ResistancePlus® MG

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

CE 0123 IVD UKCA **IVDR Certified**

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

Accessory products – Analysis software

ResistancePlus® MG (LC480)	REF 99003
ResistancePlus® MG (7500)	REF 99002
ResistancePlus® MG (CFX)	REF 99008

EC REP

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Not for sale in the USA

Contents

1 Product description

The ***Resistance****Plus*® MG kit simultaneously detects *M. genitalium* and 4 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 ***Resistance****Plus*® 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 or A2058C mutation in the 23S rRNA gene; and Readout 3 is an internal control to monitor extraction efficiency and qPCR inhibition. The ***Resistance****Plus*® MG kit utilises ***Plex****Zyme*® and ***Plex****Prime*® for specificity and superior multiplexing capability. The assay is validated on samples extracted using the MagNA Pure 96 System (Roche), MICROLAB STARlet IVD (Hamilton), QIAsymphony® SP (QIAGEN), NUCLISENS® easyMAG® (Biomérieux) and real-time detection on the Roche LightCycler® 480 Instrument II (LC480 II), the Applied Biosystems® 7500 Fast (7500 Fast), Applied Biosystems® 7500 Fast Dx (7500 Fast Dx) and the Bio-Rad CFX96™ Dx (CFX96 Dx) and CFX96 Touch™ (CFX96 Touch) Real-time PCR Detection Systems.

2 Intended use

The ***Resistance****Plus*® MG kit is a qualitative multiplexed *in vitro* diagnostic real-time PCR test for the identification of *M. genitalium* and detection of 4 mutations in the 23S rRNA gene (A2058G, A2059G, A2058T and A2058C), *Escherichia coli* numbering) that are associated with resistance to azithromycin (macrolide antibiotic). It is intended to aid in the diagnosis of *M. genitalium* and detects mutations associated with azithromycin resistance in *M. genitalium* and should be used in conjunction with clinical and other laboratory information.

The ***Resistance****Plus*® MG kit may be used with the following specimen types: male and female urine, and vaginal 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 ***Resistance****Plus*® 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[1]. 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)[1,2,3].

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[4,5]. The most common mutations are A2058G and A2059G, contributing 89% of macrolide resistance mutations in a recent study[6].

4 Kit contents

Table 1. Contents for *ResistancePlus*® MG kits

Cap colour	Contents	Description	Cat no 20001L-01 (100 reactions)	Cat no 2000125 (25 reactions)
Blue	***Plex*** Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, $MgCl_2$, DNA polymerase and buffer	1 x 1 mL	1 x 250 µL
Brown	MG+23S Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of *M. genitalium* and 23S rRNA mutations	1 x 100 µL	1 x 25 µL
White	Control Mix 1, 20x	Mix containing oligonucleotides^ for amplification and detection of internal control assay for LC480 II	1 x 100 µL	1 x 25 µL
Red	Internal Control Cells#	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

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

Table 2. Contents for *ResistancePlus*® MG(550) kits				
Cap colour	**Contents**	**Description**	**Cat no 2000201 (100 reactions)**	**Cat no 2000225 (25 reactions)**
Blue	***Plex*** Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, $MgCl_2$, DNA polymerase and buffer	1 x 1 mL	1 x 250 µL
Brown	MG+23S Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of *M. genitalium* and 23S rRNA mutations	1 x 100 µL	1 x 25 µL
White	Control Mix 2, 20x	Mix containing oligonucleotides^ 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#	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

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

Table 3. Contents for *ResistancePlus*® MG(675) kits				
Cap colour	**Contents**	**Description**	**Cat no 2000301 (100 reactions)**	**Cat no 2000325 (25 reactions)**
Blue	***Plex*** Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, $MgCl_2$, DNA polymerase and buffer	1 x 1 mL	1 x 250 µL
Brown	MG+23S Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of *M. genitalium* and 23S rRNA mutations	1 x 100 µL	1 x 25 µL
White	Control Mix 3, 20x	Mix containing oligonucleotides^ 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#	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

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

5 Shipping and storage

- The components of the ***Resistance****Plus*® 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

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 ***Resistance****Plus*® 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 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 ***Resistance****Plus*® 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 for further support.

7 Associated Products and Consumables

Positive Control Material

- ***Resistance****Plus*® 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 1000 uL (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

- ***Plex****PCR*® 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® Multitest swab specimen collection kit (Hologic, Cat no PRD-03546)
- 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. ***Plex****PCR*® is a qPCR technology utilising ***Plex****Zyme*® enzymes that detect and report the amplified product through the generation of a fluorescent signal (**Figure 1**). ***Plex****Prime*® primers for specific amplification of mutant sequences which is coupled with mutant specific ***Plex****Zyme*® detection (**Figure 2**).

Plex*Zyme*® enzymes are catalytic DNA complexes composed of two DNA oligos referred to as "Partial Enzymes". Each Partial Enzyme has a target-specific region, a catalytic core and a universal probe binding region. When the target product is present, the two Partial Enzymes bind adjacently to form the active ***Plex****Zyme*® which has catalytic activity to cleave a labelled probe. Cleavage separates the fluorophore and quencher dyes, producing a fluorescent signal that can be monitored in real time. ***Plex****Zyme*® enzymes have additional specificity compared to alternate detection technologies, since two Partial Enzymes are required to bind for detection. ***Plex****Zyme*® enzymes are also multiple turnover enzymes, and multiple probes can be cleaved during each PCR cycle, resulting in a strong and sensitive signal. ***Plex****Zyme*® assays are highly sensitive and specific and are ideally suited for the multiplexed detection of pathogens.

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

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

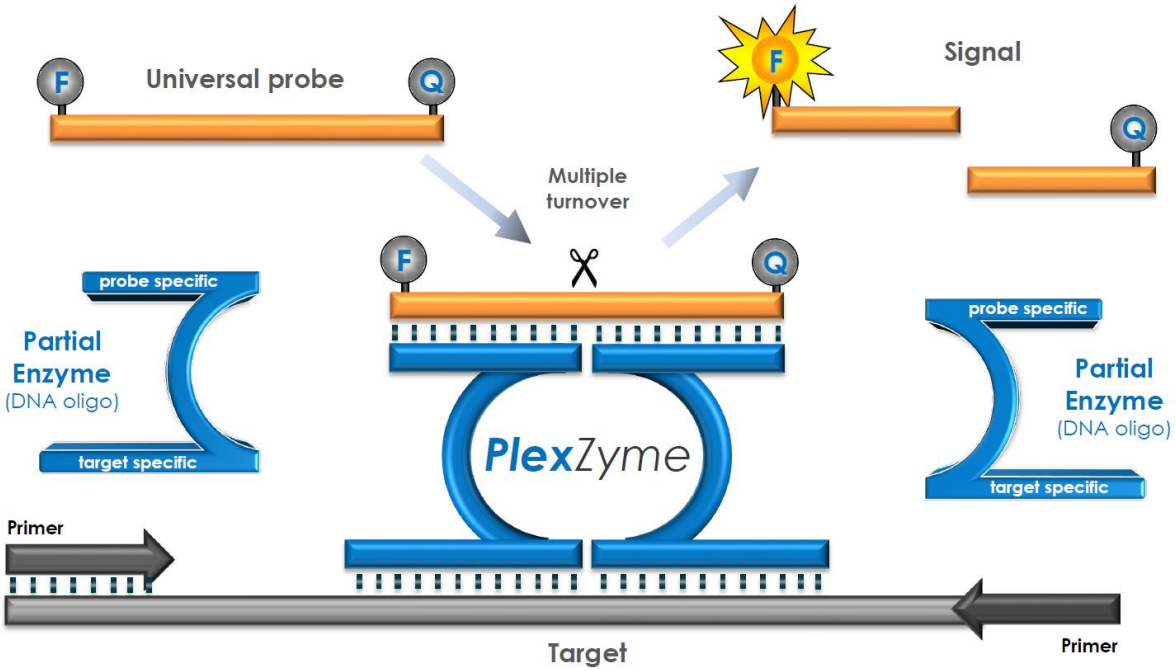
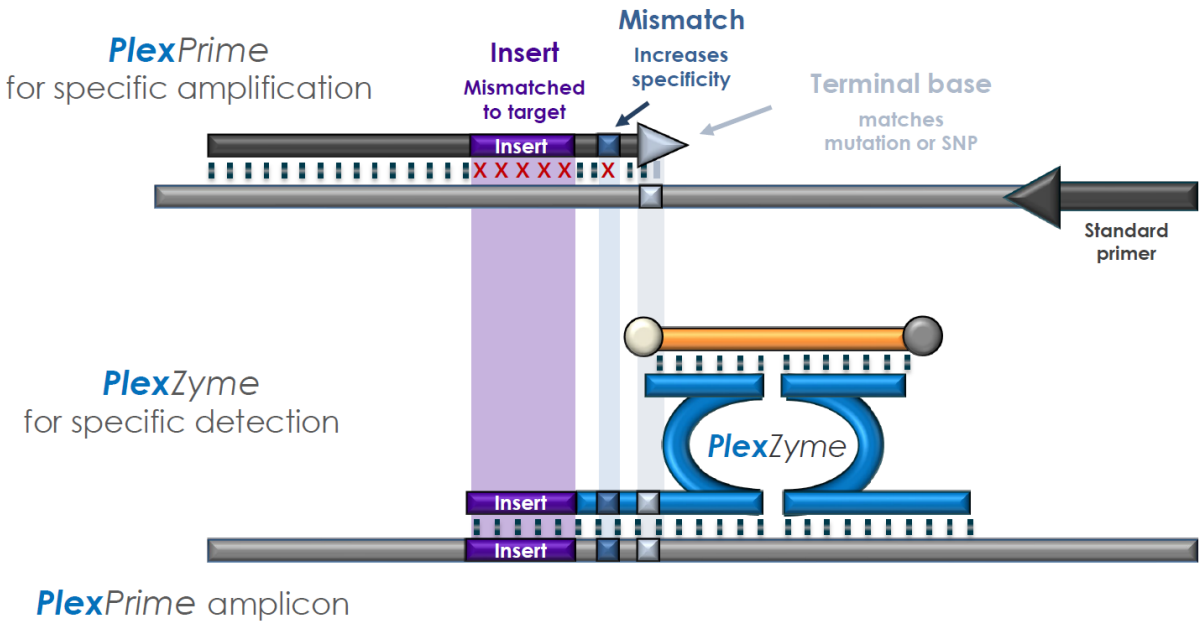
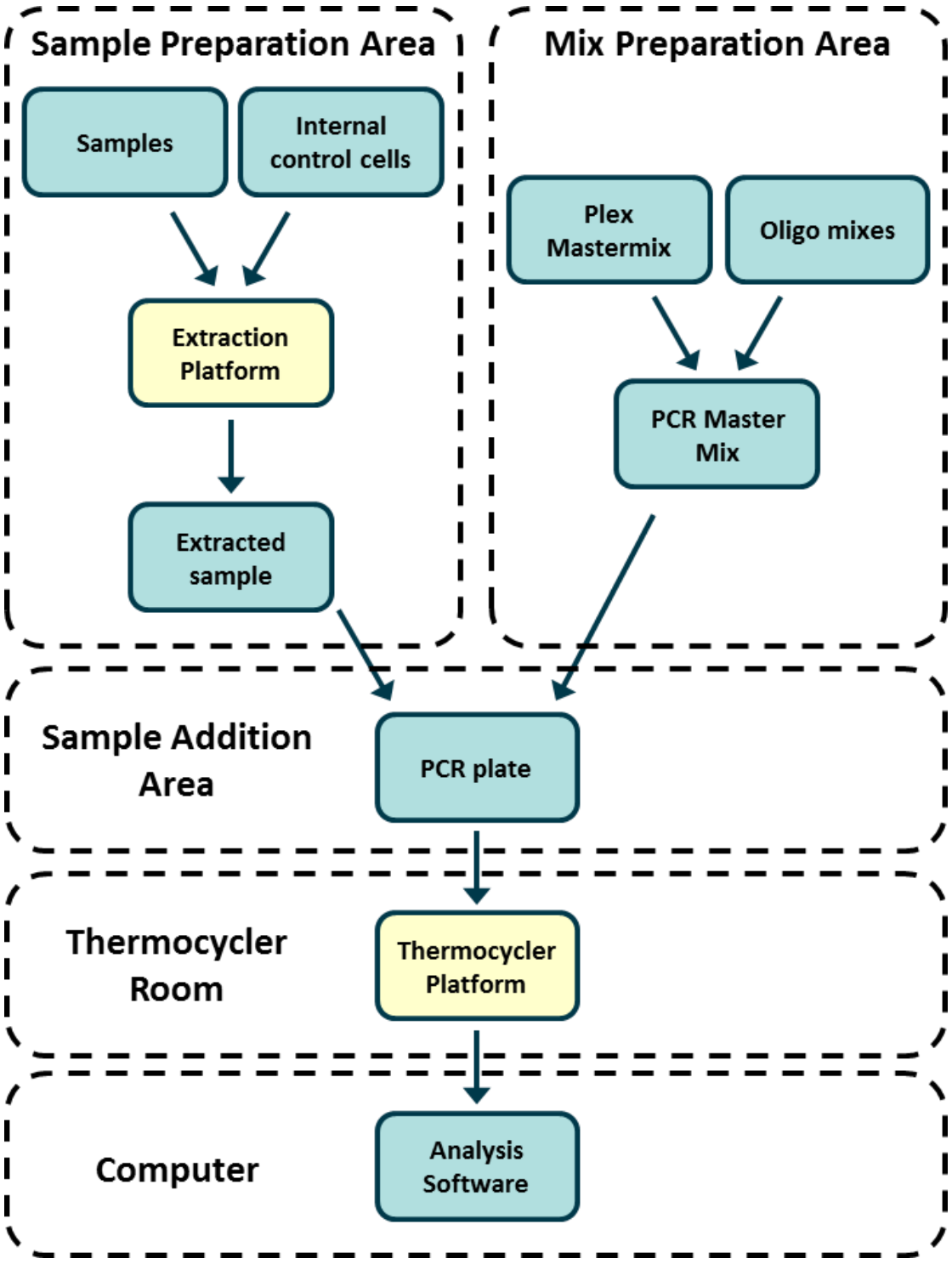


Figure 2. Schematic representation of the *PlexPrime*® primer coupled with *PlexZyme*® detection. The *PlexPrime*® primer specifically amplifies mutant sequence and *PlexZyme*® enzymes specifically detect the amplicon.



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 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 ***Resistance****Plus*® 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 20-50 mL 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 clinician and patient collected vaginal swab specimens. Due to the variability, refer to the manufacturers package insert for appropriate 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 and vaginal 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.

10.1.5 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 (Hologic, Cat no 301040). Please note that clinical performance of this collection device has only been demonstrated with specimens extracted using the MagNA Pure 96 instrument and MagNA Pure 96 DNA and Viral NA Large Volume Kit. Refer to **Section 10.2** and **Section 16.1.5** for further details.

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 2 mL 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® Multitest swab specimen collection kit (Hologic, Cat no PRD-03546) collection, transport and storage

Directions are summarized below for the collection and transport of vaginal swab specimens with the Aptima® Multitest swab specimen collection kit (Hologic, Cat no PRD-03546). Please note that clinical performance of this collection device has only been demonstrated with specimens extracted using the MagNA Pure 96 instrument and MagNA Pure 96 DNA and Viral NA Large Volume Kit. Refer to **Section 10.2** and **Section 16.1.5** for further details.

10.1.6.1 Vaginal swab specimen collection, transport and storage

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

10.1.11 Validated sample extracts

Sample extracts validated for use include:

- cobas® x480 (from CT/NG protocol)

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[a]	MagNA Pure 96 DNA and Viral NA Small Volume Kit	200 µL	Pathogen Universal 200	50 µL or 100 µL
MagNA Pure 96[a]	MagNA Pure 96 DNA and Viral NA Large Volume Kit	1000 µL^	Viral NA Universal LV 1000 3.1	100 µL
MICROLAB STARlet IVD[b]	STARMag 96 x 4 Universal Cartridge kit (Seegene)	200 µL	10 µL diluted Internal Control Cells added per sample Select 'Pause before PCR setup' to perform sample extraction only	100 µL
QIAsymphony SP[c]	DSP Virus/Pathogen Mini Kit	200 µL	Complex200_V6_DSP	110 µL
NucliSENS® easyMAG®[d]	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

[a] See 10.3.1 for how to use the internal control with the MagNA Pure 96

[b] See 10.3.2 for how to use the internal control with the STARlet IVD

[c] See 10.3.3 for how to use the internal control with the QIAsymphony SP

[d] See 10.3.4 for how to use the internal control with the NucliSENS® easyMAG®

^ Clinical performance of specimens collected with Aptima® collection kits have only been demonstrated with this extraction protocol. Refer to Section 16.1.5 for further details.

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)			
***Internal Control Cells* (RED) (µL)**	**1x PBS (µL)**	**Total volume (µL)**	**Volume added to sample (µL)**
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)			
Internal Control Cells **(RED) (µL)**	**1x PBS (µL)**	**Total volume (µL)**	**Volume added to sample (µL)**
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)		
Internal Control Cells **(RED) (µL)**	**1x PBS (µL)**	**Total volume (µL)**
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					
Tube type	**Number of samples**	**Volume of diluted IC Cells (µL)**	**Stock carrier RNA (µL)**	**Buffer AVE (µL)**	**Total volume (µL)**
-	1	10	3	107	120
2 mL	1 + void volume^	40	12	428	480
14 mL	1 + void volume#	60	18	642	720

^ 2 mL tube requires 3 additional samples (360 µL) to account for void volume

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)			
Internal Control Cells **(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

Prepare the Master 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.

Table 11. Master Mix		
Reagent	**Concentration**	**Volume per 20 µL reaction (µL)**
Nuclease Free Water (**NEUTRAL**)	N/A	3.0
Plex Mastermix (**BLUE**)	2x	10.0
MG+23S Mix (**BROWN**)	20x	1.0
Control Mix♯ (**WHITE**)	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.

11 Programming and analysis

Details for programming and analysis are described in the **Section 19 - Section 22**.

The ***Resistance****Plus*® MG kit has three channels for detection of *M. genitalium*, 23S rRNA mutation and Internal Control (**Table 12**).

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

12 Interpretation of results

Data interpretation requires the ***Resistance****Plus*® MG analysis software. While ***Plex****Prime*® 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 ***Resistance****Plus*® 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 23**.

See **Table 13** 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 for more information.

Table 13. *ResistancePlus*® MG analysis software

Cat no	Analysis software*	Real-time PCR instrument
99003	***Resistance****Plus*® MG (LC480)	LC480 II
99002	***Resistance****Plus*® MG (7500)	7500 Fast and 7500 Fast Dx
99008	***Resistance****Plus*® 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 ***Resistance****Plus*® MG assay targets the *MgPa* gene for *M. genitalium* and mutations at positions 2058 and 2059 in the 23S rRNA gene (A2058G, A2059G, A2058T and A2058C, *E. coli* numbering) that are associated with resistance to azithromycin (macrolide-based antibiotic).
- The ***Resistance****Plus*® MG assay has been shown to cross-react with the *M. genitalium*, 23S rRNA A2059C mutant sequences.
- The ***Resistance****Plus*® MG clinical performance studies summarised in Section 16.1 include testing with male urine, female urine and vaginal swabs. Additional specimen types including rectal, cervical, endocervical, urethral, penile, penile meatal and pharyngeal swabs have also been tested, however there are currently limited data supporting the use of these specimen types.
- The ***Resistance****Plus*® 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 ***Resistance****Plus*® 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***® MG kit includes an internal control to monitor extraction efficiency and qPCR inhibition (**Section 10.3**).

The ***ResistancePlus***® 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***® MG Positive Controls. A known negative specimen is recommended to be used as a negative control.

15 *ResistancePlus*® MG Positive Control instructions

The ***ResistancePlus***® MG Positive Control kit contains positive control material for *M. genitalium* 23S rRNA mutants and a *M. genitalium* wild type 23S rRNA (**Table 14**).

Table 14. Contents for *ResistancePlus*® 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 *M. genitalium*, 23S rRNA wild type	1 x 50 µL
Green	MG, 23S rRNA A2058G	Positive control template for the detection of *M. genitalium*, 23S rRNA A2058G mutation	1 x 50 µL
Red	MG, 23S rRNA A2059G	Positive control template for the detection of *M. genitalium*, 23S rRNA A2059G mutation	1 x 50 µL
Blue	MG, 23S rRNA A2058T	Positive control template for the detection of *M. genitalium*, 23S rRNA A2058T mutation	1 x 50 µL
Yellow	MG, 23S rRNA A2058C	Positive control template for the detection of *M. genitalium*, 23S rRNA A2058C 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***® MG analysis software, refer to **Section 23.9** 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. 144 samples were selected for inclusion in the study. The 144 samples consisted of 84 male urine, 33 female urine, 14 vaginal swabs and 13 high vaginal swabs. To determine performance of the ***Resistance****Plus*® 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[7], and 23S rRNA mutant detection was compared to Sanger sequencing[8]. The ***Resistance****Plus*® 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[9]. For 23S rRNA mutant detection, Sanger sequencing was taken as the true result. Resolved results and sensitivity and specificity of the ***Resistance****Plus*® MG kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 15**. 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 16**. The 23S rRNA mutation analysis is shown in **Table 17**.

Table 15. Clinical evaluation of the *ResistancePlus*® MG kit (Clinical Study 1)

		***M. genitalium* detection 16S rRNA qPCR**				**23S rRNA mutant detection Sequencing**	
		Positive	**Negative**			**Mutant**	**Wild type**
***ResistancePlus*® MG**	**Positive**	83	0		**Mutant detected**	52	2
	Negative	1	58^		**Mutant not detected**	2	21
Sensitivity		98.8% (95% CI 93.5-100.0%)			**Sensitivity**	96.3% (95% CI 87.3-99.6%)	
Specificity		100.0% (95% CI 93.8-100.0%)			**Specificity**	91.3% (95% CI 72.0-98.9%)	

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

^ The ***Resistance****Plus*® MG kit detected 1 true *M. genitalium* negative using composite reference, table represents resolved results

Table 16. Clinical result analysis in accordance to specimen^ (Clinical Study 1)			
Specimen	**Expected *M. genitalium* negative**	**Expected *M. genitalium* 23S rRNA wild type**	**Expected *M. genitalium* 23S rRNA mutant**
Male urine	28/28	8/10[1]	41/42[1]
Female urine	12/13	11/11	4/6[2]
Vaginal swab	8/8	1/1	2/2[3]
High vaginal swab	9/9	1/1	4/4[4]

Mutant – 23S rRNA mutation in A2058G, A2059G, A2058Tand A2058C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

^ 2 female urine, 3 male urine, 1 vaginal swab excluded as sequencing failed and mutant status could not be determined

[1] 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

[2] Female urine: 1 A2058G, 3 A2059G correctly detected; 2 A2059G miscalled as *M. genitalium* detected, mutant not detected

[3] Vaginal swab: 2 A2059G correctly detected

[4] High vaginal swab: 3 A2058G, 1 A2059G correctly detected

Table 17. *M. genitalium* 23S rRNA mutation analysis (Clinical Study 1)	
Reference result^	***ResistancePlus*® MG result**
Wild type	21/33[1]
A2058G	22/23[2]
A2059G	26/28[3]
A2058T	3/3

^ For *M. genitalium* positive samples only

[1] Wild type: 2 Male urine miscalled as *M. genitalium* mutant detected

[2] A2058G: 1 Male urine miscalled as *M. genitalium* not detected

[3] 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 ABI 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 ***Resistance****Plus*® $MG_{(550)}$ kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 18**. Analysis of 23S rRNA mutation detection only includes samples where the mutant status could be determined.

Table 18. Clinical evaluation of the *ResistancePlus*® $MG_{(550)}$ kit (Clinical Study 2)

		***M. genitalium* detection 16S rRNA qPCR**				**23S rRNA mutant detection Sequencing**	
		Positive	**Negative**			**Mutant**	**Wild type**
***ResistancePlus*® MG**	**Positive**	79	0^		**Mutant detected**	47	1
	Negative	2	43#		**Mutant not detected**	4	19
Sensitivity		97.5% (95% CI 91.4-99.7%)			**Sensitivity**	92.2% (95% CI 81.1-97.8%)	
Specificity		100.0% (95% CI 91.8-100.0%)			**Specificity**	95.0% (95% CI 75.1-99.9%)	

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

^ The ***Resistance****Plus*® $MG_{(550)}$ kit detected 1 true *M. genitalium* positive using reference test, table represents resolved results

The ***Resistance****Plus*® $MG_{(550)}$ 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, collected with the multi-Collect Specimen Collection Kit (Abbott). The 137 samples consisted of 110 male urine, 11 female urine, 15 vaginal swabs, 1 urethral/vaginal swab and 1 vaginal/cervical swab. To determine performance of the ***Resistance****Plus*® 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 ***Resistance****Plus*® 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 ***Resistance****Plus*® MG kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 19**. One sample was 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 20**. The 23S rRNA mutation analysis is shown in **Table 21**.

Table 19. Clinical evaluation of the *ResistancePlus*® MG kit (Clinical Study 3)

		***M. genitalium* detection 16S rRNA qPCR**				**23S rRNA mutant detection Sequencing**	
		Positive	**Negative**			**Mutant**	**Wild type**
***ResistancePlus*® MG**	**Positive**	76	0		**Mutant detected**	52	1
	Negative	3	57^		**Mutant not detected**	5	19
Sensitivity		96.2% (95% CI 89.3-99.2%)			**Sensitivity**	91.2% (95% CI 80.7-97.1%)	
Specificity		100.0% (95% CI 93.7-100.0%)			**Specificity**	95.0% (95% CI 75.1-99.9%)	

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

^ Table represents resolved results

Table 20. Clinical result analysis in accordance to specimen (Clinical Study 3)			
Specimen	**Expected *M. genitalium* negative**	**Expected *M. genitalium* wild type**	**Expected *M. genitalium* 23S rRNA mutant**
Male urine	45/45	17/18[1]	38/45[1]
Female urine	4/4	1/1	6/6[2]
Vaginal swab	6/6	1/1	8/8[3]
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 and A2058C positions (*E. coli* numbering); Wild type – absence of mutation in these positions

[1] Male urine: 1 *M. genitalium* wild type miscalled as *M. genitalium* mutant detected, 4 A2058G, 32 A2059G, 1 A2058T, 1 A2058C correctly detected; 1 A2058G and 1 A2059G miscalled as *M. genitalium* not detected, 3 A2058G and 2 A2059G miscalled as *M. genitalium* mutant not detected

[2] Female urine: 2 A2058G, 4 A2059G correctly detected

[3] Vaginal swab: 1 A2058G, 7 A2059G correctly detected

Table 21. *M. genitalium* 23S rRNA mutation analysis (Clinical Study 3)	
Reference result^	***ResistancePlus*® MG result**
Wild type	19/20[1]
A2058G	7/10[2]
A2059G	43/45[3]
A2058T	1/1
A2058C	1/1

^ For *M. genitalium* positive samples only

[1] Wild type: 1 Male urine miscalled as *M. genitalium* mutant detected

[2] A2058G: 3 Male urine miscalled as *M. genitalium* mutant not detected

[3] A2059G: 2 Male urine 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 ***Resistance****Plus*® MG(675) kit for the detection of *M. genitalium* and azithromycin resistance-associated mutations in retrospective samples collected between December 2017- April 2018. Samples were collected using the DeltaSwab ViCUM® (Deltalab, Spain) for swabs or Vacumed® Urine (FL medical, Italy) for urine. The 86 samples consisted of 46 urines and 40 vaginal 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™ STI Essential (Seegene) as well as to the ***Resistance****Plus*® MG kit (SpeeDx) on the LC480 II for both *M. genitalium* detection and 23S rRNA status. The sensitivity and specificity of the ***Resistance****Plus*® MG(675) kit for *M. genitalium* detection compared to Allplex™ STI Essential (Seegene) is shown in **Table 22**. The sensitivity and specificity of the ***Resistance****Plus*® MG(675) compared to ***Resistance****Plus*® MG is as shown in **Table 23**. Analysis of results in accordance to specimen type is shown in **Table 24**.

Table 22. Comparison of *ResistancePlus*® MG(675) kit with Allplex™ STI essential (Clinical Study 4)

		***M. genitalium* detection** **Allplex™ STI Essential**	
		Positive	**Negative**
***ResistancePlus*® MG(675)**	**Positive**	40	0
	Negative	0	46
Sensitivity		100.0% (95% CI 91.2-100.0%)	
Specificity		100.0% (95% CI 92.3-100.0%)	

Table 23. Clinical evaluation of the *ResistancePlus*® MG(675) kit (Clinical Study 4)

		***M. genitalium* detection** ***ResistancePlus*® MG (LC480 II)**				**23S rRNA mutant detection#** ***ResistancePlus*® MG (LC480 II)**	
		Positive	**Negative**			**Mutant detected**	**Mutant not detected**
***ResistancePlus*® MG(675)**	**Positive**	40	0		**Mutant detected**	20	0
	Negative	0	46		**Mutant not detected**	1	20
Sensitivity		100.0% (95% CI 91.2-100.0%)			**Sensitivity**	100.0% (95% CI 83.2-100.0%)	
Specificity		100.0% (95% CI 92.3-100.0%)			**Specificity**	100.0% (95% CI 83.2-100.0%)	

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

1 sample excluded from analysis as this was sequenced as mixed wild-type and mutant

Table 24. Clinical result analysis in accordance to specimen (Clinical Study 4)

Specimen	**Expected *M. genitalium* negative**	**Expected *M. genitalium* 23S rRNA wild type**	**Expected *M. genitalium* 23S rRNA mutant**
Male urine	26/26	5/5	15/15
Female vaginal swab	20/20	15/15	5/5

16.1.5 Clinical Study 5

A retrospective clinical study was conducted at Royal Women's Hospital (RWH), Melbourne, Australia using Aptima® collected urine and swabs from June 2017-November 2017. Matched patient specimens were collected as neat urine (routine sample) or with the Aptima® Urine Specimen Collection kit (Hologic), or as dry swab (routine sample) or with the Aptima® Unisex Swab Specimen Collection kit (Hologic). The 147 samples consisted of 122 urines and 25 vaginal swabs. To determine the performance of Aptima® collected samples with the ***Resistance****Plus*® MG kit, *M. genitalium* and 23S rRNA mutant detection was compared to the clinical diagnostic results obtained from the ***Resistance****Plus*® MG kit (SpeeDx) using the routine sample. Testing of Aptima® 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 ***Resistance****Plus*® 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 ***Resistance****Plus*® MG kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 25**. 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 26**.

Table 25. Clinical evaluation of the *ResistancePlus*® MG kit (Clinical Study 5)

		***M. genitalium* detection** ***ResistancePlus*® MG (routine sample)**				**23S rRNA mutant detection** ***ResistancePlus*® MG (routine sample)**	
		Positive	**Negative**			**Mutant**	**Wild type**
***ResistancePlus*® MG (with 1mL Aptima sample)**	**Positive**	77	3		**Mutant detected**	51	0
	Negative	3	64		**Mutant not detected**	2	24
Sensitivity		96.3% (95% CI 89.4-99.2%)			**Sensitivity**	96.2% (95% CI 87.0-99.5%)	
Specificity		95.5% (95% CI 87.5-99.1%)			**Specificity**	100.0% (95% CI 86.0-100.0%)	

Table 26. Clinical result analysis in accordance to specimen type (Clinical Study 5)

Specimen	**Expected *M. genitalium* negative**	**Expected *M. genitalium* wild type**	**Expected *M. genitalium* 23S rRNA mutant**
Urine	50/52[1]	21/22[1]	45/48[1]
Vaginal swab	14/15[2]	3/4[2]	6/6

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

[1] 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

[2] Vaginal swab: 1 *M. genitalium* negative miscalled as *M. genitalium* wild type; 1 *M. genitalium* wild type 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® x480 extracts from urine and swab samples collected from February 2017-February 2019. Specimens were collected as neat urine or with the cobas® PCR media collection kit (Roche) and extracted on the cobas® x480 (cobas® 4800, Roche) instrument using the "Full Workflow" and "CT/NG" protocol, without addition of SpeeDx Internal Control Cells. The 109 extracts consisted of 10 vaginal swabs, 5 high vaginal swabs, as well as 84 male and 10 female urine specimens.

To determine the performance of cobas® extracts with the ***Resistance****Plus*® MG(550) 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 ***Resistance****Plus*® MG(550) kit was performed on the ABI 7500 Fast Dx. The sensitivity and specificity of the ***Resistance****Plus*® MG(550) kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 27**. 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 28**. The 23S rRNA mutation analysis is shown in **Table 29**.

Table 27. Clinical evaluation of the *ResistancePlus*® MG(550) kit (Clinical Study 6)

		***M. genitalium* detection MgPa qPCR**			**23S rRNA mutant detection Sanger Sequencing**	
		Positive	**Negative**		**Mutant**	**Wild type**
***ResistancePlus*® MG(550)**	**Positive**	54	0	**Mutant detected**	37^	0
	Negative	1	51	**Mutant not detected**	0	17
Sensitivity		98.2% (95% CI 90.3-100.0%)		**Sensitivity**	100.0% (95% CI 90.5-100.0%)	
Specificity		100.0% (95% CI 93.0-100.0%)		**Specificity**	100.0% (95% CI 80.5-100.0%)	

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

Table 28. Clinical result analysis in accordance to specimen (Clinical Study 6) #

Specimen	**Expected *M. genitalium* negative**	**Expected *M. genitalium* 23S rRNA wild type**	**Expected *M. genitalium* 23S rRNA mutant**
Male urine	42/42	13/13	26/27[1]
Female urine	6/6	1/1	3/3[2]
Vaginal swab	1/1	1/1	7/7[3^]
High vaginal swab	2/2	2/2	1/1[4]

3 samples were excluded as sequencing failed and true 23S status could not be determined, including: 2 urine and 1 vaginal sample

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

[2] Female urine: 2 A2058G and 1 A2059G correctly identified

[3] Vaginal swab: 3 A2058G, 2 A2058T and 1 A2059G correctly identified; ^ 1 vaginal swab was identified as a mixture WT/A2059G

[4] High vaginal swab: 1 A2059G correctly identified

Table 29. *M. genitalium* 23S rRNA mutation analysis (Clinical Study 6)	
Reference result^	***ResistancePlus*® MG result**
Wild type	17/17
A2058G	13/13
A2059G	19/19[1]
A2058T	5/5
A2058C	-

^ For *M. genitalium* positive samples only

[1] A2059G: 1 vaginal swab mixed wild-type/A2059G correctly identified as *M. genitalium*, 23S mutation 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 19 vaginal swabs, 2 high vaginal swabs as well as 44 urine specimens.

The ***Resistance****Plus*® MG kit was performed on the LC480 II, after sample extraction on the QIAsymphony 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 ***Resistance****Plus*® 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 ***Resistance****Plus*® MG kit for *M. genitalium* detection and 23S rRNA mutant detection are shown in **Table 30**. 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 31**.

Table 30. Clinical evaluation of the *ResistancePlus*® MG kit (Clinical Study 7)							
		***M. genitalium* detection *ResistancePlus*® MG (MP96)**				**23S rRNA mutant detection *ResistancePlus*® MG (MP96)**	
		Positive	**Negative**			**Mutant**	**Wild type**
***ResistancePlus*® MG (QIAsymphony SP)**	**Positive**	36	0		**Mutant detected**	16	1
	Negative	1	27		**Mutant not detected**	1	18
Sensitivity		97.3% (95% CI 85.8-99.9%)			**Sensitivity**	94.1% (95% CI 71.3-99.9%)	
Specificity		100.0% (95% CI 87.2-100.0%)			**Specificity**	94.7% (95% CI 74.0-99.9%)	

Table 31. Clinical result analysis in accordance to specimen (Clinical Study 7) [#]			
Specimen	**Expected *M. genitalium* negative**	**Expected *M. genitalium* 23S rRNA wild type**	**Expected *M. genitalium* 23S rRNA mutant**
Male Urine	17/17	9/9	12/14[1]
Female Urine	1/1	1/2[2]	1/1
Vaginal swab	8/8[#]	7/7	3/3
High vaginal swab	1/1	1/1	-

[#] 1 vaginal swab was excluded as it produced an invalid result with the ***Resistance****Plus*® MG kit

[1] Male urine: 1 *M. genitalium* 23S rRNA mutant 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

[2] 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 ***Resistance****Plus*® MG kit on the LC480 II was assessed using quantified synthetic template for *M. genitalium* MgPa and 23S rRNA targets (A2058G, A2059G, A2058T and A2058C) 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 32**). The two lots showed good reproducibility with coefficient of variation (%CV) between 0.35-2.37% for all targets.

Table 32. Lot-to-lot variability				
	Average Cq	**STDEV**	**%CV**	**# samples**
MgPa 10,000 copies	16.9	0.15	0.89	12/12
MgPa 30 copies	25.5	0.52	2.05	12/12
A2058G 10,000 copies	20.4	0.48	2.37	12/12
A2058G 36 copies	27.8	0.43	1.54	12/12
A2059G 10,000 copies	18.0	0.06	0.35	12/12
A2059G 30 copies	25.6	0.50	1.94	12/12
A2058T 10,000 copies	18.7	0.09	0.46	12/12
A2058T 30 copies	26.2	0.30	1.14	12/12
A2058C 10,000 copies	17.7	0.13	0.75	12/12
A2058C 30 copies	25.4	0.29	1.15	12/12

To determine day-to-day variability, testing was performed over three days by one operator on the same machine (**Table 33**). The three runs showed good reproducibility between different days with coefficient of variation between 0.88-2.31% for all targets.

Table 33. 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

To determine run-to-run variability, three qPCR runs were compared, run on the same day by the same operator (**Table 34**). The three runs showed good reproducibility with coefficient of variation between 0.40-3.20% for all targets.

Table 34. 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

To determine operator variability, two runs were compared from two operators (**Table 35**). The two runs from different operators showed good reproducibility with coefficient of variation between 0.54-1.62% for all targets.

Table 35. 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

To determine instrument variability, two runs from two machines were compared, performed by the same operator (**Table 36**). The runs from different instruments showed good reproducibility with coefficient of variation between 0.30-2.62% for all targets.

Table 36. 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

To determine within-run variability, three experiments were compared, set up separately by the same operator running each target on the same plate (**Table 37**). The three experiments showed good reproducibility with coefficient of variation between 0.57-3.12% for all targets.

Table 37. 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

16.2.2 Analytical sensitivity

The analytical sensitivity of the ***Resistance****Plus*® MG kit on the LC480 II was determined by running limited dilution series', using quantified synthetic template for *M. genitalium* MgPa and 23S rRNA targets (A2058G, A2059G, A2058T and A2058C). The sensitivity for each target was determined as the number of copies per reaction with ≥ 95% detection shown in **Table 38**.

Table 38. Analytical sensitivity	
	Analytical Sensitivity (copies/reaction)
MgPa	10
A2058G	12
A2059G	10
A2058T	10
A2058C	10

16.2.3 Analytical specificity

This study was conducted to evaluate the ***Resistance****Plus*® 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 x 10^6 genomes/mL, unless otherwise stated. Viral strains were tested at 1 x 10^5 genomes/mL, unless otherwise stated. All other organisms were tested at the concentrations stated. All 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 39**).

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

Table 39. Microorganisms tested for analytical specificity					
Organism	**Concentration (genomes/mL)**	**Organism**	**Concentration (genomes/mL)**	**Organism**	**Concentration (genomes/mL)**
Actinomyces israelii	1 x 10^6	HIV-1^	1 x 10^3	*Mycoplasma pirum* (2)*	1 x 10^6
Atopobium vaginae	1 x 10^6	HPV type 18 (HeLa cells)^	1 x 10^5	*Mycoplasma pneumoniae* (6)*	1 x 10^6
Bacterioides fragilis	1 x 10^6	*Klebsiella oxytoca*	1 x 10^6	*Mycoplasma primatum*	1 x 10^6
Bifidobacterium adolescentis	1 x 10^6	*Lactobacillus acidophilus*	1 x 10^6	*Mycoplasma salivarium*	1 x 10^6
Campylobacter jejuni	1 x 10^6	*Lactobacillus crispatus*	1 x 10^6	*Neisseria gonorrhoeae*	1 x 10^6
Candida albicans	1 x 10^5	*Lactobacillus jensenii*	1 x 10^6	*Pentatrichomonas hominis*#	1 x 10^5
Candida glabrata	1 x 10^6	*Lactobacillus vaginalis*	1 x 10^6	*Peptostreptococcus anaerobius*	1 x 10^6
Candida parapsilosis	1 x 10^6	*Listeria monocytogenes*	1 x 10^6	*Prevotella bivia*	1 x 10^6
Candida tropicalis	1 x 10^5	*Mobiluncus curtisii*	1 x 10^6	*Propionibacterium acnes*	1 x 10^5
Chlamydia trachomatis	1 x 10^6	*Mycobacterium smegmatis*	1 x 10^5	*Proteus mirabilis*	1 x 10^6
Clostridium perfringens	1 x 10^6	*Mycoplasma alvi*	1 x 10^6	*Proteus vulgaris*	1 x 10^6
Corynebacterium genitalium	1 x 10^6	*Mycoplasma amphoriforme* (2)*	1 x 10^6	*Pseudomonas aeruginosa*	1 x 10^6
Enterobacter aerogenes	1 x 10^6	*Mycoplasma arginini*	1 x 10^6	*Staphylococcus aureus*	1 x 10^6
Enterobacter cloaceae	1 x 10^6	*Mycoplasma buccale*	1 x 10^6	*Staphylococcus saprophyticus*	1 x 10^6
Enterococcus fecalis	1 x 10^6	*Mycoplasma fermentans*	1 x 10^6	*Streptococcus agalactiae*	1 x 10^6
Fusobacterium nucleatum	1 x 10^6	*Mycoplasma gallisepticum*	1 x 10^4	*Streptococcus pyogenes*	1 x 10^6
Gardnerella vaginalis	1 x 10^6	*Mycoplasma hominis*	1 x 10^6	*Trichomonas vaginalis*#	1 x 10^5
Haemophilus ducreyi	1 x 10^6	*Mycoplasma lipohilum*	1 x 10^4	*Ureaplasma urealyticum*	1 x 10^5
Herpes simplex virus I	1 x 10^6	*Mycoplasma orale*	1 x 10^6		
Herpes simplex virus II	1 x 10^6	*Mycoplasma penetrans*	1 x 10^6		

* number in brackets denotes the number of strains tested

^ quantified as PFU/mL

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 ***Resistance****Plus*® 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 40** and **Table 41**.

Table 40. 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	--	10^5 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 41. 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

Table 41. Potentially interfering substances in vaginal swab samples		
Class/Substance	**Product name**	**Test concentration**
	Estrace® (estradiol vaginal cream, USP 0.01%)	0.25% w/v
Leukocytes	--	10^5 cells/mL
Intravaginal hormones	--	7 mg/ml Progesterone + 0.07 mg/ml Beta Estradiol

16.2.5 Cross-reactivity to other 23S rRNA mutations

Cross-reactivity of the ***Resistance****Plus*® MG kit was assessed using quantified synthetic template for *M. genitalium* MgPa and 23S rRNA targets (A2059C) at 10,000 and 45 copies per reaction. The results demonstrated that the ***Resistance****Plus*® MG test cross-reacts to the *M. genitalium* A2059C 23S rRNA target with a 100% hit rate.

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

18 References

1. Taylor-Robinson D, Jensen JS. *Mycoplasma genitalium*: from Chrysalis to multicolored butterfly. Clin Microbiol Rev. 2011;24:498–514.
2. Manhart LE, Broad JM, Golden MR. Mycoplasma genitalium: should we treat and how? Clin Infect Dis. 2011 Dec;53 Suppl 3:S129-42.
3. Cazanave C, Manhart LE, Bébéar C. Mycoplasma genitalium, an emerging sexually transmitted pathogen. Med Mal Infect. 2012 Sep;42(9):381-92
4. Jensen JS, Bradshaw CS, Tabrizi SN, Fairley CK, Hamasuna R. Azithromycin treatment failure in Mycoplasma genitalium-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. Clin Infect Dis. 2008 Dec 15;47(12):1546-53.
5. Jensen JS. Chapter 8: Protocol for the Detection of Mycoplasma genitalium by PCR from Clinical Specimens and Subsequent Detection of Macrolide Resistance-Mediating Mutations in Region V of the 23S rRNA Gene in Diagnosis of Sexually Transmitted Diseases: Methods and Protocols, Methods in Molecular Biology, vol. 903, Science+Business Media New York 2012.
6. Bissessor M, Tabrizi SN, Twin J, Abdo H, Fairley CK, Chen MY, Vodstrcil LA, Jensen JS, Hocking JS, Garland SM, Bradshaw CS. Macrolide resistance and azithromycin failure in a Mycoplasma genitalium-infected cohort and response of azithromycin failures to alternative antibiotic regimens. Clin Infect Dis. 2015 Apr 15;60(8):1228-36.
7. Twin J, Taylor N, Garland SM, Hocking JS, Walker J, Bradshaw CS, Fairley CK, Tabrizi SN. Comparison of two Mycoplasma genitalium real-time PCR detection methodologies. J Clin Microbiol. 2011 Mar;49(3):1140-2.
8. Twin J, Jensen JS, Bradshaw CS, et al. Transmission and selection of macrolide resistant Mycoplasma genitalium infections detected by rapid high resolution melt analysis. PLoS One 2012; 7:e35593.
9. Jensen JS, Bjornelius E, Dohn B, Lidbrink P. Use of Taqman 5' nuclease real-time PCR for quantitative detection of Mycoplasma genitalium DNA in males with and without urethritis who were attendees at a sexually transmitted disease clinic. J Clin Microbiol. 2004 42:683-692.

19 Appendix 1: LightCycler® 480 instrument II

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

The ***Resistance****Plus*® MG kit contains dyes for the LightCycler® 480 Instrument II. The ***Plex****PCR*® 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) shown in **Table 42.**

Table 42. Filter combinations^

LC480 II	440-488	465-510	533-580	533-610	533-640	618-660

^ These Filter Combinations are the default names for the channels

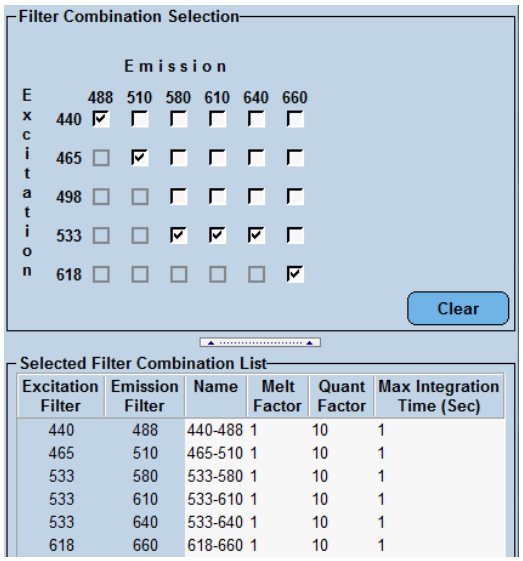
Set the **Selected Filter Combination List** for all channels as:

Melt Factor: 1

Quant Factor: 10

Max Integration Time (sec): 1

Figure 3. Custom SpeeDx Detection Format



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

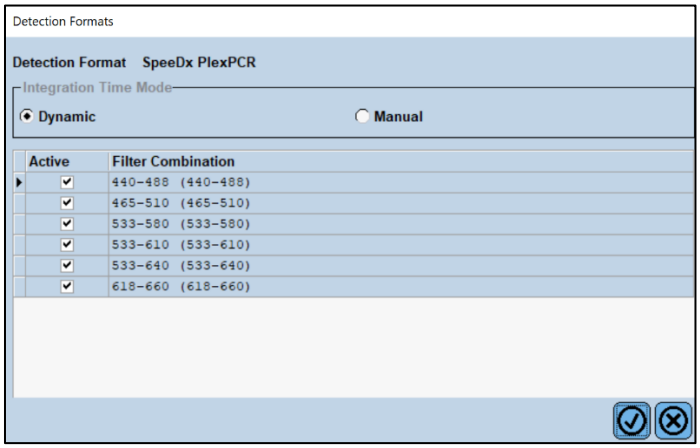
For **Detection Format** select the custom '**SpeeDx PlexPCR**' (**Figure 4**)

Select **Customize** >

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

Select all Active **Filter Combinations** shown in **Figure 4.**

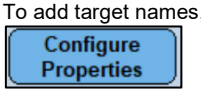
Figure 4. Customize Detection Format



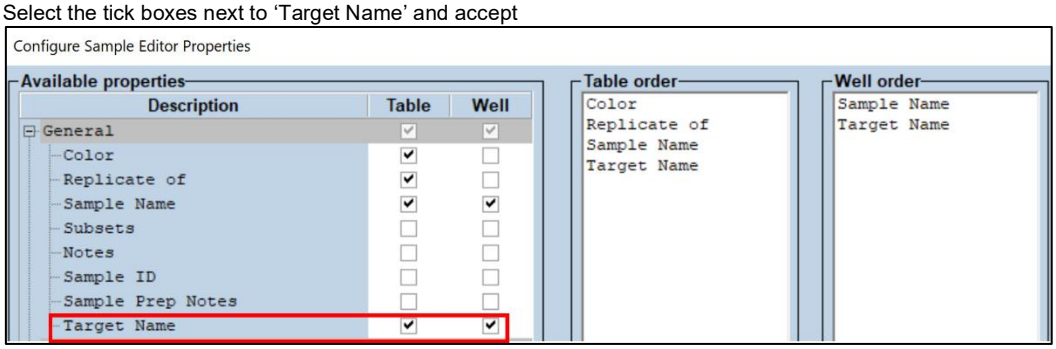
To enable automated sample detection in the analysis software, assign nametags to the wells on the plate

Open the **Sample Editor** module

To add target names, select **Configure Properties**



Select the tick boxes next to 'Target Name' and accept



Edit the **Target Name** for each channel to match the Target Instrument Reference defined in the Lab Configuration > Assays menu of the analysis software and shown in **Table 43.**

Table 43. Channels for *ResistancePlus*® MG targets

Target Name	MgPa	23S rRNA mutation	IC
LC480 II channel	465-510	533-580	533-640

To assign nametags, select the well

Edit **Sample Name** to match the nametag defined in Lab Configuration > Assays menu of the analysis software (see **Section 23.3)**

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 44** and **Figure 5**). Additional nametags can be defined for both regular samples and controls within the analysis software.

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

Table 44. Sample nametags for analysis software

Sample type	Default Prefix (in analysis software)
Regular sample	No default – user defined
Negative control	NC
No template control	NTC
Positive control (MG, 23S rRNA mutant type) (Pa)	Pa
Positive control (MG, 23S rRNA wild type) (Pb)	Pb

Figure 5. Sample Editor – Assigning nametags to wells

Pos	Filter combination	Color	Repl Of	Sample Name	Target Name
A1	465-510 (465-510)			NC	MgPa
A1	533-580 (533-580)			NC	23S rRNA mutation
A1	533-640 (533-640)			NC	IC
A2	465-510 (465-510)			NTC	MgPa
A2	533-580 (533-580)			NTC	23S rRNA mutation
A2	533-640 (533-640)			NTC	IC
A3	465-510 (465-510)			Pa	MgPa
A3	533-580 (533-580)			Pa	23S rRNA mutation
A3	533-640 (533-640)			Pa	IC
A4	465-510 (465-510)			Pb	MgPa
A4	533-580 (533-580)			Pb	23S rRNA mutation
A4	533-640 (533-640)			Pb	IC

Set **Reaction Volume** > 20 µL

Create the following Program in **Table 45** (shown in more detail in **Figure 6 – Figure 9):**

Table 45. Thermocycling Program

Program name	Cycles	Target °C	Hold	Ramp rate (°C/s)≠
Polymerase activation	1	95°C	2 min	4.4
Touch down cycling$^{\delta}$: Step down -0.5°C/cycle	10	95°C	5 s	4.4
		61°C – 56.5°C$^{\delta}$	30 s	2.2
Quantification cycling^{+}: Acquisition/Detection	40	95°C	5 s	4.4
		52°C^{+}	40 s	2.2
Cooling	1	40°C	30 s	2.2

≠ Default ramp rate (96 well plate)

$^{\delta}$ **Step size:** -0.5°C/Cycle, **Sec Target:** 56°C

$^{+}$ **Analysis mode:** Quantification, **Acquisition mode**: Single

Figure 6. Thermocycling Program – Polymerase activation

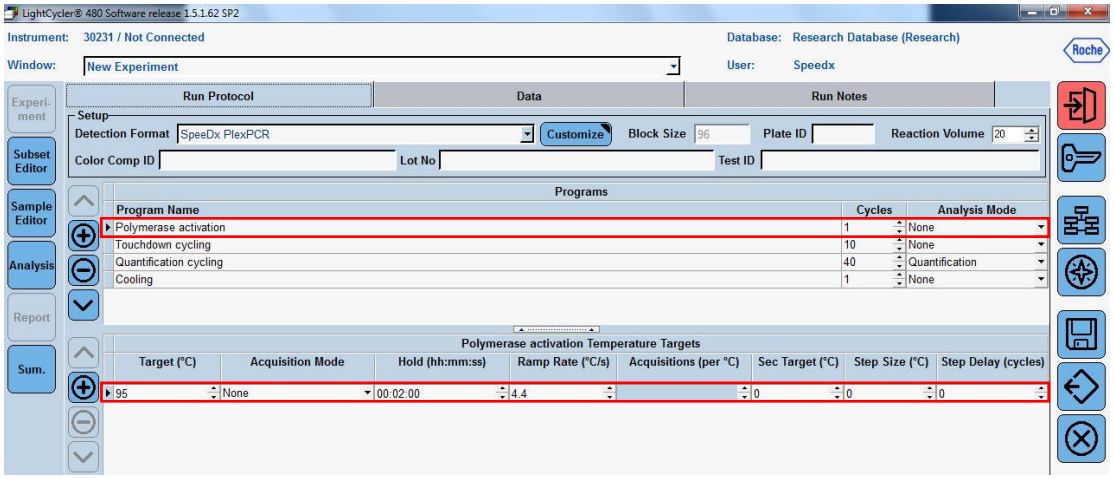


Figure 7. Thermocycling Program – Touchdown cycling

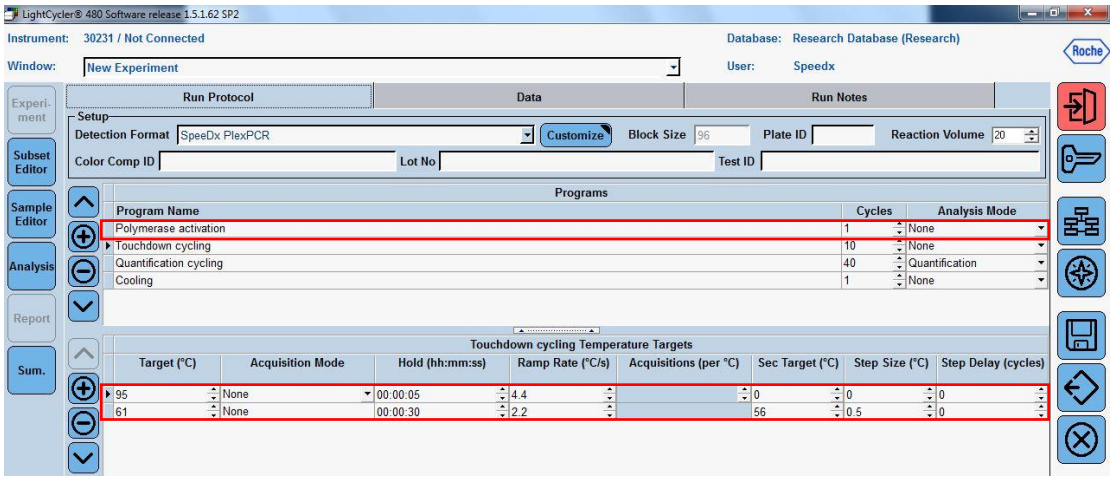


Figure 8. Thermocycling Program – Quantification cycling

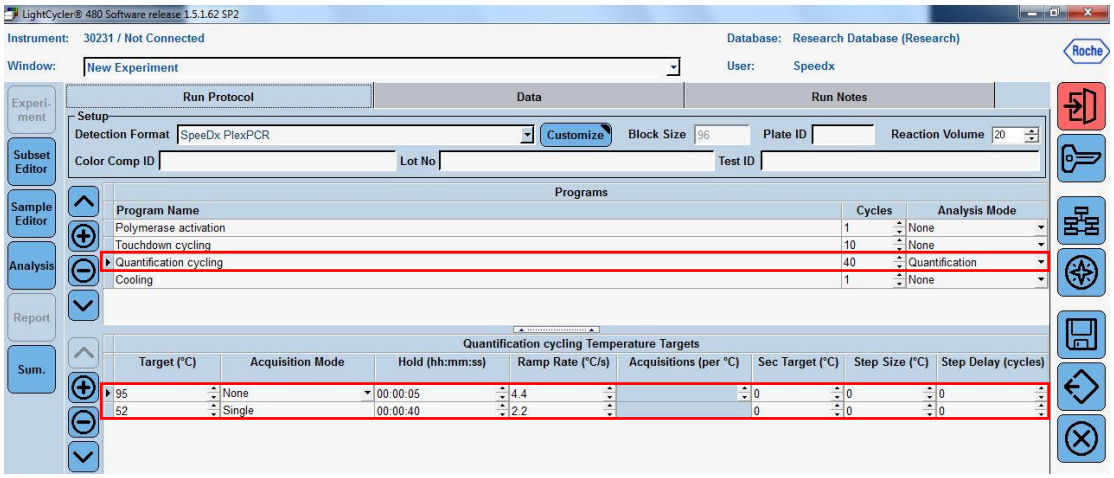
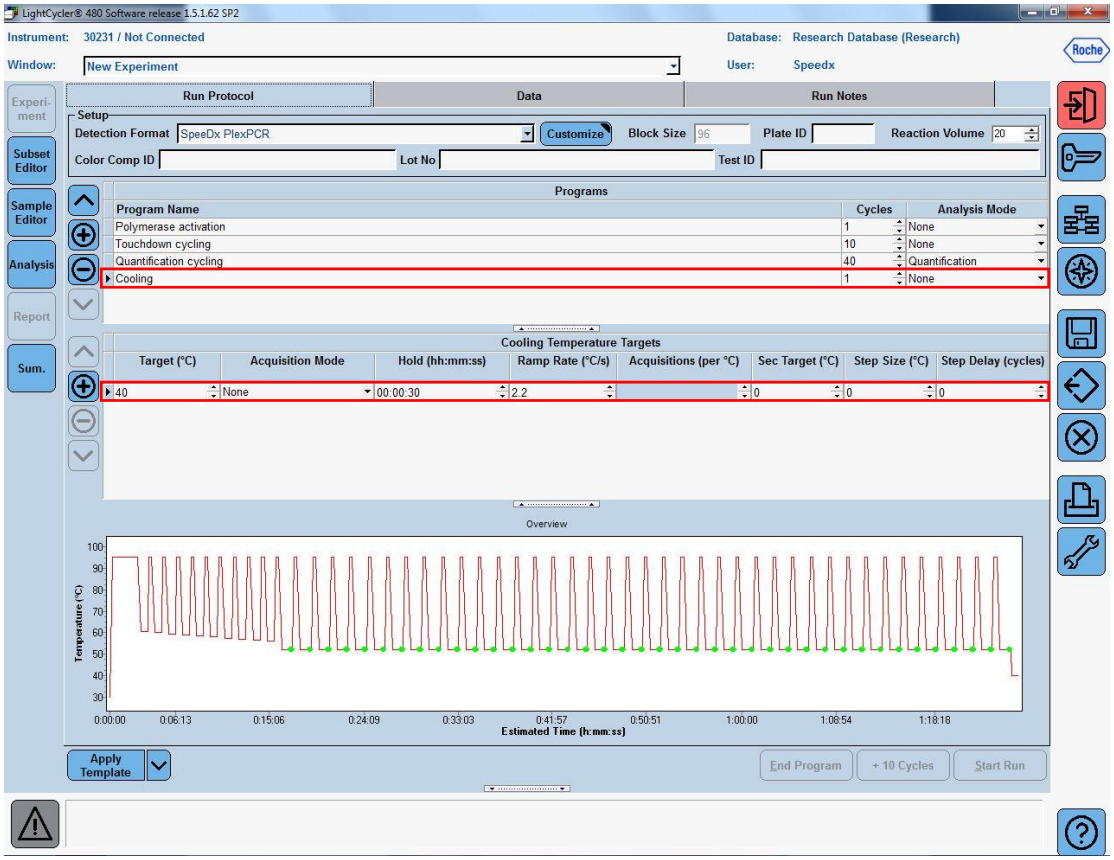


Figure 9. Thermocycling Program – Cooling



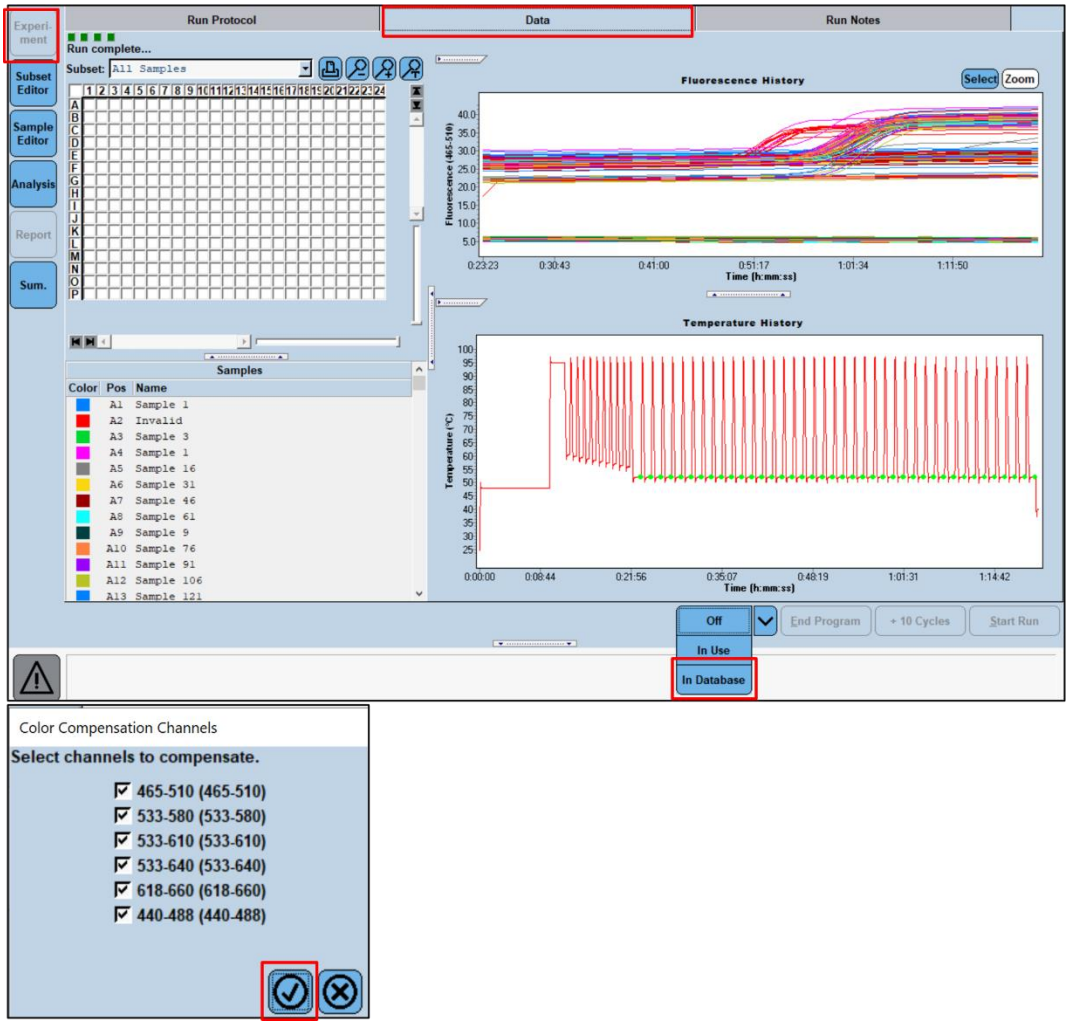
> **Start Run**

When the cycling program has finished, attach the CC object to the run file as shown in **Figure 10** and export as a .IXO file for analysis in the ***ResistancePlus***® MG analysis software. Refer to **Section 19.2** for instructions on how to create the CC Object and store this within the LightCycler 480 software database.

Select **Experiment > Data**

Click the drop-down arrow next to **Colour Comp (Off)** and select **In Database**

Figure 10. Attaching the CC object to the run file



Select the appropriate CC Object, ensure all channels are selected and select the **tick** icon 

Select the **Save** icon 

Select the **Export** icon 

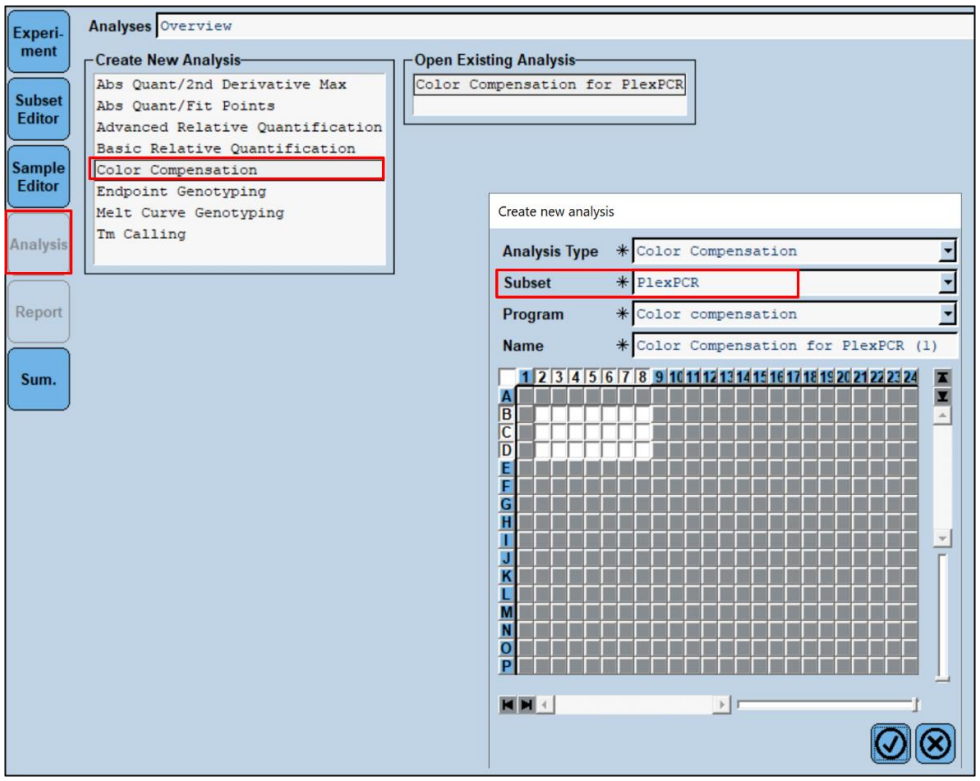
Save in an easily identifiable location

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.

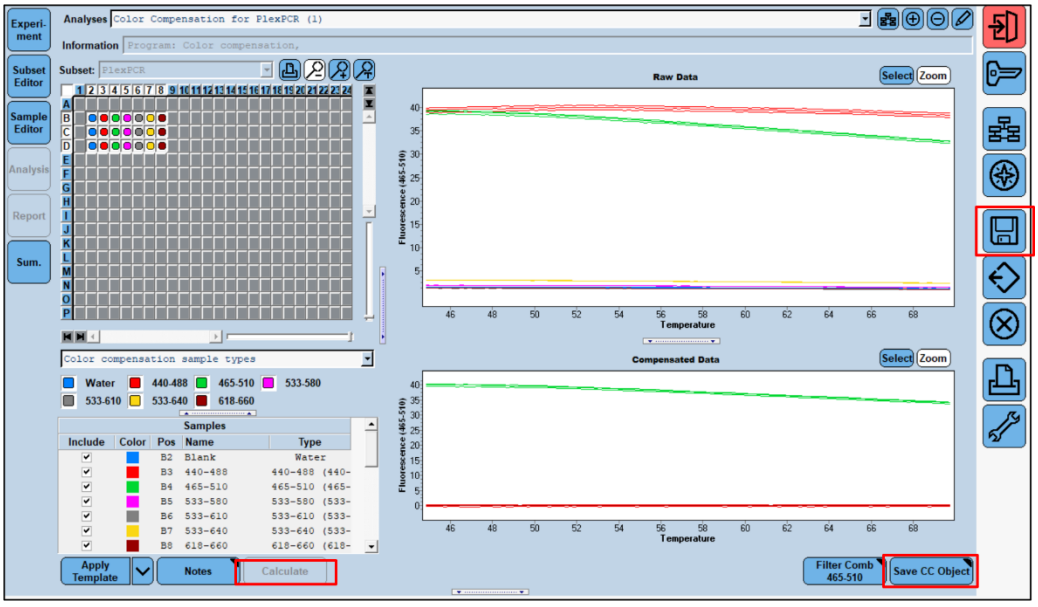
Analyse the Colour Compensation file via **Analysis > Colour Compensation** and select the correct subset, shown in **Figure 11.**

Figure 11. Analysis – Colour Compensation



Select **Calculate** (**Figure 12**).

Figure 12. Calculate and save CC Object



Refer to the PlexPCR Colour Compensation Instructions for Use (IF-IV0001) for further details to ensure the Colour Compensation file has been created correctly.

Select **Save** 

19.3 Interpretation of results

Data interpretation requires the ***Resistance****Plus*® MG (LC480) analysis software. The analysis software can be supplied on request. Please contact tech@speedx.com.au for more information.

Refer to **Section 23** for instructions for using the ***Resistance****Plus*® MG (LC480) analysis software.

20 Appendix 2: Applied Biosystems® 7500 Fast

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

The ***ResistancePlus***® $MG_{(550)}$ kit contains dyes for the Applied Biosystems® (ABI) 7500 Fast. Default dye calibrations are used for all channels. Custom calibration is not required.

20.1 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**

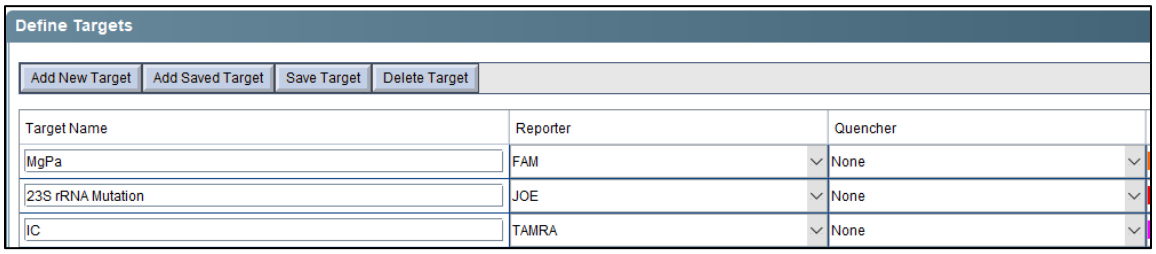
In **Define Targets and Samples** tab **>**

Define Targets as shown below in **Table 46** and **Figure 13** (define colours as required)

Table 46. Define Targets

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

Figure 13. Define Targets and Samples



Define Samples (define colours as required)

To enable automated sample detection in the analysis software, ensure the Target Name (shown in **Table 46)** matches the Target Instrument Reference defined in the **Lab Configuration > Assays** menu of the analysis software

In addition, sample nametags will also need to be assigned to the wells on the plate

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

In **Define Targets and Samples** tab **>**

Define Samples

Edit **Sample Name** to match the nametag defined in the **Lab Configuration > Assays** menu of the analysis software (**Section 23.3**)

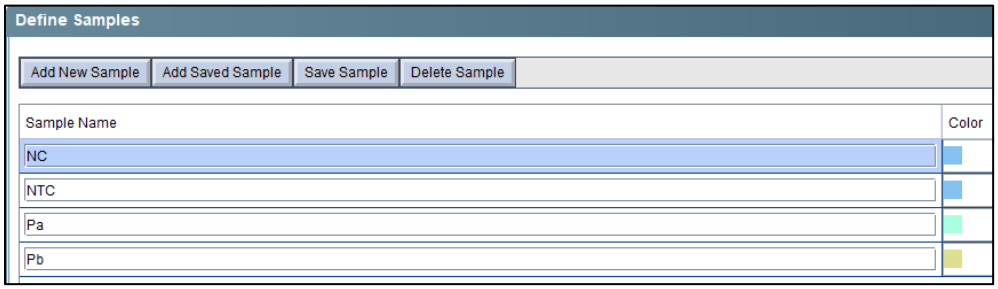
Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 47** and **Figure 14**). Additional nametags can be defined for both regular samples and controls within the analysis software.

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

Table 47. Sample nametags for analysis software

Sample type	Default Prefix (in analysis software)
Regular sample	No default – user defined
Negative control	NC
No template control	NTC
Positive control (MG, 23S rRNA mutant type) (Pa)	Pa
Positive control (MG, 23S rRNA wild type) (Pb)	Pb

Figure 14. 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 in **Table 48** (shown in more detail in Graphical View (**Figure 15** and **Figure 16**) and Tabular View (**Figure 17**):

Table 48. 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/cycleδ	10	95°C	5 s	100%
		61°C – 56.5°Cδ	30 s	100%
Quantification cycling+: Acquisition/Detection	40	95°C	5 s	100%
		52°C+	40 s	100%

≠ Default ramp rate

δ Enable AutoDelta: -0.5°C/cycle

+ Collect data on hold

Figure 15. Run method – Graphical view

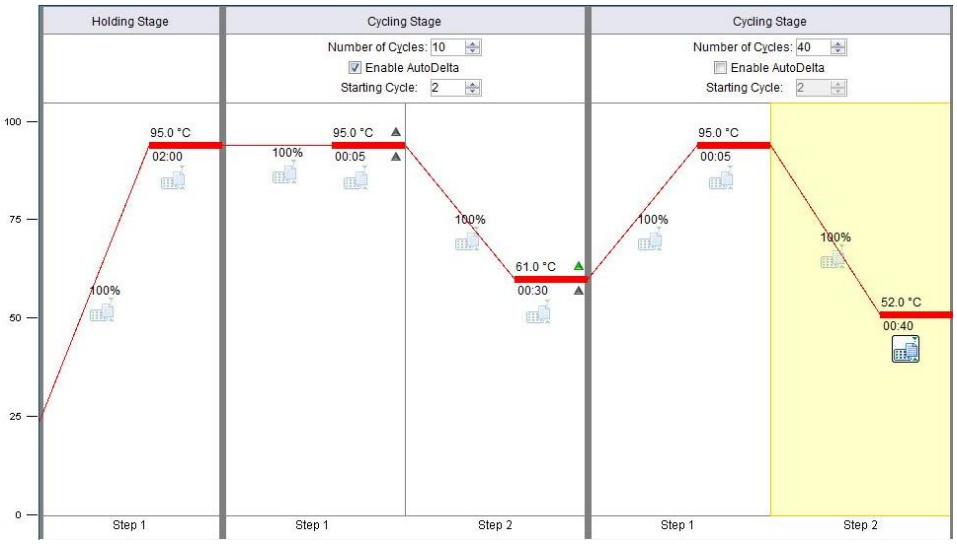


Figure 16. Run method – Graphical view – Enable AutoDelta

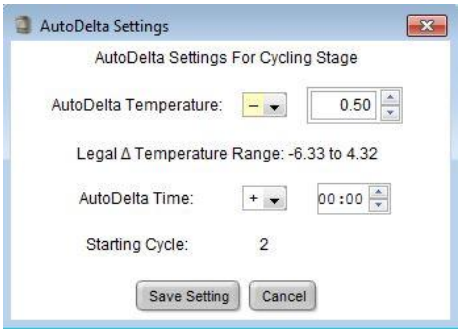
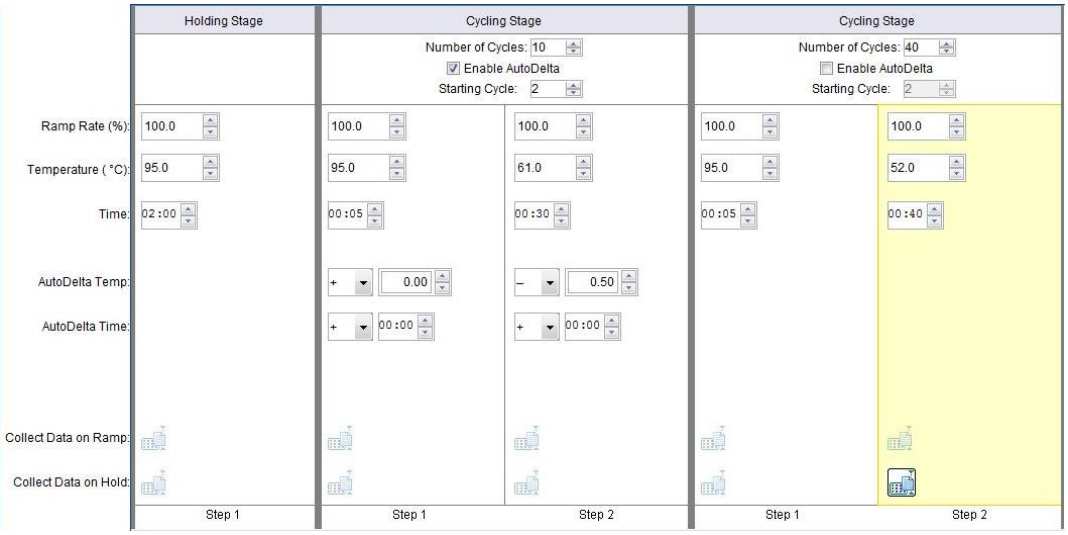


Figure 17. Run method – Tabular view



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

Select **Start Run**

20.2 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 for more information.

Refer to **Section 23** for instructions for using the ***ResistancePlus*®** MG (7500) analysis software.

21 Appendix 3: Applied Biosystems 7500 Fast Dx

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

The ***ResistancePlus***® $MG_{(550)}$ kit contains dyes for the Applied Biosystems® (ABI) 7500 Fast Dx. Default dye calibrations are used for all channels. Custom calibration is not required.

21.1 Programming the Applied Biosystems® 7500 Fast Dx

Select Create New Document

In **New Document Wizard** select the following (**Figure 18**):

Assay > Standard Curve (Absolute Quantification)

Container > 96-Well Clear

Template > Blank document

Run mode > Standard 7500

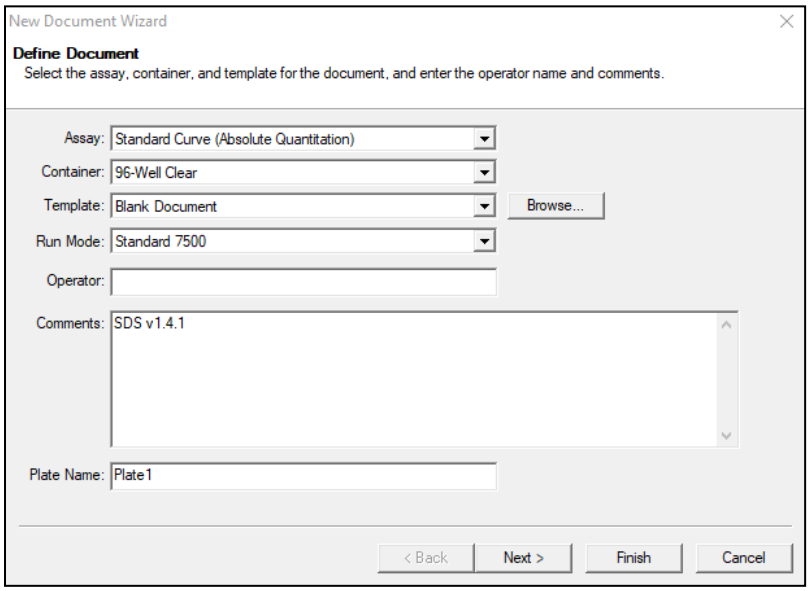
Operator > Enter Operator's name

Comments > Enter any comments or additional notes for the run file

Plate Name > Assign a unique name to the run file

Select **Next**

Figure 18. New Document Wizard window



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

Define detectors as shown below (define colours as required) (**Table 49** and **Figure 19**)

Table 49. 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**

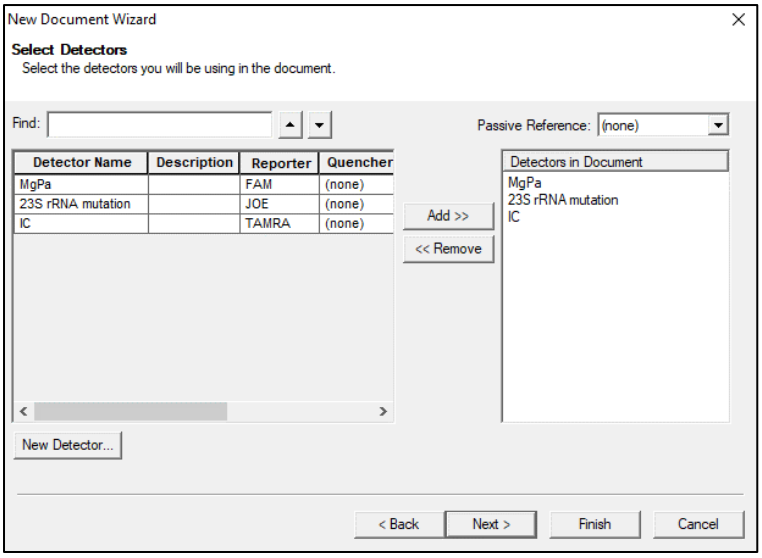
Figure 19. New Detector window

Select Detectors (**Figure 20**)

Select detectors and **Add** to Document

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

Figure 20. 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, ensure the Detector Name (shown in **Table 49**) matches the Target Instrument Reference defined in the **Lab Configuration > Assays** menu of the analysis software

In addition, sample nametags will also need to be assigned to the wells on the plate

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

Edit **Sample Name** to match the nametag defined in the **Lab Configuration > Assays** menu of the analysis software (see **Section 23.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 50**). Additional nametags can be defined for both regular samples and controls within the analysis software.

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	Default Prefix_ (in analysis software)
Regular sample	No default – user defined
Negative control	NC
No template control	NTC
Positive control (MG, 23S rRNA mutant type) (Pa)	Pa
Positive control (MG, 23S rRNA wild type) (Pb)	Pb

In **Instrument** tab

In **Settings** box

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

Create the following thermal cycler protocol (**Table 51** and **Figure 21** and **Figure 22**)

Table 51. 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/cycleδ	10	95°C	5 s	100%
		61°C – 56.5°Cδ	30 s	100%
Quantification cycling+: Acquisition/Detection	40	95°C	5 s	100%
		52°C+	40 s	100%

≠ *Default ramp rate*

δ *Enable AutoDelta: -0.5°C/cycle*

\+ *Collect data on hold*

Figure 21. Thermal Cycler Protocol – Thermal profile

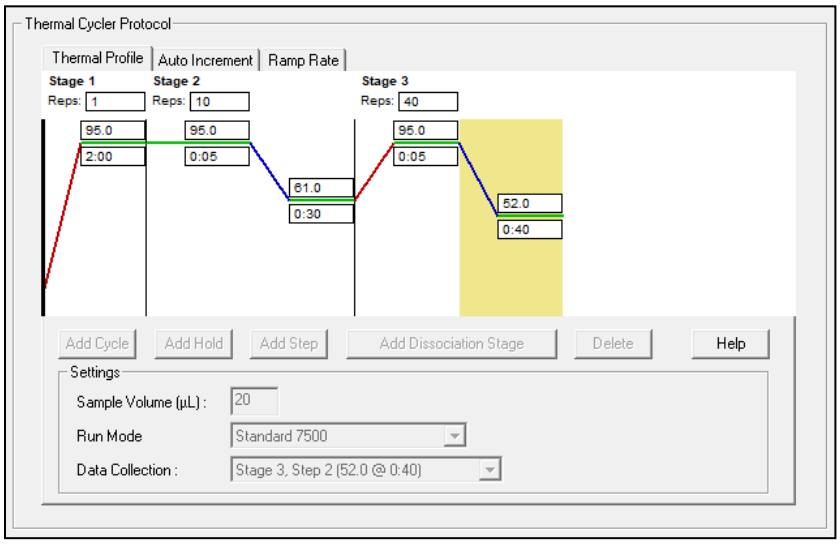
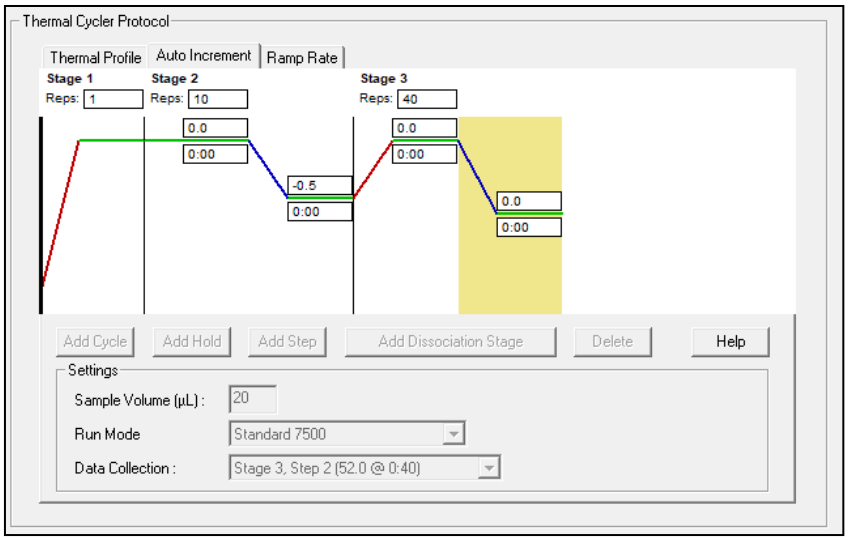


Figure 22. Thermal Cycler Protocol – Auto increment



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 for more information.

Refer to **Section 23** for instructions for using the ***ResistancePlus*®** MG (7500) analysis software.

22 Appendix 4: 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***® $MG_{(675)}$ kit contains dyes for the CFX96 Real-Time PCR System. Default dye calibrations are used for all channels. Custom calibration is not required.

22.1 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 23**):

Set **Sample Volume** > 20 µL

Create the following thermocycling program (**Table 52**) 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 24**).

Table 52. Thermocycling Program

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

[δ] **Step options** > Increment: -0.5°C/cycle

[+] **Add Plate Read to Step**

Figure 23. Thermocycling Protocol – Graphical view

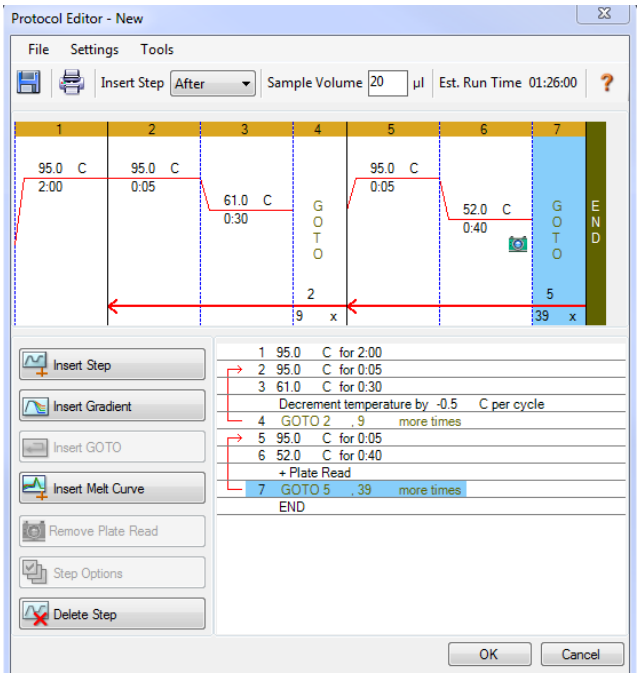
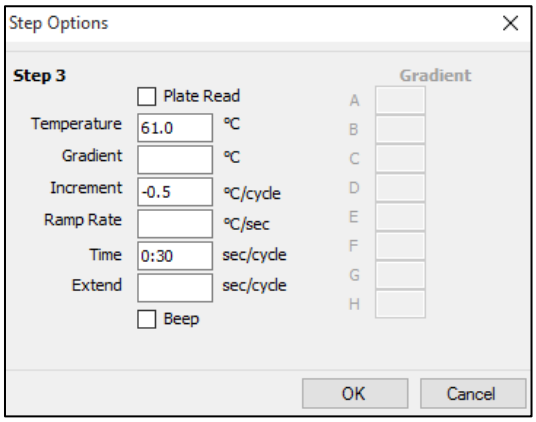


Figure 24. Step options



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 53**)

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

Save plate

Table 53. Channels for *ResistancePlus*® MG(675) targets

Target Name	MgPa	23S	IC
CFX-96 Touch/Dx channel	FAM	HEX	Quasar 705

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

Select Block

Start Run

To enable automated sample detection in the analysis software, ensure the Target Name and channel (shown in **Table 53**) matches the Target Instrument Reference defined in the **Lab Configuration > Assays** menu of the analysis software

In addition, sample nametags will also need to be assigned to the wells on the plate

Open the **Plate Setup** module

Select well

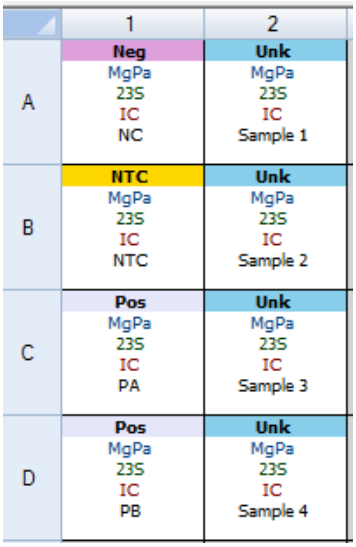
Edit **Sample Name** to match the nametag defined in the **Lab Configuration > Assays** module of the analysis software (see **Section 23.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 54** and **Figure 25**). Additional nametags can be defined for both regular samples and controls within the analysis software.

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

Table 54. Sample nametags for analysis software	
Sample type	**Default Prefix_ (in analysis software)**
Regular sample	No default – user defined
Negative control	NC
No template control	NTC
Positive control (MG, 23S rRNA mutant type) (Pa)	Pa
Positive control (MG, 23S rRNA wild type) (Pb)	Pb

Figure 25. Sample editor – Assigning nametags to wells



22.2 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 for more information.

Refer to **Section 23** for instructions for using the ***ResistancePlus***® MG (CFX) analysis software.

23 Appendix A: Result interpretation

Data interpretation requires the ***Resistance****Plus*® MG analysis software. While ***Plex****Prime*® 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 ***Resistance****Plus*® MG analysis software automates the data interpretation of amplification results and streamlines workflow.

See **Table 55** 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 for more information.

Table 55. *ResistancePlus*® MG analysis software

Cat no	Analysis software*	Real-time PCR instrument
99003	***Resistance****Plus*® MG (LC480)	LC480 II
99002	***Resistance****Plus*® MG (7500)	7500 Fast and 7500 Fast Dx
99008	***Resistance****Plus*® 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.

23.1 FastFinder platform – Minimum IT requirements

The analysis software is available within the FastFinder platform (https://www.ugentec.com/fastfinder/analysis). It is recommended that customers access the software platform from a secure and trusted network and computer. The minimum IT requirements for access and use of the FastFinder platform are listed below.

Hardware requirements

Internet Connection Cable or DSL

Min. screen resolution: 1366x768 pixels, optimal 1920 x 1080 pixels or higher

Supported browsers

- Microsoft Edge 88 or newer
- Firefox 83 or newer
- Google Chrome 88 or newer.

Firewall requirements

The following hosts must be reachable over HTTPS (port 443):

*.ugentec.app

*.fastfinder.app

*.pendo.io

*.fonts.gstatic.com

*.googleapis.com

*.msecnd.net

*.visualstudio.com

*.browser-update.org

*.blob.core.windows.net

*.powerbi.com

*.analysis.windows.net

*.pbideldicated.windows.net

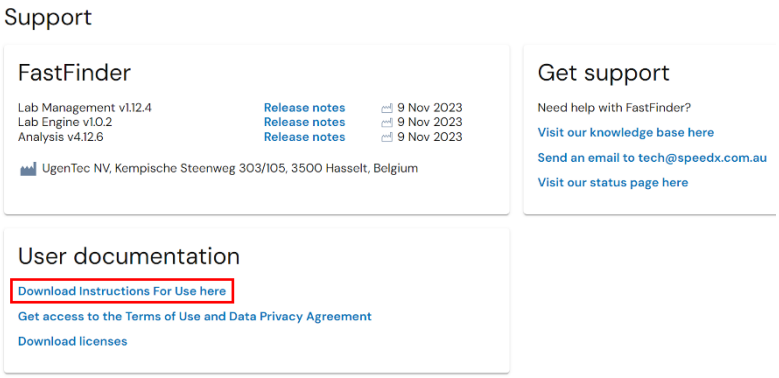
*.content.powerapps.com

If required, firewall exceptions will have to be configured for these hosts. In order to access all content of in-app user guides, the host *.player.vimeo.com must also be reachable.

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

To access the Support menu

- Select Support from the list of menu options on the left-hand side panel
- Select **Download Instructions For Use here** within the **User Documentation** section



23.2 Assay plug-in (new user)

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

FastFinder can be accessed directly through a web browser by logging in with your unique username and password at https://customer.fastfinder.app.

- Select **Lab Configuration > Assays** from the left-hand menu
- Select **Add New Assay**
  - > For LC480 II > Select **ResistancePlus MG (LC480)** 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
- Select **Import Selected**

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

> In **General tab**

> Navigate to the Status

> Select 

to activate or deactivate the version of the assay

23.3 Sample naming

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

Select Lab **Configuration > Assays** from the left-hand menu

- In the **General tab**, navigate to the **Sample types** table nametags (prefix), select ⊕ to add a new nametag
  - > Add desired word, acronym or letter to text box
  - > Default nametags are provided for the controls. These can be removed by selecting the × next to the nametag

- 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 **7500 Fast** see **Section 20** for instructions on programming sample nametags in the run file
  - > For **7500 Fast Dx** see **Section 21** for instructions on programming sample nametags in the run file
  - > For **CFX96 Dx** and **CFX96 Touch** see **Section 22** 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.

23.4 Analysis

Select **Analyses** from the left-hand menu to start a new analysis

Select **+ Create New Analysis** from the top right of the screen

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

- Select run (data) file from the relevant folder
  - > Select **Open**

The analysis will appear within the **Open Tab** as a new row within the table

- If all nametags have been applied and read correctly, the status will appear as **Ready for review**
- If the assay information needs to be manually assigned to the wells, the status will appear as **Manual PCR setup required**

Assign the assay information to the plate manually if sample naming has not been set up in the Lab **Configuration > Assays** menu or sample names/targets have not been applied in the instrument software

Select the runfile from the **Open tab** within the **Analyses** menu

The Plate Configuration will be displayed within the **PCR setup tab** for the open analysis

- For **LC480 II** > Select **ResistancePlus MG (LC480)**

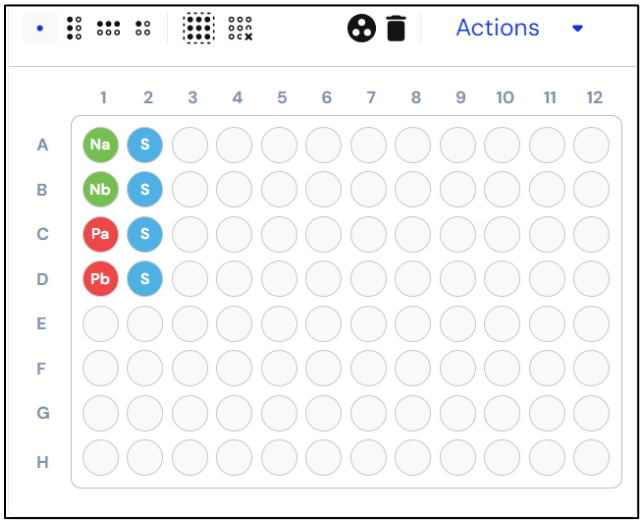
- For **7500 Fast** and **7500 Fast Dx** > Select **ResistancePlus MG (7500)**

- For **CFX96 Dx** and **CFX96 Touch** > Select **ResistancePlus MG (CFX)**

- Select wells and assign as:
  - > Regular Sample (S)
  - > Negative Control (Na)
  - > No Template Control (Nb)
  - > Positive Control (MG 23S rRNA mutant) (Pa)
  - > Positive Control (MG 23S rRNA wild type) (Pb)

To assign wells on the plate, either:

- Click and drag the coloured symbols to place them on the plate
- Select one or multiple wells (use Ctrl and shift keys) and then click the relevant coloured symbols to assign to selection.



- Select **Analyze**

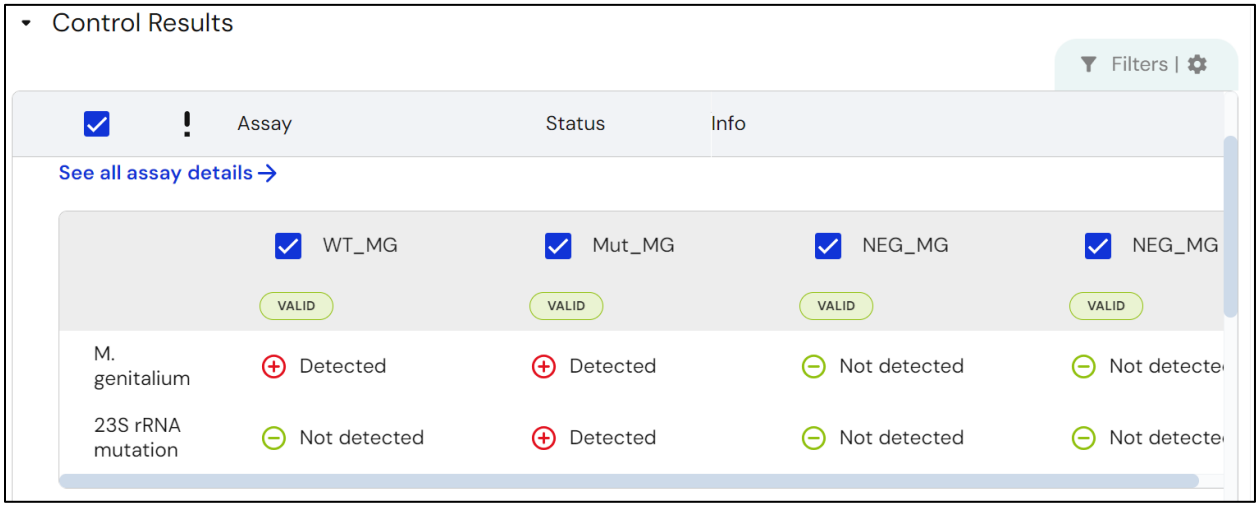
23.5 Results

See **Table 57** for a summary of possible reported sample results.

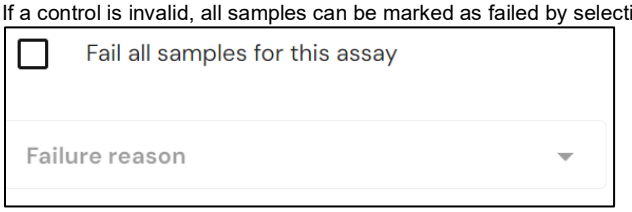
NOTE: It is highly recommended that amplification curves should be confirmed for all positive samples.

23.5.1 Summary Tab

Control results for every assay are shown at the top-left of the Summary tab, allowing evaluation of control validity for the run. More details can be found by expanding this block, displaying the details per control.

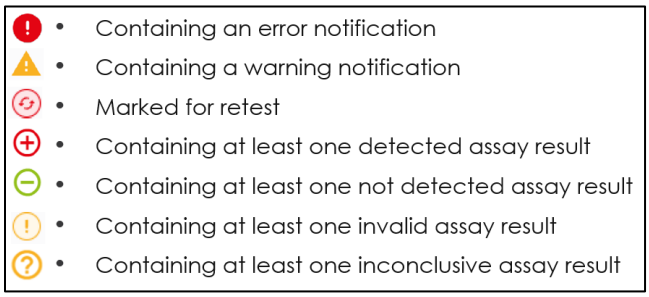


If a control is invalid, all samples can be marked as failed by selecting **Fail all samples for this assay**

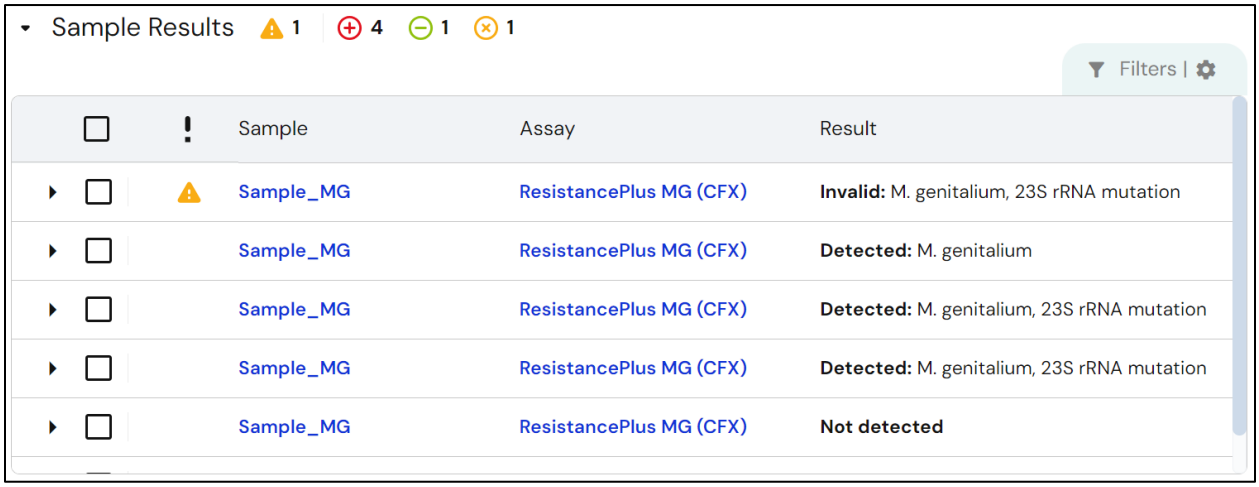


A failure reason needs to be chosen from the dropdown menu

Sample results are shown at the bottom-left of the Summary tab. Next to the header, additional icons may provide a high-level overview of the analysis results as well as indicating the total number of samples corresponding to a particular icon.

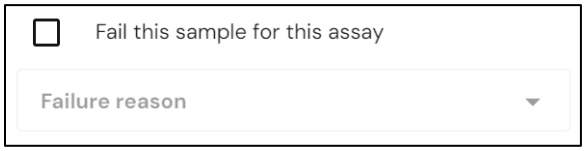


Each sample is displayed as a row within the sample results table.



The drop-down menu offers more details on each target result and Cq per sample (Refer to the examples shown in **Section 23.10**).

Individual samples can be marked as failed if desired (e.g. if the sample is Invalid) by selecting **Fail this sample for this assay**



A failure reason needs to be chosen from the dropdown menu

Fluorescence graphs can be viewed at the top-right of the Summary tab

A plate layout can be viewed at the bottom-right of the Summary tab

Example information and warning notifications are summarized below in **Table 56.**

Table 56. Example information and warning notifications for the ResistancePlus® MG analysis software*

Sample Type	Error	Notification
Assay target notifications		
Regular Sample	Invalid – IC failure	Warning: IC invalid. Re-extract and re-test sample.
	Valid but control invalid – Invalid control warning on regular sample with valid result	Warning: Invalid control present. Re-extract and re-test the sample.
Negative Control	Invalid - Contamination	Warning: Possible contamination detected.
No Template Control		
Gene target notifications		
Regular Sample	Target Cq outside cut-off	Info: Cq outside cutoff
Positive Control	Invalid – Target not detected	Warning: Expected reaction did not occur in control.
Negative Control	Invalid - Contamination	Warning: Possible contamination
	Invalid – IC not detected	Warning: IC not detected
	Invalid – IC Cq outside cut-off	Warning: Cq outside cutoff
No Template Control	Invalid - Contamination	Warning: Possible contamination

Table 56. Example information and warning notifications for the ResistancePlus® MG analysis software*		
Sample Type	**Error**	**Notification**
Regular Sample or Control	Uncertain fluorescence signal	Warning: Uncertain fluorescence signal. Review required.
	Cq detected with low fluorescence	dRn end fluorescence below cut-off

*The examples listed here may not be applicable for all assay plug-ins. Refer to the FastFinder Instructions For Use for all possible notifications, accessible from the Support menu

23.5.2 Details Tab

All targets are shown for each sample as separate rows within the table on the left-hand side. Selecting one or more rows will display the corresponding fluorescence curves on the graph at the top-right and will also highlight the wells within the plate layout shown at the bottom-right.

Select **Filters** to display results according to parameters such as assay name, sample type, target and result.

To finalise analysis and prevent further user edits

> Select **Authorize**

> Select **Authorize** again to confirm

- To assign a second review

> Select **Actions**, **Assign label** and **Second Review**

- To assign the analysis to a different user

> Select **Actions** and **Assign User**

> Select the appropriate user from the drop-down list

- To reject the analysis

> Select **Actions** and **Discard Analysis**

> Add a comment and select **Discard** to confirm

23.6 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 **Summary** or **Details** tab
- From the amplification graph menu > Select 

> Select the check box for the curve of interest and select **Mark as reference**

This reference curve will now appear linked to the assay in the **Lab Configuration > Assays** menu within the **PCR tab** and can be inactivated at any time.

23.7 Exporting results

- To export results from an individual authorised run as either a CSV or PDF file:

> Select **Actions > Downloads** in the top-right corner

> Select either of the following report types: **Analysis (CSV)** or **Analysis (PDF)**

- To export results from multiple previously authorised runs as a single CSV file:

> Navigate to the **Archive > Sample Results** menu

> Use the filters at the top of the page to display the results of interest (the CSV file is limited to a maximum of 10,000 results)

> Select **Export CSV** in the top-right corner

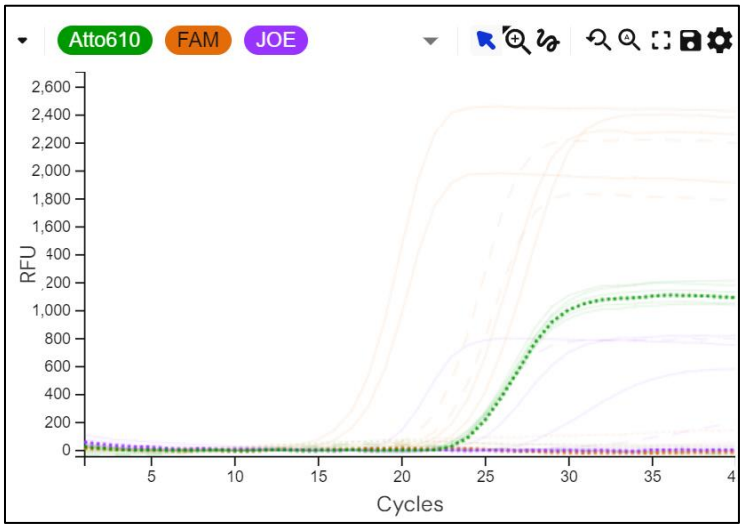
23.8 Retrieving authorized analyses

- All authorized analyses are available by selecting **Archive > Analysis Results**. Select a row to return to the results overview for that particular analysis
- All authorized regular samples are stored within the **Archive > Sample Results** menu. Selecting a sample will display additional information including the analysis name and the result details
- The individual target results for all authorized regular samples and controls are stored within the **Archive > Target Results** menu. Selecting a target will highlight this on the fluorescence graph. Selecting the Analysis Name will return to the results overview for that particular analysis.

23.9 Control Example Graphs

The following examples show the amplification curves (baseline-corrected amplification curves) and the Results overview from the **ResistancePlus MG (LC480)** analysis software for control sample types.

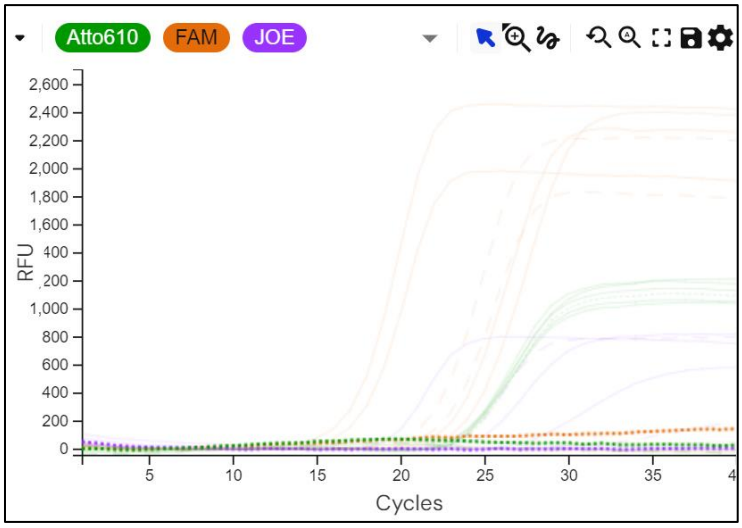
23.9.1 *M. genitalium* negative control (Na) (negative specimen)



IC **MgPa** **23S rRNA mutation**

Sample	Assay	Result
Na	ResistancePlus MG (LC480)	**Valid**
	M. genitalium	Not detected
	23S rRNA mutation	Not detected

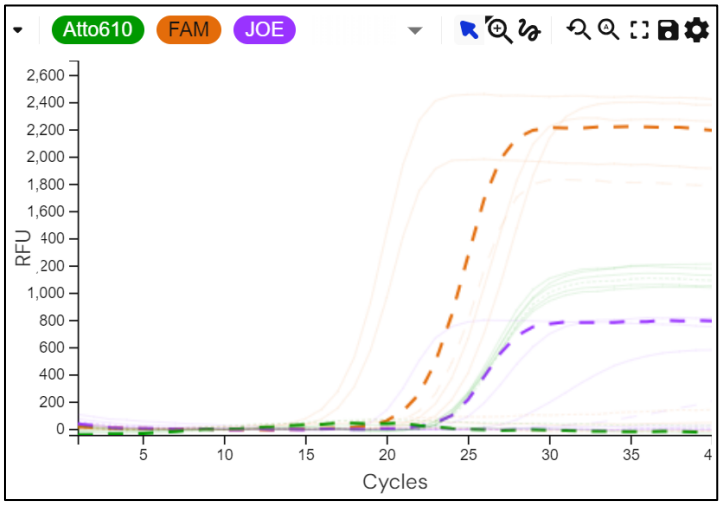
23.9.2 No Template Control (Nb)



IC MgPa 23S rRNA mutation

Sample	Assay	Result
Nb	ResistancePlus MG (LC480)	**Valid**
	M. genitalium — Not detected	
	23S rRNA mutation — Not detected	

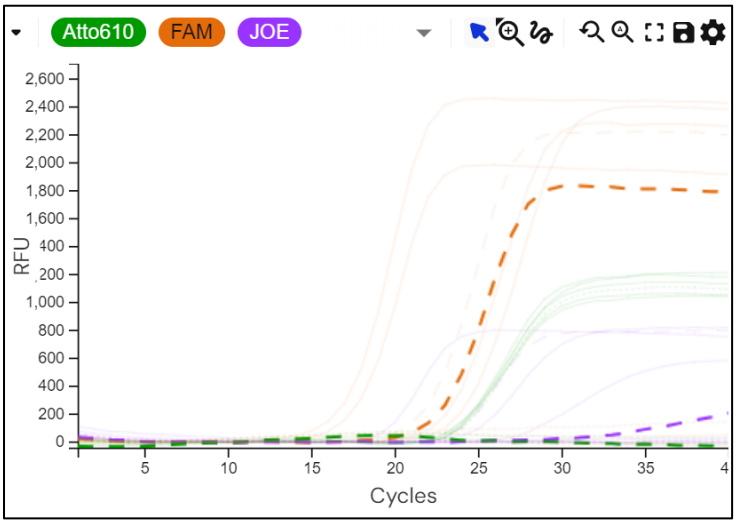
23.9.3 *M. genitalium*, 23S rRNA mutant control (Pa)



IC MgPa 23S rRNA mutation

Sample	Assay	Result
Pa	ResistancePlus MG (LC480)	**Valid**
	M. genitalium ⊕ Detected	
	23S rRNA mutation ⊕ Detected	

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



IC MgPa 23S rRNA mutation

Sample	Assay	Result
Pb	ResistancePlus MG (LC480)	**Valid**
	M. genitalium ⊕ Detected	
	23S rRNA mutation ⊖ Not detected	

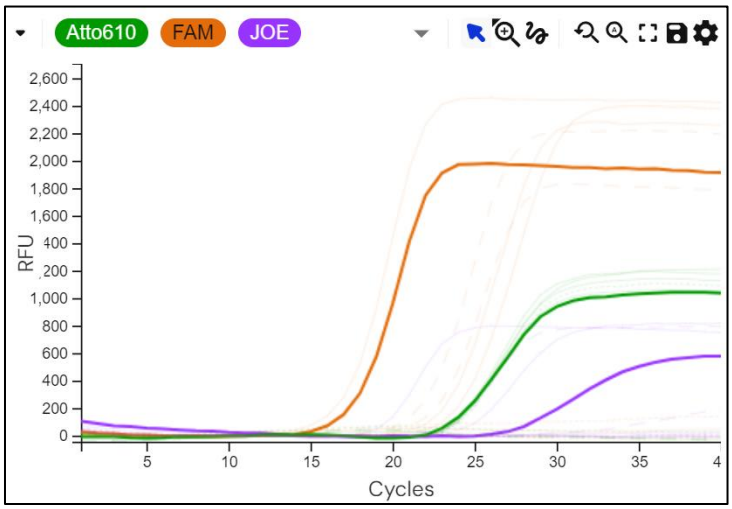
23.10 Examples

Example results for the ***ResistancePlus***® MG analysis software are shown in **Table 57.**

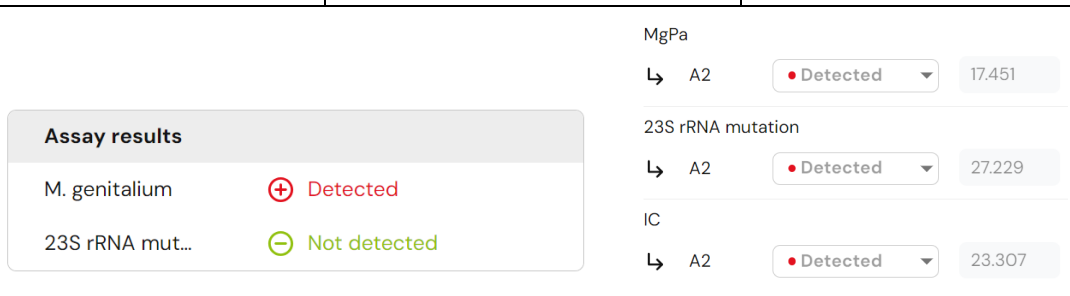
Table 57. Example results for interpretation of ResistancePlus® MG analysis software			
	Sample	**Assay**	**Result**
	Sample 101	ResistancePlus MG (LC480)	**Not detected**
	Sample 102	ResistancePlus MG (LC480)	**Detected:** M. genitalium
	Sample 103	ResistancePlus MG (LC480)	**Detected:** M. genitalium, 23S rRNA mutation
[1]	Sample 104	ResistancePlus MG (LC480)	**Invalid:** M. genitalium, 23S rRNA mutation

[1] A sample interpreted as Invalid will be flagged with ⚠ Warning: IC invalid. Re-extract and re-test sample.

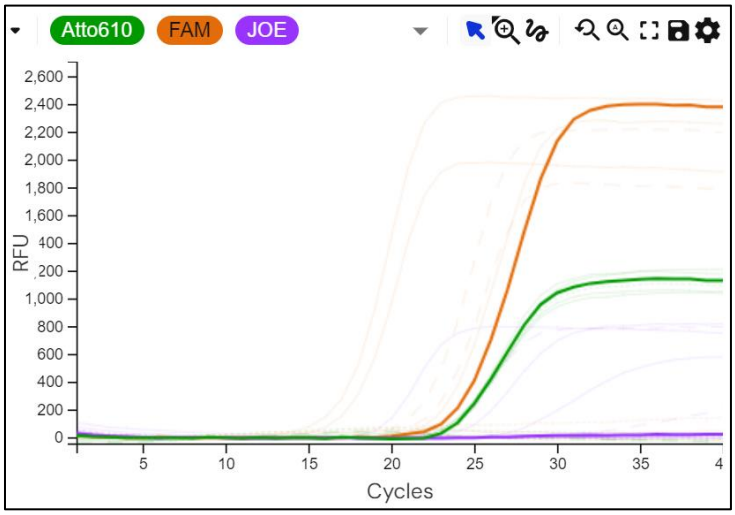
23.10.1 Example 1. High copy *M. genitalium*, 23S rRNA wild type sample



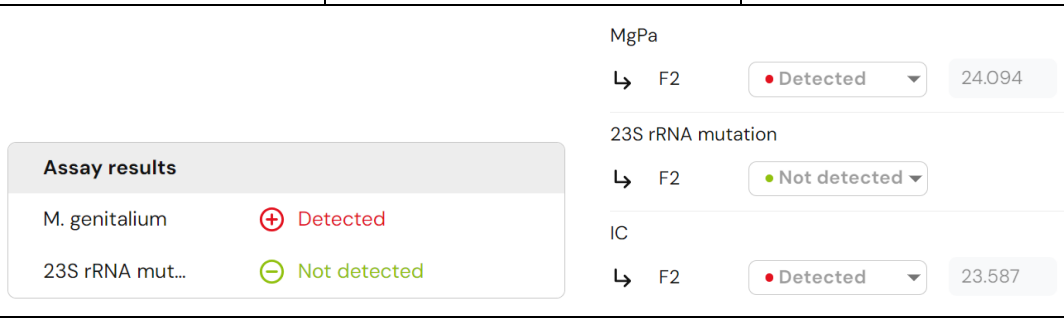
Sample	**Assay**	**Result**
Sample 105	ResistancePlus MG (LC480)	**Detected:** M. genitalium



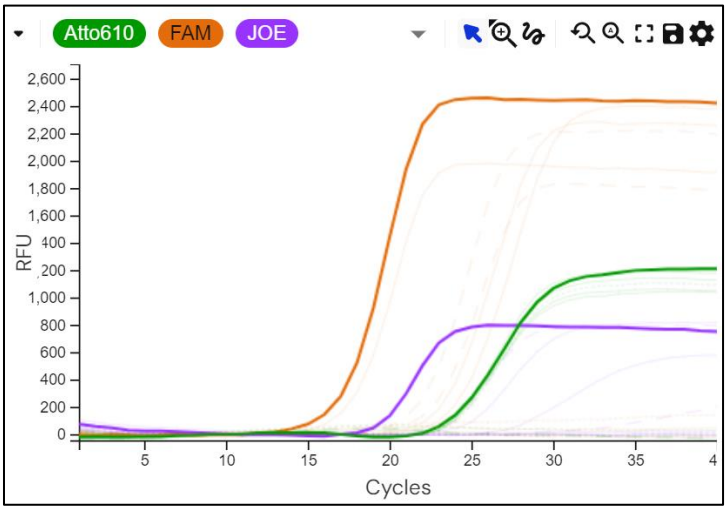
23.10.2 Example 2. Low copy *M. genitalium*, 23S rRNA wild type sample



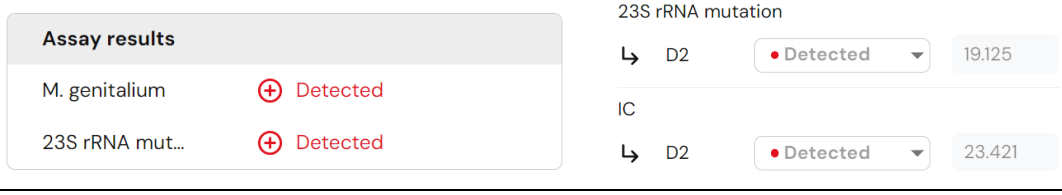
Sample	Assay	Result
Sample 106	ResistancePlus MG (LC480)	**Detected:** M. genitalium



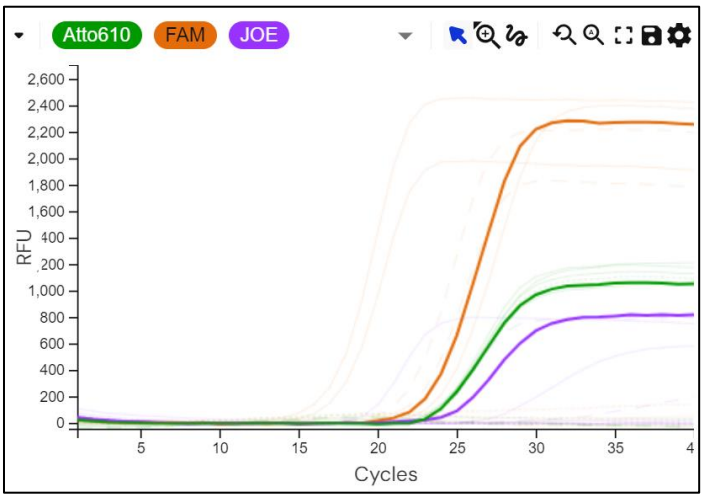
23.10.3 Example 3. High copy *M. genitalium*, 23S rRNA mutant sample



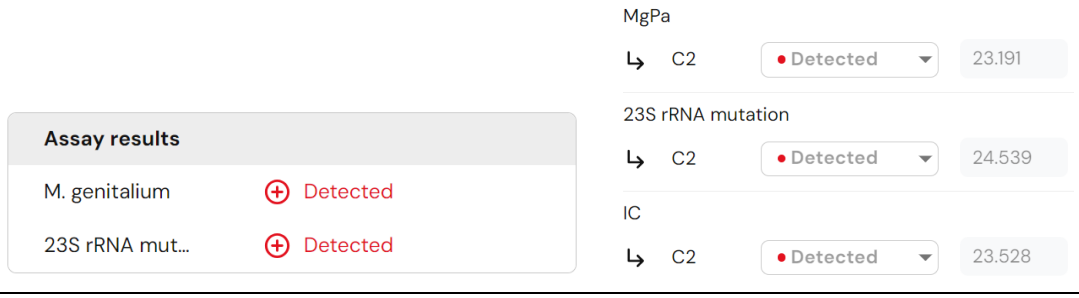
Sample	Assay	Result
Sample 107	ResistancePlus MG (LC480)	**Detected:** M. genitalium, 23S rRNA mutation



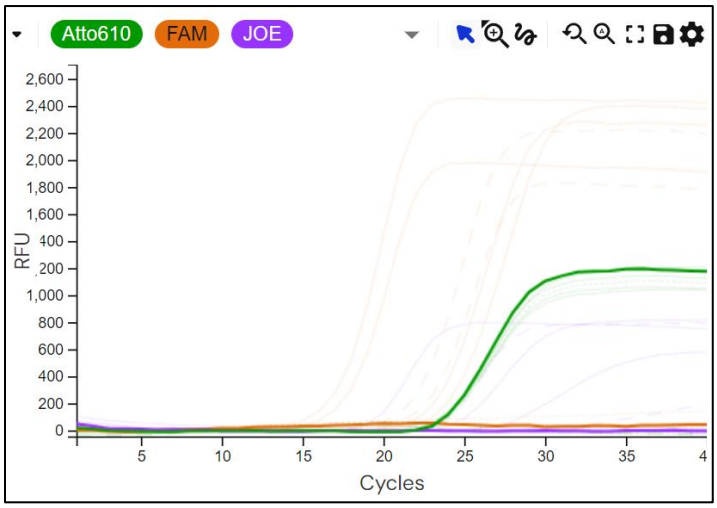
23.10.4 Example 4. Low copy *M. genitalium*, 23S rRNA mutant sample



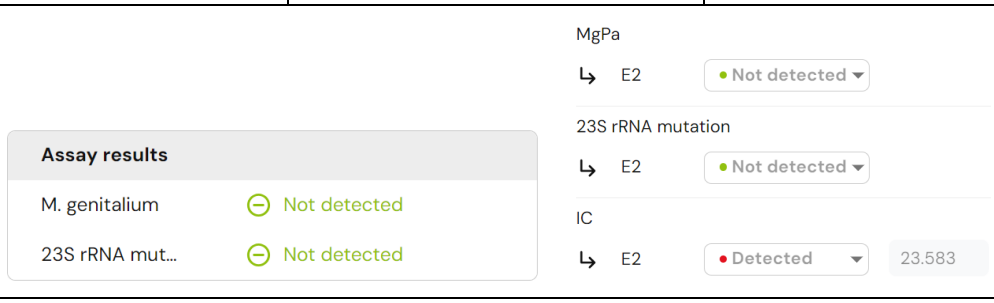
Sample	Assay	Result
Sample 108	ResistancePlus MG (LC480)	**Detected:** M. genitalium, 23S rRNA mutation



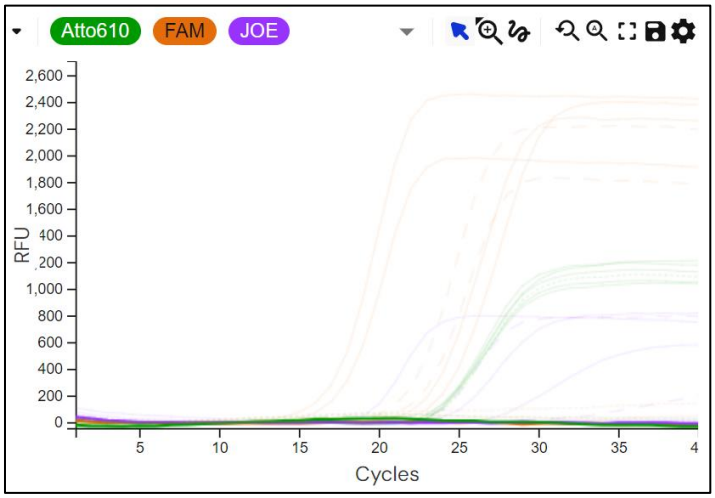
23.10.5 Example 5. Negative sample



Sample	Assay	Result
Sample 109	ResistancePlus MG (LC480)	**Not Detected**

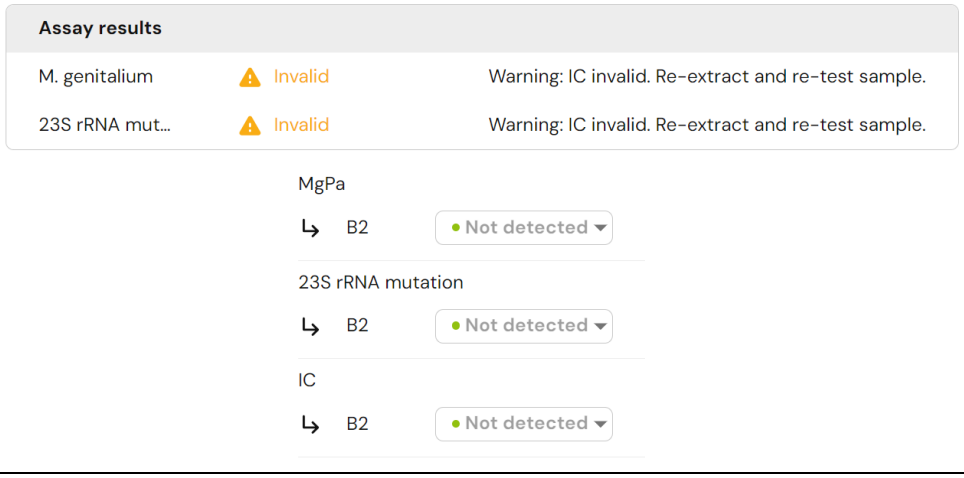


23.10.6 Example 6. Invalid sample



IC MgPa 23S rRNA mutation

Sample	Assay	Result
Sample 110	ResistancePlus MG (LC480)	**Invalid:** M. genitalium, 23S rRNA mutation



In this example, the IC has not amplified and therefore has not been detected. For IC invalid samples, re-extract the sample and then repeat test.

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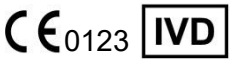


European Conformity
For *In Vitro* Diagnostic Use

Catalogue number

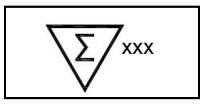
Batch code

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

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