



ResistancePlus® GC

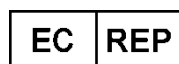
Multiplex real-time PCR assay for the detection of *Neisseria gonorrhoeae* and markers associated with susceptibility and resistance to ciprofloxacin



Product	Platform	Size (reactions)	Catalogue no.
<i>ResistancePlus</i> ® GC ₍₆₁₀₎	LC480 II	100	REF 2011001
<i>ResistancePlus</i> ® GC ₍₆₁₀₎	LC480 II	25	REF 2011025
<i>ResistancePlus</i> ® GC ₍₅₅₀₎	ABI 7500 Fast ABI 7500 Fast Dx	100	REF 2013001
<i>ResistancePlus</i> ® GC ₍₅₅₀₎	ABI 7500 Fast ABI 7500 Fast Dx	25	REF 2013025
<i>ResistancePlus</i> ® GC ₍₆₇₅₎	CFX96 Dx CFX96 Touch	100	REF 2015001
<i>ResistancePlus</i> ® GC ₍₆₇₅₎	CFX96 Dx CFX96 Touch	25	REF 2015025

Accessory products – Analysis software

<i>ResistancePlus</i> ® GC (LC480)	REF 99010
<i>ResistancePlus</i> ® GC (7500)	REF 99009
<i>ResistancePlus</i> ® GC (CFX)	REF 99015



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1 Product description

The **ResistancePlus**[®] GC kit simultaneously detects the bacterium *Neisseria gonorrhoeae* (GC) and the *gyrA* S91 (wild type) or *gyrA* S91F (mutant) markers that are associated with susceptibility or resistance to the fluoroquinolone antibiotic, ciprofloxacin.

The **ResistancePlus**[®] GC kit is a 1-well real-time PCR multiplex consisting of 5 readouts: Readout 1) GC *opa* gene, Readout 2) GC *porA* gene, Readout 3) *gyrA* S91 (wild type), Readout 4) *gyrA* S91F (mutant), and Readout 5) Internal control (to monitor extraction efficiency and PCR inhibition). The **ResistancePlus**[®] GC kit utilises **PlexZyme**[®] and **PlexPrime**[®] technology for specificity and superior multiplexing capability.

The assay is compatible with samples extracted using the MagNA Pure 96 System (Roche), EZ1 Advanced XL (Qiagen), and real-time detection on the LightCycler[®] 480 Instrument II (LC480 II, Roche), Applied Biosystems[®] 7500 Fast (7500 Fast), Applied Biosystems[®] 7500 Fast Dx (7500 Fast Dx), the Bio-Rad CFX96 Dx (CFX96 Dx) and CFX96 Touch[™] (CFX96 Touch) Real-time PCR Detection Systems.

2 Intended use

The **ResistancePlus**[®] GC kit is a qualitative real-time PCR assay for the detection of *Neisseria gonorrhoeae* and *gyrA* S91 wild type and *gyrA* S91F mutant DNA markers that are associated with susceptibility or resistance to ciprofloxacin.

The **ResistancePlus**[®] GC kit is intended to aid in the diagnosis of *Neisseria gonorrhoeae* and *gyrA* status and should be used in conjunction with clinical and other laboratory information.

The **ResistancePlus**[®] GC kit may be used with the following specimen types: male and female urine, anal, rectal, vaginal and pharyngeal swabs, from symptomatic and asymptomatic patients.

The **ResistancePlus**[®] GC 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

Neisseria gonorrhoeae (GC) is the aetiological agent of the sexually transmitted infection gonorrhoea, and is a common cause of urethritis in men, and cervicitis, pelvic inflammatory disease (PID), and reproductive complications (infertility and ectopic pregnancy) in women¹. Antimicrobial resistant GC is a global public health concern, and standard management using empiric treatment is being challenged by the spread of resistance and limited alternate treatment options. Individualised treatment guided by antibiotic susceptibility information, has been proposed as a strategy to re-instigate the use of antibiotics no longer recommended for empiric treatment, while also conserving last-line therapies.

Ciprofloxacin, a fluoroquinolone antibiotic, is an excellent option for individualised treatment. While it was abandoned from empirical use in most regions from the 2000's², large proportions of gonorrhoea cases remain susceptible. In addition, the *gyrA* S91 wild type and *gyrA* S91F mutation markers are highly predictive of ciprofloxacin susceptibility and resistance, respectively^{3,4,5}. Molecular detection of the *gyrA* S91 wild type and *gyrA* S91F markers can therefore be used to guide effective ciprofloxacin treatment in patients with susceptible infections.

4 Kit contents

Table 1. <i>ResistancePlus</i> [®] GC ₍₆₁₀₎ kit contents				
Cap colour	Contents	Description	Cat no 2011001 (100 reactions [*])	Cat no 2011025 (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	GC+ <i>gyrA</i> Mix, 20x	Mix containing oligonucleotides [^] for amplification and detection of <i>N. gonorrhoeae</i> , <i>gyrA</i> S91 and <i>gyrA</i> S91F	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 **PlexPrime**[®] primers), **PlexZyme**[®] enzymes and fluorescent probe

^{*} Sufficient for 100 reactions when using 20 µL reaction volume; Sufficient for 64 reactions when using 30 µL reaction volume

^{**} Sufficient for 25 reactions when using 20 µL reaction volume; Sufficient for 16 reactions when using 30 µL reaction volume

Table 2. <i>ResistancePlus</i> [®] GC ₍₅₅₀₎ kit contents				
Cap colour	Contents	Description	Cat no 2013001 (100 reactions [*])	Cat no 2013025 (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	GC+ <i>gyrA</i> Mix, 20x	Mix containing oligonucleotides [^] for amplification and detection of <i>N. gonorrhoeae</i> , <i>gyrA</i> S91 and <i>gyrA</i> S91F	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 **PlexPrime**[®] primers), **PlexZyme**[®] enzymes and fluorescent probe

^{*} Sufficient for 100 reactions when using 20 µL reaction volume; Sufficient for 64 reactions when using 30 µL reaction volume

^{**} Sufficient for 25 reactions when using 20 µL reaction volume; Sufficient for 16 reactions when using 30 µL reaction volume

Table 3. <i>ResistancePlus</i> [®] GC ₍₆₇₅₎ kit contents				
Cap colour	Contents	Description	Cat no 2015001 (100 reactions*)	Cat no 2015025 (25 reactions**)
Blue	<i>Plex</i> 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	GC+gyrA Mix, 20x	Mix containing oligonucleotides [^] for amplification and detection of <i>N. gonorrhoeae</i> , <i>gyrA</i> S91 and <i>gyrA</i> S91F	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 CFX 96 Dx and CFX 96 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 *PlexPrime*[®] primers), *PlexZyme*[®] enzymes and fluorescent probe

* Sufficient for 100 reactions when using 20 µL reaction volume; Sufficient for 64 reactions when using 30 µL reaction volume

** Sufficient for 25 reactions when using 20 µL reaction volume; Sufficient for 16 reactions when using 30 µL reaction volume

5 Shipping and storage

- The components of the *ResistancePlus*[®] GC kits are shipped on dry ice or ice gel packs. All components should be stored between -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.

6 Warnings and precautions

6.1 General

- For *in vitro* diagnostic use only.
- Carefully read these Instructions for Use prior to use. Closely follow procedures as described to ensure reliability of test results. Any deviation from these procedures may affect test performance.
- Users should be adequately trained in the use of the *ResistancePlus*[®] GC 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 **ResistancePlus**[®] GC 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

- **ResistancePlus**[®] GC Control (Cat no 95003)

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 System Fluid (external) (Roche, Cat no 06640729001)
- MagNA Pure 96 Processing Cartridge (Roche, Cat no 06241603001)
- MagNA Pure 96 Pure tip 1000 µL (Roche, Cat no 6241620001)
- MagNA Pure 96 Output Plate (Roche, Cat no 06241611001)
- MagNA Pure Sealing Foil (Roche, Cat no 06241638001)

For Qiagen EZ1 Advanced XL Instrument

- EZ1 DSP Virus Kit (Cat no 62724)
- EZ1 Advanced XL DSP Virus Card (Cat no 9018703)

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 LightCycler® 480 Instrument II

- **PlexPCR**® Colour Compensation (CC) kit (Cat no 90001)
- LightCycler® 480 Multiwell Plate 96 (Roche, Cat no 04729692001)
- LightCycler® 480 Sealing Foil (Roche, Cat no 04729757001)

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

- Aptima® urine collection kit (Hologic, Cat no 301040)
- Aptima® Multitest swab collection kit (Hologic, Cat no PRD-03546)
- cobas® PCR media (Roche, Cat no 06466281190)
- cobas® PCR Urine Sample Kit (Roche, Cat no 05170486190)
- cobas® PCR Media Uni Swab Sample Kit (Roche, Cat no 07958030190)

8 Principle of the technology

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

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

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

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

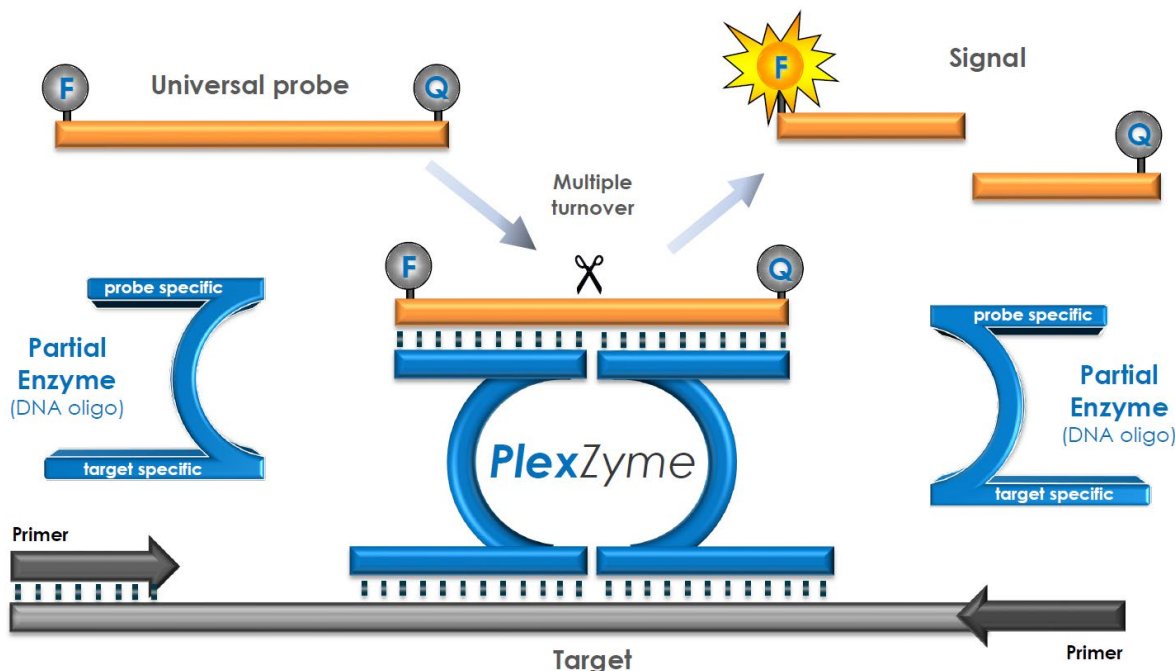
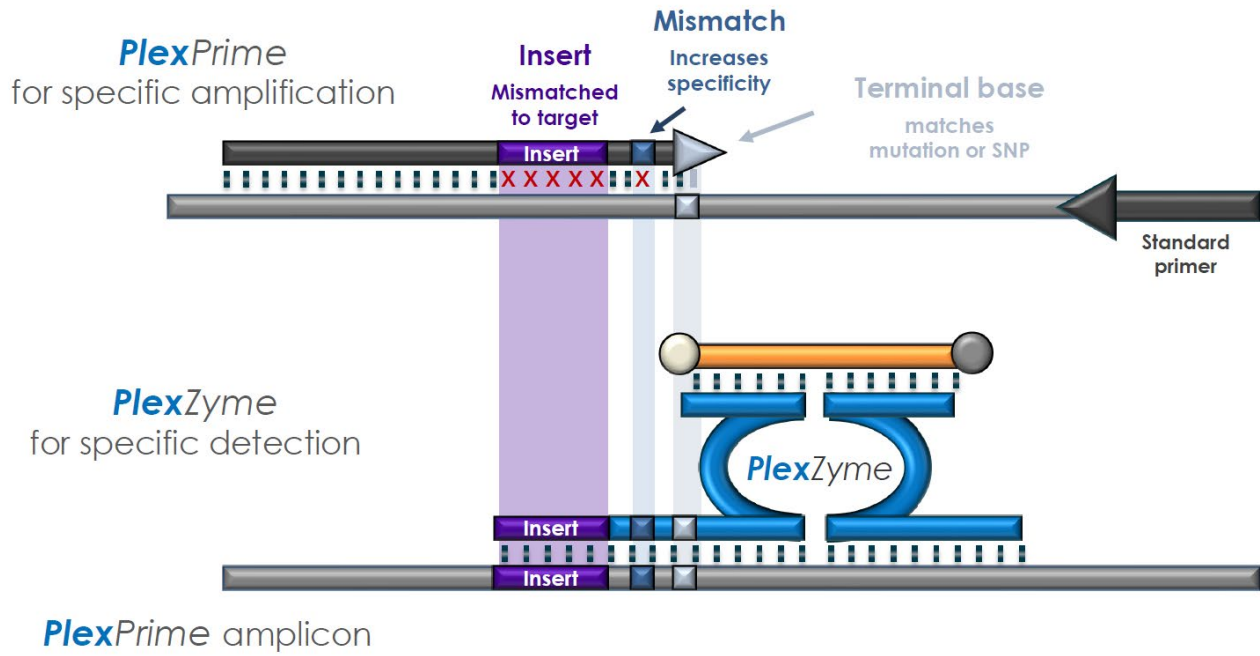
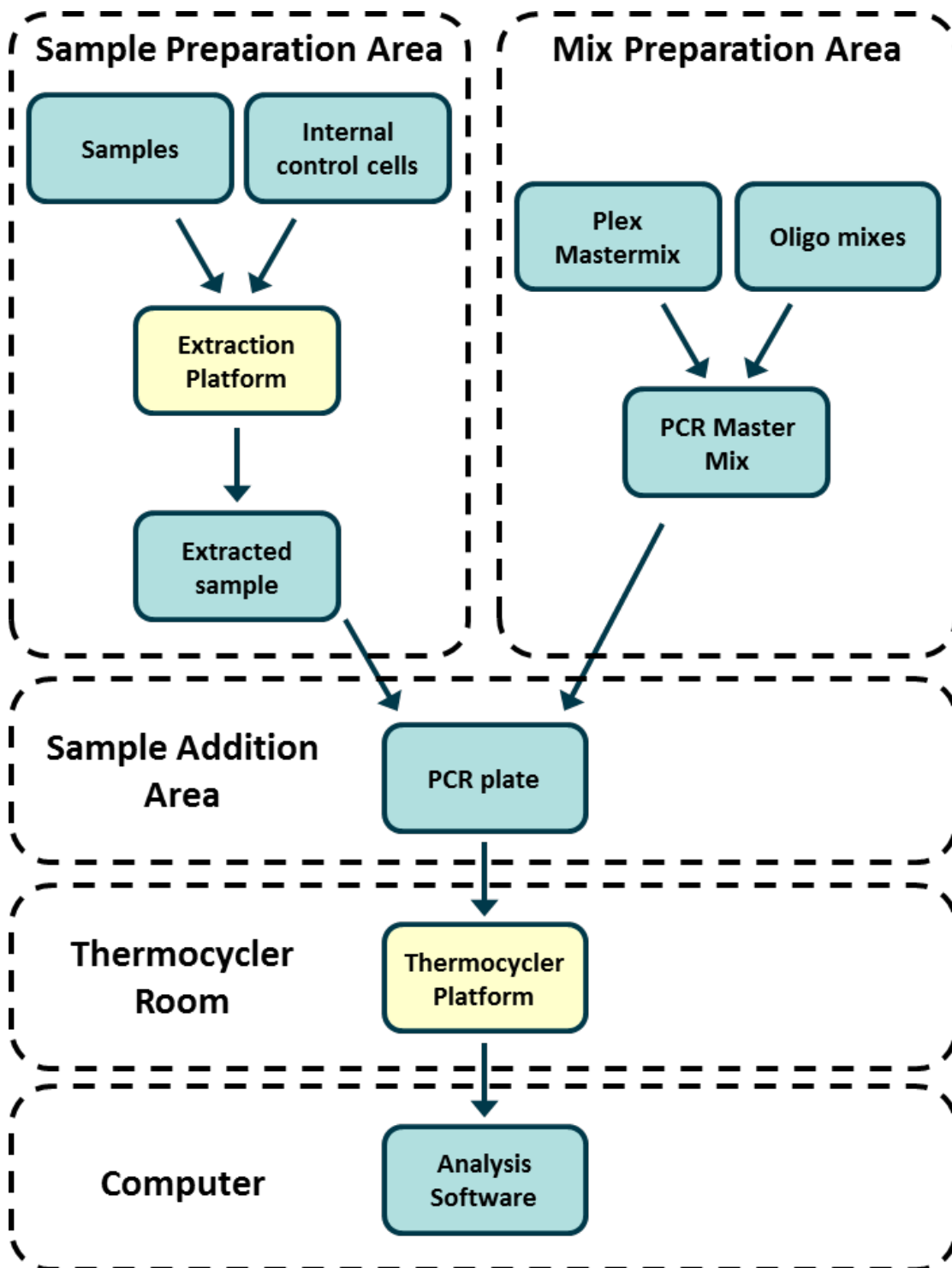


Figure 2. Schematic representation of the *PlexPrime*[®] primer coupled with *PlexZyme*[®] detection. The *PlexPrime*[®] primer specifically amplifies the mutant sequence and *PlexZyme*[®] enzymes specifically detect the amplicon.



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

N. gonorrhoeae isolates have also been validated for use with the **ResistancePlus**[®] GC kit.

10.1.1 Validated sample collection devices

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

Sample collection devices that have been validated with the **ResistancePlus**[®] GC 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 Aptima[®] Urine Collection Kit (Hologic, Cat no 301040) collection, transport and storage

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

1. Use of a clear sterile urine collection cup, free of any preservatives or transport media is recommended for patient self-collection.
2. Patient is directed to provide 20-30 mL of first void urine into provided urine collection cup. Female patients should not cleanse the labial area prior to providing the specimen.
3. Using the pipette and transport tube included in Aptima[®] Urine Collection Kit, transfer 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.

10.1.4 Aptima[®] Multitest swab collection kit (Hologic, Cat no PRD-03546) collection, transport and storage

Directions are summarized below for the collection and transport of vaginal, anal/rectal and pharyngeal swab specimens with the Aptima[®] Multitest swab specimen collection kit (Hologic, Cat no PRD-03546)

10.1.4.1 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.

10.1.4.2 Anal/rectal 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 rectum about 1-2 inches (3-5 cm) past the anal margin and gently rotate the swab clockwise for 5 to 10 seconds. 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.

10.1.4.3 Pharyngeal 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 throat ensuring contact with bilateral tonsils (if present) and the posterior pharyngeal wall, then withdraw the swab without touching the inside of the cheeks or tongue.
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.

10.1.5 cobas® PCR Urine Sample Kit (Roche, Cat no 05170486190) collection, transport and storage

Directions are summarized below for the collection and transport of male and female urine with cobas® PCR Urine Sample Kit (Roche, Cat no 05170486190).

1. Transfer the first catch urine collected into the cobas® PCR Media tube using the provided disposable pipette. (Note: If the urine specimen cannot be transferred immediately, it can be stored at 2°C to 30°C for up to 24 hours.)
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.
5. If transfer to a preservation media, refer to manufacture
6. Refer to manufacturer's instructions for detailed storage optimization.

10.1.6 cobas® PCR Media Uni Swab Sample Kit (Roche, Cat no 07958030190) collection, transport and storage

Directions are summarized below for the collection and transport of rectal and vaginal clinician collection specimen with cobas® PCR Media Uni Swab Sample Kit (Roche, Cat no 07958030190).

1. To collect specimen by rectal swabs: hold the swab with the scoreline above your hand and insert the swab about 3 to 5 cm into the anal canal. Gently turn the swab for about 5-10 seconds while running the swab against the walls of the rectum. If the swab is grossly contaminated with feces, discard and repeat the collection. Withdraw the swab carefully. Do not let the swab touch any surface before placing it into the collection tube.
2. To collect the specimen by vaginal swab (clinician collected): In one hand, hold the swab with the scoreline above your hand and insert the swab about 5 cm (2 inches) into the vaginal opening. Gently turn the swab for about 30 seconds while rubbing the swab against the walls of the vagina. Withdraw the swab carefully. Do not let the swab touch any surface before placing it into the collection tube.

10.1.7 Validated sample extracts

Sample extracts validated for use include:

- cobas® x480 extracts

10.2 Sample processing

The *ResistancePlus*® GC 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
EZ1 Advanced XL ^a	EZ1 DSP Virus Kit	200 µL	“On board” workflow	90 µL
MagNA Pure 96 ^b	MagNA Pure 96 DNA and Viral NA Small Volume Kit	200 µL	Pathogen Universal 200	50 µL or 100 µL

^a See 10.3.1 for how to use the internal control on the EZ1

^b See 10.3.2 for how to use the internal control on the MagNA Pure 96

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 (**Table 6**). 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 EZ1 Advanced XL

Add 5 µL *Internal Control cells* (**RED**) to each sample and load into the sample vessel on the instrument.

10.3.2 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 and 20 µL is automatically added to each sample (default).

Note: Do NOT store diluted Internal Control Cells

Table 5. Dilution of Internal Control Cells for MagNA Pure 96 (1 in 200 dilution)			
<i>Internal Control Cells</i> (RED) (µL)	1x PBS (µL)	Total volume (µL)	Volume added to sample (µL)
18	3582	3600	20

10.4 Processing Isolates

Prepare culture to a 1.0 McFarland standard and heat at 100°C for 8 minutes. Once the isolate boilate has returned to room temperature, briefly vortex and spin down. Dilute boilate 1 in 10 using PCR-grade or distilled water. Processed boilate is then treated as an extracted sample.

10.4.1 Storage of processed isolates

Store boilates at -20°C for up to 1 month or -70°C for long term storage.

10.5 Preparation of real-time PCR

The **ResistancePlus**[®] GC kit can be tested at a final reaction volume of 20 μ L or optionally be tested at a final reaction volume of 30 μ L if sensitivity is a concern. Refer to **Section 28** for instructions for testing at a final reaction volume of 30 μ L.

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

10.5.1 Master Mix preparation

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

For a 20 μ L reaction volume, 15 μ L Master Mix and 5 μ L sample is required. Prepare Master Mix as outlined in **Table 6**.

- Pipette the Master Mix into the PCR plate and then add extracted sample to the reaction.
- Positive and negative controls should be run on each plate.
- Seal, then centrifuge the plate and transfer to thermocycler.

Table 6. Master Mix		
Reagent	Concentration	Volume per 20 μ L reaction (μ L)
Nuclease Free Water (Neutral)	N/A	3.0
Plex Mastermix (BLUE)	2x	10.0
GC+ <i>gyrA</i> Mix (BROWN)	20x	1.0
Control Mix* (WHITE)	20x	1.0
Total volume (μ L)		15.0
Add 5 μ L sample for a final volume of 20 μ L		

* 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

10.5.2 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 26**.

The **ResistancePlus**[®] GC kit uses five channels for detection of *N. gonorrhoeae* via the *opa* and *porA* genes, *gyrA* S91 wild type, *gyrA* S91F mutation and Internal Control (**Table 7**).

Table 7. Channels for ResistancePlus [®] GC targets					
qPCR Instrument	<i>N. gonorrhoeae</i> <i>gyrA</i> S91 (wild type)	<i>N. gonorrhoeae</i> <i>porA</i>	<i>N. gonorrhoeae</i> <i>gyrA</i> S91F (mutant)	Internal Control	<i>N. gonorrhoeae</i> <i>opa</i>
	CHANNEL A	CHANNEL B	CHANNEL C	CHANNEL D	CHANNEL E
7500 Fast	FAM	JOE	Texas Red	TAMRA	Cy5
7500 Fast Dx	FAM	JOE	Texas Red	TAMRA	Cy5
LC480 II	465-510	533-580	533-610	533-640	618-660
CFX96 Dx	FAM	HEX	Texas Red	Quasar 705	Cy5
CFX96 Touch	FAM	HEX	Texas Red	Quasar 705	Cy5

Details for each instrument including programming and analysis are described in the **Appendices**.

12 Interpretation of results

Data interpretation requires the **ResistancePlus**[®] GC analysis software. While **PlexPrime**[®] primers offer greater specificity than other allele-specific primers, some non-specific amplification from the **ResistancePlus**[®] GC assay may be seen in samples that contain high concentrations of *N. gonorrhoeae* with *gyrA* S91 wild type in the *gyrA* S91F target channel, and vice versa. The **ResistancePlus**[®] GC analysis software automates the data interpretation of amplification results and streamlines workflow. Instructions for how to use the analysis software are described in **Section 27**.

See **Table 8** 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 8. ResistancePlus [®] GC analysis software		
Cat no	Analysis software*	Real-time PCR instrument
99010	ResistancePlus [®] GC (LC480)	LC480 II
99009	ResistancePlus [®] GC (7500)	7500 Fast, 7500 Fast Dx
99015	ResistancePlus [®] GC (CFX)	CFX96 Dx and CFX96 Touch

* Refer to the website <https://plexpcr.com/resistanceplus-gc/resources> to ensure you are using the most current version of analysis software.

13 Limitations

- The **ResistancePlus**[®] GC assay should only be performed by personnel trained in the procedure and should be performed in accordance to these Instructions for Use.
- Reliable results are dependent on adequate specimen collection transport, storage, and processing. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
- The **ResistancePlus**[®] GC 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 *N. gonorrhoeae* and *gyrA* S91F mutation will affect the positive and negative predictive values for the assay.
- The **ResistancePlus**[®] GC clinical performance studies summarised in **Section 16.1** include testing with male urine, female urine anal, rectal, vaginal and pharyngeal swabs. Additional specimen types including cervical, endocervical, urethral, and eye swabs have also been tested, however there are currently limited data supporting the use of these specimen types.
- 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 marker 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**[®] GC kit includes an internal control to monitor extraction efficiency and qPCR inhibition (**Section 10.3**).

The **ResistancePlus**[®] GC Control kit (PI-IV0015) is recommended as positive control material for nucleic acid amplification. Refer to **Section 15** for instructions to use the **ResistancePlus**[®] GC Control kit.

15 ResistancePlus® GC Control instructions

The **ResistancePlus®** GC Control kit contains positive control material for *gyrA* wild type and *gyrA* S91F mutant, and negative amplification control material (**Table 9**).

Cap colour	Contents	Description	Quantity (10 reactions)
White	GC, <i>gyrA</i> wild type	Positive control template for the detection of <i>N. gonorrhoeae</i> , <i>gyrA</i> wild type	1 x 100 µL
Green	GC, <i>gyrA</i> S91F mutant	Positive control template for the detection of <i>N. gonorrhoeae</i> , <i>gyrA</i> S91F mutation	1 x 100 µL
Black	Negative Control	Negative control	1 x 100 µL

15.1 Instructions for use

Prepare qPCR reactions as described in **Section 10** or **Section 28.1** using positive control material as sample.

Data interpretation requires the **ResistancePlus®** GC analysis software, refer to **Section 27.10** for example results.

16 Performance characteristics

16.1 Clinical performance

16.1.1 Clinical Study 1

A clinical performance study for the **ResistancePlus®** GC₍₅₅₀₎ kit was conducted at the University of Queensland Centre for Clinical Research (UQCCR), Australia. 212 cobas® 4800 (Roche) extracts were collected between May-August 2018 from Townsville Sexual Health Services, Australia, consisting of 82 *N. gonorrhoeae* positive and 130 *N. gonorrhoeae* negative extracts, based on clinical laboratory results. Samples consisted of 101 male, 110 female specimens as well as 1 gender unspecified specimen, and included 6 pharyngeal swabs, 16 rectal swabs, 4 penile swabs, 32 vaginal swabs, 19 endocervical swabs, 3 urethral swabs, 1 peritoneal swab, 1 genital swab site-unspecified, 10 site-unspecified swabs as well as 120 urine specimens. The cobas® extracts were tested with the **ResistancePlus®** GC₍₅₅₀₎ kit on the Applied Biosystems® 7500 Fast Dx Instrument in a 30 µL reaction volume.

To determine performance of the **ResistancePlus®** GC₍₅₅₀₎ kit, *N. gonorrhoeae* detection was compared to cobas® CT/NG results (with positives confirmed by a *opa/porA* real-time PCR test⁶), and *gyrA* detection was compared to Sanger sequencing^{7,8}. Results are summarised in **Table 10**. Analysis of *gyrA* mutation detection only includes samples where the mutant status could be determined (9 samples failed sequencing). Overall, the **ResistancePlus®** GC₍₅₅₀₎ *gyrA* characterisation rate was 92.7% (76/82; 6 samples were reported as *N. gonorrhoeae* positive but *gyrA* indeterminate by the **ResistancePlus®** GC₍₅₅₀₎ kit (2 male pharyngeal swabs, 2 female site-unspecified, 1 female vaginal swab and 1 female urine; only 1/6 was successfully sequenced and was a S91 wild type (female vaginal swab)). Analysis of results in accordance to specimen type is shown in **Table 11**.

		<i>N. gonorrhoeae</i> detection cobas® CT/NG		<i>gyrA</i> detection Sequencing		
		Positive*	Negative	S91F mutant	S91 wild type	
ResistancePlus® GC	Positive	82	1	Mutant detected	2	0
	Negative	0	129	Mutant not detected	0	70
Sensitivity		100.0% (95% CI 95.6-100.0%)		Sensitivity		100.0% (95% CI 15.8-100.0%)
Specificity		99.2%(95% CI 95.8-99.9%)		Specificity		100.0% (95% CI 94.9-100.0%)

* *N. gonorrhoeae* positives confirmed by *opa/porA* real-time PCR

Table 11. Clinical result analysis in accordance to specimen type (Clinical study 1)

Specimen	Expected <i>N. gonorrhoeae</i> negative	Expected <i>N. gonorrhoeae gyrA</i> wild type	Expected <i>N. gonorrhoeae gyrA</i> S91F mutant
Male urine	62/62	20/20	1/1
Female urine	13/13	19/19	1/1
Male rectal swab	13/14 ¹	1/1	-
Male penile	-	4/4	-
Male urethral swab	-	3/3	-
Male pharyngeal swab	-	1/1	-
Female pharyngeal swab	1/1	2/2	-
Endocervical swab	13/13	6/6	-
Vaginal swab	23/23	8/9 ²	-
Female peritoneal swab	-	1/1	-
Female genital swab	1/1	-	-
Female site-unspecified	3/3	3/3	-
Gender unknown, urine	-	1/1	-

¹ Male rectal swab: 1 *N. gonorrhoeae* negative called as *N. gonorrhoeae gyrA* indeterminate

² Vaginal swab: 1 *N. gonorrhoeae gyrA* wild type called as *N. gonorrhoeae gyrA* indeterminate

16.1.2 Clinical Study 2

A clinical performance study for the **ResistancePlus**[®] GC₍₅₅₀₎ kit was conducted at the Molecular Microbiology Laboratory, Royal Prince Alfred Hospital, Australia. Samples were collected between May-July 2018, using Aptima[®] urine and Aptima[®] unisex swab specimen collection kits, consisting of 164 *N. gonorrhoeae* positive and 55 *N. gonorrhoeae* negative samples. The 219 samples consisted of 179 male and 35 female specimens, including 80 pharyngeal swabs, 70 rectal swabs, 8 vaginal swabs, 4 cervical swabs, 2 urethral swabs, 1 eye swab and 1 site unspecified swab as well as 53 urine specimens. The samples were extracted on the EZ1 Advanced XL (Qiagen).

16.1.2.1 Performance of the **ResistancePlus**[®] GC₍₅₅₀₎ kit on the 7500 Fast

To determine performance of the **ResistancePlus**[®] GC₍₅₅₀₎ kit, *N. gonorrhoeae* detection was compared to Aptima[®] Combo 2 results (with positives confirmed by the Aptima[®] *Neisseria gonorrhoeae* assay), and *gyrA* detection was compared to Sanger sequencing^{7,8}. The **ResistancePlus**[®] GC₍₅₅₀₎ kit was run on the 7500 Fast in a 30 µL reaction volume, and results are summarised in **Table 12**. Analysis of *gyrA* mutation detection only includes samples where the mutant status could be determined (a subset of 146 samples were sequenced; 34 samples failed sequencing). Overall, the **ResistancePlus**[®] GC₍₅₅₀₎ *gyrA* characterisation rate was 90.7% (136/150; 14 samples were reported as *N. gonorrhoeae* positive but *gyrA* indeterminate by the **ResistancePlus**[®] GC₍₅₅₀₎ kit (1 female urine, 4 male rectal swabs, 9 male pharyngeal swabs; 11 samples failed sequencing, 3 samples were not available for sequencing). Analysis of results in accordance to specimen type is shown in **Table 13**.

Table 12. Clinical evaluation of the *ResistancePlus*[®] GC₍₅₅₀₎ kit (Clinical study 2)

		<i>N. gonorrhoeae</i> detection Aptima [®] Combo 2		<i>gyrA</i> detection Sequencing		
		Positive*	Negative	S91F mutant	S91 wild type	
<i>ResistancePlus</i> [®] GC ₍₅₅₀₎	Positive	150	0	Mutant detected	31	0
	Negative	14 [^]	50	Mutant not detected	0	81
Sensitivity		91.5% (95% CI 86.1-95.3%)		Sensitivity		100.0% (95% CI 88.8-100.0%)
Specificity		100.0% (95% CI 92.9-100.0%)		Specificity		100.0% (95% CI 95.6-100.0%)

* *N. gonorrhoeae* positives confirmed by Aptima[®] *Neisseria gonorrhoeae* assay

[^] 10/14 false negative samples were also *N. gonorrhoeae* negative with an in-house porA qPCR assay

Table 13. Clinical result analysis in accordance to specimen type (Clinical study 2)

Specimen	Expected <i>N. gonorrhoeae</i> negative	Expected <i>N. gonorrhoeae</i> <i>gyrA</i> wild type	Expected <i>N. gonorrhoeae</i> <i>gyrA</i> S91F mutant
Male urine	14/14	19/19	3/3
Female urine	8/8	1/1	1/1
Male rectal swab	9/9	33/33	11/11
Female rectal swab	-	1/1	-
Male urethral swab	1/1	-	1/1
Male pharyngeal swab	7/7	25/25	7/7
Female pharyngeal swab	3/3	-	4/4
Cervical swab	3/3	1/1	-
Vaginal swab	4/4	-	4/4
Female eye Swab	-	1/1	-
Female site unspecified swab	1/1	-	-

16.1.2.2 Performance of the *ResistancePlus*[®] GC₍₆₁₀₎ at 30 µL reaction on the LC480 II

To determine performance of the *ResistancePlus*[®] GC₍₆₁₀₎ kit, *N. gonorrhoeae* detection was compared to Aptima[®] Combo 2 results (with positives confirmed by the Aptima[®] *Neisseria gonorrhoeae* assay), and *gyrA* detection was compared to Sanger sequencing^{7,8}. The *ResistancePlus*[®] GC₍₆₁₀₎ kit was run on the LC480 II in a 30 µL reaction volume, and results are summarised in **Table 14**. Analysis of *gyrA* mutation detection only includes samples where the mutant status could be determined (a subset of 144 samples were sequenced; 32 samples failed sequencing; 1 sample was *N. gonorrhoeae* positive but *gyrA* indeterminate by the *ResistancePlus*[®] GC₍₆₁₀₎ kit). Overall, the *ResistancePlus*[®] GC₍₆₁₀₎ *gyrA* characterisation rate was 90.1% (135/150; 15 samples were reported as *N. gonorrhoeae* positive but *gyrA* indeterminate by the *ResistancePlus*[®] GC₍₆₁₀₎ kit (1 female urine, 6 male rectal swabs, and 8 male pharyngeal swabs; only 1/15 was successfully sequenced and was a S91 wild type (male pharyngeal throat swab)).

Table 14. Clinical evaluation of the <i>ResistancePlus</i> [®] GC ₍₆₁₀₎ kit (Clinical study 2)							
		<i>N. gonorrhoeae</i> detection Aptima [®] Combo 2		<i>gyrA</i> detection Sequencing			
		Positive*	Negative		S91F mutant	S91 wild type	
<i>ResistancePlus</i> [®] GC ₍₆₁₀₎	Positive	150	0	Mutant detected	31	0	
	Negative	14 [^]	54	Mutant not detected	0	80	
Sensitivity		91.5% (95% CI 86.1-95.3%)		Sensitivity			100.0% (95% CI 88.8-100.0%)
Specificity		100.0% (95% CI 93.4-100.0%)		Specificity			100.0% (95% CI 93.3-99.9%)

* *N. gonorrhoeae* positives confirmed by Aptima[®] *Neisseria gonorrhoeae* assay

[^] 8/14 false negative samples were also *N. gonorrhoeae* negative with an in-house porA qPCR assay

16.1.2.3 Performance of the *ResistancePlus*[®] GC₍₆₁₀₎ at 30 µL and 20 µL reaction volumes

A subset of samples were compared at 30 µL and 20 µL reaction volumes with the *ResistancePlus*[®] GC₍₆₁₀₎ kit on the LC480 II. Results are summarised in **Table 15**. Analysis of *gyrA* mutation detection only includes samples where the mutant status could be determined (9 samples were *N. gonorrhoeae* positive but *gyrA* indeterminate when tested at both reaction volumes, with an additional 12 samples for 20 µL reaction volume only, and 1 sample at 30 µL reaction volume only).

Table 15. Comparison of <i>ResistancePlus</i> [®] GC ₍₆₁₀₎ kit in 30 µL and 20 µL reaction volumes (Clinical study 2)							
		<i>N. gonorrhoeae</i> detection <i>ResistancePlus</i> [®] GC ₍₆₁₀₎ (30 µL)		<i>gyrA</i> detection <i>ResistancePlus</i> [®] GC ₍₆₁₀₎ (30 µL)			
		Positive	Negative		Mutant detected	Mutant not detected	
<i>ResistancePlus</i> [®] GC ₍₆₁₀₎ (20 µL)	Positive	117	4	Mutant detected	27	0	
	Negative	5	56	Mutant not detected	0	68	
Sensitivity		95.6% (95% CI 90.7-98.7%)		Sensitivity			100.0% (95% CI 78.2-100.0%)
Specificity		93.3% (95% CI 83.8-98.2%)		Specificity			100.0% (95% CI 94.7-100.0%)

16.1.3 Clinical Study 3

Performance of the *ResistancePlus*[®] GC₍₅₅₀₎ kit on *N. gonorrhoeae* clinical isolates was evaluated at the University of Queensland Centre for Clinical Research (UQCCR), Australia. 30 *N. gonorrhoeae* clinical isolates collected in New South Wales (NSW) as part of an Australian national surveillance study in 2014, consisting of isolates from both females (n=2) and males (n=28), collected from the cervix (n=2), rectum (n=5), pharynx (n=4) and urine (n=19). Of the 30 isolates, 15 had the *gyrA* S91F genotype and were resistant to ciprofloxacin and 15 were wild type S91 and were susceptible to ciprofloxacin. The isolates were processed following the boilate method (**Section 10.4**), and tested on the 7500 Fast Dx Instruments in a 30 µL reaction volume.

The performance of the *ResistancePlus*[®] GC₍₅₅₀₎ kit for *gyrA* detection was compared to the genotype as determined by whole genome sequencing (WGS), and ciprofloxacin phenotype as determined by antibiotic susceptibility testing (AST)⁹. Results are summarised in **Table 16**.

Table 16. Evaluation of the *ResistancePlus*[®] GC₍₅₅₀₎ kit on *N. gonorrhoeae* clinical isolates (Clinical study 3)

		Genotype		Phenotype			
		WGS		Ciprofloxacin AST			
		<i>gyrA</i> S91F mutant	<i>gyrA</i> wild type		Resistant (MIC > 1.0 mg/L)	Susceptible (MIC < 1.0 mg/L)	
<i>ResistancePlus</i> [®] GC ₍₅₅₀₎	Mutant detected	15	0	Mutant detected	15	0	
	Mutant not detected	0	15	Mutant not detected	0	15	
Sensitivity		100.0% (95% CI 78.2-100.0%)		Sensitivity		100.0% (95% CI 78.2-100.0%)	
Specificity		100.0% (95% CI 78.2-100.0%)		Specificity		100.0% (95% CI 78.2-100.0%)	

16.1.4 Clinical Study 4

The performance of the *ResistancePlus*[®] GC₍₆₇₅₎ kit was assessed on cobas[®] collected samples (cobas[®] PCR Urine Sample Kit and cobas[®] PCR Media Uni Swab Sample Kit) at PathWest Laboratory Medicine, QEII Medical Centre, Western Australia.

91 *N. gonorrhoeae* positive clinical samples and 42 *N. gonorrhoeae* negative samples, which had been collected from May 2018 to September 2019, were tested. Samples were collected from 92 males and 41 females, and consisted of 18 pharyngeal swabs, 36 anal swabs, 5 penile swabs, 19 vaginal swabs, 6 cervical swabs, 7 urethral swabs and 42 urine specimens. The samples were extracted using the MagNA Pure 96 with a 100 µL elution volume and tested on the CFX96 Touch instrument as a 20 µL reaction volume.

The performance of the *ResistancePlus*[®] GC₍₆₇₅₎ kit for *N. gonorrhoeae* detection was compared to cobas[®] CT/NG (performed according to the manufacturer's instructions) and *gyrA* detection was compared to an in-house Sanger sequencing assay. Results are summarised in **Table 17**. All samples were successfully sequenced. Overall, the *ResistancePlus*[®] GC₍₆₇₅₎ *gyrA* characterisation rate was 98.9% (90/91); 1 male pharyngeal sample was reported as *N. gonorrhoeae* positive but *gyrA* indeterminate by the *ResistancePlus*[®] GC₍₆₇₅₎ kit. Sanger sequencing determined this specimen to be S91 wildtype. Analysis of results in accordance to specimen type is shown in **Table 18**.

Table 17. Clinical evaluation of the *ResistancePlus*[®] GC₍₆₇₅₎ kit (Clinical study 4)

		<i>N. gonorrhoeae</i> detection		<i>gyrA</i> detection			
		cobas [®] CT/NG		Sequencing			
		Positive	Negative		S91F mutant	S91 wild type	
<i>ResistancePlus</i> [®] GC	Positive	91	2	Mutant detected	41	0	
	Negative	0	40	Mutant not detected	0	49	
Sensitivity		100.0% (95% CI 96.0-100.0%)		Sensitivity		100.0% (95% CI 91.4-100.0%)	
Specificity		95.2% (95% CI 83.8-99.4%)		Specificity		100.0% (95% CI 92.6-100.0%)	

Table 18. Clinical result analysis in accordance to specimen type (Clinical study 4)

Specimen	Expected <i>N. gonorrhoeae</i> negative	Expected <i>N. gonorrhoeae gyrA</i> wild type	Expected <i>N. gonorrhoeae gyrA</i> S91F mutant
Male urine	2/2	15/15	14/14
Female urine	6/6	5/5	-
Male anal swab	10/11 ¹	10/10	12/12
Female anal swab	2/2	1/1	-
Male pharyngeal swab	2/3 ²	3/4 ³	9/9
Female pharyngeal swab	-	1/1	1/1
Male urethral swab	-	6/6	1/1
Penile swab	-	3/3	2/2
Cervical swab	5/5	1/1	-
Vaginal swab	13/13	4/4	2/2

¹ 1 male anal swab was false positive "*N. gonorrhoeae* detected, *gyrA* mutation detected."

² 1 male pharyngeal swab was false positive "*N. gonorrhoeae* detected, *gyrA* indeterminate."

³ 1 *gyrA* S91 wild type male pharyngeal swab was called "*N. gonorrhoeae* detected, *gyrA* indeterminate."

16.2 Analytical performance

16.2.1 Reproducibility and repeatability (7500 Fast and 7500 Fast Dx)

The reproducibility of the **ResistancePlus**[®] GC₍₅₅₀₎ kit was assessed using quantified extracted genomic material, *N. gonorrhoeae gyrA* wild type strain (ATCC:700825; FA 1090) and *gyrA* S91F mutant strain (clinical isolate), tested at 3x LOD. Experiments utilizing 20 µL reaction volumes were performed on the 7500 Fast and experiments utilizing 30 µL reaction volumes were performed on the 7500 Fast Dx.

To determine the lot-to-lot variability, two lots were tested, run on one machine, performed by one operator. For the 30 µL reaction (**Table 19**), the two lots showed good reproducibility with coefficient of variation (%CV) between 0.61-1.88%.

Table 19. Lot-to-lot variability (30 µL reaction volume)					
Strain	Target	Average Cq	STDEV	%CV	# Samples
<i>N. gonorrhoeae</i> <i>gyrA</i> wild type	<i>opa</i>	23.61	0.31	1.29	12/12
	<i>porA</i>	25.06	0.19	0.75	12/12
	<i>gyrA</i> S91 wild type	24.34	0.22	0.91	12/12
<i>N. gonorrhoeae</i> <i>gyrA</i> mutant	<i>opa</i>	23.65	0.14	0.61	12/12
	<i>porA</i>	25.37	0.31	1.21	12/12
	<i>gyrA</i> S91F mutant	29.25	0.55	1.88	12/12

For lot-to-lot variability for the 20 µL reaction (Table 20), the two lots showed good reproducibility with coefficient of variation (%CV) between 0.61-1.40%.

Table 20. Lot-to-lot variability (20 µL reaction volume)					
Strain	Target	Average Cq	STDEV	%CV	# Samples
<i>N. gonorrhoeae</i> <i>gyrA</i> wild type	<i>opa</i>	23.03	0.14	0.61	12/12
	<i>porA</i>	24.78	0.23	0.92	12/12
	<i>gyrA</i> S91 wild type	24.26	0.26	1.09	12/12
<i>N. gonorrhoeae</i> <i>gyrA</i> mutant	<i>opa</i>	22.52	0.16	0.70	12/12
	<i>porA</i>	24.54	0.27	1.10	12/12
	<i>gyrA</i> S91F mutant	28.64	0.40	1.40	12/12

To determine the day-to-day variability, testing was performed over three days, by one operator on the same machine. For the 30 µL reaction (Table 21), the three runs showed good reproducibility with coefficient of variation (%CV) between 1.13-2.31%.

Table 21. Day-to-day variability (30 µL reaction volume)					
Strain	Target	Average Cq	STDEV	%CV	# Samples
<i>N. gonorrhoeae</i> <i>gyrA</i> wild type	<i>opa</i>	24.08	0.56	2.31	18/18
	<i>porA</i>	25.31	0.39	1.54	18/18
	<i>gyrA</i> S91 wild type	24.88	0.45	1.80	18/18
<i>N. gonorrhoeae</i> <i>gyrA</i> mutant	<i>opa</i>	23.95	0.27	1.15	18/18
	<i>porA</i>	25.66	0.45	1.75	18/18
	<i>gyrA</i> S91F mutant	29.83	0.34	1.13	18/18

For day-to-day variability for the 20 µL reaction (Table 22), the three runs performed over the 3 days showed good reproducibility with coefficient of variation (%CV) between 0.65-3.23%.

Table 22. Day-to-day variability (20 µL reaction volume)					
Strain	Target	Average Cq	STDEV	%CV	# Samples
<i>N. gonorrhoeae</i> <i>gyrA</i> wild type	<i>opa</i>	23.24	0.29	1.26	18/18
	<i>porA</i>	24.99	0.16	0.65	18/18
	<i>gyrA</i> S91 wild type	24.88	0.56	2.25	18/18
<i>N. gonorrhoeae</i> <i>gyrA</i> mutant	<i>opa</i>	23.13	0.75	3.23	18/18
	<i>porA</i>	25.18	0.71	2.81	18/18
	<i>gyrA</i> S91F mutant	28.92	0.77	2.65	18/18

To determine the run-to-run variability, three qPCR runs were compared, run on the same day by the same operator. For the 30 µL reaction (Table 23), the three runs showed good reproducibility with coefficient of variation (%CV) between 0.69-1.78%.

Table 23. Run-to-run variability (30 µL reaction volume)					
Strain	Target	Average Cq	STDEV	%CV	# Samples
<i>N. gonorrhoeae</i> <i>gyrA</i> wild type	<i>opa</i>	24.37	0.43	1.76	18/18
	<i>porA</i>	25.45	0.38	1.50	18/18
	<i>gyrA</i> S91 wild type	25.10	0.45	1.78	18/18
<i>N. gonorrhoeae</i> <i>gyrA</i> mutant	<i>opa</i>	24.11	0.17	0.69	18/18
	<i>porA</i>	25.91	0.33	1.28	18/18
	<i>gyrA</i> S91F mutant	30.06	0.34	1.12	18/18

For run-to-run variability for the 20 µL reaction (Table 24), the three runs showed good reproducibility with coefficient of variation (%CV) between 0.83-1.30%.

Table 24. Run-to-run variability (20 µL reaction volume)					
Strain	Target	Average Cq	STDEV	%CV	# Samples
<i>N. gonorrhoeae</i> <i>gyrA</i> wild type	<i>opa</i>	23.10	0.30	1.30	18/18
	<i>porA</i>	24.79	0.22	0.87	18/18
	<i>gyrA</i> S91 wild type	25.16	0.22	0.88	18/18
<i>N. gonorrhoeae</i> <i>gyrA</i> mutant	<i>opa</i>	22.61	0.21	0.94	18/18
	<i>porA</i>	24.67	0.21	0.83	18/18
	<i>gyrA</i> S91F mutant	28.51	0.31	1.08	18/18

To determine the operator variability, two runs were compared from two operators. For the 30 µL reaction (Table 25), the two runs performed by different operators showed good reproducibility with coefficient of variation (%CV) between 1.21-2.71%.

Table 25. Operator variability (30 μ L reaction volume)					
Strain	Target	Average Cq	STDEV	%CV	# Samples
<i>N. gonorrhoeae</i> <i>gyrA</i> wild type	<i>opa</i>	24.08	0.65	2.71	12/12
	<i>porA</i>	25.38	0.46	1.79	12/12
	<i>gyrA</i> S91 wild type	24.81	0.52	2.11	12/12
<i>N. gonorrhoeae</i> <i>gyrA</i> mutant	<i>opa</i>	23.97	0.31	1.30	12/12
	<i>porA</i>	25.75	0.50	1.93	12/12
	<i>gyrA</i> S91F mutant	29.83	0.36	1.21	12/12

For operator variability for the 20 μ L reaction (Table 26), the two runs performed by different operators showed good reproducibility with coefficient of variation (%CV) between 0.48-2.23%.

Table 26. Operator variability (20 μ L reaction volume)					
Strain	Target	Average Cq	STDEV	%CV	# Samples
<i>N. gonorrhoeae</i> <i>gyrA</i> wild type	<i>opa</i>	23.07	0.13	0.56	12/12
	<i>porA</i>	24.91	0.14	0.56	12/12
	<i>gyrA</i> S91 wild type	24.67	0.55	2.23	12/12
<i>N. gonorrhoeae</i> <i>gyrA</i> mutant	<i>opa</i>	22.63	0.11	0.48	12/12
	<i>porA</i>	24.72	0.22	0.88	12/12
	<i>gyrA</i> S91F mutant	28.45	0.34	1.20	12/12

To determine the instrument variability, two runs performed on two different instruments were compared. For the 30 μ L reaction (Table 27), the two runs performed on different instruments showed good reproducibility with coefficient of variation (%CV) between 1.20-3.42%.

Table 27. Instrument variability (30 μ L reaction volume)					
Strain	Target	Average Cq	STDEV	%CV	# Samples
<i>N. gonorrhoeae</i> <i>gyrA</i> wild type	<i>opa</i>	24.52	0.54	2.19	12/12
	<i>porA</i>	25.42	0.39	1.52	12/12
	<i>gyrA</i> S91 wild type	25.35	0.43	1.70	12/12
<i>N. gonorrhoeae</i> <i>gyrA</i> mutant	<i>opa</i>	24.21	0.76	3.12	12/12
	<i>porA</i>	25.92	0.89	3.42	12/12
	<i>gyrA</i> S91F mutant	29.72	0.36	1.20	12/12

For instrument variability for the 20 μ L reaction (Table 28), the two runs performed showed good reproducibility with coefficient of variation (%CV) between 0.58-1.73%.

Table 28. Instrument variability (20 µL reaction volume)					
Strain	Target	Average Cq	STDEV	%CV	# Samples
<i>N. gonorrhoeae</i> <i>gyrA</i> wild type	<i>opa</i>	22.96	0.25	1.11	12/12
	<i>porA</i>	24.74	0.31	1.27	12/12
	<i>gyrA</i> S91 wild type	24.95	0.43	1.73	12/12
<i>N. gonorrhoeae</i> <i>gyrA</i> mutant	<i>opa</i>	22.59	0.13	0.58	12/12
	<i>porA</i>	24.55	0.29	1.18	12/12
	<i>gyrA</i> S91F mutant	28.58	0.28	0.97	12/12

16.2.2 Reproducibility and repeatability (CFX96 Dx and CFX96 Touch)

The reproducibility of the **ResistancePlus**[®] GC₍₆₇₅₎ kit was assessed using quantified extracted genomic material, *N. gonorrhoeae gyrA* wild type strain (ATCC:700825; FA 1090) and *gyrA* S91F mutant strain (clinical isolate), tested at 3x LOD. Each material was tested in replicates of ten twice daily over three days, by two operators using two different lots of the **ResistancePlus**[®] GC₍₆₇₅₎ kit and two different CFX96 instruments (one CFX96 Dx, and one CFX96 Touch). All replicates were tested with 20 µL reaction volume.

To determine the lot-to-lot variability, two lots were tested, run on two instruments, performed by two operators, over three days. The two lots showed good reproducibility with coefficient of variation (%CV) between 0.15-3.59% (**Table 29**).

Table 29. Lot-to-lot variability					
Strain	Target	Average Cq	STDEV	%CV	# Samples
<i>N. gonorrhoeae</i> <i>gyrA</i> wild type	<i>opa</i>	22.34	0.03	0.15	60/60
	<i>porA</i>	24.03	0.13	0.53	60/60
	<i>gyrA</i> S91 wild type	24.92	0.89	3.59	60/60
<i>N. gonorrhoeae</i> <i>gyrA</i> mutant	<i>opa</i>	23.07	0.16	0.71	60/60
	<i>porA</i>	24.91	0.13	0.52	60/60
	<i>gyrA</i> S91F mutant	29.30	0.28	0.94	60/60

To determine the day-to-day variability, two lots were tested on two instruments, performed by two operators, over three days. The two lots showed good reproducibility with coefficient of variation (%CV) between 0.15-3.59% (**Table 30**).

Table 30. Day-to-day variability					
Strain	Target	Average Cq	STDEV	%CV	# Samples
<i>N. gonorrhoeae</i> <i>gyrA</i> wild type	<i>opa</i>	22.34	0.23	1.01	60/60
	<i>porA</i>	24.03	0.19	0.78	60/60
	<i>gyrA</i> S91 wild type	24.92	0.03	0.14	60/60
<i>N. gonorrhoeae</i> <i>gyrA</i> mutant	<i>opa</i>	23.07	0.11	0.46	60/60
	<i>porA</i>	24.91	0.04	0.15	60/60
	<i>gyrA</i> S91F mutant	29.30	0.26	0.89	60/60

16.2.3 Analytical sensitivity

The analytical sensitivity of the **ResistancePlus**[®] GC kit was established on the 7500 Fast Dx using 30 µL reaction volume by running a limited dilution series, using quantified extracted genomic material, *N. gonorrhoeae gyrA* wild type strain (ATCC:700825; FA 1090) and *N. gonorrhoeae gyrA* S91F mutant strain (clinical isolate). The sensitivity for each target was determined as the number of genomes per reaction detected with ≥ 95% of replicates performed (**Table 31**). The LOD (genomes per reaction) of each representative strain of *N. gonorrhoeae* was confirmed for the 7500 Fast, LC480 II, CFX96 Dx and CFX96 Touch, for a 30 µL reaction volume, and confirmed for the 7500 Fast, 7500 Fast Dx, LC480 II, CFX96 Dx and CFX96 Touch for a 20 µL reaction volume.

Table 31. Analytical sensitivity	
Strain	Limit of detection (genomes/reaction)
<i>N. gonorrhoeae gyrA</i> wild type	15
<i>N. gonorrhoeae gyrA</i> mutant	15

16.2.4 Inclusivity

All inclusivity strains were tested using 20 µL and 30 µL reaction volumes. Each inclusivity strain of *N. gonorrhoeae* was tested at 1x LOD genomes per reaction. All testing using 20 µL reaction volumes was performed on the 7500 Fast and all testing using 30 µL reaction volumes was performed on the 7500 Fast Dx. All inclusivity strains achieved ≥ 95% detection (**Table 32**).

Table 32. Analytical inclusivity			
Strain	<i>gyrA</i> status	20 µL Reaction volume	30 µL Reaction volume
		# Replicates	# Replicates
WHO B	S91F mutant	20/20	20/20
WHO C	S91 wild type	20/20	20/20
WHO F	S91 wild type	20/20	20/20
WHO G	S91F mutant	20/20	20/20
WHO K	S91F mutant	20/20	20/20
WHO L	S91F mutant	19/20	20/20
WHO M	S91F mutant	20/20	20/20
WHO N	S91F mutant	19/20	19/20
WHO P	S91 wild type	20/20	20/20
WHO X	S91F mutant	20/20	20/20
WHO Z	S91F mutant	20/20	20/20
FC428	S91F mutant	20/20	20/20

16.2.5 Analytical specificity

The **ResistancePlus**[®] GC kit was designed to be specific for the target organism *N. gonorrhoeae* as well as markers for resistance/susceptibility, by checking for homology to non-target organisms in public sequence databases. All testing using 20 µL reaction volumes was performed on the 7500 Fast and all testing using 30 µL reaction volumes was performed on the 7500 Fast Dx. Each organism was tested at the stated concentration. Specificity testing for selected organisms did not show cross-reactivity (**Table 33**).

Table 33. Analytical specificity	
Organism	Test concentration (copies/reaction)
<i>Candida albicans</i> (3153A)	10 ⁴
<i>Chlamydia trachomatis</i> (LGV II 434)	10 ⁴
<i>Chlamydomydia pneumoniae</i> (CM-1)	10 ⁴
Cytomegalovirus (AD169-BAC isolate)	10 ⁴
<i>Enterococcus faecalis</i> (Portland)	10 ⁶
Epstein-Barr virus (Human herpes virus 4)	10 ⁴
<i>Escherichia coli</i> (Crooks)	10 ⁶
<i>Haemophilus influenzae</i> (Rd KW20)	10 ⁶
Herpes simplex virus 1 (McIntyre)	10 ⁴
Herpes simplex virus 2 (MS)	10 ⁴
<i>Klebsiella oxytoca</i> (Flugge) Lautrop	10 ⁶
<i>Legionella pneumophila</i> (Philadelphia-1)	10 ⁴
<i>Listeria monocytogenes</i> (Li 23)	10 ⁶
<i>Moraxella osloensis</i> (clinical isolate)	10 ⁵
<i>Mycoplasma genitalium</i> (G37)	10 ⁴
<i>Mycoplasma hominis</i> (ZK-CU2)	10 ⁴
<i>Mycoplasma pneumoniae</i> (FH strain of Eaton Agent)	10 ⁶
<i>Neisseria cinerea</i> (clinical isolate)	10 ⁵
<i>Neisseria flavescens</i> (clinical isolate)	10 ⁵
<i>Neisseria lactamica</i> (clinical isolate)	10 ⁴
<i>Neisseria meningitidis</i> (clinical isolate)	10 ⁵
<i>Neisseria mucosa</i> (clinical isolate)	10 ⁵
<i>Neisseria polysaccharea</i> (clinical isolate)	10 ⁵
<i>Neisseria sicca</i> (clinical isolate)	10 ⁵
<i>Neisseria subflava</i> (clinical isolate)	10 ⁵
<i>Neisseria weaverii</i> (clinical isolate)	10 ⁵
<i>Pseudomonas aeruginosa</i> (PAO1-LAC)	10 ⁶
<i>Salmonella typhimurium</i>	10 ⁴
<i>Staphylococcus aureus</i>	10 ⁴
<i>Streptococcus agalactiae</i> (2603V/R)	10 ⁵
<i>Streptococcus pneumoniae</i> (R6)	10 ⁴
<i>Streptococcus salivarius</i> (275 (DSM 20560))	10 ⁶

Table 33. Analytical specificity	
Organism	Test concentration (copies/reaction)
<i>Treponema pallidum</i> (Nichols strain)	10 ³
<i>Trichomonas vaginalis</i>	10 ⁴
<i>Ureaplasma parvum</i>	10 ⁴
<i>Ureaplasma urealyticum</i> (960)	10 ³

16.2.6 Competitive interference.

To study competitive interference, the **ResistancePlus**[®] GC kit was tested in contrived samples simulating co-infections. Testing was performed with each potential interfering organism mixed with each representative strain of a *N. gonorrhoeae gyrA* wild type strain (ATCC:700825; FA 1090) and *N. gonorrhoeae gyrA* S91F mutant strain (clinical isolate) at 3x LOD. All testing using 20 µL reaction volumes was performed on the 7500 Fast and all testing using 30 µL reaction volumes was performed on the 7500 Fast Dx. Results indicated that none of these organisms interfered with detection of each representative strain of *N. gonorrhoeae* at the stated concentrations (**Table 34**).

Table 34. Competitive interference			
Organism	Concentration (copies/reaction)	# Samples detected	
		<i>N. gonorrhoeae</i> S91 wild type strain (3x LOD)	<i>N. gonorrhoeae</i> S91F mutant strain (3x LOD)
<i>Moraxella osloensis</i> (clinical isolate)	10 ⁵	3/3	3/3
<i>Neisseria cinerea</i> (clinical isolate)	10 ⁵	3/3	3/3
<i>Neisseria flavescens</i> (clinical isolate)	10 ⁵	3/3	3/3
<i>Neisseria lactamica</i> (clinical isolate)	10 ⁴	3/3	3/3
<i>Neisseria meningitidis</i> (clinical isolate)	10 ⁵	3/3	3/3
<i>Neisseria mucosa</i> (clinical isolate)	10 ⁵	3/3	3/3*
<i>Neisseria polysaccharea</i> (clinical isolate)	10 ⁵	3/3	3/3
<i>Neisseria sicca</i> (clinical isolate)	10 ⁵	3/3	3/3
<i>Neisseria subflava</i> (clinical isolate)	10 ⁴	3/3	3/3*
<i>Neisseria weaverii</i> (clinical isolate)	10 ⁵	3/3	3/3

**N. gonorrhoeae* S91F mutant strains may be called as *N. gonorrhoeae* S91 wild type when non-target organism is present in a co-infection above the stated concentration.

16.2.7 Potentially interfering substances

The effect of potential interfering substances on the **ResistancePlus**[®] GC kit was assessed in contrived samples through the performance of the Internal Control, which monitors extraction and qPCR inhibition. Whole blood added to negative samples (PBS only) was tested, and the High Abnormal MAS[®] Urinalysis (Thermo Scientific) was tested as a urine control sample which contains high abnormal urine metabolites (bilirubin, blood, creatinine, crystals, glucose, hCG, ketones, leukocyte esterase, microalbumin, nitrite, osmolality, pH, potassium, protein, red blood cells, sodium, specific gravity, urobilinogen, and white blood cells). Samples were extracted with the *Internal Control Cells* and assessed with the Internal Control assay. A minor shift ($\Delta Cq < 0.5$) in the Internal Control signal was observed in the presence of the substances which did not affect detection (**Table 35**).

Substance	Concentration	IC Average Cq	STDEV	Δ Cq	# Samples detected
--	--	26.7	0.09	--	3/3
Whole Blood	10% (v/v)	27.1	0.07	0.44	3/3
Urinalysis	n/a	26.7	0.18	0.01	3/3

17 Customer and technical support

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

Tel: +61 2 9209 4170, Email: tech@speedx.com.au

18 References

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19 Appendix 1: LightCycler® 480 II programming for reaction volume of 20 µL

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

The **ResistancePlus®** GC₍₆₁₀₎ kit contains dyes for the LightCycler® 480 Instrument II. The **PlexPCR®** Colour Compensation kit (Cat no 90001) must be run and applied for LC480 II analysis (see **Section 19.2**). This kit can be supplied on request.

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

Detection Format

Create a custom **Detection Format**

Open Tools > Detection Formats

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

For **Filter Combination Selection** select the following (Excitation-Emission) shown in **Table 36**:

Table 36. 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

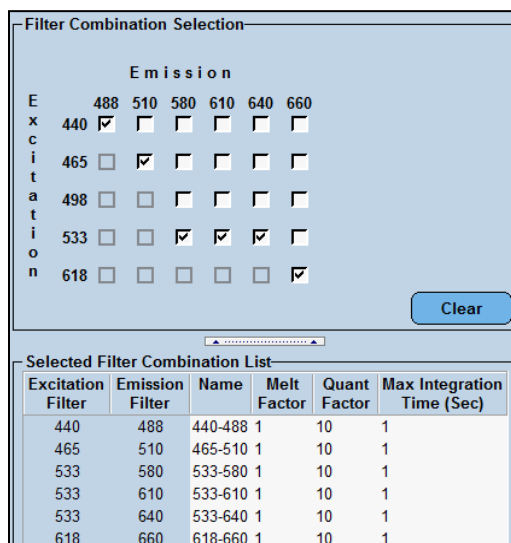
Set the **Selected Filter Combination List** for all channels as:

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

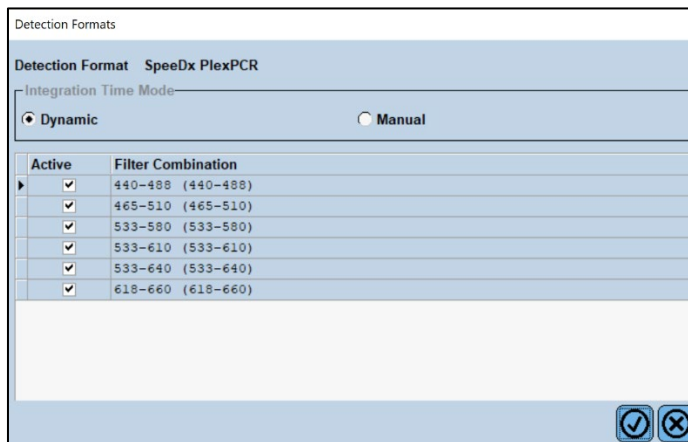
For **Detection Format** select the custom 'SpeedX PlexPCR' (**Figure 4**)

Select **Customize >**

Select **Integration Time Mode > Dynamic**

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

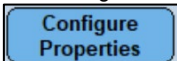
Figure 4. Customize Detection Format



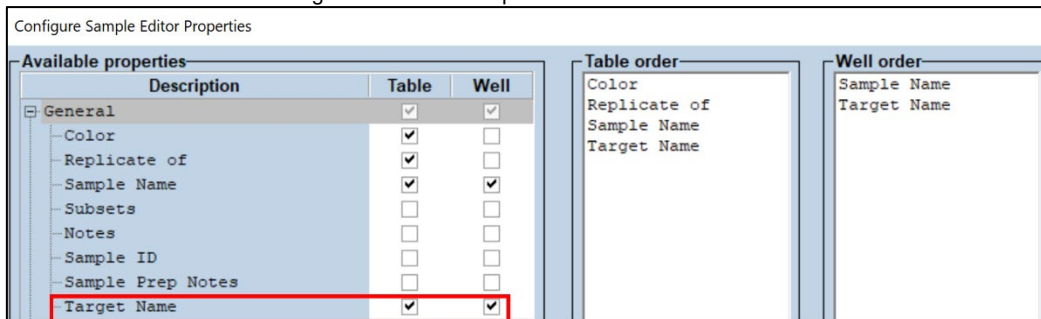
To enable automated sample detection in the analysis software, assign nametags to the wells on the plate

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 37**.

Table 37. Channels for ResistancePlus® GC targets					
Target Name	gyrA-S91	porA	gyrA-S91F	IC	opa
LC480 II channel	465-510	533-580	533-610	533-640	618-660

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 27.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 38** 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 38. Sample nametags for analysis software

Sample Type	Default Prefix_ (in analysis software)
Regular sample	No default – user defined
Negative Control	NC
Positive Control (MT, GC gyrA S91F) (Pa)	Pa
Positive Control (WT, GC gyrA S91) (Pb)	Pb

Figure 5. Sample Editor – Assigning nametags to wells

Pos	Filter combination	Color	Repl Of	Sample Name	Target Name
A1	465-510 (465)	Blue		NC	gyrA-S91
A1	533-580 (533)	Blue		NC	porA
A1	533-610 (533)	Blue		NC	gyrA-S91F
A1	533-640 (533)	Blue		NC	IC
A1	618-660 (618)	Blue		NC	opa
A2	465-510 (465)	Red		Pa	gyrA-S91
A2	533-580 (533)	Red		Pa	porA
A2	533-610 (533)	Red		Pa	gyrA-S91F
A2	533-640 (533)	Red		Pa	IC
A2	618-660 (618)	Red		Pa	opa
A3	465-510 (465)	Green		Pb	gyrA-S91
A3	533-580 (533)	Green		Pb	porA
A3	533-610 (533)	Green		Pb	gyrA-S91F
A3	533-640 (533)	Green		Pb	IC
A3	618-660 (618)	Green		Pb	opa

For a 20 µL reaction

Set **Reaction Volume** > 20 µL

Create the following Program in **Table 39** (shown in more detail in **Figure 6 - Figure 9**):

Table 39. Thermocycling Program				
Program Name	Cycles	Target °C	Hold	Ramp Rate (°C/s) [‡]
Polymerase activation	1	95°C	2 min	4.4
Touch down cycling [§] : Step down -0.5°C/cycle	10	95°C	5 s	4.4
		61°C – 56.5°C [§]	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)

[§] **Step size:** -0.5°C/Cycle, **Sec Target:** 56°C

⁺ **Analysis mode:** Quantification, **Acquisition mode:** Single

Figure 6. Thermocycling Program (20 µL reaction) – Polymerase activation

LightCycler® 480 Software release 1.5.1.62 SP2
 Instrument: 30231 / Not Connected Database: Research Database (Research)
 Window: New Experiment User: Speedx

Run Protocol | Data | Run Notes

Setup
 Detection Format: SpeedX PlexPCR Block Size: 96 Plate ID: Reaction Volume: 20
 Color Comp ID: Lot No: Test ID:

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

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

Figure 7. Thermocycling Program (20 µL reaction) – Touchdown cycling

LightCycler® 480 Software release 1.5.1.62 SP2
 Instrument: 30231 / Not Connected Database: Research Database (Research)
 Window: New Experiment User: Speedx

Run Protocol | Data | Run Notes

Setup
 Detection Format: SpeedX PlexPCR Block Size: 96 Plate ID: Reaction Volume: 20
 Color Comp ID: Lot No: Test ID:

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

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

Figure 8. Thermocycling Program (20 µL reaction) – Quantification cycling

LightCycler® 480 Software release 1.5.1.62 SP2
 Instrument: 30231 / Not Connected Database: Research Database (Research)
 Window: New Experiment User: Speedx

Run Protocol | Data | Run Notes

Setup
 Detection Format: SpeedX PlexPCR Block Size: 96 Plate ID: Reaction Volume: 20
 Color Comp ID: Lot No: Test ID:

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

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

Figure 9. Thermocycling Program (20 μ L reaction) – Cooling

Experiment	Run Protocol	Data	Run Notes																								
Setup	Detection Format: SPEEDX PLEX PCR	Block Size: 96	Plate ID: B1350213																								
Subset Editor	Color Comp ID: []	Lot No: []	Reaction Volume: 20 μ L																								
Sample Editor	<table border="1"> <thead> <tr> <th>Program Name</th> <th>Cycles</th> <th>Analysis Mode</th> </tr> </thead> <tbody> <tr> <td>Polymerase activation</td> <td>1</td> <td>None</td> </tr> <tr> <td>Touch down cycling</td> <td>10</td> <td>None</td> </tr> <tr> <td>Quantification cycling</td> <td>40</td> <td>Quantification</td> </tr> <tr> <td>Cooling</td> <td>1</td> <td>None</td> </tr> </tbody> </table>			Program Name	Cycles	Analysis Mode	Polymerase activation	1	None	Touch down cycling	10	None	Quantification cycling	40	Quantification	Cooling	1	None									
Program Name	Cycles	Analysis Mode																									
Polymerase activation	1	None																									
Touch down cycling	10	None																									
Quantification cycling	40	Quantification																									
Cooling	1	None																									
Analysis	<table border="1"> <thead> <tr> <th colspan="8">Cooling Temperature Targets</th> </tr> <tr> <th>Target (°C)</th> <th>Acquisition Mode</th> <th>Hold (hh:mm:ss)</th> <th>Ramp Rate (°C/s)</th> <th>Acquisitions (per °C)</th> <th>Sec Target (°C)</th> <th>Step Size (°C)</th> <th>Step Delay (cycles)</th> </tr> </thead> <tbody> <tr> <td>40</td> <td>None</td> <td>00:00:30</td> <td>2.2</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> </tr> </tbody> </table>			Cooling Temperature Targets								Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	40	None	00:00:30	2.2	0	0	0	0
Cooling Temperature Targets																											
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)																				
40	None	00:00:30	2.2	0	0	0	0																				
Report																											
Sum.																											

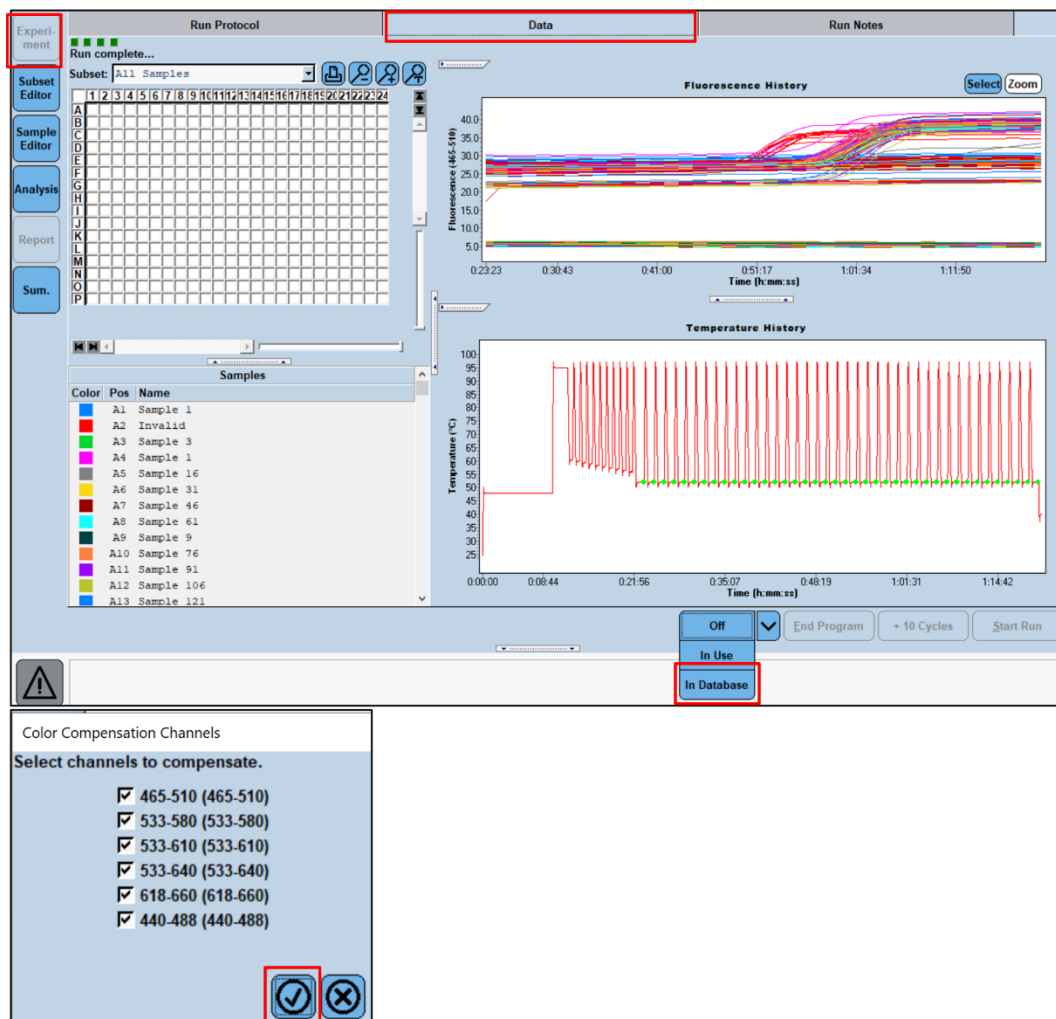
> **Start Run**

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**[®] GC 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



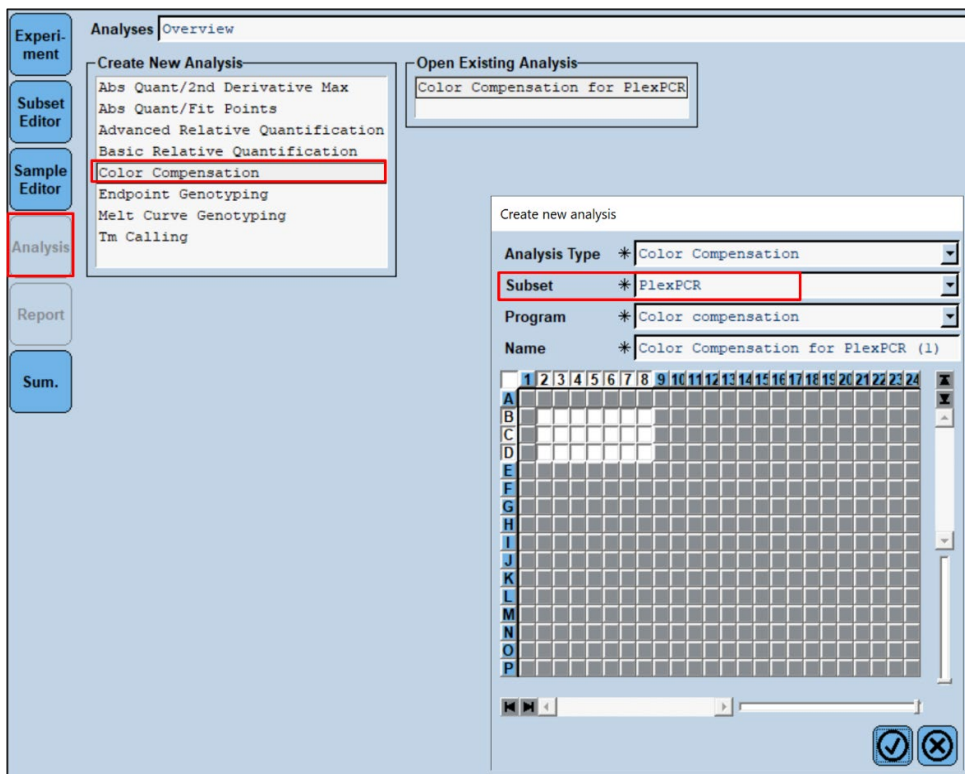
Save in an easily identifiable location

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.*

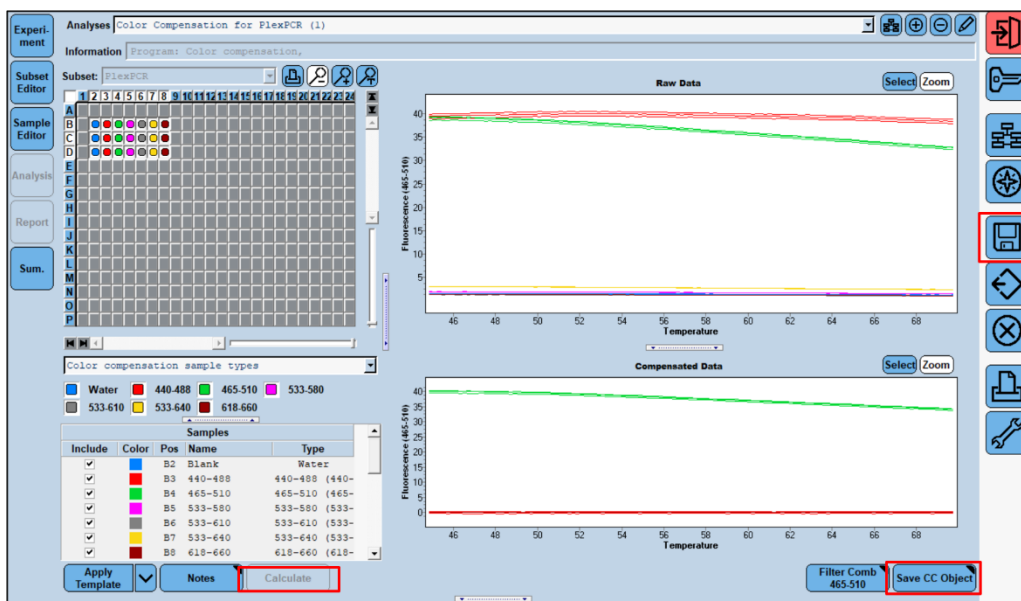
Analyse the Colour Compensation file via **Analysis > Colour Compensation** and select the correct subset, shown in **Figure 11**.

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 **ResistancePlus**[®] GC (LC480) analysis software. The analysis software can be supplied on request, contact tech@speedx.com.au for more information.

Refer to **Section 27** for instructions on using the **ResistancePlus**[®] GC (LC480) analysis software.

20 Appendix 2: LightCycler® 480 II programming for reaction volume of 30 µL

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

The **ResistancePlus®** GC₍₆₁₀₎ kit contains dyes for the LightCycler® 480 Instrument II. The **PlexPCR®** Colour Compensation kit (Cat no 90001) must be run and applied for LC480 II analysis (see **Section 20.2**). This kit can be supplied on request.

20.1 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 13**)

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

Table 40. Filter Combinations ^A						
LC480 II	440-488	465-510	533-580	533-610	533-640	618-660

^A These Filter Combinations are the default names for the channels

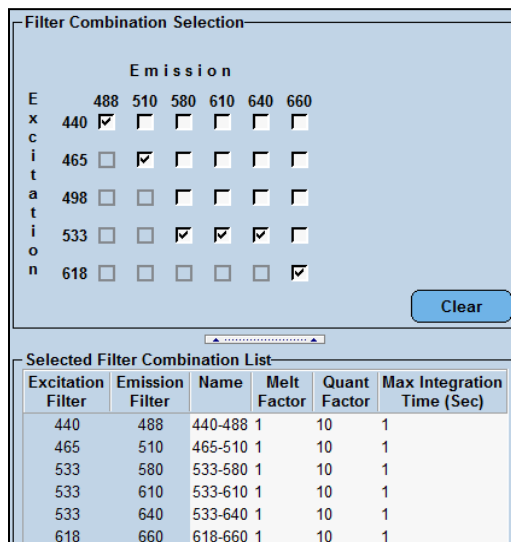
Set the **Selected Filter Combination List** for all channels as:

Melt Factor: 1

Quant Factor: 10

Max Integration Time (sec): 1

Figure 13. 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

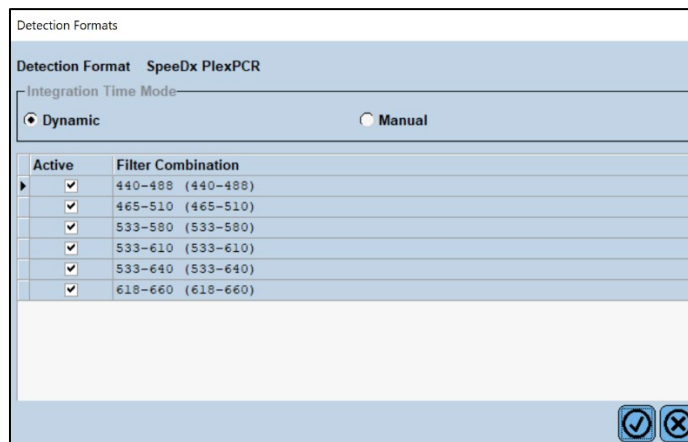
For **Detection Format** select the custom 'SpeedX PlexPCR' (**Figure 14**)

Select **Customize >**

Select **Integration Time Mode > Dynamic**

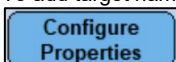
Select all Active **Filter Combinations** shown in **Figure 14**

Figure 14. Customize Detection Format

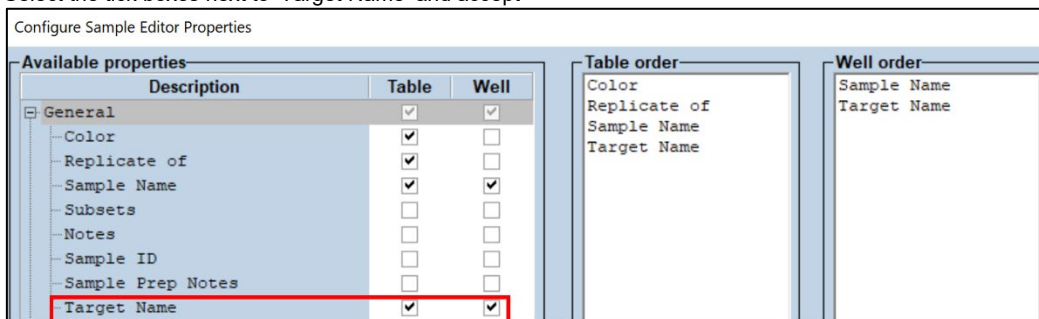


To enable automated sample detection in the analysis software, add target names and assign nametags to the wells on the plate
Open the **Sample Editor** module

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 41**.

Table 41. Channels for ResistancePlus® GC targets					
Target Name	gyrA-S91	porA	gyrA-S91F	IC	opa
LC480 II channel	465-510	533-580	533-610	533-640	618-660

Edit **Sample Name** to match the nametag defined in Lab Configuration > Assays menu of the analysis software (see **Section 27.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 42** and **Figure 15**). 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 42. Sample nametags for analysis software	
Sample Type	Default Prefix_ (in analysis software)
Regular sample	No default – user defined
Negative Control	NC
Positive Control (MT, GC gyrA S91F) (Pa)	Pa
Positive Control (WT, GC gyrA S91) (Pb)	Pb

Figure 15. Sample Editor – Assigning nametags to wells

Pos	Filter combination	Color	Repl Of	Sample Name	Target Name
A1	465-510 (465)	Blue		NC	gyrA-S91
A1	533-580 (533)	Blue		NC	porA
A1	533-610 (533)	Blue		NC	gyrA-S91F
A1	533-640 (533)	Blue		NC	IC
A1	618-660 (618)	Blue		NC	opa
A2	465-510 (465)	Red		Pa	gyrA-S91
A2	533-580 (533)	Red		Pa	porA
A2	533-610 (533)	Red		Pa	gyrA-S91F
A2	533-640 (533)	Red		Pa	IC
A2	618-660 (618)	Red		Pa	opa
A3	465-510 (465)	Green		Pb	gyrA-S91
A3	533-580 (533)	Green		Pb	porA
A3	533-610 (533)	Green		Pb	gyrA-S91F
A3	533-640 (533)	Green		Pb	IC
A3	618-660 (618)	Green		Pb	opa

For a 30 µL reaction

Set **Reaction Volume** > 30 µL

Create the following Program in **Table 43** (shown in more detail in **Figure 16 – Figure 19**):

Table 43. Thermocycling Program				
Program Name	Cycles	Target °C	Hold	Ramp Rate (°C/s)*
Polymerase activation	1	95°C	2 min	4.4
Touch down cycling [⊖] : Step down -0.5°C/cycle	10	95°C	5 s	4.4
		61°C – 56.5°C [⊖]	30 s	2.2
Quantification cycling ⁺ : Acquisition/Detection	50	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)

[⊖] Step size: -0.5°C/Cycle, Sec Target: 56°C

⁺ Analysis mode: Quantification, Acquisition mode: Single

Figure 16. Thermocycling Program (30 µL reaction) – Polymerase activation

Program Name	Cycles	Analysis Mode
Polymerase activation	1	None
Touchdown cycling	10	None
Quantification cycling	50	Quantification
Cool down	1	None

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

Figure 17. Thermocycling Program (30 μ L reaction) – Touchdown cycling

Program Name	Cycles	Analysis Mode
Polymerase activation	1	None
Touchdown cycling	10	None
Quantification cycling	50	Quantification
Cool down	1	None

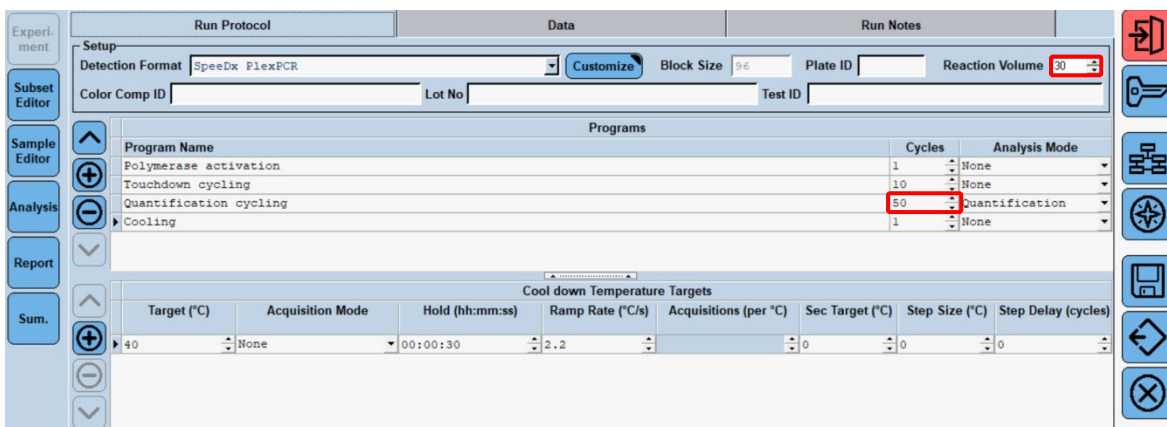
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:00:05	4.4	0	0	0	0
61	None	00:00:30	2.2	56	0.5	0	0

Figure 18. Thermocycling Program (30 μ L reaction) – Quantification cycling

Program Name	Cycles	Analysis Mode
Polymerase activation	1	None
Touchdown cycling	10	None
Quantification cycling	50	Quantification
Cool down	1	None

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

Figure 19. Thermocycling Program (30 µL reaction) – Cooling

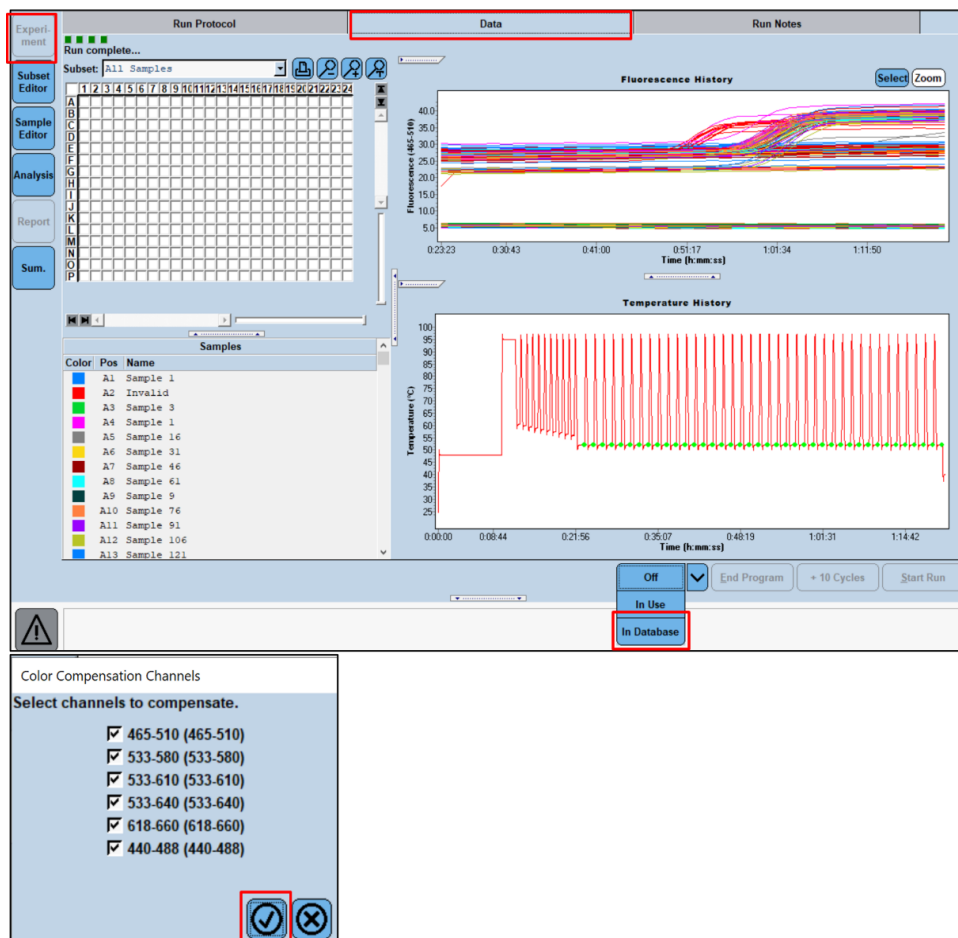



When the cycling program has finished, attach the CC object to the run file as shown in **Figure 20** and export as a .IXO file for analysis in the **ResistancePlus®** GC analysis software. Refer to **Section 20.2** for instructions on how to create the CC Object and store this within the LightCycler 480 software database.

Select **Experiment > Data**

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

Figure 20. 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 

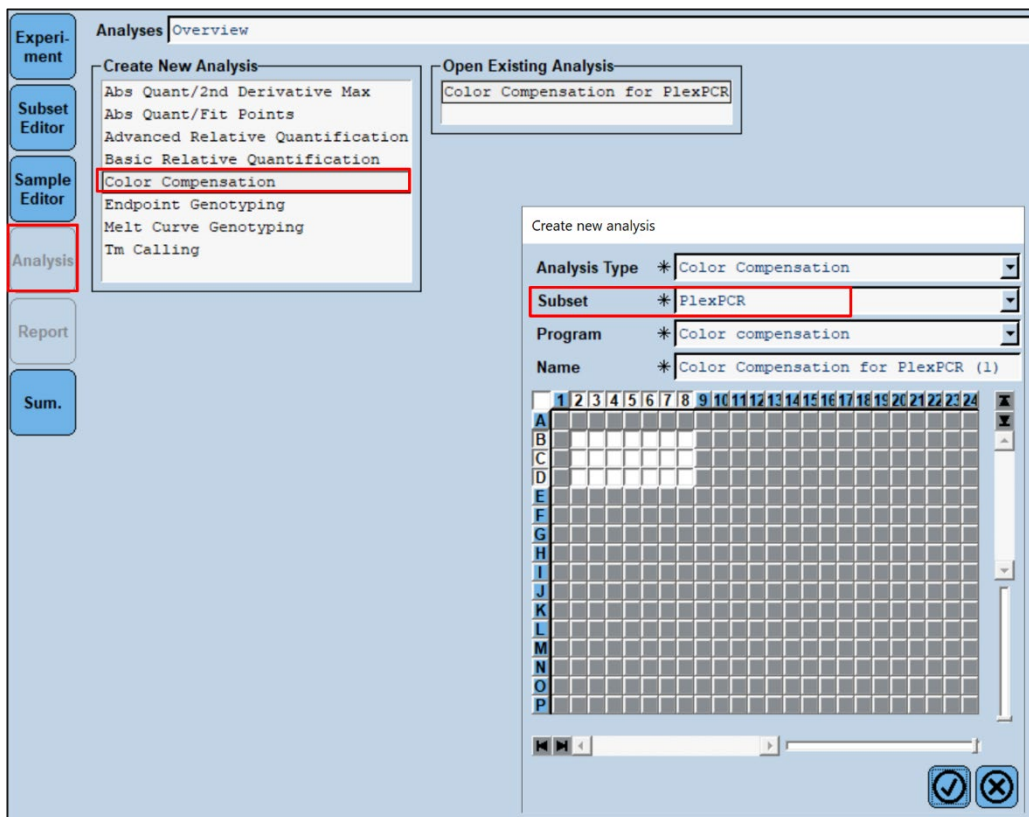
Save in an easily identifiable location

20.2 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.

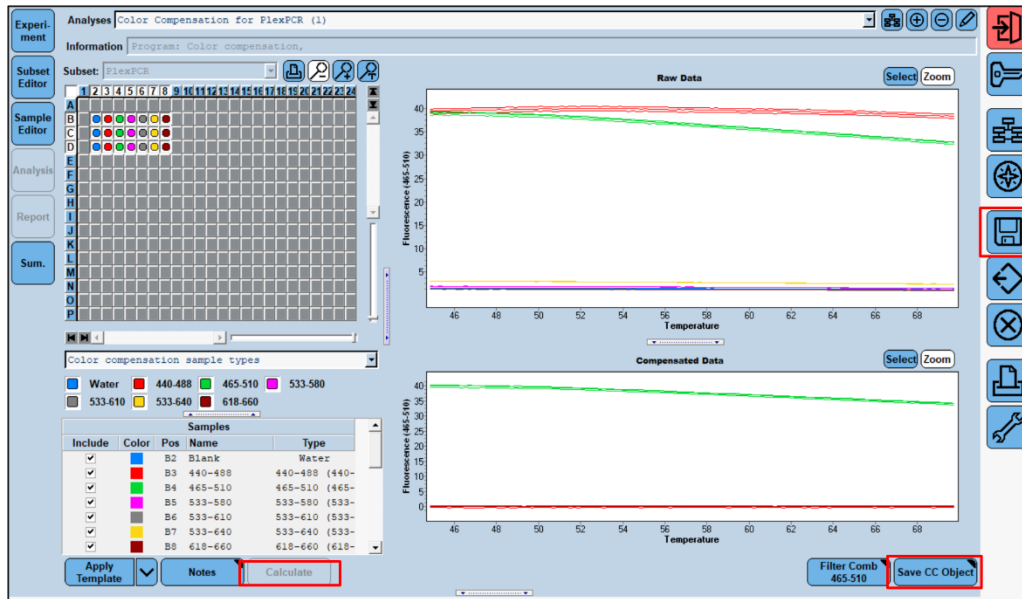
Analyse the Colour Compensation file via **Analysis > Colour Compensation** and select the correct subset, shown in **Figure 21**.

Figure 21. Analysis – Colour Compensation



Select **Calculate** (Figure 22).

Figure 22. 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** 

20.3 Interpretation of results

Data interpretation requires the **ResistancePlus**[®] GC (LC480) analysis software. The analysis software can be supplied on request, contact tech@speedx.com.au for more information.

Refer to **Section 27** for instructions on using the **ResistancePlus**[®] GC (LC480) analysis software.

21 Appendix 3: Applied Biosystems® 7500 Fast programming for reaction volume 20 µL

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

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

21.1 Programming the Applied Biosystems® 7500 Fast

Select **Advanced Setup**

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

Name the experiment

Instrument > 7500 Fast (96 Wells)

Type of experiment > Quantitation – Standard Curve

Reagents > Other

Ramp Speed > Standard

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

In **Define Targets and Samples** tab >

Define Targets as shown below in **Table 44** and **Figure 23** (define colours as required)

Target Name	Reporter	Quencher
gyrA-S91	FAM	None
porA	JOE	None
IC	TAMRA	None
gyrA-S91F	Texas Red	None
opa	Cy5	None

Figure 23. Define Targets and Samples

Define Targets			
Add New Target	Add Saved Target	Save Target	Delete Target
Target Name	Reporter	Quencher	
gyrA-S91	FAM	None	
porA	JOE	None	
IC	TAMRA	None	
gyrA-S91F	TEXAS RED	None	
opa	CY5	None	

Define Samples (define colours as required)

To enable automated sample detection in the analysis software, ensure the Target Name (shown in **Table 44**) 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

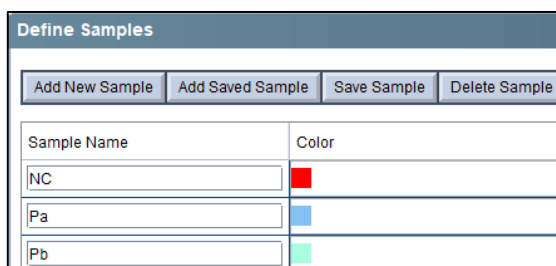
Edit **Sample Name** to match the nametag defined in the **Lab Configuration > Assays** menu of the analysis software (**Section 27.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 45** and **Figure 24**). 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 45. Sample nametags for analysis software	
Sample Type	Default Prefix_ (in analysis software)
Regular sample	No default – user defined
Negative Control	NC
Positive Control (MT, GC gyrA S91F) (Pa)	Pa
Positive Control (WT, GC gyrA S91) (Pb)	Pb

Figure 24. 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**

For a 20 µL reaction;

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

Create the following Program (shown in more detail in Graphical View (**Table 46** and **Figure 25 – Figure 26**) and Tabular View (**Figure 27**):

Table 46. Thermocycling Program				
Program Name	Cycles	Target °C	Hold	Ramp*
Polymerase activation	1	95°C	2 min	100%
Touch down cycling: Step down -0.5°C/cycle ⁵	10	95°C	5 s	100%
		61°C – 56.5°C ⁵	30 s	100%
Quantification cycling*: Acquisition/Detection	40	95°C	5 s	100%
		52°C*	40 s	100%

* Default ramp rate

- ◊ Enable AutoDelta: -0.5°C/cycle
- + Collect data on hold

Figure 25. Run method (20 µl reaction) – Graphical View

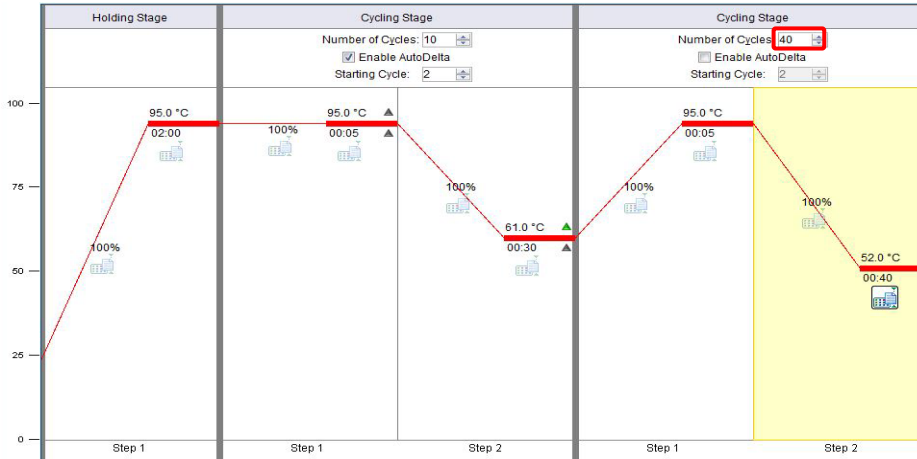


Figure 26. Run method (20 µL reaction) – Graphical View – Enable AutoDelta

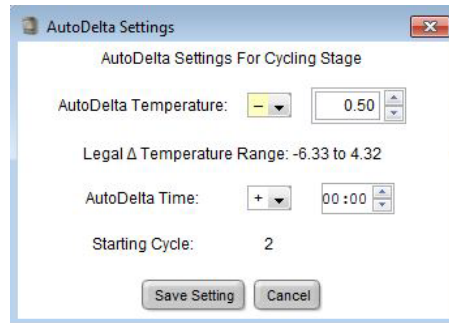


Figure 27. Run method (20 µL reaction) – Tabular View

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

In **Setup** > open **Run Method**
 Select **Start Run**

21.2 Interpretation of results

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

Refer to **Section 27** for instructions on using the **ResistancePlus**[®] GC (7500) analysis software.

22 Appendix 4: Applied Biosystems® 7500 Fast programming for reaction volume 30 µL

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

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

22.1 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 47** and **Figure 28** (define colours as required)

Target Name	Reporter	Quencher
gyrA-S91	FAM	None
porA	JOE	None
IC	TAMRA	None
gyrA-S91F	Texas Red	None
opa	Cy5	None

Figure 28. Define Targets and Samples

Define Targets		
Add New Target	Add Saved Target	Save Target
	Delete Target	
Target Name	Reporter	Quencher
gyrA-S91	FAM	None
porA	JOE	None
IC	TAMRA	None
gyrA-S91F	TEXAS RED	None
opa	CY5	None

Define Samples (define colours as required)

To enable automated sample detection in the analysis software, ensure the Target Name (shown in **Table 47**) 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

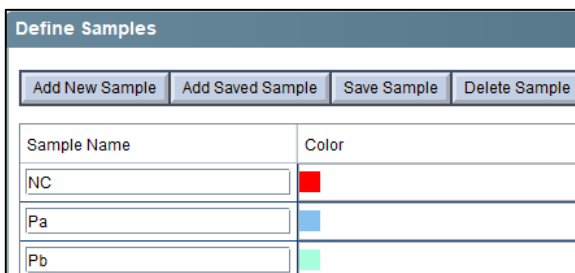
Edit **Sample Name** to match the nametag defined in the **Lab Configuration > Assays** menu of the analysis software (see **Section 27.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 48** and **Figure 29**). 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 48. Sample nametags for analysis software	
Sample Type	Default Prefix_ (in analysis software)
Regular sample	No default – user defined
Negative Control	NC
Positive Control (MT, GC gyrA S91F) (Pa)	Pa
Positive Control (WT, GC gyrA S91) (Pb)	Pb

Figure 29. 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**

For a 30 µL reaction;

Set **Reaction Volume Per Well** > 30 µL

Create the following Program (shown in more detail in Graphical View (**Table 49** and **Figure 29 – Figure 30**) and Tabular View (**Figure 32**):

Table 49. Thermocycling Program				
Program Name	Cycles	Target °C	Hold	Ramp#
Polymerase activation	1	95°C	2 min	100%
Touch down cycling:	10	95°C	5 s	100%
Step down -0.5°C/cycle ⁵		61°C – 56.5°C ⁵	30 s	100%
Quantification cycling*: Acquisition/Detection	50	95°C	5 s	100%
		52°C ⁺	40 s	100%

⁵ Default ramp rate

◊ Enable AutoDelta: -0.5°C/cycle

+ Collect data on hold

Figure 30. Run method (30 µl reaction) – Graphical View

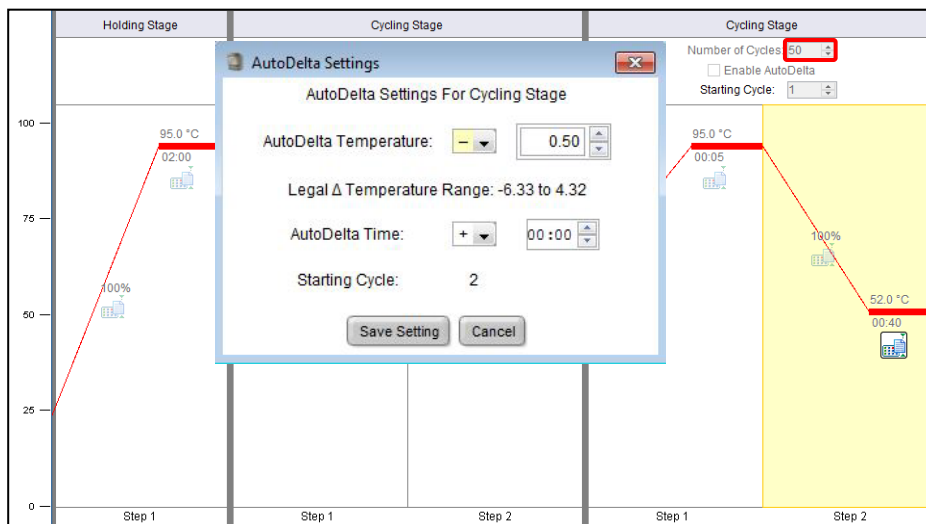


Figure 31. Run method (30 µL reaction) – Graphical View – Enable AutoDelta

Figure 32. Run method (30 µL reaction) – Tabular View

	Holding Stage	Cycling Stage		Cycling Stage	
		Number of Cycles: 10 <input checked="" type="checkbox"/> Enable AutoDelta Starting Cycle: 1		Number of Cycles: 50 <input type="checkbox"/> Enable AutoDelta Starting Cycle: 1	
Ramp Rate (%)	100.0	100.0	100.0	100.0	100.0
Temperature (°C)	95.0	95.0	61.0	95.0	52.0
Time	02:00	00:05	00:30	00:05	00:40
AutoDelta Temp.		+ 0.00	- 0.50		
AutoDelta Time		+ 00:00	+ 00:00		
Collect Data on Ramp:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Collect Data on Hold:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
	Step 1	Step 1	Step 2	Step 1	Step 2

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

Select **Start Run**

22.2 Interpretation of results

Data interpretation requires the **ResistancePlus® GC (7500)** analysis software. The analysis software can be supplied on request, contact tech@speedx.com.au for more information.

Refer to **Section 27** for instructions on using the **ResistancePlus® GC (7500)** analysis software.

23 Appendix 5: Applied Biosystems® 7500 Fast Dx programming for reaction volume 20 µL

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

The **ResistancePlus®** GC₍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.

23.1 Programming the Applied Biosystems® 7500 Fast Dx (using run file template)

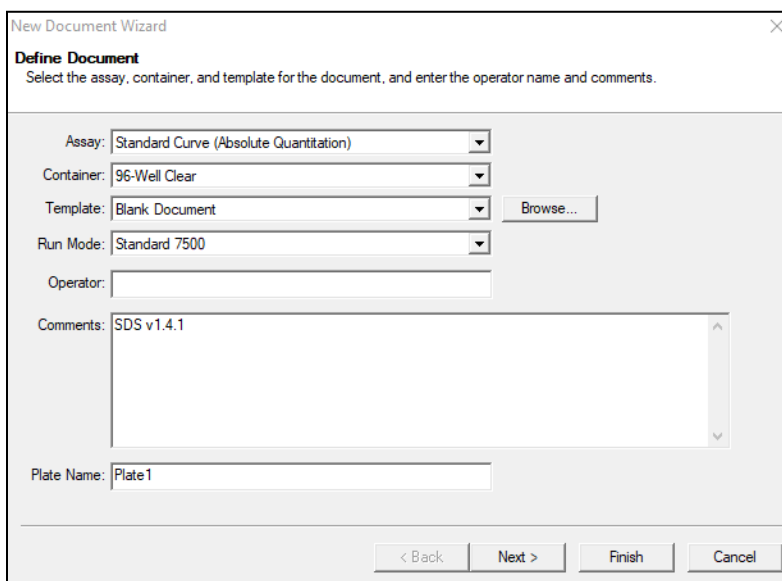
Select Create New Document

In **New Document Wizard** select the following (**Figure 33**):

- 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 33. New Document Wizard window



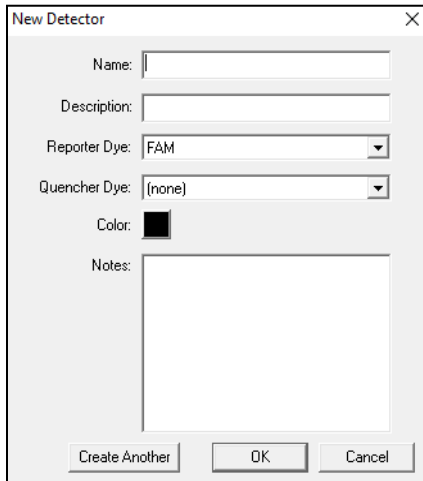
In **Select Detectors** > select **New Detector**

Define detectors as shown below (define colours as required) (**Table 50** and **Figure 34**)

Table 50. Define Detectors			
Detectors	Detector Name	Reporter Dye	Quencher
Detector 1	<i>gyrA</i> -S91	FAM	None
Detector 2	<i>porA</i>	JOE	None
Detector 3	IC	TAMRA	None
Detector 4	<i>gyrA</i> -S91F	Texas Red	None
Detector 5	<i>opa</i>	Cy5	None

Select **OK**

Figure 34. New Detector window

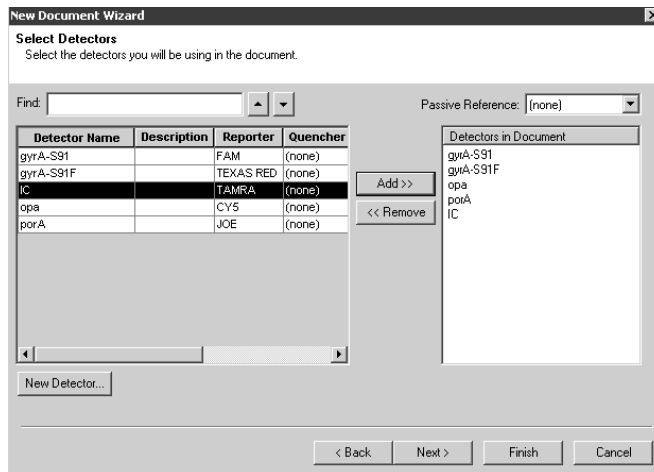


Select Detectors (Figure 35)

Select detectors and **Add** to Document

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

Figure 35. Select Detectors window



Detector Name	Description	Reporter	Quencher
gyrA-S91		FAM	(none)
gyrA-S91F		TEXAS RED	(none)
IC		TAMRA	(none)
opa		CY5	(none)
potA		JOE	(none)

In **Set Up** sample plate >

Select wells and assign 4 detectors to the selected wells

- gyrA-S91
- gyrA-S91F
- opa
- potA
- IC

Note: Only 4 detectors can be added to a well within the 7500 Fast System SDS Software; all detectors added to the programme are reported when analysed in the analysis software (see Section 27)

Select **Next**

To enable automated sample detection in the analysis software, ensure the Detector Name (shown in **Table 50**) 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 27.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 51**). 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 51. Sample nametags for analysis software	
Sample Type	Default Prefix_ (in analysis software)
Regular sample	No default – user defined
Negative Control	NC
Positive Control (MT, GC gyrA S91F) (Pa)	Pa
Positive Control (WT, GC gyrA S91) (Pb)	Pb

In **Instrument** tab

In **Settings** box

For a 20 µL reaction, set **Sample Volume (µL)**: Enter 20 µL

Create the following Thermal Cycler Protocol (**Table 52** and **Figure 36– Figure 37**)

Table 52. Thermal Cycler Protocol				
Program Name	Cycles	Target °C	Hold	Ramp [‡]
Polymerase activation	1	95°C	2 min	100%
Touch down cycling: Step down -0.5°C/cycle [§]	10	95°C	5 s	100%
		61°C – 56.5°C [§]	30 s	100%
Quantification cycling ⁺ : Acquisition/Detection	40	95°C	5 s	100%
		52°C ⁺	40 s	100%

[‡] Default ramp rate

[§] Enable AutoDelta: -0.5°C/cycle

⁺ Collect data on hold

Figure 36. Thermal Cycler Protocol (20 μ L reaction) – Thermal Profile

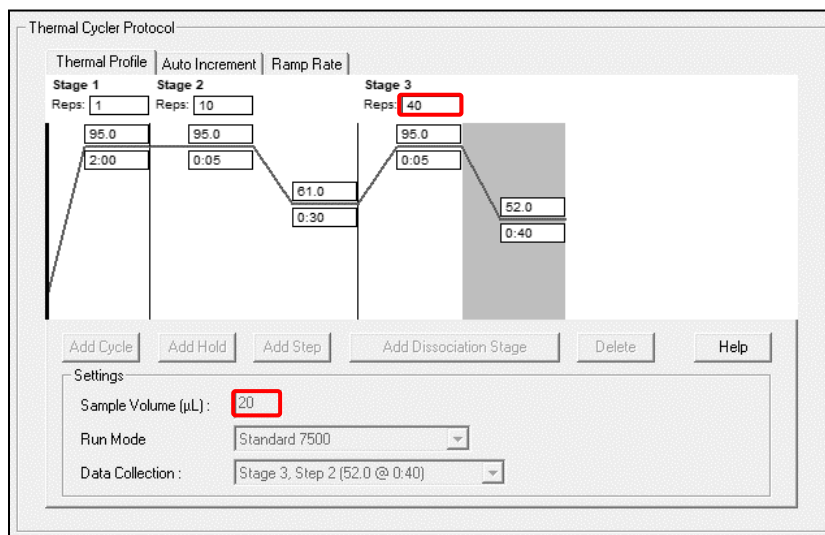
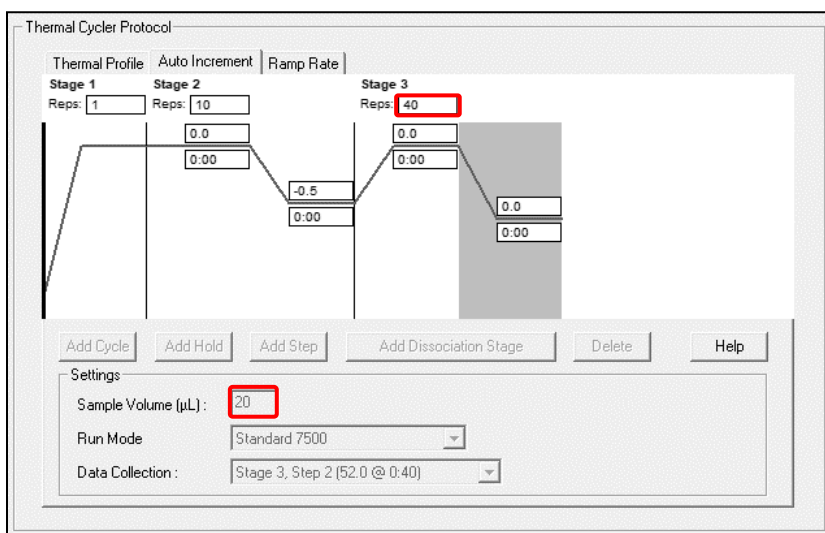


Figure 37. Thermal Cycler Protocol (20 μ L reaction) – Auto Increment



23.2 Interpretation of results

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

Refer to **Section 27** for instructions on using the **ResistancePlus**[®] GC (7500) analysis software.

24 Appendix 6: Applied Biosystems® 7500 Fast Dx programming for reaction volume 30 µL

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

The **ResistancePlus®** GC₍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.

24.1 Programming the Applied Biosystems® 7500 Fast Dx (using run file template)

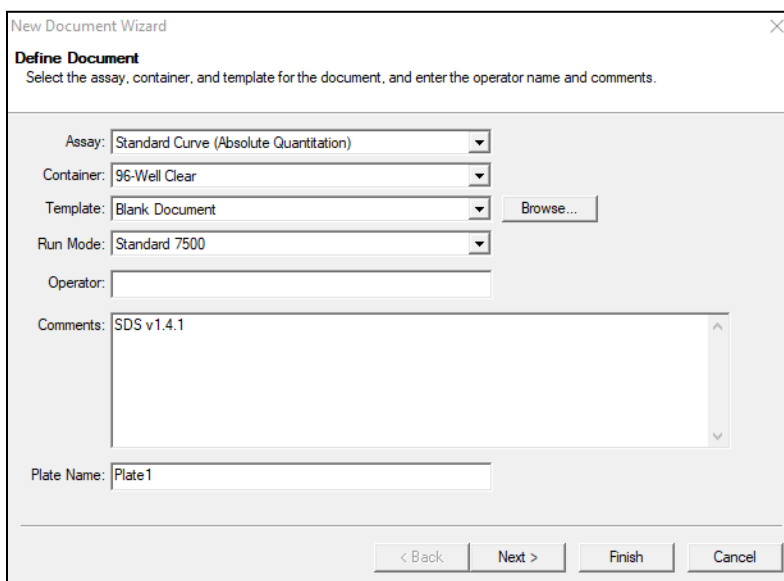
Select Create New Document

In **New Document Wizard** select the following (**Figure 38**):

- Assay** > Standard Curve (Absolute Quantitation)
- 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 38. New Document Wizard window



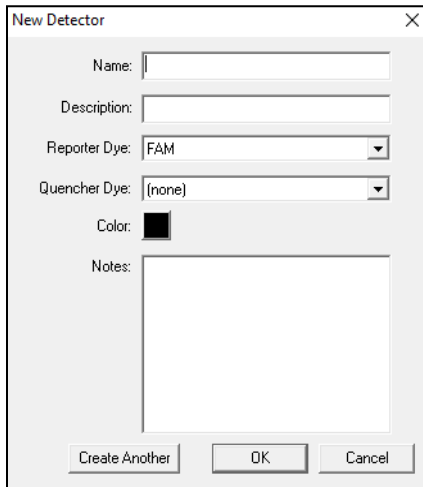
In **Select Detectors** > select **New Detector**

Define detectors as shown below (define colours as required) (**Table 53** and **Figure 39**)

Table 53. Define Detectors			
Detectors	Detector Name	Reporter Dye	Quencher
Detector 1	gyrA-S91	FAM	None
Detector 2	porA	JOE	None
Detector 3	IC	TAMRA	None
Detector 4	gyrA-S91F	Texas Red	None
Detector 5	opa	Cy5	None

Select **OK**

Figure 39. New Detector window

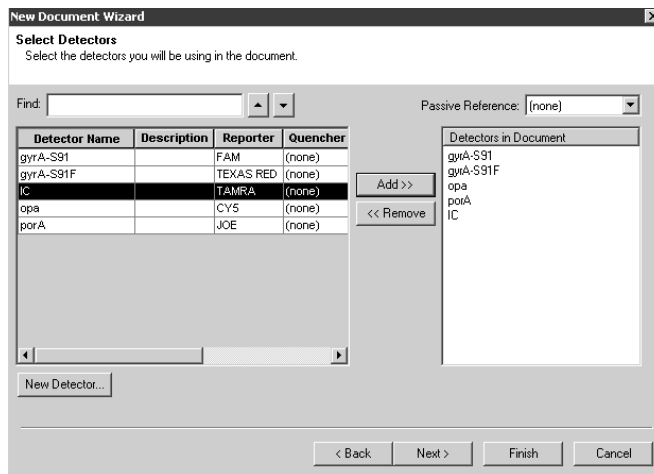


Select Detectors (Figure 40)

Select detectors and **Add** to Document

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

Figure 40. Select Detectors window



Detector Name	Description	Reporter	Quencher
gyrA-S91		FAM	(none)
gyrA-S91F		TEXAS RED	(none)
IC		TAMRA	(none)
opa		CYS	(none)
porA		JOE	(none)

In **Set Up** sample plate >

Select wells and assign 4 detectors to the selected wells

- gyrA-S91
- gyrA-S91F
- opa
- porA
- IC

Note: Only 4 detectors can be added to a well within the 7500 Fast System SDS Software; all detectors added to the programme are reported when analysed in the analysis software (see Section 27)

Select **Next**

To enable automated sample detection in the analysis software, ensure the Detector Name (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

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 27.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Table 54**). 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
Positive Control (MT, GC gyrA S91F) (Pa)	Pa
Positive Control (WT, GC gyrA S91) (Pb)	Pb

Select **Next**

In **Instrument** tab

In **Settings** box

For a 30 μ L reaction, set **Sample Volume (μ L)**: Enter 30 μ L

Create the following Thermal Cycler Protocol (**Table 55** and **Figure 41** and **Figure 42**)

Table 55. Thermal Cycler Protocol				
Program Name	Cycles	Target °C	Hold	Ramp [≠]
Polymerase activation	1	95°C	2 min	100%
Touch down cycling: Step down -0.5°C/cycle [⊖]	10	95°C	5 s	100%
		61°C – 56.5°C [⊖]	30 s	100%
Quantification cycling*: Acquisition/Detection	50	95°C	5 s	100%
		52°C ⁺	40 s	100%

[≠] Default ramp rate

[⊖] Enable AutoDelta: -0.5°C/cycle

⁺ Collect data on hold

Figure 41. Thermal Cycler Protocol (30 µL reaction) – Thermal Profile

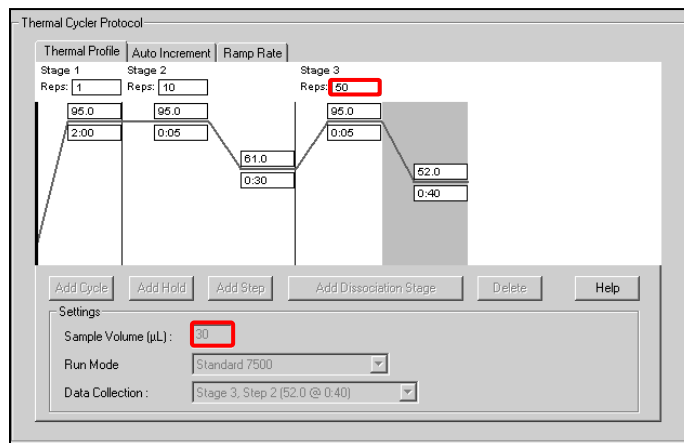
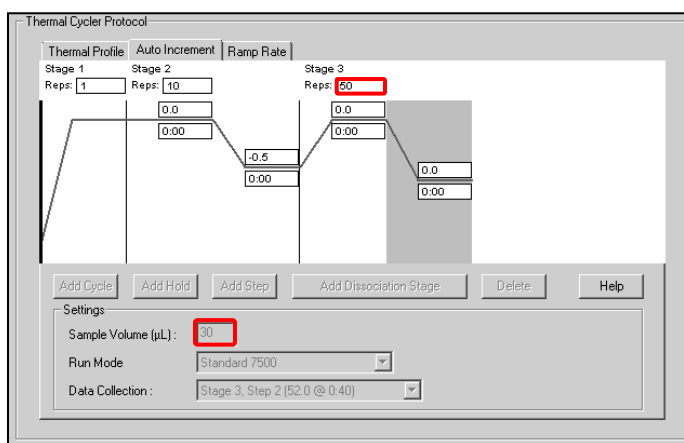


Figure 42. Thermal Cycler Protocol (30 µL reaction) – Auto Increment



24.2 Interpretation of results

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

Refer to **Section 27** for instructions on using the **ResistancePlus**[®] GC (7500) analysis software.

25 Appendix 7: Bio-Rad CFX96™ Dx & Touch programming for reaction volume of 20 µL

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

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

25.1 Programming the CFX96™ Dx or Touch Real-time PCR System

Select **View > Open Run Setup**

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

In the **Protocol Editor** (see **Figure 43**)

Set **Sample Volume** > 20 µL

Create the following thermocycling program in **Table 56** 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 44**).

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

⁵ **Step options > Increment: -0.5°C/cycle**

⁺ **Add Plate Read to Step**

Figure 43. Thermocycling Protocol – Graphical view

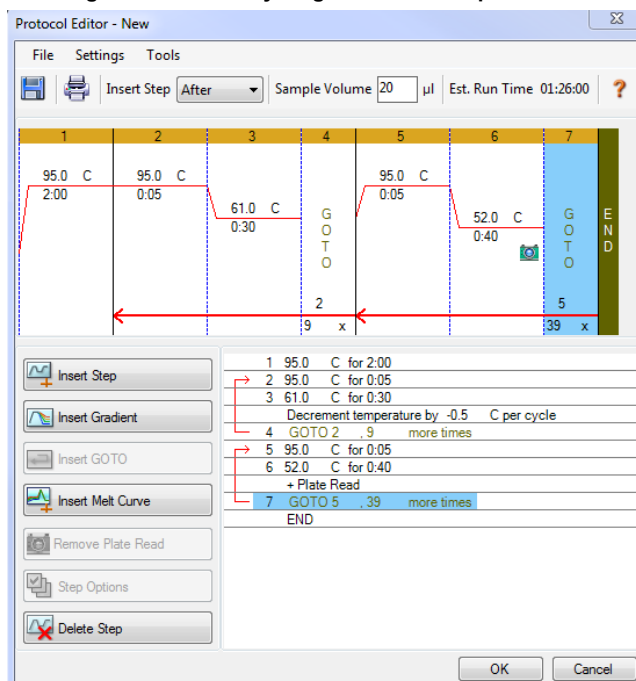


Figure 44. Step Options

In **Run Setup > Plate** tab

Select **Create New**

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

Set **Scan mode > All channels**

Select **Fluorophores > FAM, HEX, Texas Red, Quasar 705, Cy 5** (see **Table 57**)

Select wells containing samples and assign **Sample Type** and check **Load** for fluorophores (FAM, HEX, Quasar 705, Texas Red, Cy5.)

Save plate

Table 57. Channels for ResistancePlus® GC ₍₆₇₅₎ targets					
Target Name	gyrA-S91	porA	gyrA-S91F	IC	opa
CFX-96 channel	FAM	HEX	Texas Red	Quasar 705	Cy5

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 57**) 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 27.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Figure 45**). 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 58. Sample nametags for analysis software	
Sample Type	Default Prefix_ (in analysis software)
Regular sample	No default – user defined
Negative Control	NC
Positive Control (GC gyrA S91F mutant) (Pa)	Pa
Positive Control (GC gyrA S91 wild type) (Pb)	Pb

Figure 45. Sample Editor – Assigning nametags to wells

	1	2
A	Neg	Unk
	gyrA-S91	gyrA-S91
	porA	porA
	gyrA-S91F	gyrA-S91F
	opa	opa
B	IC	IC
	NC	Sample 1
	Pos	Unk
	gyrA-S91	gyrA-S91
	porA	porA
C	gyrA-S91F	gyrA-S91F
	opa	opa
	IC	IC
	Pb	Sample 3

25.2 Interpretation of results

Data interpretation requires the **ResistancePlus®** GC (CFX) analysis software. The analysis software can be supplied on request, contact tech@speedx.com.au for more information.

Refer to **Section 27** for instructions for using the **ResistancePlus®** GC (CFX) analysis software.

26 Appendix 8: Bio-Rad CFX96™ Dx & Touch programming for reaction volume of 30 µL

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

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

26.1 Programming the CFX96™ Dx or Touch Real-time PCR System

Select **View** > Open **Run Setup**

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

In the **Protocol Editor** (see **Figure 46**):

Set **Sample Volume** > 30 µL

Create the following thermocycling program in **Table 59** 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 47**).

Table 59. 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	50	95°C	5 s
		52°C ⁺	40 s

^δ Step options > Increment: -0.5°C/cycle

⁺ Add Plate Read to Step

Figure 46. Thermocycling Protocol – Graphical view

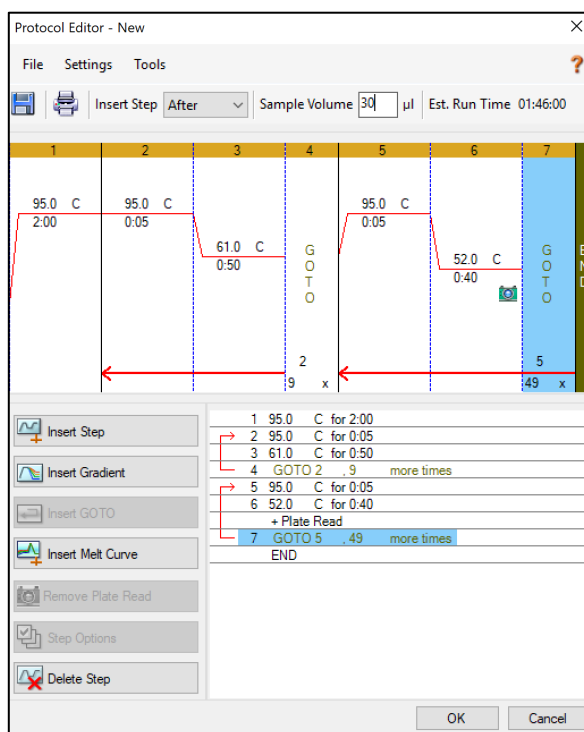
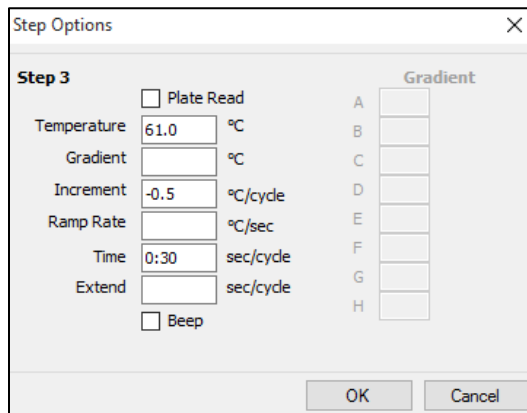


Figure 47. Step Options



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, Texas Red, Cy5** (see **Table 60**)

Select wells containing samples and assign **Sample Type** and check **Load** for fluorophores (FAM, HEX, Quasar 705, Texas Red and Cy5)

Save plate

Table 60. Channels for <i>ResistancePlus</i> [®] GC ₍₆₇₅₎ targets					
Target Name	gyrA-S91	porA	gyrA-S91F	IC	opa
CFX-96 channel	FAM	HEX	Texas Red	Quasar 705	Cy5

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 60**) 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 27.3**)

Samples should be labelled with the nametag as a Prefix. Default nametags are provided for the control reactions (as shown in **Figure 48**). 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 61. Sample nametags for analysis software	
Sample Type	Default Prefix_ (in analysis software)
Regular sample	No default – user defined
Negative Control	NC
Positive Control (GC gyrA S91F mutant) (Pa)	Pa
Positive Control (GC gyrA S91 wild type) (Pb)	Pb

Figure 48. Sample Editor – Assigning nametags to wells

	1	2
A	Neg	Unk
	gyrA-S91	gyrA-S91
	porA	porA
	gyrA-S91F	gyrA-S91F
	opa	opa
	IC	IC
B	Pos	Unk
	gyrA-S91	gyrA-S91
	porA	porA
	gyrA-S91F	gyrA-S91F
	opa	opa
	IC	IC
C	Pos	Unk
	gyrA-S91	gyrA-S91
	porA	porA
	gyrA-S91F	gyrA-S91F
	opa	opa
	IC	IC
	Pb	Sample 3

26.2 Interpretation of results

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

Refer to **Section 27** for instructions for using the **ResistancePlus**[®] GC (CFX) analysis software.

27 Appendix A: Result interpretation

Data interpretation requires the **ResistancePlus®** GC analysis software. See **Table 62** for the appropriate analysis software for each real-time PCR instrument. The analysis software can be supplied on request. Please contact tech@speedx.com.au for more information.

Cat no	Analysis software*	Real-time PCR instrument
99010	ResistancePlus® GC (LC480)	LC480 II
99009	ResistancePlus® GC (7500)	7500 Fast and 7500 Fast Dx
99015	ResistancePlus® GC (CFX)	CFX96 Dx and CFX96 Touch

* Refer to the website <https://plexpcr.com/resistanceplus-gc/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.

27.1 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

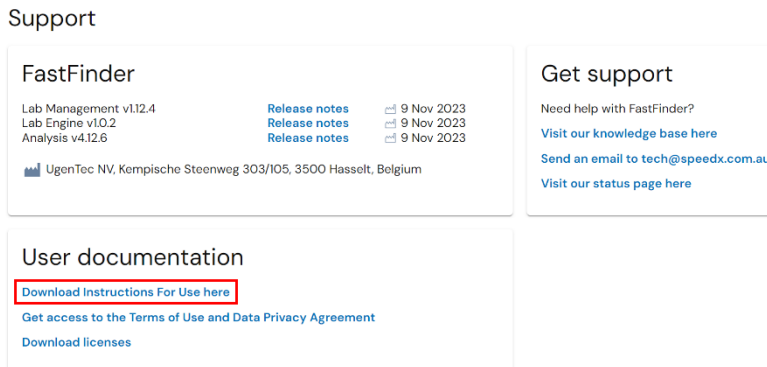
*.content.powerapps.com

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



Support

FastFinder

Lab Management v1.12.4 [Release notes](#) 9 Nov 2023
 Lab Engine v1.0.2 [Release notes](#) 9 Nov 2023
 Analysis v4.12.6 [Release notes](#) 9 Nov 2023

UgenTec NV, Kempische Steenweg 303/105, 3500 Hasselt, Belgium

Get support

Need help with FastFinder?
[Visit our knowledge base here](#)
[Send an email to tech@speedx.com.au](mailto:tech@speedx.com.au)
[Visit our status page here](#)

User documentation

[Download Instructions For Use here](#)
[Get access to the Terms of Use and Data Privacy Agreement](#)
[Download licenses](#)

27.2 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 GC (LC480)** from the list
 - > For 7500 Fast and 7500 Fast Dx > Select **ResistancePlus GC (7500)** from the list
 - > For CFX96 Dx and CFX96 Touch > Select **ResistancePlus GC (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 **Active** to activate or deactivate the version of the assay

27.3 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** and **Section 20** for instructions on programming sample nametags in the run file
 - > For **7500 Fast** see **Section 21** and **Section 22** for instructions on programming sample nametags in the run file
 - > For **7500 Fast Dx** see **Section 23** and **Section 24** for instructions on programming sample nametags in the run file
 - > For **CFX96 Dx** and **CFX96 Touch** see **Section 25** and **Section 26** 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.

27.4 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 GC (LC480)**



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



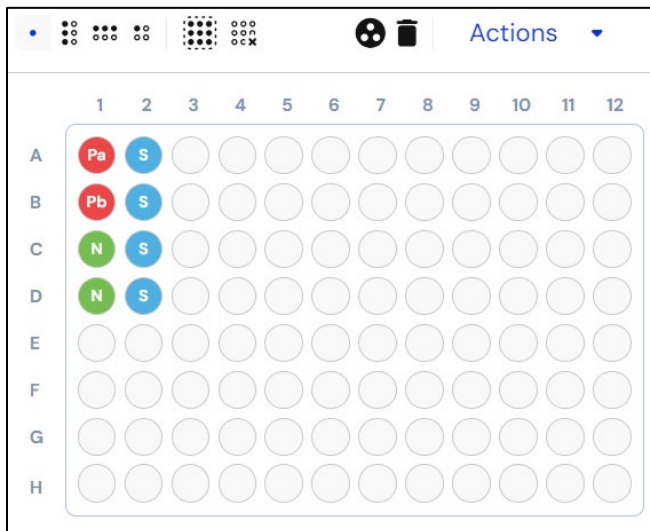
- For **CFX96 Dx** and **CFX96 Touch** > Select **ResistancePlus GC (CFX)**



- Select wells and assign as:
 - > Regular Sample (S)
 - > Negative Control (N)
 - > Positive Control (GC gyrA S91F mutant) (Pa)
 - > Positive Control (GC gyrA S91 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**

27.5 Results

See **Table 64** for a summary of possible reported sample results.

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

27.5.1 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.

Control Results

<input checked="" type="checkbox"/>	↓	!	Assay	Status	Info				
<input checked="" type="checkbox"/>			ResistancePlus GC (L...	IVD	VALID				
See all assay details →									
<input checked="" type="checkbox"/>			NC	<input checked="" type="checkbox"/>	PB	<input checked="" type="checkbox"/>	PA		
			VALID	VALID	VALID				
			N. gonorrhoeae	⊖ Not detected	⊕ Detected	⊕ Detected			
			gyrA mutation	⊖ Not detected	⊖ Not detected	⊕ Detected			

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

Fail all samples for this assay

Failure reason ▼

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.

- ! • Containing an error notification
- ⚠ • Containing a warning notification
- ↺ • Marked for retest
- ⊕ • Containing at least one detected assay result
- ⊖ • Containing at least one not detected assay result
- ! • Containing at least one invalid assay result
- ? • Containing at least one inconclusive assay result

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

Sample Results ▲ 2 ● 3 ● 1 ● 2			Filters ⚙	
<input type="checkbox"/>	↓ !	Sample	Assay	Result
▶ <input type="checkbox"/>	▲	Sample 4 ↻	ResistancePlus GC (LC480)	Invalid: N. gonorrhoeae, gyrA mutation
▶ <input type="checkbox"/>	▲	Sample 5 ↻	ResistancePlus GC (LC480)	Detected: N. gonorrhoeae - Invalid: gyrA mutat...
▶ <input type="checkbox"/>		Sample 1 ↻	ResistancePlus GC (LC480)	Detected: N. gonorrhoeae, gyrA mutation
▶ <input type="checkbox"/>		Sample 2 ↻	ResistancePlus GC (LC480)	Detected: N. gonorrhoeae
▶ <input type="checkbox"/>		Sample 3 ↻	ResistancePlus GC (LC480)	Not detected

The drop-down menu offers more details on each target result and Cq per sample (Refer to the examples shown in **Section 27.10**). Individual samples can be marked as failed if desired (e.g. if the sample is Invalid) by selecting **Fail this sample for this assay**

Fail this sample for this assay

Failure reason ▼

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 63**.

Table 63. Example information and warning notifications for the ResistancePlus® GC 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.
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
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

27.5.2 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

27.6 **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.

27.7 **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

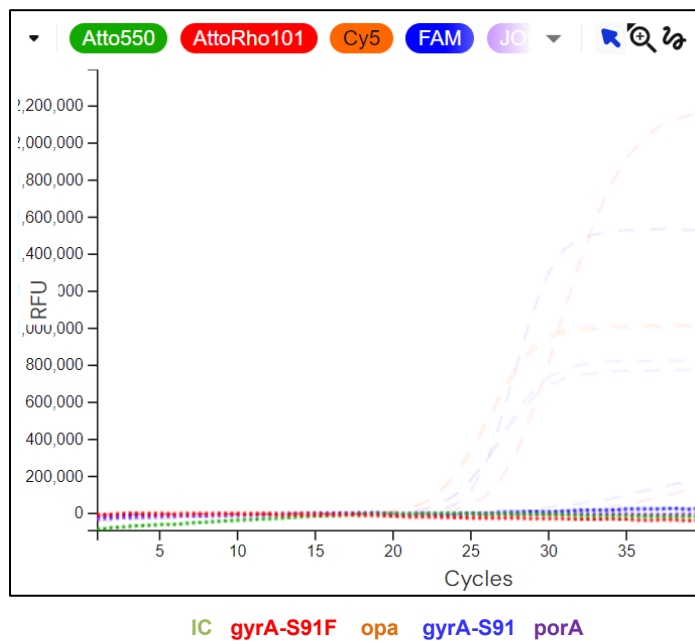
27.8 Retrieving authorized analyses

- 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.

27.9 Control example graphs

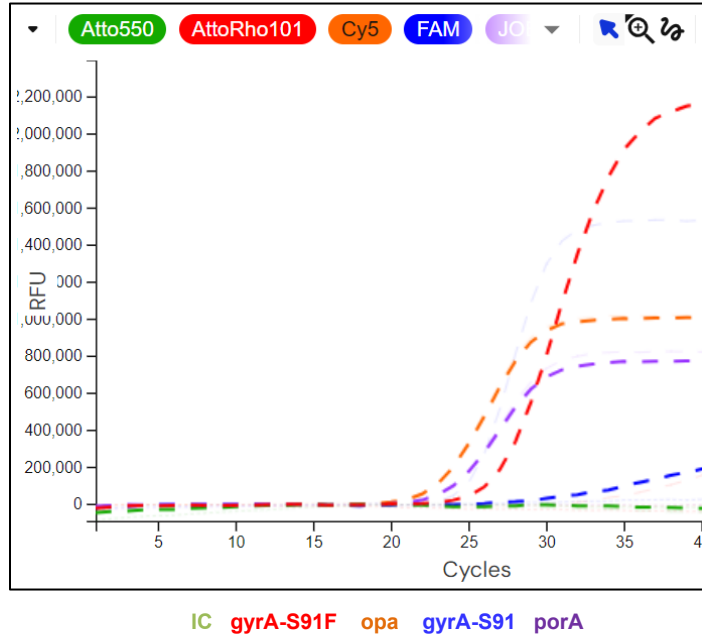
The following examples show the amplification curves (baseline-corrected amplification curves) and the Results overview from the **ResistancePlus GC (LC480)** analysis software for control sample types.

27.9.1 Negative Control (N)



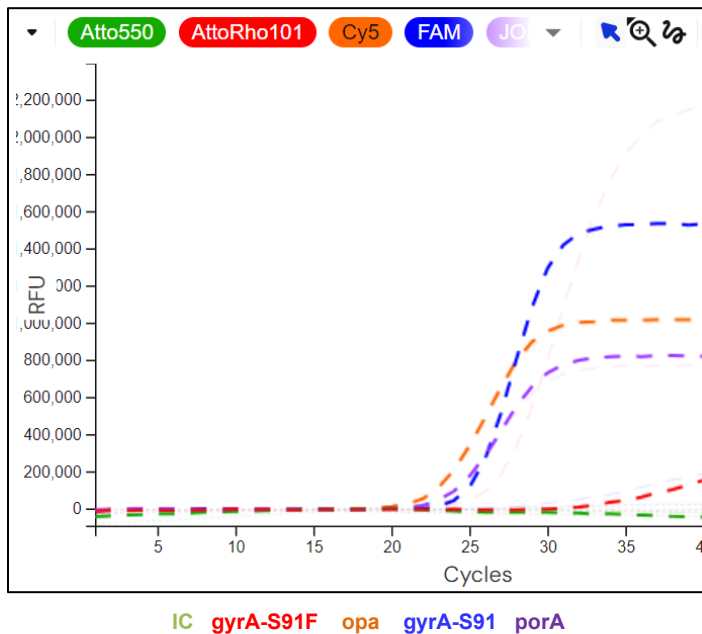
Sample	Assay	Result
N	ResistancePlus GC (LC480)	Valid
	N. gonorrhoeae	⊖ Not detected
	gyrA mutation	⊖ Not detected

27.9.2 Positive Control (GC *gyrA* S91F mutant) (Pa)



Sample	Assay	Result
Pa	ResistancePlus GC (LC480)	Valid
	<i>N. gonorrhoeae</i>	⊕ Detected
	<i>gyrA</i> mutation	⊕ Detected

27.9.3 Positive Control (GC *gyrA* S91 wild type) (Pb)



Sample	Assay	Result
Pb	ResistancePlus GC (LC480)	Valid
<p>N. gonorrhoeae ⊕ Detected</p> <p>gyrA mutation ⊖ Not detected</p>		

27.10 Examples

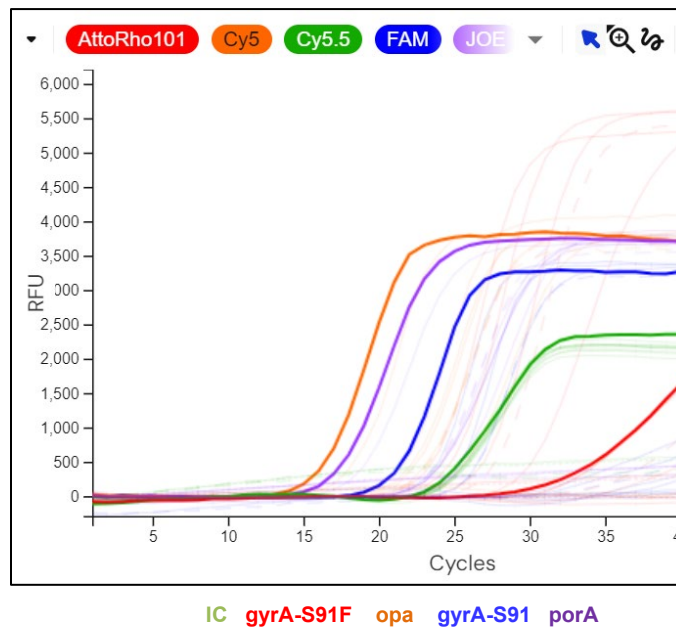
Example results for the **ResistancePlus®** GC analysis software are shown in **Table 64**.

Table 64. Example results for interpretation of ResistancePlus® GC analysis software			
	Sample	Assay	Result
	Sample 101	ResistancePlus GC (LC480)	Not detected
	Sample 102	ResistancePlus GC (LC480)	Detected: N. gonorrhoeae
	Sample 103	ResistancePlus GC (LC480)	Detected: N. gonorrhoeae, gyrA mutation
¹	Sample 104	ResistancePlus GC (LC480)	Detected: N. gonorrhoeae - Invalid: gyrA mutation
²	Sample 106	ResistancePlus GC (LC480)	Invalid: N. gonorrhoeae, gyrA mutation

¹ Samples displaying this result will be flagged with ⚠ Warning : gyrA mutation indeterminate.

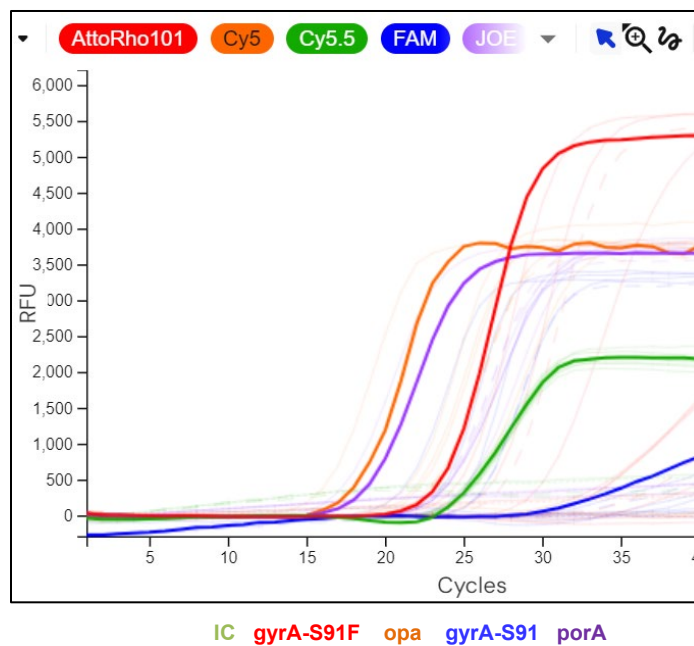
² A sample interpreted as Invalid will be flagged with ⚠ Warning: IC invalid. Re-extract and re-test sample.

27.10.1 High copy *N. gonorrhoeae* positive, *gyrA* wild type



Sample	Assay	Result
Sample 107	ResistancePlus GC (LC480)	Detected: N. gonorrhoeae
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>Assay results</p> <p>N. gonorrhoeae ⊕ Detected</p> <p>gyrA mutation ⊖ Not detected</p> </div> <div style="width: 50%;"> <p>opa ↳ B2 ● Detected 16.065</p> <p>gyrA-S91 ↳ B2 ● Detected 20.733</p> <p>porA ↳ B2 ● Detected 17.106</p> <p>IC ↳ B2 ● Detected 23.995</p> <p>gyrA-S91F ↳ B2 ● Detected 32.766</p> </div> </div>		

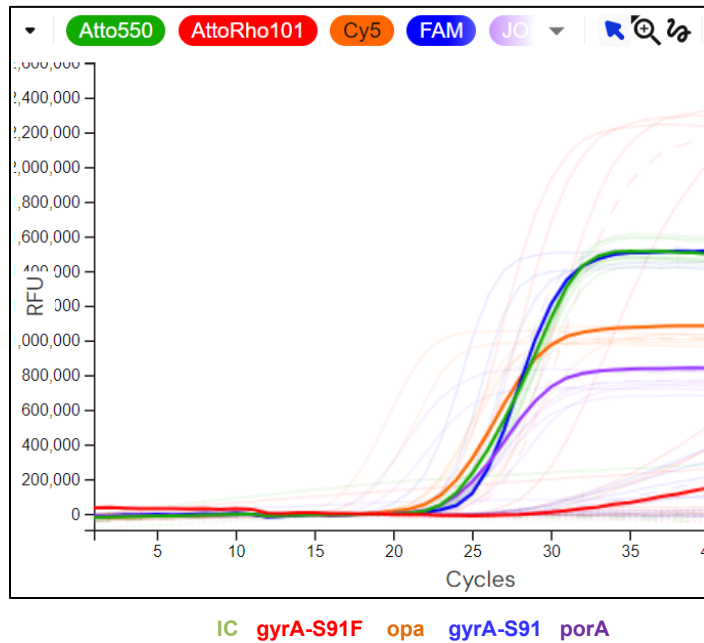
27.10.2 High copy N. gonorrhoeae positive, gyrA mutant



Sample	Assay	Result
Sample 108	ResistancePlus GC (LC480)	Detected: N. gonorrhoeae, gyrA mutation

<p>Assay results</p> <p>N. gonorrhoeae + Detected</p> <p>gyrA mutation + Detected</p>	opa	F2	Detected	18.168
	gyrA-S91	F2	Detected	32.432
	porA	F2	Detected	18.424
	IC	F2	Detected	24.084
	gyrA-S91F	F2	Detected	23.44

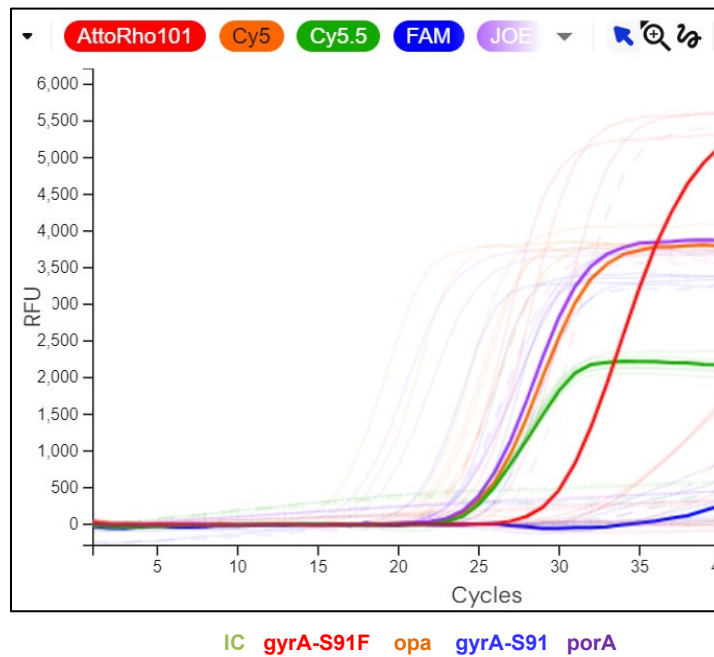
27.10.3 Low copy N. gonorrhoeae positive, gyrA wild type



Sample	Assay	Result
Sample 109	ResistancePlus GC (LC480)	Detected: N. gonorrhoeae

<p>Assay results</p> <p>N. gonorrhoeae ⊕ Detected</p> <p>gyrA mutation ⊖ Not detected</p>		<p>opa ↳ G2 ● Detected 22.814</p> <p>gyrA-S91 ↳ G2 ● Detected 24.797</p> <p>porA ↳ G2 ● Detected 23.543</p> <p>IC ↳ G2 ● Detected 24.143</p> <p>gyrA-S91F ↳ G2 ● Detected 34.95</p>
--	--	--

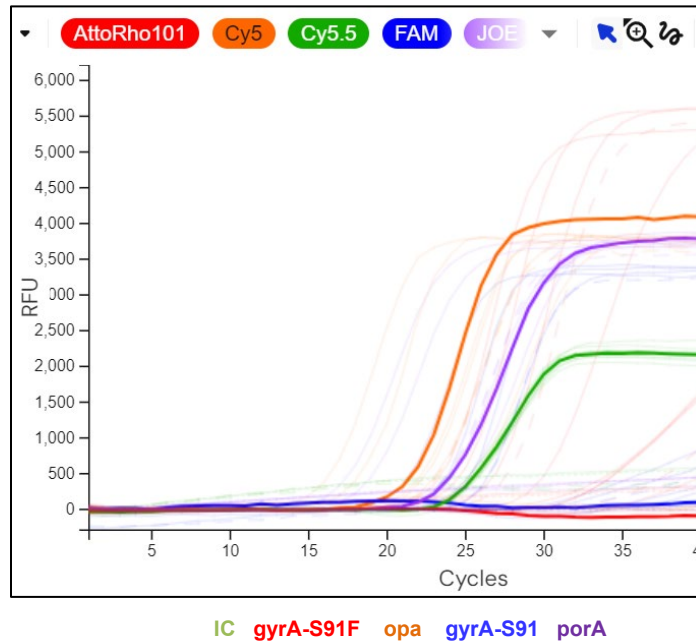
27.10.4 Low copy N. gonorrhoeae positive, gyrA mutant



Sample	Assay	Result
Sample 110	ResistancePlus GC (LC480)	Detected: N. gonorrhoeae, gyrA mutation

<p>Assay results</p> <p>N. gonorrhoeae ⊕ Detected</p> <p>gyrA mutation ⊕ Detected</p>	opa	↳ C3	• Detected	25.091
	gyrA-S91	↳ C3	• Not detected	
	porA	↳ C3	• Detected	24.884
	IC	↳ C3	• Detected	24.517
	gyrA-S91F	↳ C3	• Detected	29.801

27.10.5 N. gonorrhoeae positive, gyrA indeterminate



Sample	Assay	Result
Sample 111	ResistancePlus GC (LC480)	Detected: N. gonorrhoeae - Invalid: gyrA mutation

Assay results

N. gonorrhoeae	⊕ Detected	
gyrA mutation	⚠ Invalid	Warning: gyrA mutation indeterminate.

opa

↳ B3 ● Detected ▼ 21.381

gyrA-S91

↳ B3 ● Not detected ▼

porA

↳ B3 ● Detected ▼ 23.717

IC

↳ B3 ● Detected ▼ 24.158

gyrA-S91F

↳ B3 ● Not detected ▼

28 Appendix B: Instructions for testing *ResistancePlus*[®] GC at 30 μ L reaction volume

The *ResistancePlus*[®] GC kit can optionally be tested at a final reaction volume of 30 μ L if sensitivity is a concern.

Refer to **Section 10.5** for instructions for testing at a final reaction volume of 20 μ L.

28.1 Preparation of PCR

28.1.1 Master mix preparation – Samples extracted with SpeedX Internal Control Cells

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

For a 30 μ L reaction volume, 20 μ L of Master Mix and 10 μ L sample is required. Prepare Master Mix as outlined in **Table 65**.

- Pipette the Master Mix into the PCR plate and then add extracted sample to the reaction.
- Positive and negative controls should be run on each plate.
- Seal, then centrifuge the plate and transfer to thermocycler.

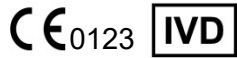
Table 65. Master Mix - 30 μ L reaction		
Reagent	Concentration	Volume per 30 μ L reaction (μ L)
Nuclease Free Water	N/A	2.0
<i>Plex</i> Mastermix (BLUE)	2x	15.0
GC+ <i>gyrA</i> Mix (BROWN)	20x	1.5
Control Mix* (WHITE)	20x	1.5
Total volume (μ L)		20.0
Add 10 μL sample for a final volume of 30 μ L		

* The Control Mix included in each kit is specific to the PCR instrument used; refer to **Table 1**, **Table 2** and **Table 3** for the correct Control Mix to use

28.1.2 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.

29 Glossary



European Conformity
For *In Vitro* Diagnostic Use



Catalogue number



Batch code



Authorised Representative
In the European Community



Manufacturer



Date of manufacture



Temperature limitation



Contains sufficient for
xxx determinations



Use by Date



Importer



United Kingdom Conformity
Assessment Mark

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