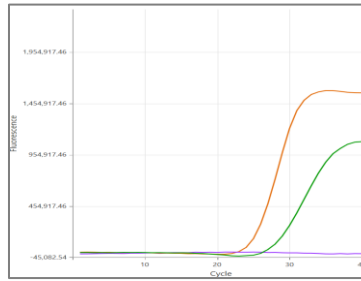


CHANNEL A CHANNEL B CHANNEL C

Well	Name	Assay	Result	Cq values	Overall results
D2	Sample 12	ResistancePlus MG	Positive	CHANNEL A: 16.34 CHANNEL B: 26.59 CHANNEL C: 26.00	<b>Sample 12 - Positive</b> M. genitalium detected, 23S rRNA mutation not detected



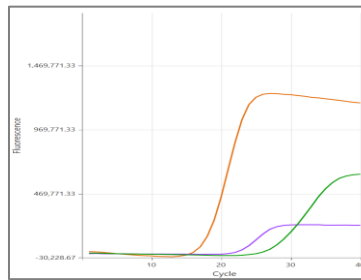
24.12.2 Example 2. Low copy *M. genitalium*, 23S rRNA wild type sample



CHANNEL A CHANNEL B CHANNEL C

Well	Name	Assay	Result	Cq values	Overall results
F1	Sample 6	ResistancePlus MG	Positive	CHANNEL A: 29.30 CHANNEL C: 28.11	<b>Sample 6 - Positive</b> M. genitalium detected, 23S rRNA mutation not detected

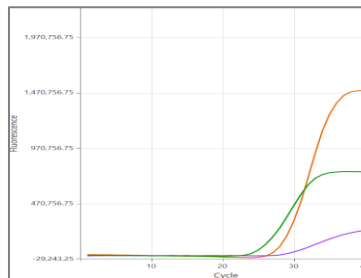
24.12.3 Example 3. High copy *M. genitalium*, 23S rRNA mutant sample



CHANNEL A CHANNEL B CHANNEL C

Well	Name	Assay	Result	Cq values	Overall results
G3	Sample 9	ResistancePlus MG	Positive	CHANNEL A: 18.08 CHANNEL B: 22.31 CHANNEL C: 28.03	<b>Sample 9 - Positive</b> M. genitalium, 23S rRNA mutation detected

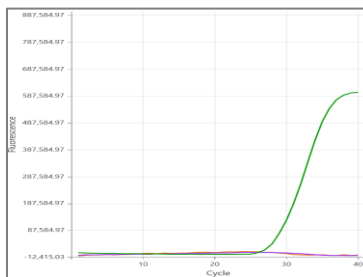
24.12.4 Example 4. Low copy *M. genitalium*, 23S rRNA mutant sample



CHANNEL A CHANNEL B CHANNEL C

Well	Name	Assay	Result	Cq values	Overall results
E3	Sample 21	ResistancePlus MG	Positive	CHANNEL A: 29.08 CHANNEL B: 29.23 CHANNEL C: 26.13	<b>Sample 21 - Positive</b> M. genitalium, 23S rRNA mutation detected

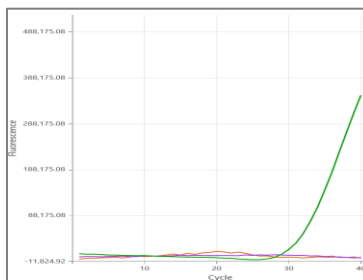
24.12.5 Example 5. Negative sample



CHANNEL A CHANNEL B CHANNEL C

Well	Name	Assay	Result	Cq values	Overall results
E3	Sample 73	ResistancePlus MG	Negative	CHANNEL C: 29.23	Sample 73 - Negative M. genitalium not detected, IC valid

24.12.6 Example 6. Invalid sample



CHANNEL A CHANNEL B CHANNEL C

Well	Name	Assay	Result	Cq values	Overall results
E3	Sample 35	ResistancePlus MG	Invalid	CHANNEL C: 31.16	Sample 35 - Invalid IC invalid, repeat test

In this example, the IC signal is outside the channel cut-off. For IC invalid samples, re-extract the sample and then repeat test.

24.12.7 Example 7. Samples to resolve – Negative signal

In this example, CHANNEL B (JOE) was flagged for resolution with the software suggesting the sample is Negative (Figure 30).

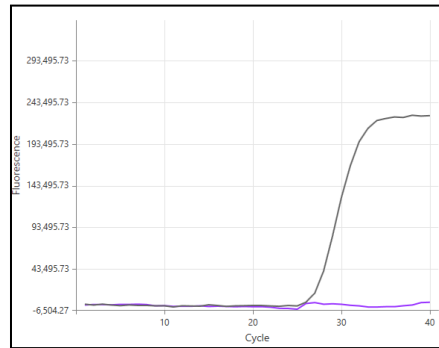
Figure 30. Samples to resolve as seen in the analysis software Resolve menu

Target	Channel	Cq	Curve result	Info	
MgPa	FAM	21.32	Positive	M. genitalium detected	
23S rRNA mutation	JOE	—	Negative	Mutant not detected	
IC	TAMRA	27.31	Positive		

To determine appropriate action to take to resolve, another sample or control can be plotted for signal comparison

- Select  to plot a positive reference curve (previously saved) for CHANNEL B (JOE)
- Select  to plot a positive control from the run
- Select  to plot a negative control from the run

**CHANNEL B**



After inspection of the amplification curves (above) it can be seen that there is no amplification in the channel.

The result is resolved by selecting the  icon, to confirm the Negative suggestion from the software. The resolved result is shown in **Figure 31** below.

**Figure 31. Resolved result as seen in the analysis software Resolve menu**

Target	Channel	Cq	Result	Info	
MgPa	FAM	21.32	Positive	M. genitalium detected	
23S rRNA mutation	JOE	—	Negative	Mutant not detected	
IC	TAMRA	27.31	Positive		

24.12.8 Example 8. Samples to resolve – Inconclusive signal

In this example, CHANNEL B (JOE) was flagged for resolution with the software suggesting sample is Positive (**Figure 32**).

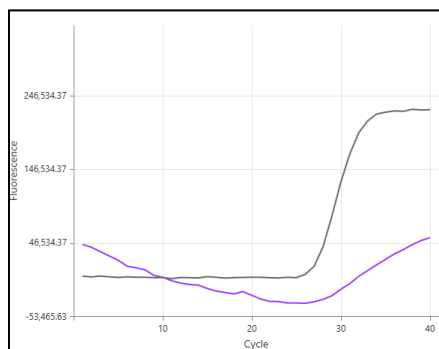
**Figure 32. Samples to resolve as seen in the analysis software Resolve menu**

Target	Channel	Cq	Curve result	Info
MgPa	FAM	26.27	Positive	M. genitalium detected
23S rRNA mutation	JOE	28.11	Positive	Mutant detected
IC	TAMRA	28.92	Positive	

To determine appropriate action to take to resolve, plot another sample or control for signal comparison

- Select **Ref** to plot a positive reference curve (previously saved) for CHANNEL B (JOE)
- Select **P** to plot a positive control from the run
- Select **N** to plot a negative control from the run

**CHANNEL B**



After inspection of the amplification curves (above) there is potential amplification in the channel.

It is recommended to resolve to Inconclusive, by selecting the icon, and selecting the Inconclusive option from the drop-down menu. Comments can be added to the sample audit trail. The sample should be re-extracted and re-tested. The resolved result is shown in **Figure 33** below.

Refer to **Table 63**, Sample 7, for how results are displayed before and after resolution in the **Results Overview** tab.

**Figure 33. Resolved result as seen in the analysis software Resolve menu**

Target	Channel	Cq	Result	Info
MgPa	FAM	26.27	Positive	M. genitalium detected
23S rRNA mutation	JOE	28.11	Inconclusive	Mutant detected
IC	TAMRA	28.92	Positive	

## 25 Glossary



European Conformity  
For *In Vitro* Diagnostic Use



Catalogue number



Batch code



Authorised Representative  
In the European Community



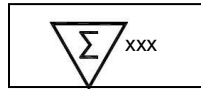
Manufacturer



Date of manufacture



Temperature limitation



Contains sufficient for  
xxx determinations



Use by Date



Importer



United Kingdom Conformity Assessment Mark

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