SpeeDx

SpeeDx®
Diagnostics Defining Therapy

ResistancePlus® GC

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

CE 0123 IVD UKCA

Product	Platform	Size (reactions)	Catalogue no.
ResistancePlus® GC(610)	LC480 II z 480	100	REF **2011001**
ResistancePlus® GC(610)	LC480 II z 480	25	REF **2011025**
ResistancePlus® GC(550)	ABI 7500 Fast ABI 7500 Fast Dx	100	REF **2013001**
ResistancePlus® GC(550)	ABI 7500 Fast ABI 7500 Fast Dx	25	REF **2013025**
ResistancePlus® GC(675)	CFX96 IVD CFX96 Touch	100	REF **2015001**
ResistancePlus® GC(675)	CFX96 IVD CFX96 Touch	25	REF **2015025**

Accessory products – Analysis software

ResistancePlus® GC (LC480)	REF **99010**
ResistancePlus® GC (z480)	REF **99019**
ResistancePlus® GC (7500)	REF **99009**
ResistancePlus® GC (CFX)	REF **99015**

EC REP

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Not for sale in the USA

Contents

1 Product description

The ***Resistance****Plus*® 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 ***Resistance****Plus*® 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 ***Resistance****Plus*® GC kit utilises ***Plex****Zyme*® and ***Plex****Prime*® 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), cobas® z 480 analyser (z 480), Applied Biosystems® 7500 Fast (7500 Fast), Applied Biosystems® 7500 Fast Dx (7500 Fast Dx), the Bio-Rad CFX96™ IVD (CFX96 IVD) and CFX96 Touch™ (CFX96 Touch) Real-time PCR Detection Systems.

2 Intended use

The ***Resistance****Plus*® 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 ***Resistance****Plus*® 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 ***Resistance****Plus*® 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 ***Resistance****Plus*® 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 women[1] . 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's[2] , 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. *ResistancePlus*® 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+gyrA Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of *N. gonorrhoeae*, *gyrA* S91 and *gyrA* 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 and z 480	1 x 100 µl	1 x 25 µl
Red	Internal Control Cells#	Internal control cells containing internal control DNA template to monitor extraction and amplification efficiency	1 x 500 µl	1 x 100 µl
Neutral	Nuclease Free Water	PCR grade water	1 x 1 ml	1 x 1 ml

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

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

* 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. *ResistancePlus*® 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+*gyrA* Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of *N. gonorrhoeae*, *gyrA* S91 and *gyrA* 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 ***Plex****Prime*® primers), ***Plex****Zyme*® 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. *ResistancePlus*® GC$_{(675)}$ kit contents				
Cap colour	**Contents**	**Description**	**Cat no 2015001 (100 reactions*)**	**Cat no 2015025 (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+gyrA Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of *N. gonorrhoeae*, *gyrA* S91 and *gyrA* 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 IVD 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 ***Plex****Prime*® primers), ***Plex****Zyme*® 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 ***Resistance****Plus*® 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.
- Any serious incident shall be reported to SpeeDx by contacting tech@speedx.com.au

6 Warnings and precautions

6.1 General

- For *in vitro* diagnostic use only.
- Carefully read these Instructions for Use prior to use. Closely follow procedures as described to ensure reliability of test results. Any deviation from these procedures may affect test performance.
- Users should be adequately trained in the use of the ***Resistance****Plus*® 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 ***Resistance****Plus*® 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 Materials required but not provided

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)

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 1000uL (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 and cobas® z480 analyser

- ***Plex****PCR*® 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™ IVD 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)

Negative Control Material

- Known negative sample (provided by end user) (The known negative specimen is a negative process control that is either a previously tested negative sample or negative matrix that can be utilized throughout the process to ensure extraction and amplification worked appropriately as it has IC in it.)

Sample Collection Devices

- Aptima® urine collection kit (Hologic, Cat no 301040)
- Aptima® unisex swab specimen collection kit (Hologic, Cat no 301041)
- 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 Materials provided as a separate accessory product

Positive Material

- ***Resistance****Plus*® GC Control (Cat no 95003)

9 Principle of the technology

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

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

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

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

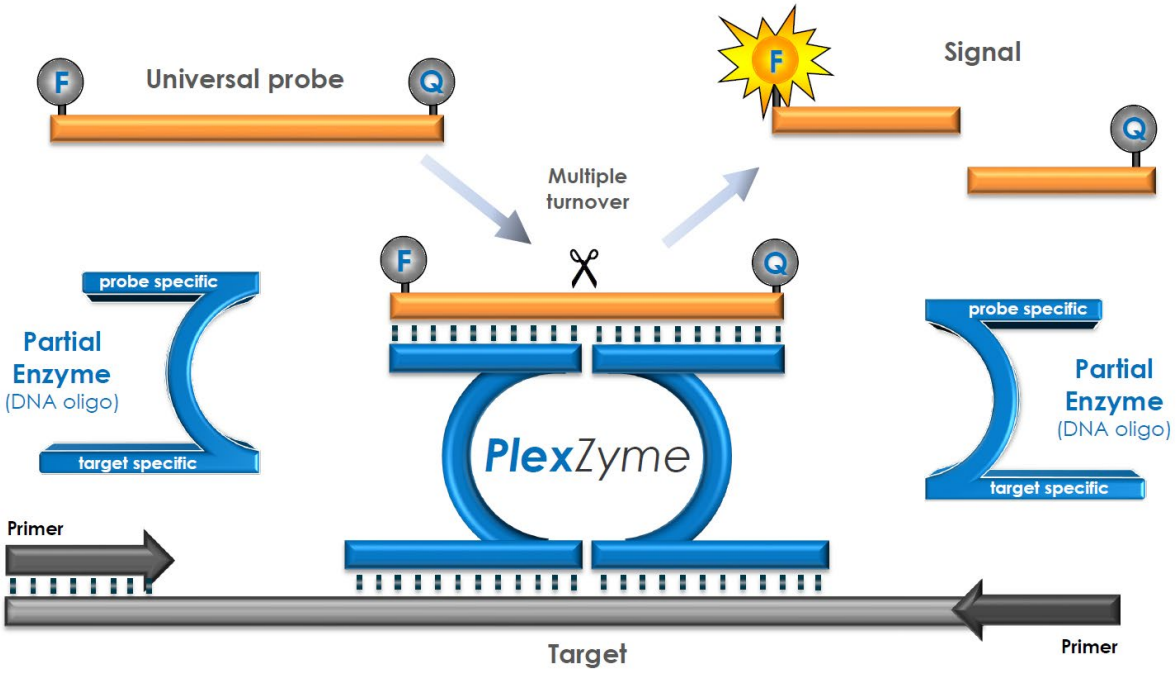
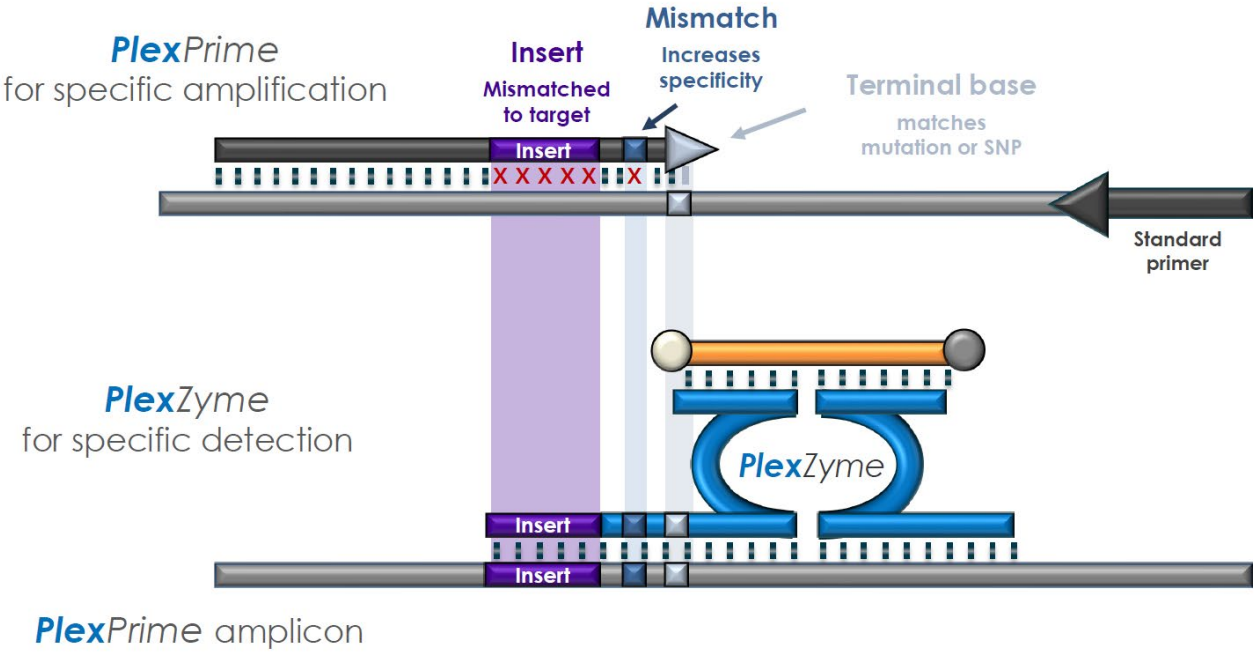
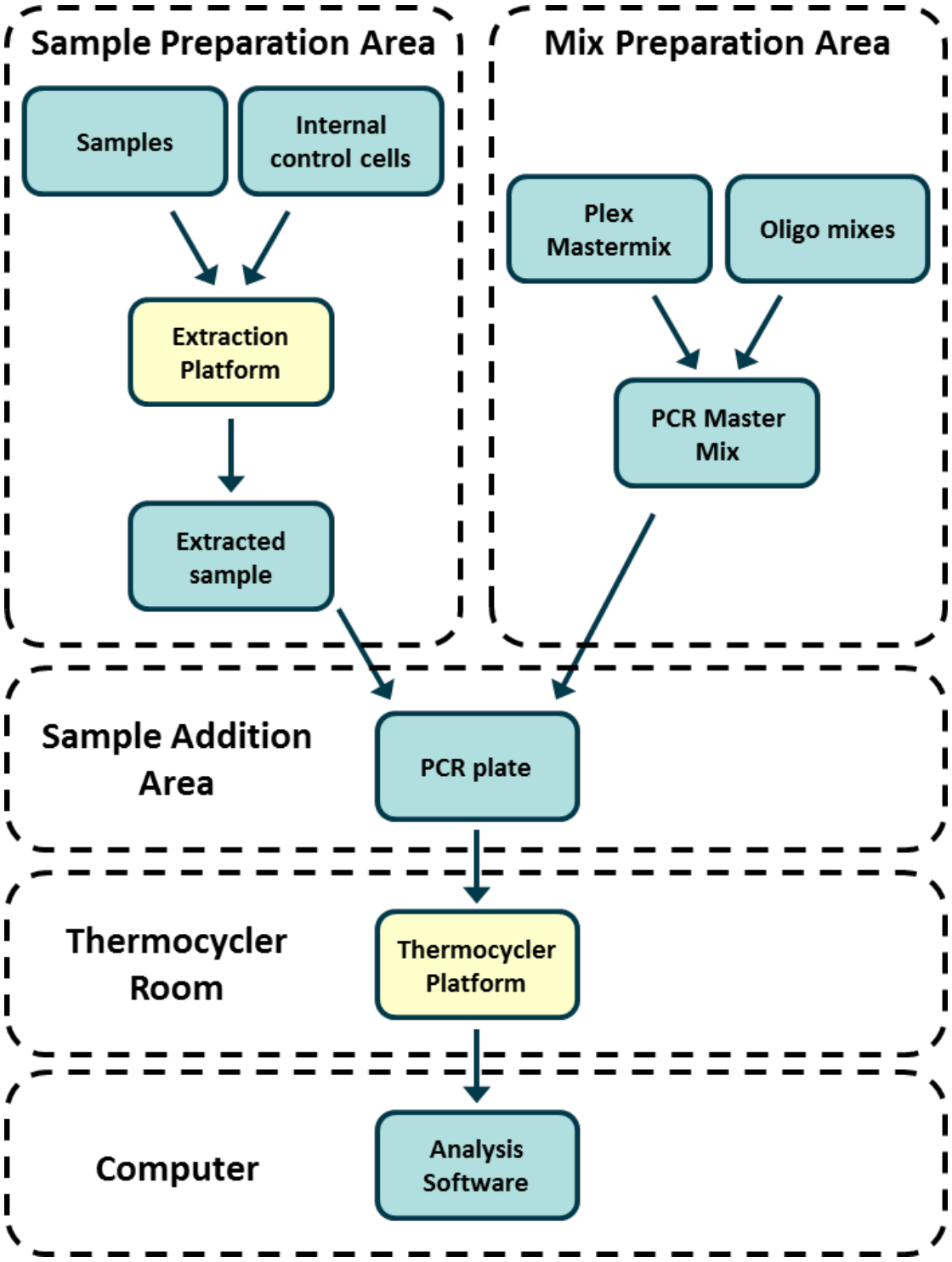


Figure 2. Schematic representation of the *PlexPrime*® primer coupled with *PlexZyme*® detection. The *PlexPrime*® primer specifically amplifies the mutant sequence and *PlexZyme*® enzymes specifically detect the amplicon.



10 Procedure overview



11 Detailed procedure

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

11.1 Sample collection, transport and storage

Male and female urine, and anal, rectal, cervical, endocervical, vaginal, urethral, pharyngeal and eye 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 ***Resistance****Plus*® GC kit.

11.1.1 Validated sample collection devices

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

Sample collection devices that have been validated with the ***Resistance****Plus*® 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.

N. gonorrhoeae isolates have also been validated for use with the ***Resistance****Plus*® GC kit.

11.1.2 Neat urine collection, transport and storage

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

11.1.3 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 2mL of urine with the pipette into the uncapped specimen transport tube. Proper urine volume line must fall within the black fill lines on the urine transport tube. Urine must be transferred from the clear sterile urine cup to the Aptima urine specimen tube within 24 hours of collection.
4. Re-cap the urine transport tube tightly.
5. After collection, processed urine specimens in the Aptima urine specimen transport tube should be transported and stored at 2°C to 30°C and store at 2°C to 30°C until tested. Refer to manufacturer's instructions for detailed storage optimization.

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

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

11.1.4.1 Endocervical swab specimen collection, transport and storage

1. Remove excess mucus from the cervical os and surrounding mucosa using the cleaning swab (white shaft swab in the package with red printing). Discard this swab. Note: To remove excess mucus from the cervical os, a large-tipped swab (not provided) may be used.
2. Insert the specimen collection swab (blue shaft swab in the package with the green printing) into the endocervical canal.
3. Gently rotate the swab clockwise for 10 to 30 seconds in the endocervical canal to ensure adequate sampling.
4. Withdraw the swab carefully; avoid any contact with the vaginal mucosa.

5. Remove the cap from the swab specimen transport tube and immediately place the specimen collection swab into the transport tube.
6. Carefully break the swab shaft against the side of the tube at the score line and discard the top portion of the swab shaft; use care to avoid splashing of contents.
7. Re-cap the swab specimen transport tube tightly. After collection, transport and store the swab in the swab specimen transport tube at 2°C to 30°C until tested.

11.1.4.2 Male urethral swab specimen collection, transport and storage

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

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

The Aptima® Multitest swab collection kit may be used for various clinician and patient collection specimen. Due to the variability, refer to manufacturer package insert for appropriate specimen types and collection methods.

11.1.6 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.

11.1.7 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.

11.1.8 Validated sample extracts

Sample extracts validated for use include:

- cobas® x480 extracts

11.2 Sample processing

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

See **Section 11.3** 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 11.3.1 for how to use the internal control on the EZ1

[b] See 11.3.2 for how to use the internal control on the MagNA Pure 96

11.3 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.

11.3.1 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.

11.3.2 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)			
***Internal Control Cells* (RED) (µl)**	**1x PBS (µl)**	**Total volume (µl)**	**Volume added to sample (µl)**
18	3582	3600	20

11.4 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.

11.4.1 Storage of processed isolates

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

11.5 Preparation of real-time PCR

The ***Resistance****Plus*® GC kit can be tested at a final reaction volume of 20 µl or optionally be tested at a final reaction volume of 30 µl if sensitivity is a concern. Refer to **Section 30** for instructions for testing at a final reaction volume of 30 µl.

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

11.5.1 Master Mix preparation

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

For a 20 µl reaction volume, 15 µl Master Mix and 5 µl 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 µl reaction (µl)**
Nuclease Free Water (**Neutral**)	N/A	3.0
Plex Mastermix (**BLUE**)	2x	10.0
GC+*gyrA* Mix (**BROWN**)	20x	1.0
Control Mix[≠] (**WHITE**)	20x	1.0
Total volume (µl)		15.0
Add 5 µl sample for a final volume of 20 µl		

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

11.5.2 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.

12 Programming and analysis

Details for programming and analysis are described in the **Section 20 – Section 28**.

The ***Resistance****Plus*® 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	***N. gonorrhoeae gyrA* S91 (wild type)**	***N. gonorrhoeae porA***	***N. gonorrhoeae gyrA* S91F (mutant)**	***N. gonorrhoeae opa***	**Internal Control**
	CHANNEL A	**CHANNEL B**	**CHANNEL C**	**CHANNEL E**	**CHANNEL D**
7500 Fast	FAM	JOE	Texas Red	Cy5	TAMRA
7500 Fast Dx	FAM	JOE	Texas Red	Cy5	TAMRA
LC480 II	465-510	533-580	533-610	618-660	533-640
z 480	465-510	540-580	540-610	610-670	540-645
CFX96 IVD	FAM	HEX	Texas Red	Cy5	Quasar 705
CFX96 Touch	FAM	HEX	Texas Red	Cy5	Quasar 705

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

13 Interpretation of results

Data interpretation requires the ***Resistance****Plus*® GC analysis software. While ***Plex****Prime*® primers offer greater specificity than other allele-specific primers, some non-specific amplification from the ***Resistance****Plus*® 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 ***Resistance****Plus*® 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 29**.

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	***Resistance****Plus*® GC (LC480)	LC480 II
99019	***Resistance****Plus*® GC (z480)	z 480
99009	***Resistance****Plus*® GC (7500)	7500 Fast, 7500 Fast Dx
99015	***Resistance****Plus*® GC (CFX)	CFX96 IVD 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.

14 Limitations

- The ***Resistance****Plus*® 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 ***Resistance****Plus*® 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.
- 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.

15 Quality control

The ***Resistance****Plus*® GC kit includes an internal control to monitor extraction efficiency and qPCR inhibition (**Section 11.3**).

The ***Resistance****Plus*® GC Control kit (PI-IV0015) is recommended as positive control material for nucleic acid amplification. Refer to **Section 16** for instructions to use the ***Resistance****Plus*® GC Control kit.

16 *ResistancePlus*® GC Control instructions

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

Table 9. Contents for *ResistancePlus*® GC Control kit (Cat no 95003)

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

16.1 Instructions for use

Prepare qPCR reactions as described in **Section 11** or **Section 30.1** using positive control material as sample.

Data interpretation requires the ***Resistance****Plus*® GC analysis software, refer to **Section 29.11** for example results.

17 Performance characteristics

17.1 Clinical performance

17.1.1 Clinical Study 1

A clinical performance study for the ***Resistance****Plus*® $GC_{(550)}$ 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 ***Resistance****Plus*® $GC_{(550)}$ kit on the Applied Biosystems® 7500 Fast Dx Instrument in a 30 µl reaction volume.

To determine performance of the ***Resistance****Plus*® $GC_{(550)}$ kit, *N. gonorrhoeae* detection was compared to cobas® CT/NG results (with positives confirmed by a *opa*/*porA* real-time PCR test[6]), 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 ***Resistance****Plus*® $GC_{(550)}$ *gyrA* characterisation rate was 92.7% (76/82; 6 samples were reported as *N. gonorrhoeae* positive but *gyrA* indeterminate by the ***Resistance****Plus*® $GC_{(550)}$ 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**.

Table 10. Clinical evaluation of the *ResistancePlus*® $GC_{(550)}$ kit (Clinical study 1)

		***N. gonorrhoeae* detection cobas® CT/NG**			***gyrA* 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 *N. gonorrhoeae* negative**	**Expected *N. gonorrhoeae gyrA* wild type**	**Expected *N. gonorrhoeae gyrA* 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

17.1.2 Clinical Study 2

A clinical performance study for the ***Resistance****Plus*® 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).

17.1.2.1 Performance of the ***Resistance****Plus*® GC$_{(550)}$ kit on the 7500 Fast

To determine performance of the ***Resistance****Plus*® 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 ***Resistance****Plus*® 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 ***Resistance****Plus*® GC$_{(550)}$ *gyrA* characterisation rate was 90.7% (136/150; 14 samples were reported as *N. gonorrhoeae* positive but *gyrA* indeterminate by the ***Resistance****Plus*® 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)							
		***N. gonorrhoeae* detection Aptima® Combo 2**				***gyrA* detection Sequencing**	
		Positive*	**Negative**			**S91F mutant**	**S91 wild type**
***ResistancePlus*® 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 *N. gonorrhoeae* negative**	**Expected *N. gonorrhoeae gyrA* wild type**	**Expected *N. gonorrhoeae gyrA* 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	-	-

17.1.2.2 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 *ResistancePlus*® GC(610) kit (Clinical study 2)							
		***N. gonorrhoeae* detection Aptima® Combo 2**				***gyrA* detection Sequencing**	
		Positive*	**Negative**			**S91F mutant**	**S91 wild type**
***ResistancePlus*® 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

17.1.2.3 Performance of the ***Resistance*Plus**® 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 ***Resistance*Plus**® 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 *ResistancePlus*® GC(610) kit in 30 µl and 20 µl reaction volumes (Clinical study 2)							
		***N. gonorrhoeae* detection *ResistancePlus*® GC(610) (30 µl)**				***gyrA* detection *ResistancePlus*® GC(610) (30 µl)**	
		Positive	**Negative**			**Mutant detected**	**Mutant not detected**
***ResistancePlus*® 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%)	

17.1.3 Clinical Study 3

Performance of the ***Resistance*Plus**® 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 11.4**), and tested on the 7500 Fast Dx Instruments in a 30 µl reaction volume.

The performance of the ***Resistance*Plus**® 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 WGS				Phenotype Ciprofloxacin AST	
		gyrA S91F mutant	*gyrA* wild type			Resistant (MIC > 1.0 mg/L)	Susceptible (MIC < 1.0 mg/L)
ResistancePlus® 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%)	

17.1.4 Clinical Study 4

The performance of the ***Resistance****Plus*® 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 ***Resistance****Plus*® 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 ***Resistance****Plus*® 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 ***Resistance****Plus*® 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)							
		N. gonorrhoeae detection cobas® CT/NG				*gyrA* detection Sequencing	
		Positive	Negative			S91F mutant	S91 wild type
ResistancePlus® 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 *N. gonorrhoeae* negative**	**Expected *N. gonorrhoeae gyrA* wild type**	**Expected *N. gonorrhoeae gyrA* 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."

17.1.5 Clinical Study 5

A clinical performance study for the ***Resistance****Plus*® $GC_{(610)}$ kit on contrived samples was conducted at the Queensland Paediatric Infectious Disease Group (QPID), Queensland, Australia. The samples consisted of 90 female urogenital (vaginal) swabs and 90 male first void urine samples collected in cobas® PCR media. The specimen types were separated into "Panels" A and B. Each panel consisted of 30 negative samples, 30 *N. gonorrhoeae* positive for wild-type (*gyra* S91S genotype), and 30 *N. gonorrhoeae* positive samples for a mutant (*gyra* S91F genotype). Within these panels were 10 of each low, mid and high positive samples for both the wild-type and mutant strains. The internal control was added prior to extraction and extracted using the MagNAPure96 DNA and Viral NA Small Volume kit. All samples were extracted using the MagNA Pure 96 with a 100 µl elution volume and tested on the z 480 instrument as a 20 µl reaction volume.

The average Cq and standard deviation for the IC assay in each sample type is shown in **Table 19**. One vaginal swab sample extracted with IC was found to be invalid (see **Table 19**.). This sample was re-run, and the IC was valid on repeat. This gives an IC invalid rate of 0.55% for Panels A and B.

Table 19. Performance of the Internal Control in collected *N. gonorrhoeae* positive specimens				
		Cq Average	**Standard deviation**	**Total number**
Resistance*Plus*® $GC_{(610)}$	**All samples**	25.19	0.51	181
	Vaginal swabs only (Panel A)	25.05	0.52	91
	Urine only (Panel B)	25.32	0.47	90
IC invalid rate		0.55% (1/181)		

17.1.5.1 Performance of the ***Resistance****Plus*® $GC_{(610)}$ kit

The ***Resistance****Plus*® $GC_{(610)}$ kit demonstrated clinical performance of >95% PPA, NPA and ORA for GC and *gyrA*-S91F mutation detection when compared to the expected result. The performance of the ***Resistance****Plus*® $GC_{(610)}$ is summarised in **Table 20** and **Table 21** below.

Table 20. Clinical evaluation of the *ResistancePlus*® GC(610) kit with contrived sample extracts – Vaginal Swab Samples (Panel A) (Clinical study 5)							
***N. gonorrhoeae* detection**^		**Expected Result**			***gyrA* mutant/wildtype detection**^	**Expected Result**	
		Positive	**Negative**			**S91F mutant**	**S91 Wild type**
***ResistancePlus*® GC(610)**	**Positive**	60	1^		**Mutant detected**	30	0
	Negative	0	29		**Mutant not detected**	0	30
PPA		100.00% (95% CI 94.04-100.00)			**PPA**	100.00% (95% CI 88.43-100.00)	
NPA		96.67% (95% CI 80.4-99.8)			**PPA**	100.00% (95% CI 88.43-100.00)	
ORA		98.89% (95% CI 93.9-99.97)			**ORA**	100.00% (95% CI 94.04-100.00)	

^One vaginal swab sample incorrectly called positive for GC and indeterminate for gyrA-S91F, the expected result was negative.

Table 21. Clinical evaluation of the *ResistancePlus*® GC(610) kit with contrived sample extracts – Urine Samples (Panel B) (Clinical study 5)							
***N. gonorrhoeae* detection**˙		**Expected Result**			***gyrA* mutant/wildtype detection**˙	**Expected Result**	
		Positive	**Negative**			**S91F mutant**	**S91 Wild type**
***ResistancePlus*® GC(610)**	**Positive**	60	0		**Mutant detected**	29	0
	Negative	0	30		**Mutant not detected**	0	31
PPA		100.00% (95% CI 94.04-100.00)			**PPA**	100.00% (95% CI 88.06-100.00)	
NPA		100.00% (95% CI 88.43-100.00)			**PPA**	100.00% (95% CI 88.78-100.00)	
ORA		100.00% (95% CI 95.98-100.00)			**ORA**	100.00% (95% CI 94.04-100.00)	

17.2 Analytical performance

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

The reproducibility of the ***Resistance****Plus*® 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 22**), the two lots showed good reproducibility with coefficient of variation (%CV) between 0.61-1.88%.

Table 22. Lot-to-lot variability (30 µl reaction volume)					
Strain	**Target**	**Average Cq**	**STDEV**	**%CV**	**# Samples**
***N. gonorrhoeae gyrA* wild type**	***opa***	23.61	0.31	1.29	12/12
	porA	25.06	0.19	0.75	12/12
	***gyrA* S91 wild type**	24.34	0.22	0.91	12/12
***N. gonorrhoeae gyrA* mutant**	***opa***	23.65	0.14	0.61	12/12
	porA	25.37	0.31	1.21	12/12
	***gyrA* S91F mutant**	29.25	0.55	1.88	12/12

For lot-to-lot variability for the 20 µl reaction (**Table 23**), the two lots showed good reproducibility with coefficient of variation (%CV) between 0.61-1.40%.

Table 23. Lot-to-lot variability (20 µl reaction volume)					
Strain	**Target**	**Average Cq**	**STDEV**	**%CV**	**# Samples**
***N. gonorrhoeae gyrA* wild type**	***opa***	23.03	0.14	0.61	12/12
	porA	24.78	0.23	0.92	12/12
	***gyrA* S91 wild type**	24.26	0.26	1.09	12/12
***N. gonorrhoeae gyrA* mutant**	***opa***	22.52	0.16	0.70	12/12
	porA	24.54	0.27	1.10	12/12
	***gyrA* 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 24**), the three runs showed good reproducibility with coefficient of variation (%CV) between 1.13-2.31%.

Table 24. Day-to-day variability (30 µl reaction volume)					
Strain	**Target**	**Average Cq**	**STDEV**	**%CV**	**# Samples**
***N. gonorrhoeae gyrA* wild type**	***opa***	24.08	0.56	2.31	18/18
	porA	25.31	0.39	1.54	18/18
	***gyrA* S91 wild type**	24.88	0.45	1.80	18/18
***N. gonorrhoeae gyrA* mutant**	***opa***	23.95	0.27	1.15	18/18
	porA	25.66	0.45	1.75	18/18
	***gyrA* S91F mutant**	29.83	0.34	1.13	18/18

For day-to-day variability for the 20 µl reaction (**Table 25**), the three runs performed over the 3 days showed good reproducibility with coefficient of variation (%CV) between 0.65-3.23%.

Table 25. Day-to-day variability (20 µl reaction volume)					
Strain	**Target**	**Average Cq**	**STDEV**	**%CV**	**# Samples**
***N. gonorrhoeae* *gyrA* wild type**	***opa***	23.24	0.29	1.26	18/18
	porA	24.99	0.16	0.65	18/18
	***gyrA* S91 wild type**	24.88	0.56	2.25	18/18
***N. gonorrhoeae* *gyrA* mutant**	***opa***	23.13	0.75	3.23	18/18
	porA	25.18	0.71	2.81	18/18
	***gyrA* 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 26**), the three runs showed good reproducibility with coefficient of variation (%CV) between 0.69-1.78%.

Table 26. Run-to-run variability (30 µl reaction volume)					
Strain	**Target**	**Average Cq**	**STDEV**	**%CV**	**# Samples**
***N. gonorrhoeae* *gyrA* wild type**	***opa***	24.37	0.43	1.76	18/18
	porA	25.45	0.38	1.50	18/18
	***gyrA* S91 wild type**	25.10	0.45	1.78	18/18
***N. gonorrhoeae* *gyrA* mutant**	***opa***	24.11	0.17	0.69	18/18
	porA	25.91	0.33	1.28	18/18
	***gyrA* S91F mutant**	30.06	0.34	1.12	18/18

For run-to-run variability for the 20 µl reaction (**Table 27**), the three runs showed good reproducibility with coefficient of variation (%CV) between 0.83-1.30%.

Table 27. Run-to-run variability (20 µl reaction volume)					
Strain	**Target**	**Average Cq**	**STDEV**	**%CV**	**# Samples**
***N. gonorrhoeae* *gyrA* wild type**	***opa***	23.10	0.30	1.30	18/18
	porA	24.79	0.22	0.87	18/18
	***gyrA* S91 wild type**	25.16	0.22	0.88	18/18
***N. gonorrhoeae* *gyrA* mutant**	***opa***	22.61	0.21	0.94	18/18
	porA	24.67	0.21	0.83	18/18
	***gyrA* 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 28**), the two runs performed by different operators showed good reproducibility with coefficient of variation (%CV) between 1.21-2.71%.

Table 28. Operator variability (30 µl reaction volume)					
Strain	**Target**	**Average Cq**	**STDEV**	**%CV**	**# Samples**
***N. gonorrhoeae* *gyrA* wild type**	***opa***	24.08	0.65	2.71	12/12
	porA	25.38	0.46	1.79	12/12
	***gyrA* S91 wild type**	24.81	0.52	2.11	12/12
***N. gonorrhoeae* *gyrA* mutant**	***opa***	23.97	0.31	1.30	12/12
	porA	25.75	0.50	1.93	12/12
	***gyrA* S91F mutant**	29.83	0.36	1.21	12/12

For operator variability for the 20 µl reaction **(Table 29**), the two runs performed by different operators showed good reproducibility with coefficient of variation (%CV) between 0.48-2.23%.

Table 29. Operator variability (20 µl reaction volume)					
Strain	**Target**	**Average Cq**	**STDEV**	**%CV**	**# Samples**
***N. gonorrhoeae* *gyrA* wild type**	***opa***	23.07	0.13	0.56	12/12
	porA	24.91	0.14	0.56	12/12
	***gyrA* S91 wild type**	24.67	0.55	2.23	12/12
***N. gonorrhoeae* *gyrA* mutant**	***opa***	22.63	0.11	0.48	12/12
	porA	24.72	0.22	0.88	12/12
	***gyrA* 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 µl reaction (**Table 30**), the two runs performed on different instruments showed good reproducibility with coefficient of variation (%CV) between 1.20-3.42%.

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

For instrument variability for the 20 µl reaction (**Table 31**), the two runs performed showed good reproducibility with coefficient of variation (%CV) between 0.58-1.73%.

Table 31. Instrument variability (20 µl reaction volume)					
Strain	**Target**	**Average Cq**	**STDEV**	**%CV**	**# Samples**
***N. gonorrhoeae* *gyrA* wild type**	***opa***	22.96	0.25	1.11	12/12
	porA	24.74	0.31	1.27	12/12
	***gyrA* S91 wild type**	24.95	0.43	1.73	12/12
***N. gonorrhoeae* *gyrA* mutant**	***opa***	22.59	0.13	0.58	12/12
	porA	24.55	0.29	1.18	12/12
	***gyrA* S91F mutant**	28.58	0.28	0.97	12/12

17.2.2 Reproducibility and repeatability (CFX96 IVD and CFX96 Touch)

The reproducibility of the ***Resistance****Plus*® $GC_{(675)}$ 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 ***Resistance****Plus*® $GC_{(675)}$ kit and two different CFX96 instruments (one CFX96 IVD, and one CFX96 Touch). All replicates were tested with 20 ul 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 32**).

Table 32. Lot-to-lot variability					
Strain	**Target**	**Average Cq**	**STDEV**	**%CV**	**# Samples**
***N. gonorrhoeae* *gyrA* wild type**	***opa***	22.34	0.03	0.15	60/60
	porA	24.03	0.13	0.53	60/60
	***gyrA* S91 wild type**	24.92	0.89	3.59	60/60
***N. gonorrhoeae* *gyrA* mutant**	***opa***	23.07	0.16	0.71	60/60
	porA	24.91	0.13	0.52	60/60
	***gyrA* 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 33**).

Table 33. Day-to-day variability					
Strain	**Target**	**Average Cq**	**STDEV**	**%CV**	**# Samples**
***N. gonorrhoeae* *gyrA* wild type**	***opa***	22.34	0.23	1.01	60/60
	porA	24.03	0.19	0.78	60/60
	***gyrA* S91 wild type**	24.92	0.03	0.14	60/60
***N. gonorrhoeae* *gyrA* mutant**	***opa***	23.07	0.11	0.46	60/60
	porA	24.91	0.04	0.15	60/60
	***gyrA* S91F mutant**	29.30	0.26	0.89	60/60

17.2.3 Analytical sensitivity

The analytical sensitivity of the ***Resistance****Plus*® 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 34**). The LOD (genomes per reaction) of each representative strain of *N. gonorrhoeae* was confirmed for the 7500 Fast, LC480 II, CFX96 IVD, CFX96 Touch, and z 480 for a 30 ul reaction volume, and confirmed for the 7500 Fast, 7500 Fast Dx, LC480 II, CFX96 IVD, CFX96 Touch, and z 480 for a 20 µl reaction volume.

Table 34. Analytical sensitivity

Strain	Limit of detection (genomes/reaction)
***N. gonorrhoeae gyrA* wild type**	15
***N. gonorrhoeae gyrA* mutant**	15

17.2.4 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 35**).

Table 35. Analytical inclusivity

Strain	*gyrA* 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

17.2.5 Analytical specificity

The ***Resistance****Plus*® 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 36**).

Table 36. Analytical specificity	
Organism	**Test concentration (copies/reaction)**
Candida albicans (3153A)	10^4
Chlamydia trachomatis (LGV II 434)	10^4
Chlamydophila pneumoniae (CM-1)	10^4
Cytomegalovirus (AD169-BAC isolate)	10^4
Enterococcus faecalis (Portland)	10^6
Epstein-Barr virus (Human herpes virus 4)	10^4
Escherichia coli (Crooks)	10^6
Haemophilus influenzae (Rd KW20)	10^6
Herpes simplex virus 1 (McIntyre)	10^4
Herpes simplex virus 2 (MS)	10^4
Klebsiella oxytoca (Flugge) Lautrop	10^6
Legionella pneumophila (Philadelphia-1)	10^4
Listeria monocytogenes (Li 23)	10^6
Moraxella osloensis (clinical isolate)	10^5
Mycoplasma genitalium (G37)	10^4
Mycoplasma hominis (ZK-CU2)	10^4
Mycoplasma pneumoniae (FH strain of Eaton Agent)	10^6
Neisseria cinerea (clinical isolate)	10^5
Neisseria flavescens (clinical isolate)	10^5
Neisseria lactamica (clinical isolate)	10^4
Neisseria meningitidis (clinical isolate)	10^5
Neisseria mucosa (clinical isolate)	10^5
Neisseria polysaccharea (clinical isolate)	10^5
Neisseria sicca (clinical isolate)	10^5
Neisseria subflava (clinical isolate)	10^5
Neisseria weaverii (clinical isolate)	10^5
Pseudomonas aeruginosa (PAO1-LAC)	10^6
Salmonella typhimurium	10^4
Staphylococcus aureus	10^4
Streptococcus agalactiae (2603V/R)	10^5
Streptococcus pneumoniae (R6)	10^4
Streptococcus salivarius (275 (DSM 20560))	10^6

Treponema pallidum (Nichols strain)	10^3
Trichomonas vaginalis	10^4
Ureaplasma parvum	10^4
Ureaplasma urealyticum (960)	10^3

17.2.6 Competitive interference.

To study competitive interference, the ***Resistance****Plus*® 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 37).**

Table 37. Competitive interference

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

17.2.7 Potentially interfering substances

The effect of potential interfering substances on the ***Resistance****Plus*® 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 (ΔCq < 0.5) in the Internal Control signal was observed in the presence of the substances which did not affect detection (**Table 38**).

Table 38. Potentially interfering substances

Substance	Concentration	IC Average Cq	STDEV	ΔCq	# 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

18 Customer and technical support

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

Tel: +61 2 9209 4169, Email: tech@speedx.com.au

19 References

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20 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 ***Resistance****Plus*® GC(610) kit contains dyes for the LightCycler® 480 Instrument II. The ***Plex****PCR*® Colour Compensation kit (Cat no 90001) must be run and applied for LC480 II analysis (see **Section 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 3**)

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

Table 39. 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

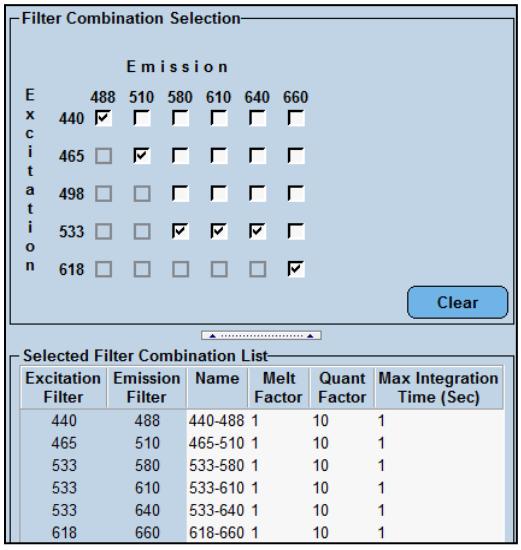
Set the **Selected Filter Combination List** for all channels as:

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** >

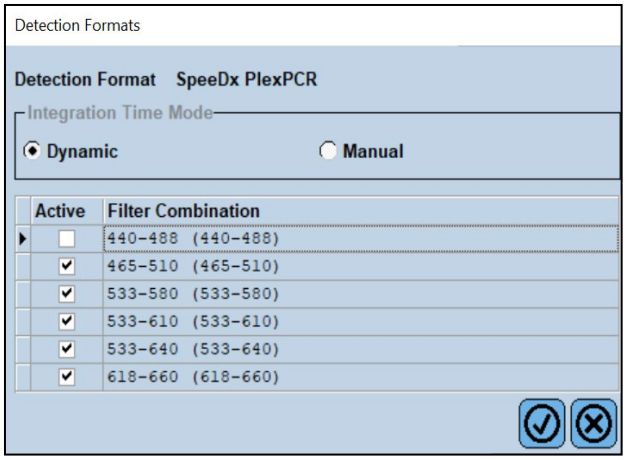
Select **Integration Time Mode** > **Dynamic**

Select the following Active **Filter Combinations** shown in **Table 40.**

Table 40. Channels for *ResistancePlus*® GC targets

Target	*N. gonorrhoeae gyrA* S91 (wild type)	*N. gonorrhoeae porA*	*N. gonorrhoeae gyrA* S91F (mutant)	Internal Control	*N. gonorrhoeae opa*
LC480 II	465-510	533-580	533-610	533-640	618-660

Figure 4. Customize Detection Format



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

Open the **Sample Editor** module

Select well

Edit **Sample Name** to match nametag defined in Assays module of the analysis software (see **Section 29.4**)

Samples are labelled as *Prefix_Suffix* (as shown in **Table 41** and **Figure 5**)

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

Table 41. Sample nametags for analysis software

Sample Type	Prefix_ (in analysis software)	_Suffix (in analysis software)	Sample Name (in LC480)
Regular sample	Sample	_GC	Sample_GC
Negative Control	NEG	_GC	NEG_GC
Positive Control (MT, GC gyrA S91F) (Pa)	Pa	_GC	Pa_GC
Positive Control (WT, GC gyrA S91) (Pb)	Pb	_GC	Pb_GC

Figure 5. Sample Editor – Assigning nametags to wells

Pos	Filter combination	Color	Repl Of	Sample Name
A1	440-488 (440			Sample 1_GC
A1	465-510 (465			Sample 1_GC
A1	533-580 (533			Sample 1_GC
A1	533-610 (533			Sample 1_GC
A1	533-640 (533			Sample 1_GC
A1	618-660 (618			Sample 1_GC
A2	440-488 (440			Pa_GC
A2	465-510 (465			Pa_GC
A2	533-580 (533			Pa_GC
A2	533-610 (533			Pa_GC
A2	533-640 (533			Pa_GC
A2	618-660 (618			Pa_GC
A3	440-488 (440			Pb_GC
A3	465-510 (465			Pb_GC
A3	533-580 (533			Pb_GC
A3	533-610 (533			Pb_GC
A3	533-640 (533			Pb_GC
A3	618-660 (618			Pb_GC
A4	440-488 (440			NEG_GC
A4	465-510 (465			NEG_GC
A4	533-580 (533			NEG_GC
A4	533-610 (533			NEG_GC
A4	533-640 (533			NEG_GC
A4	618-660 (618			NEG_GC

For a 20 µl reaction

Set **Reaction Volume** > 20 µl

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

Table 42. Thermocycling Program

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

≠ Default ramp rate (96 well plate)

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

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

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

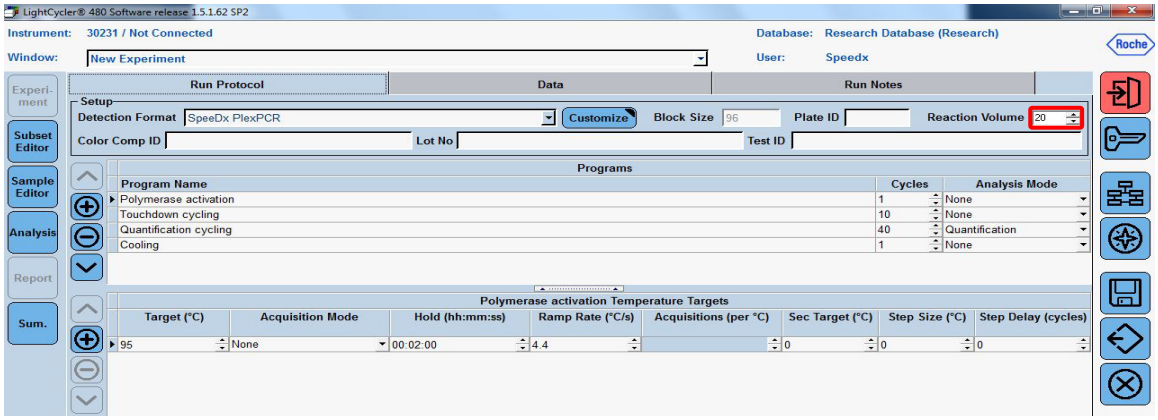


Figure 7. Thermocycling Program (20 µl reaction) – Touchdown cycling

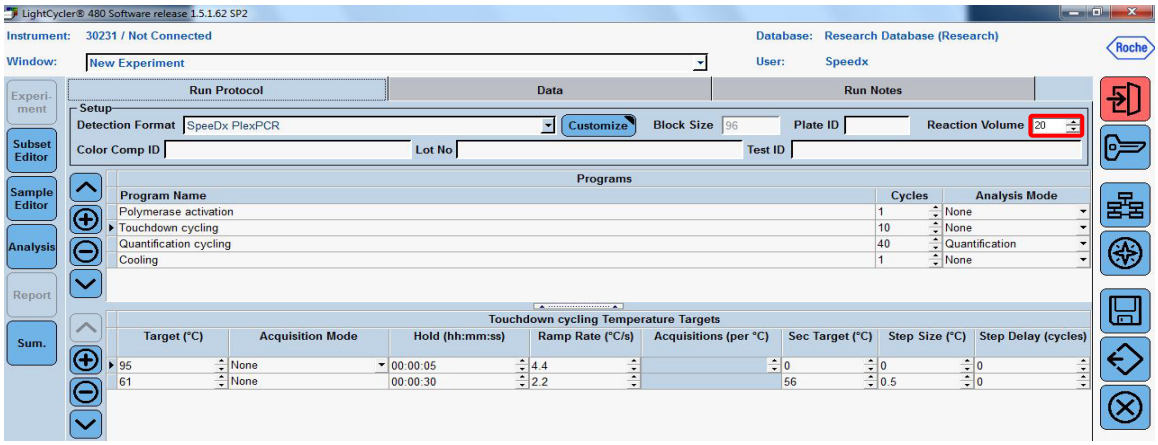


Figure 8. Thermocycling Program (20 µl reaction) – Quantification cycling

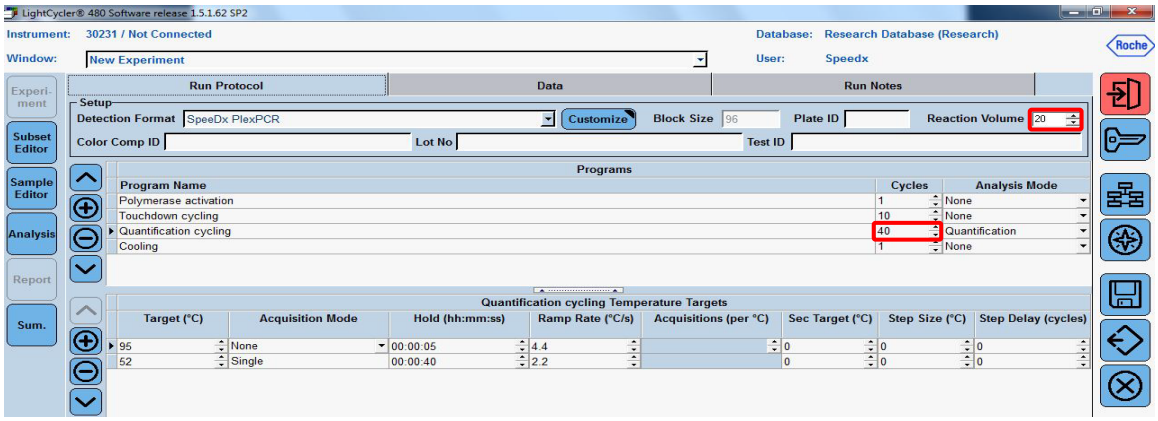
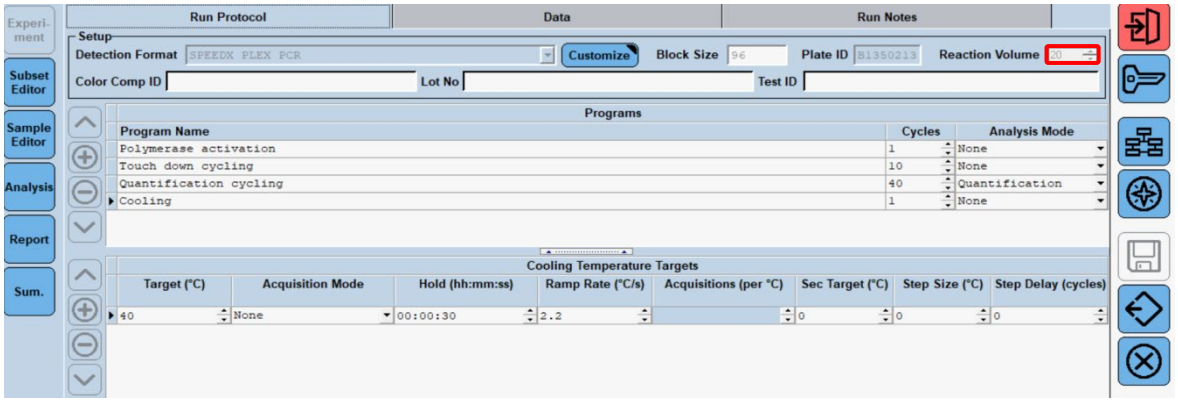


Figure 9. Thermocycling Program (20 µl reaction) – Cooling



> **Start Run**

When the cycling program has finished, export a ". ixo" file for analysis in the ***Resistance**Plus*®** GC (LC480) analysis software.

Select **Export**

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.*

For analysis using the software, the Sample Name of the colour compensation reactions must be labelled as shown in **Table 43**.

When the cycling program has finished, export a ". ixo" file for analysis in the ***Resistance**Plus*®** GC (LC480) analysis software.

Select **Export**

Save in an easily identifiable location and name as **"SpeeDx PlexPCR"**

Table 43. Sample Name for colour compensation reactions for the analysis software

Reactions	BLANK	488 mix	510 mix	580 mix	610 mix	640 mix	660 mix
Dominant Channel	Water	440-488	465-510	533-580	533-610	533-640	610-660
Sample Name	BLANK	440-488	465-510	533-580	533-610	533-640	610-660

20.3 Interpretation of results

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

Refer to **Section 29** for instructions on using the ***Resistance**Plus*®** GC (LC480) analysis software.

21 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 ***Resistance****Plus*® GC(610) kit contains dyes for the LightCycler® 480 Instrument II. The ***Plex****PCR*® Colour Compensation kit (Cat no 90001) must be run and applied for LC480 II analysis (see **Section 21.2**). This kit can be supplied on request.

21.1 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 10**)

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

Table 44. 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

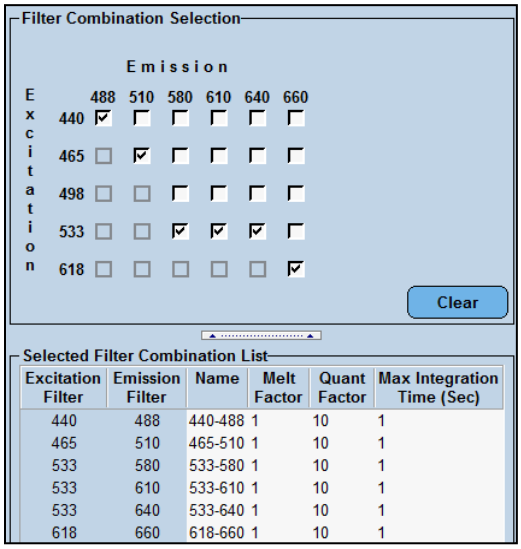
Set the **Selected Filter Combination List** for all channels as:

Melt Factor: 1

Quant Factor: 10

Max Integration Time (sec): 1

Figure 10. 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 11**)

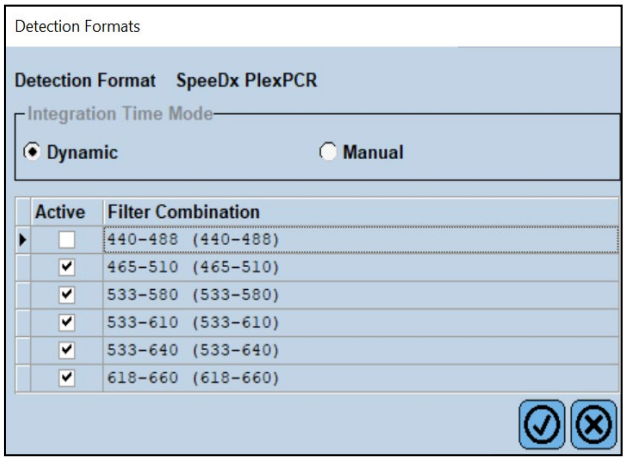
Select **Customize** >

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

Select the following Active **Filter Combinations** shown in **Table 45**

Table 45. Channels for *ResistancePlus*® GC targets					
Target	***N. gonorrhoeae gyrA* S91 (wild type)**	***N. gonorrhoeae porA***	***N. gonorrhoeae gyrA* S91F (mutant)**	**Internal Control**	***N. gonorrhoeae opa***
LC480 II	465-510	533-580	533-610	533-640	618-660

Figure 11. Customize Detection Format



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

Open the **Sample Editor** module

Select well

Edit **Sample Name** to match nametag defined in Assays module of the analysis software (see **Section 29.4**)

Samples are labelled as *Prefix_Suffix* (as shown in **Table 46** and **Figure 12**)

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

Table 46. Sample nametags for analysis software			
Sample Type	**Prefix_ (in analysis software)**	**_Suffix (in analysis software)**	**Sample Name (in LC480)**
Regular sample	Sample	_GC	Sample_GC
Negative Control	NEG	_GC	NEG_GC
Positive Control (MT, GC gyrA S91F) (Pa)	Pa	_GC	Pa_GC
Positive Control (WT, GC gyrA S91) (Pb)	Pb	_GC	Pb_GC

Figure 12. Sample Editor – Assigning nametags to wells

Pos	Filter combination	Color	Repl Of	Sample Name
A1	440-488 (440			Sample 1_GC
A1	465-510 (465			Sample 1_GC
A1	533-580 (533			Sample 1_GC
A1	533-610 (533			Sample 1_GC
A1	533-640 (533			Sample 1_GC
A1	618-660 (618			Sample 1_GC
A2	440-488 (440			Pa_GC
A2	465-510 (465			Pa_GC
A2	533-580 (533			Pa_GC
A2	533-610 (533			Pa_GC
A2	533-640 (533			Pa_GC
A2	618-660 (618			Pa_GC
A3	440-488 (440			Pb_GC
A3	465-510 (465			Pb_GC
A3	533-580 (533			Pb_GC
A3	533-610 (533			Pb_GC
A3	533-640 (533			Pb_GC
A3	618-660 (618			Pb_GC
A4	440-488 (440			NEG_GC
A4	465-510 (465			NEG_GC
A4	533-580 (533			NEG_GC
A4	533-610 (533			NEG_GC
A4	533-640 (533			NEG_GC
A4	618-660 (618			NEG_GC

For a 30 µl reaction

Set **Reaction Volume** > 30 µl

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

Table 47. Thermocycling Program

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

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

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

Figure 13. Thermocycling Program (30 µl reaction) – Polymerase activation

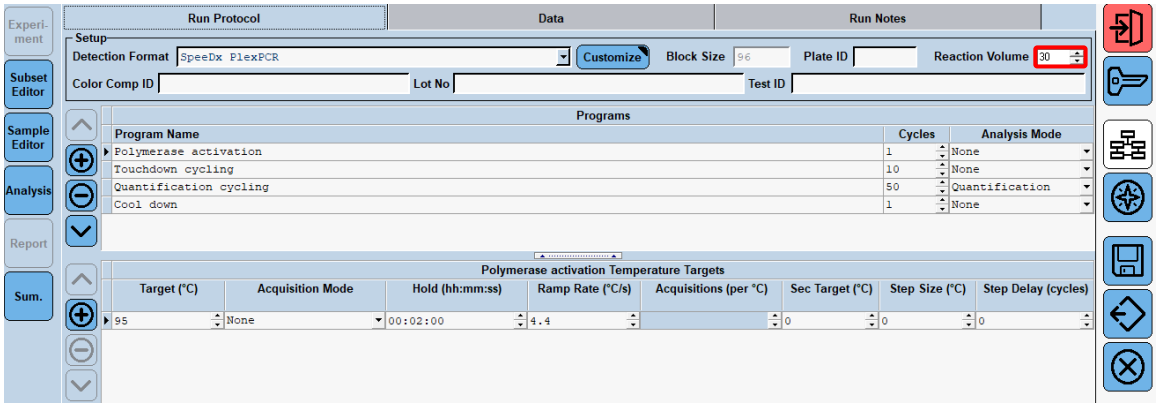


Figure 14. Thermocycling Program (30 µl reaction) – Touchdown cycling

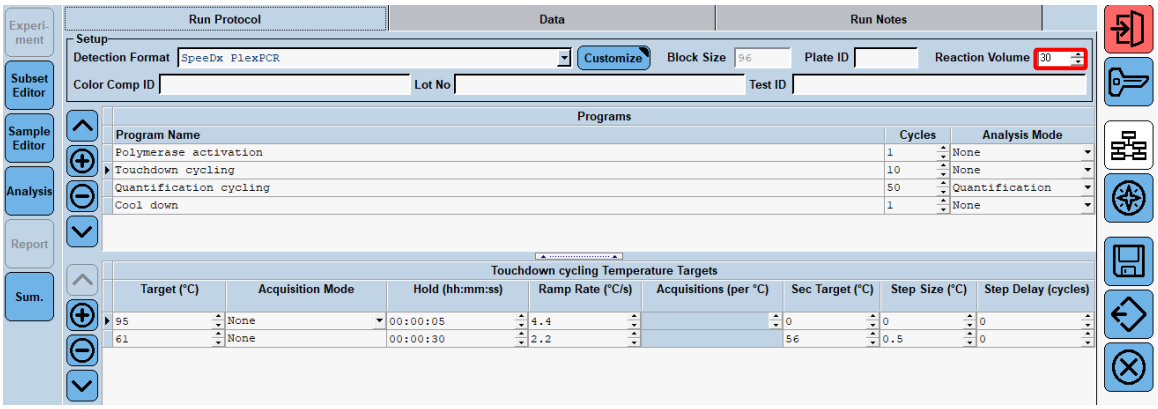


Figure 15. Thermocycling Program (30 µl reaction) – Quantification cycling

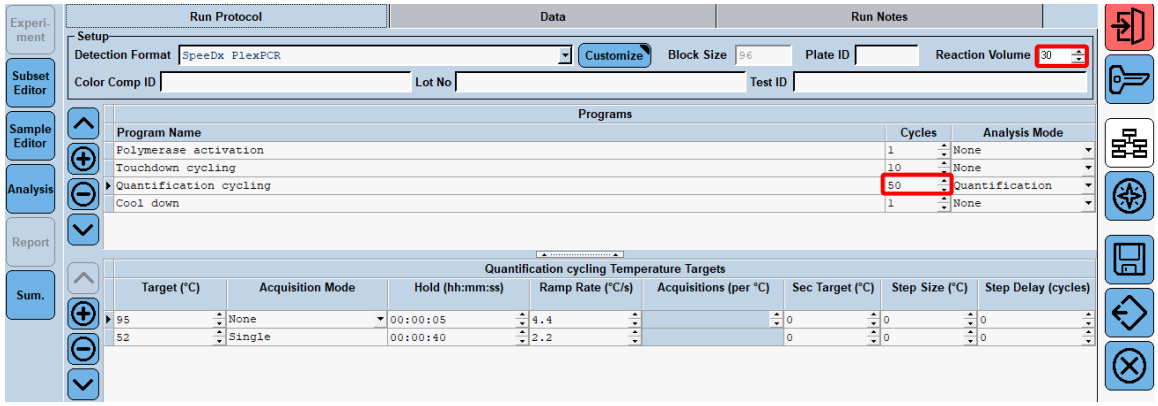
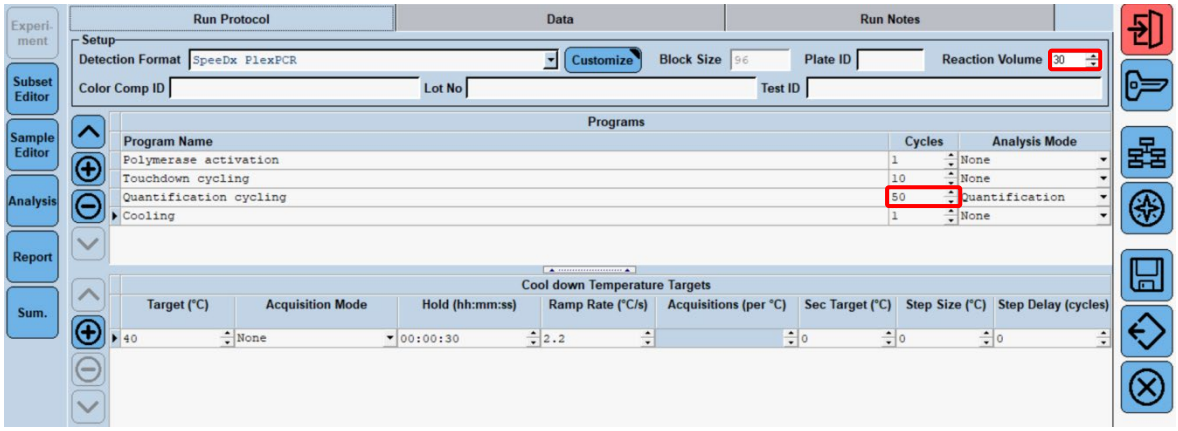


Figure 16. Thermocycling Program (30 µl reaction) – Cooling



> **Start Run**

When the cycling program has finished, export a ". ixo" file for analysis in the ***Resistance****Plus*® GC (LC480) analysis software.

Select **Export**

Save in an easily identifiable location

21.2 Colour Compensation for LightCycler® 480 Instrument II

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

For analysis using the software, the Sample Name of the colour compensation reactions must be labelled as shown in **Table 48**.

When the cycling program has finished, export a ". ixo" file for analysis in the ***Resistance****Plus*® GC (LC480) analysis software.

Select **Export**

Save in an easily identifiable location and name as **"SpeeDx PlexPCR"**

Table 48. Sample Name for colour compensation reactions for the analysis software

Reactions	BLANK	488 mix	510 mix	580 mix	610 mix	640 mix	660 mix
Dominant Channel	Water	440-488	465-510	533-580	533-610	533-640	610-660
Sample Name	BLANK	440-488	465-510	533-580	533-610	533-640	610-660

21.3 Interpretation of results

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

Refer to **Section 29** for instructions on using the ***Resistance****Plus*® GC (LC480) analysis software.

22 Appendix 2: cobas z 480 analyser programming for reaction volume 20 µl

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

The ***Resistance****Plus*® GC$_{(610)}$ kit contains dyes for the cobas z 480 analyser. The ***Plex****PCR*® Colour Compensation kit (SpeeDx Cat no 90001 / Roche Cat no 09256695001) must be run and applied for z 480 analysis (see **Section 22.2**). This kit can be supplied on request.

22.1 Programming the cobas z 480 analyser (z 480)

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 17**)

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

Table 49. Filter Combinations^

z 480	465-510	540-580	540-610	540-645	610-670

^ These Filter Combinations are the default names for the channels

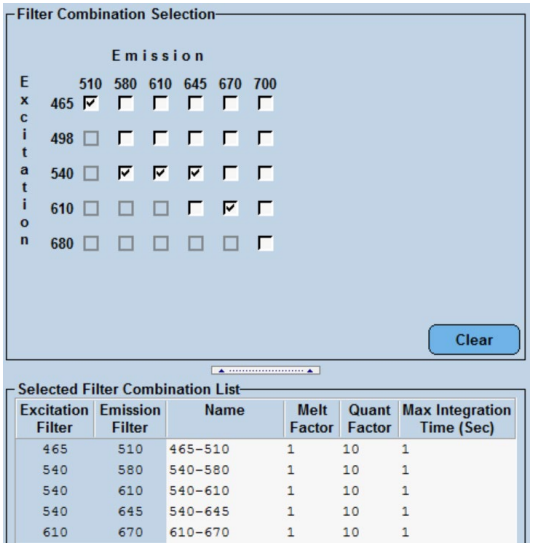
Set the **Selected Filter Combination List** for all channels as:

Melt Factor: 1

Quant Factor: 10

Max Integration Time (sec): 1

Figure 17. 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 18**)

Select **Customize** >

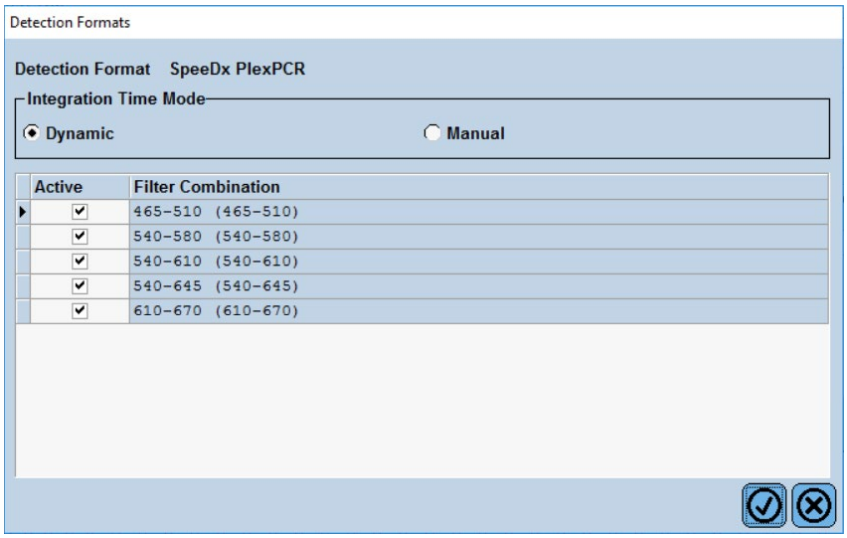
Select **Integration Time Mode** > **Dynamic**

Select the following Active **Filter Combinations** shown in **Table 50.**

Table 50. Channels for *ResistancePlus*® GC targets

Target	*N. gonorrhoeae* *gyrA* S91 (wild type)	*N. gonorrhoeae* *porA*	*N. gonorrhoeae* *gyrA* S91F (mutant)	Internal Control	*N. gonorrhoeae* *opa*
z 480	465-510	540-580	540-610	540-645	610-670

Figure 18. Customize Detection Format



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

Open the **Sample Editor** module

Select well

Edit **Sample Name** to match nametag defined in Assays module of the analysis software (see **Section 29.4**)

Samples are labelled as *Prefix_Suffix* (as shown in **Table 51** and **Figure 19**)

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	Prefix_ (in analysis software)	_Suffix (in analysis software)	Sample Name (in z 480)
Regular sample	Sample	_GC	Sample_GC
Negative Control	NEG	_GC	NEG_GC
Positive Control (MT, GC gyrA S91F) (Pa)	Pa	_GC	Pa_GC
Positive Control (WT, GC gyrA S91) (Pb)	Pb	_GC	Pb_GC

Figure 19. Sample Editor – Assigning nametags to wells

Pos	Filter combination	Color	Repl Of	Sample Name
A1	465-510 (465			Sample 1_GC
A1	540-580 (540			Sample 1_GC
A1	540-610 (540			Sample 1_GC
A1	540-645 (540			Sample 1_GC
A1	610-670 (610			Sample 1_GC
A2	465-510 (465			Pa_GC
A2	540-580 (540			Pa_GC
A2	540-610 (540			Pa_GC
A2	540-645 (540			Pa_GC
A2	610-670 (610			Pa_GC
A3	465-510 (465			Pb_GC
A3	540-580 (540			Pb_GC
A3	540-610 (540			Pb_GC
A3	540-645 (540			Pb_GC
A3	610-670 (610			Pb_GC
A4	465-510 (465			NEG_GC
A4	540-580 (540			NEG_GC
A4	540-610 (540			NEG_GC
A4	540-645 (540			NEG_GC
A4	610-670 (610			NEG_GC

Set **Reaction Volume** > 20 µl

Create the following Program (shown in more detail in **Figure 20 - Figure 23**):

Table 52. Thermocycling Program

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

≠ Default ramp rate (96 well plate)

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

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

Figure 20. Thermocycling Program (20 µl reaction) – Polymerase activation

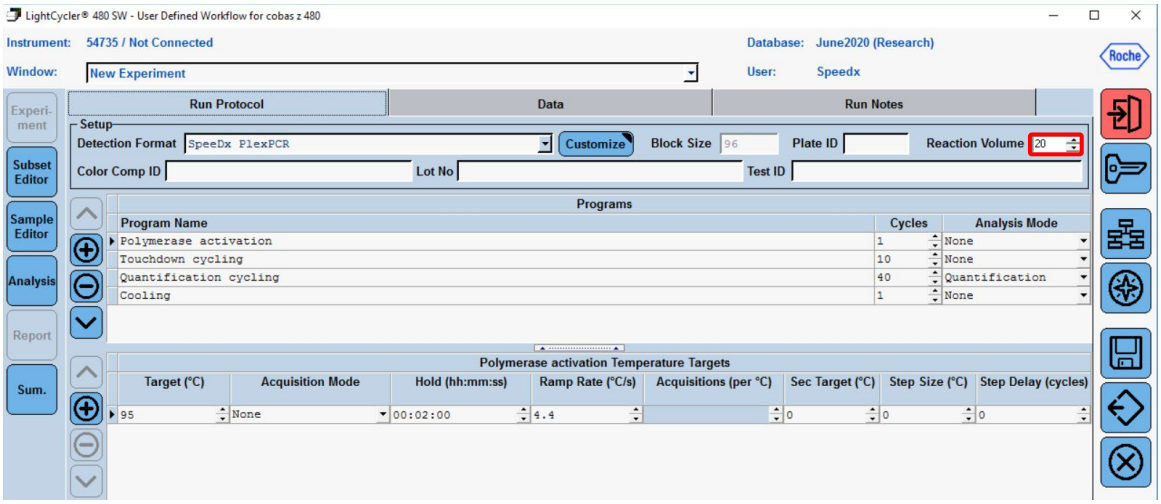


Figure 21. Thermocycling Program (20 µl reaction) – Touchdown cycling

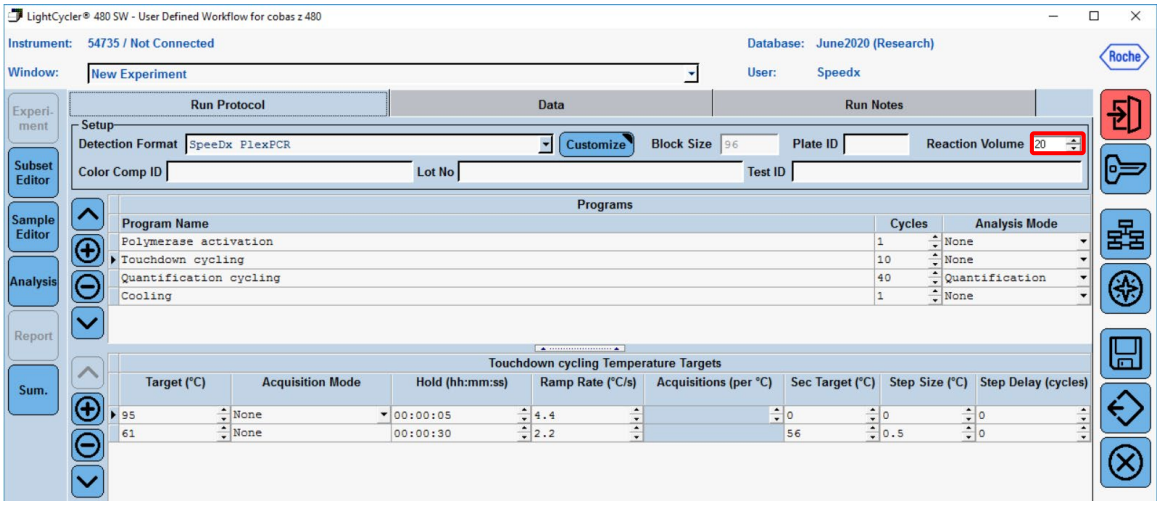


Figure 22. Thermocycling Program (20 µl reaction) – Quantification cycling

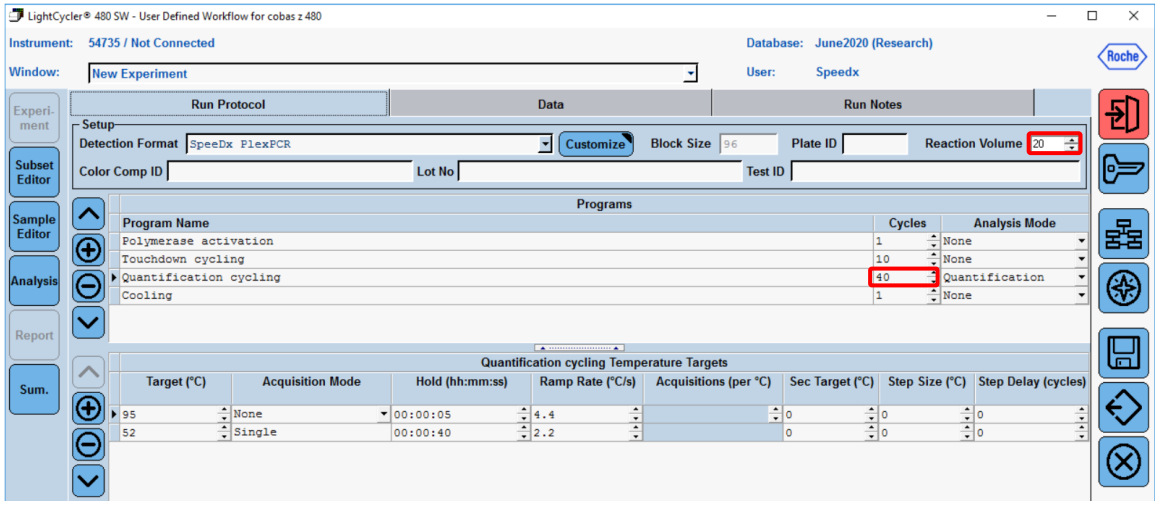
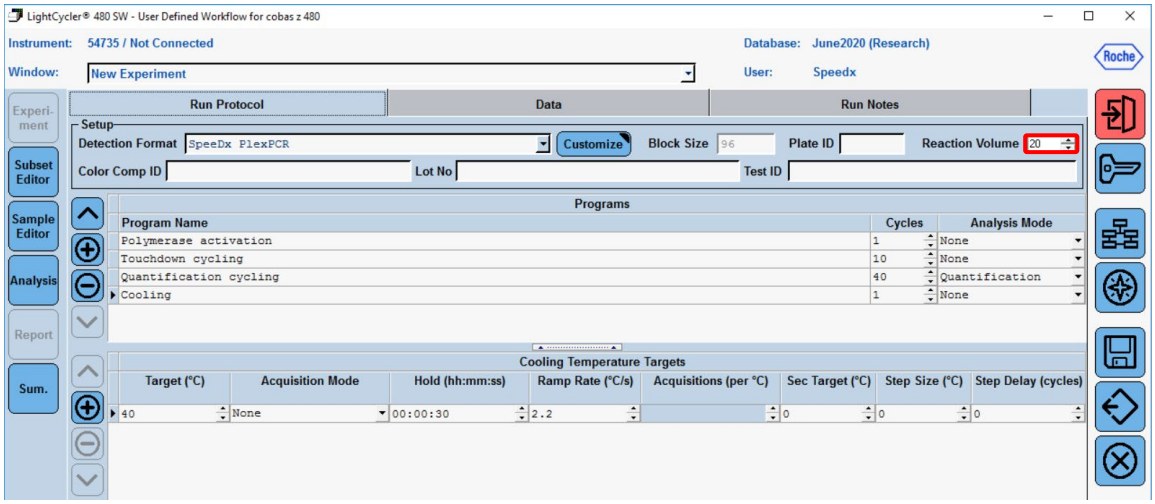


Figure 23. Thermocycling Program (20 µl reaction) – Cooling



> **Start Run**

When the cycling program has finished, export a ". ixo" file for analysis in the ***Resistance****Plus*® GC (z480) analysis software.

Select **Export**

Save in an easily identifiable location

22.2 Colour Compensation for cobas z 480 analyser

NOTE: The ***Plex****PCR*® Colour Compensation (SpeeDx Cat no 90001 / Roche Cat no 09256695001) kit must be run and applied for z480 analysis. This kit can be supplied on request.

For analysis using the software, the Sample Name of the colour compensation reactions must be labelled as shown in **Table 53**.

When the cycling program has finished, export a ". ixo" file for analysis in the ***Resistance****Plus*® GC (z480) analysis software.

Select **Export**

Save in an easily identifiable location and name as **"SpeeDx PlexPCR"**

Table 53. Sample Name for colour compensation reactions for the analysis software

Reactions	BLANK	510 mix	580 mix	610 mix	640 mix	660 mix
Dominant Channel	Water	465-510	540-580	540-610	540-645	610-670
Sample Name	BLANK	465-510	540-580	540-610	540-645	610-670

22.3 Interpretation of results

Data interpretation requires the ***Resistance****Plus*® GC (z480) analysis software. The analysis software can be supplied on request, contact your Roche representative for more information.

Refer to **Section 29** for instructions on using the ***Resistance****Plus*® GC (z480) analysis software.

23 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.

23.1 Programming the Applied Biosystems® 7500 Fast

Select **Advanced Setup**

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

Name the experiment

Instrument > 7500 Fast (96 Wells)

Type of experiment > Quantitation – Standard Curve

Reagents > Other

Ramp Speed > Standard

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

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

Define Targets as shown below (define colours as required)

Table 54. Define Targets

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

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

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

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

Define Samples Add or edit **Sample Name** to match nametags defined in Assays module of the analysis software (**Section 29.4**)

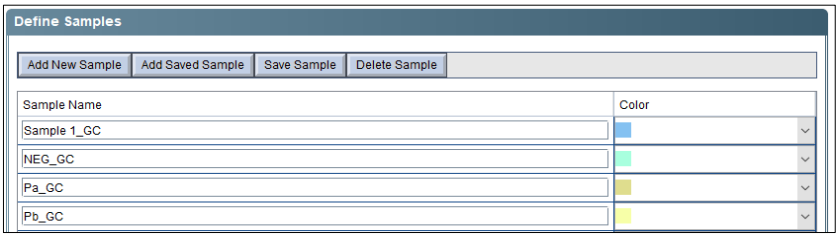
Samples are labelled as *Prefix_Suffix* (as shown in **Table 55** and **Figure 24**)

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

Table 55. Sample nametags for analysis software

Sample Type	Prefix_ (in analysis software)	_Suffix (in analysis software)	Sample Name (in LC480)
Regular sample	Sample	_GC	Sample_GC
Negative Control	NEG	_GC	NEG_GC
Positive Control (MT, GC gyrA S91F) (Pa)	Pa	_GC	Pa_GC
Positive Control (WT, GC gyrA S91) (Pb)	Pb	_GC	Pb_GC

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 56** and **Figure 25** and **Figure** 26) and Tabular View (**Figure 27**):

Table 56. Thermocycling Program

Program Name	Cycles	Target °C	Hold	Ramp≠
Polymerase activation	1	95°C	2 min	100%
Touch down cycling: Step down -0.5°C/cycleδ	10	95°C	5 s	100%
		61°C – 56.5°Cδ	30 s	100%
Quantification cycling+: Acquisition/Detection	40	95°C	5 s	100%
		52°C+	40 s	100%

≠ Default ramp rate

δ Enable AutoDelta: -0.5°C/cycle

+ Collect data on hold

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

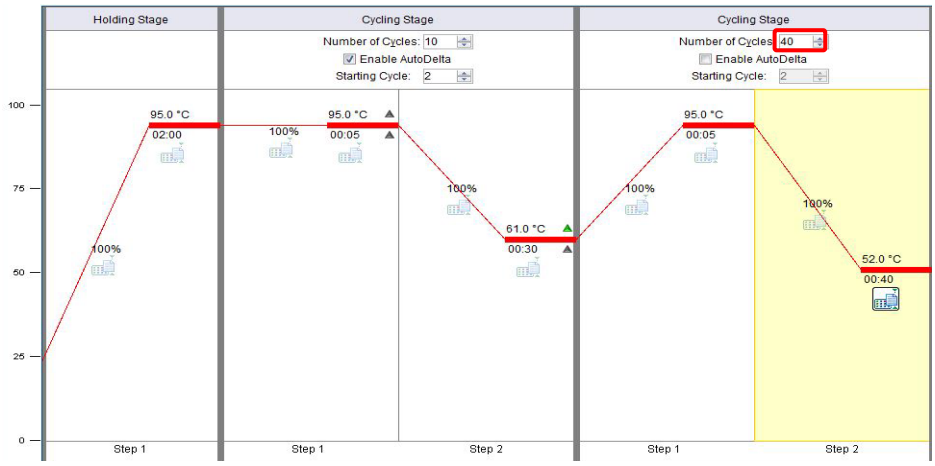


Figure 26. Run method (20 µl reaction) – Graphical View – Enable AutoDelta

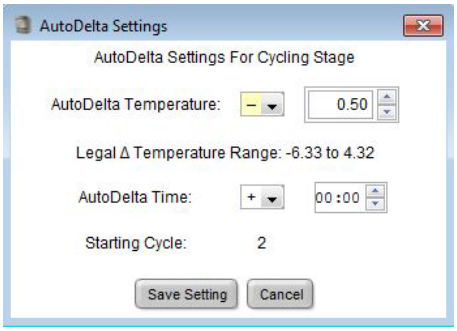
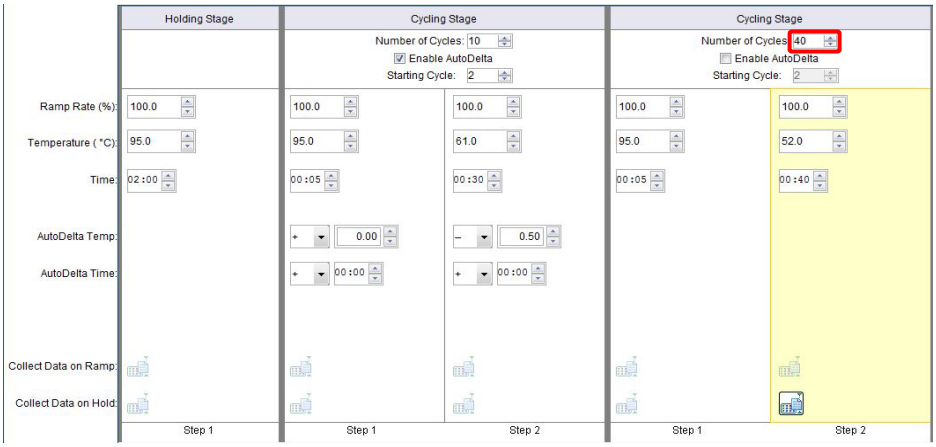


Figure 27. Run method (20 µl reaction) – Tabular View



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

Select **Start Run**

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 29** for instructions on using the ***ResistancePlus***® GC (7500) analysis software.

24 Appendix 4: Applied Biosystems® 7500 Fast programming for reaction volume 30 µl

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

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

24.1 Programming the Applied Biosystems® 7500 Fast

Select **Advanced Setup**

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

Name the experiment

Instrument > 7500 Fast (96 Wells)

Type of experiment > Quantitation – Standard Curve

Reagents > Other

Ramp Speed > Standard

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

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

Define Targets as shown below (define colours as required)

Table 57. Define Targets

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

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

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

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

Define Samples

Add or edit **Sample Name** to match nametags defined in Assays module of the analysis software (**Section 29.4**)

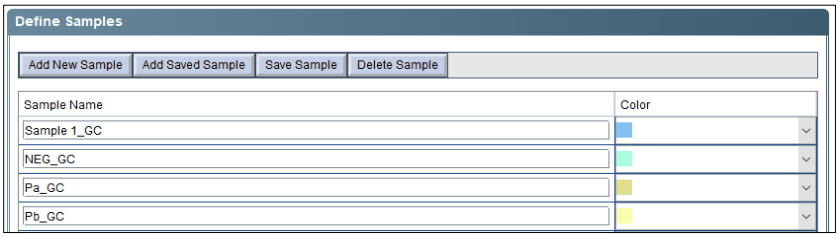
Samples are labelled as *Prefix_Suffix* (as shown in **Table 58** and **Figure 28**)

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	Prefix_ (in analysis software)	_Suffix (in analysis software)	Sample Name (in 7500 Fast)
Regular sample	Sample	_GC	Sample_GC
Negative Control	NEG	_GC	NEG_GC
Positive Control (MT, GC gyrA S91F) (Pa)	Pa	_GC	Pa_GC
Positive Control (WT, GC gyrA S91) (Pb)	Pb	_GC	Pb_GC

Figure 28. 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 59** and **Figure 29** and **Figure** 30) and Tabular View (**Figure 31**):

Table 59. Thermocycling Program

Program Name	Cycles	Target °C	Hold	Ramp≠
Polymerase activation	1	95°C	2 min	100%
Touch down cycling: Step down -0.5°C/cycleδ	10	95°C	5 s	100%
		61°C – 56.5°Cδ	30 s	100%
Quantification cycling+: Acquisition/Detection	50	95°C	5 s	100%
		52°C+	40 s	100%

≠ Default ramp rate

δ Enable AutoDelta: -0.5°C/cycle

+ Collect data on hold

Figure 29. Run method (30 µl reaction) – Graphical View

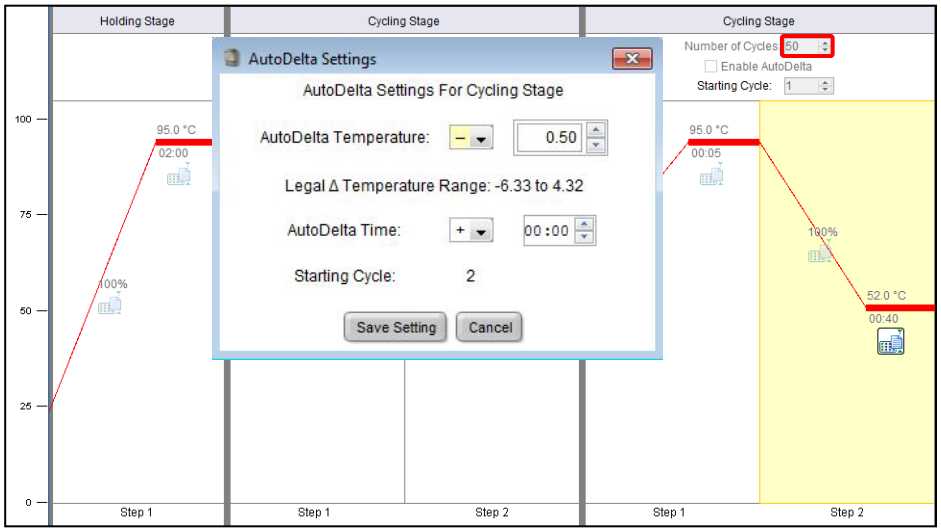
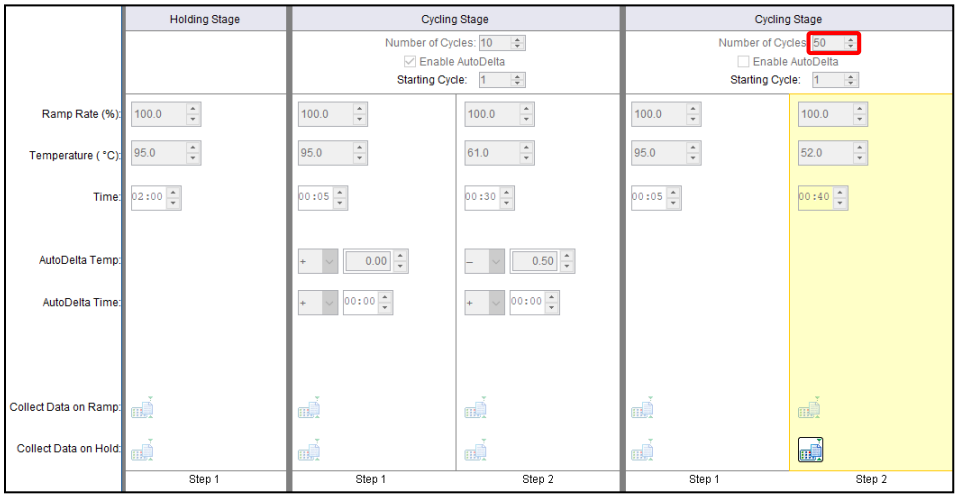


Figure 30. Run method (30 µl reaction) – Graphical View – Enable AutoDelta

Figure 31. Run method (30 µl reaction) – Tabular View



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

Select **Start Run**

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 29** for instructions on using the ***ResistancePlus***® GC (7500) analysis software.

25 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 ***Resistance****Plus*® 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.

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

Select Create New Document

In **New Document Wizard** select the following (**Figure 32**):

Assay > Standard Curve (Absolute Quantification)

Container > 96-Well Clear

Template > Blank document

Run mode > Standard 7500

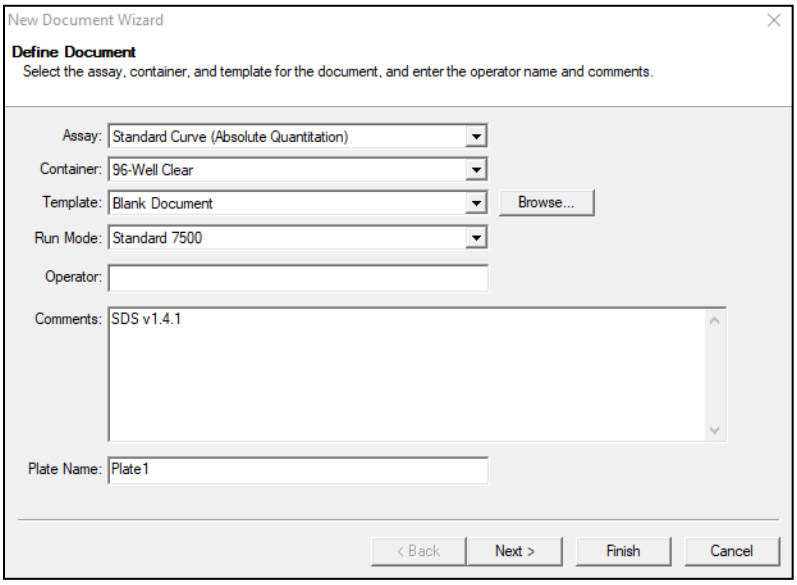
Operator > Enter Operator's name

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

Plate Name > Assign a unique name to the run file

Select **Next**

Figure 32. New Document Wizard window



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

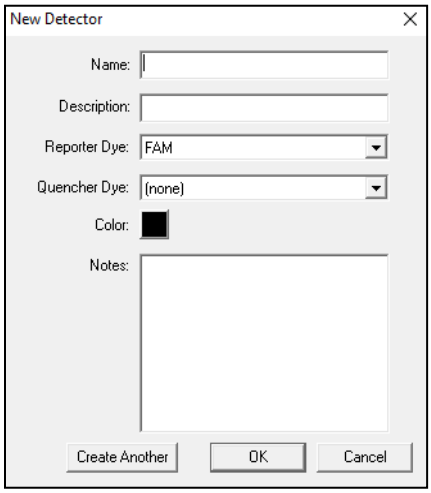
Define detectors as shown below (define colours as required) (**Table 60** and **Figure 33**)

Table 60. 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 33. New Detector window

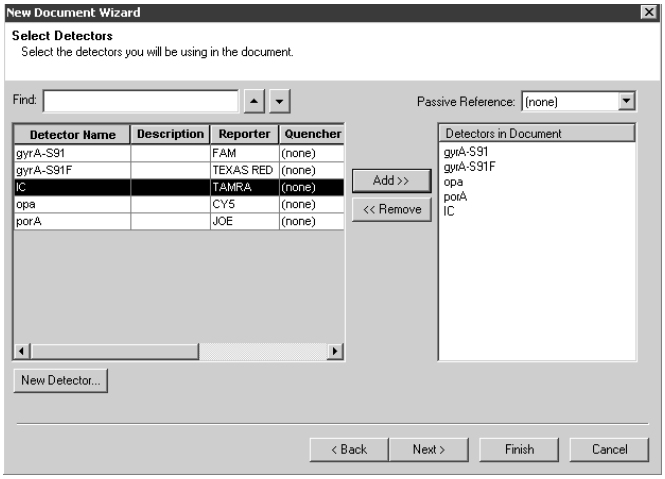


Select Detectors (**Figure 34**)

Select detectors and **Add** to Document

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

Figure 34. Select Detectors window



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 29***)*

Select **Next**

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

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

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

Edit **Sample Name** to match nametags defined in Assays module of the analysis software (**Section 29.4**)

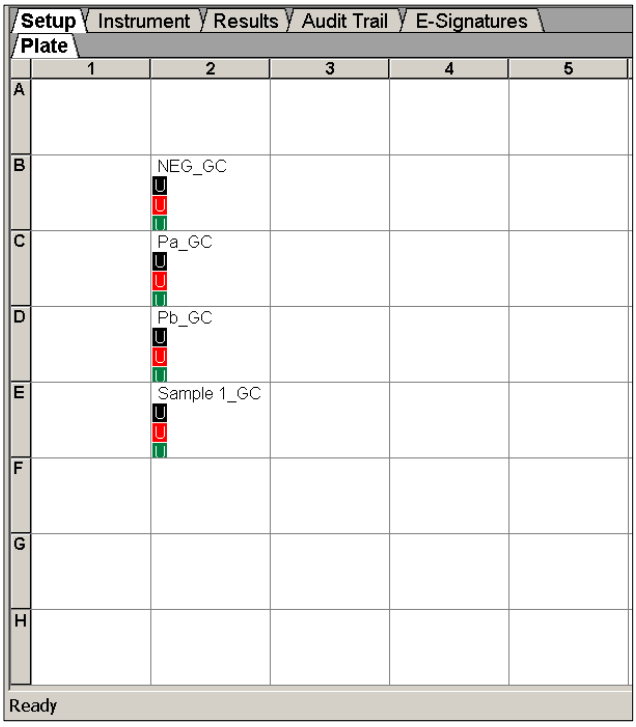
Samples are labelled as *Prefix_Suffix* (as shown in **Table 61** and **Figure 35**)

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

Table 61. Sample nametags for analysis software

Sample Type	Prefix_ (in analysis software)	Suffix_ (in analysis software)	Sample Name (in 7500 Fast Dx)
Regular sample	Sample	_GC	Sample_GC
Negative Control	NEG	_GC	NEG_GC
Positive Control (MT, GC gyrA S91F) (Pa)	Pa	_GC	Pa_GC
Positive Control (WT, GC gyrA S91) (Pb)	Pb	_GC	Pb_GC

Figure 35. Setup plate view – Assigning nametags to wells



Select **Next**

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 62** and **Figure 36** and **Figure 37)**

Table 62. Thermal Cycler Protocol				
Program Name	**Cycles**	**Target °C**	**Hold**	**Ramp≠**
Polymerase activation	1	95°C	2 min	100%
Touch down cycling: Step down -0.5°C/cycleδ	10	95°C	5 s	100%
		61°C – 56.5°Cδ	30 s	100%
Quantification cycling+: Acquisition/Detection	40	95°C	5 s	100%
		52°C+	40 s	100%

≠ Default ramp rate

δ Enable AutoDelta: -0.5°C/cycle

\+ Collect data on hold

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

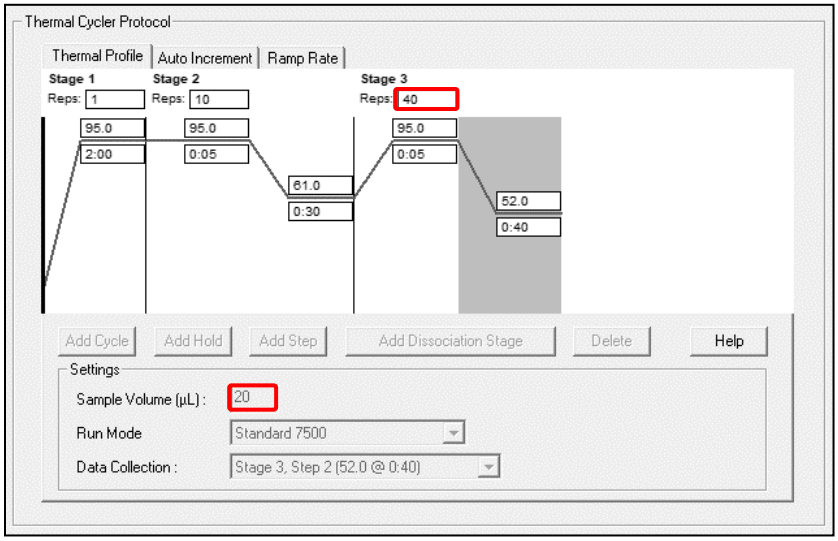
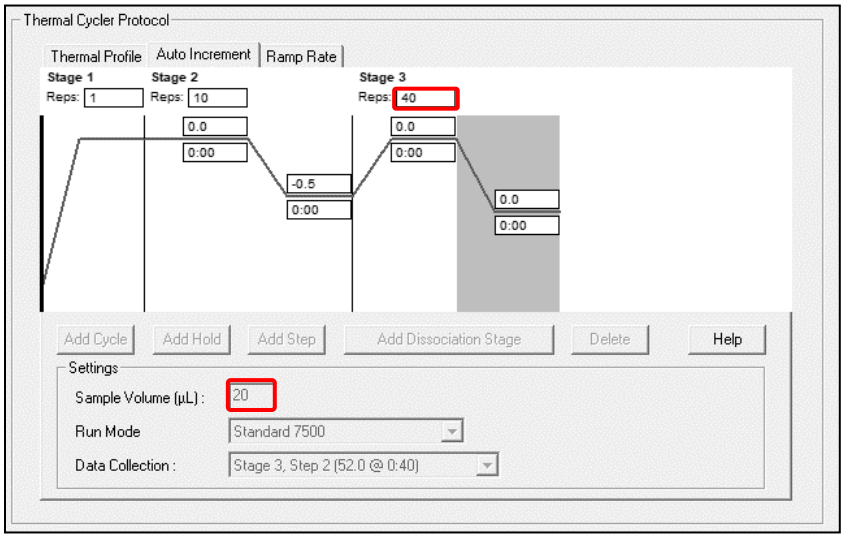


Figure 37. Thermal Cycler Protocol (20 µl reaction) – Auto Increment



25.2 Interpretation of results

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

Refer to **Section 29** for instructions on using the ***Resistance****Plus*® GC (7500) analysis software.

26 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.

26.1 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 Quantification)

Container > 96-Well Clear

Template > Blank document

Run mode > Standard 7500

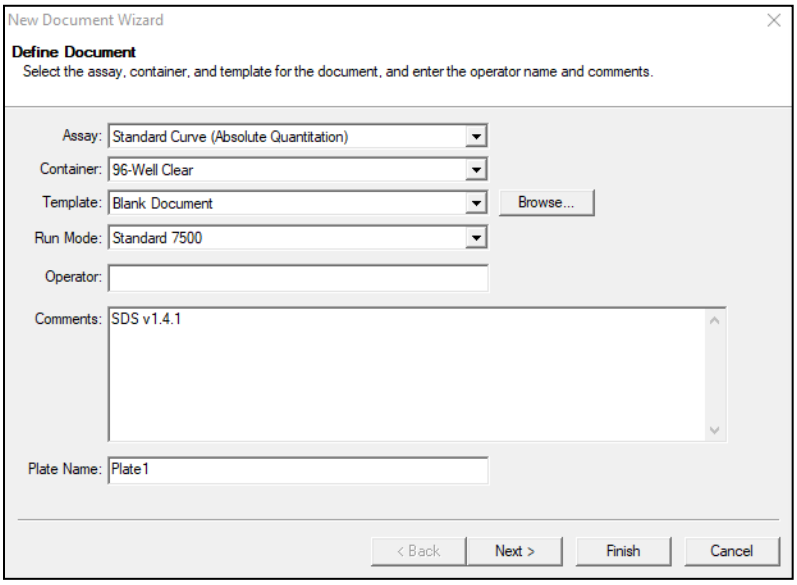
Operator > Enter Operator's name

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

Plate Name > Assign a unique name to the run file

Select **Next**

Figure 38. New Document Wizard window



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

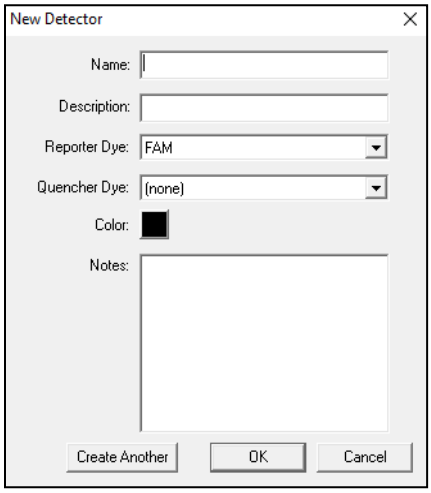
Define detectors as shown below (define colours as required) (**Table 63** and **Figure 39**)

Table 63. 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

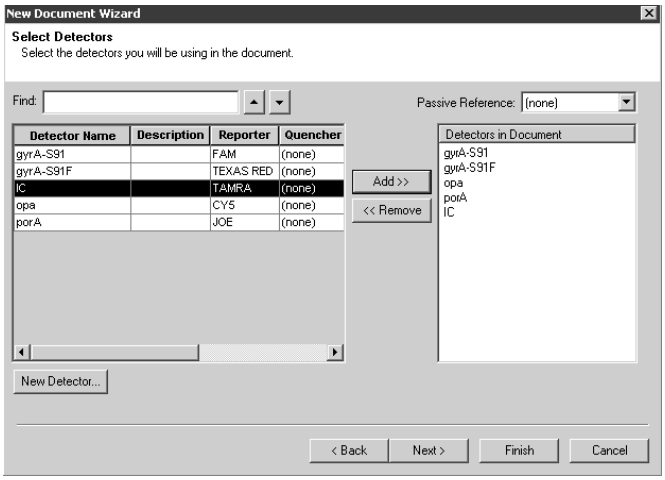


Select Detectors (**Figure 40**)

Select detectors and **Add** to Document

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

Figure 40. Select Detectors window



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 29***)*

Select **Next**

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

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

Edit **Sample Name** to match nametags defined in Assays module of the analysis software (**Section 29.4**)

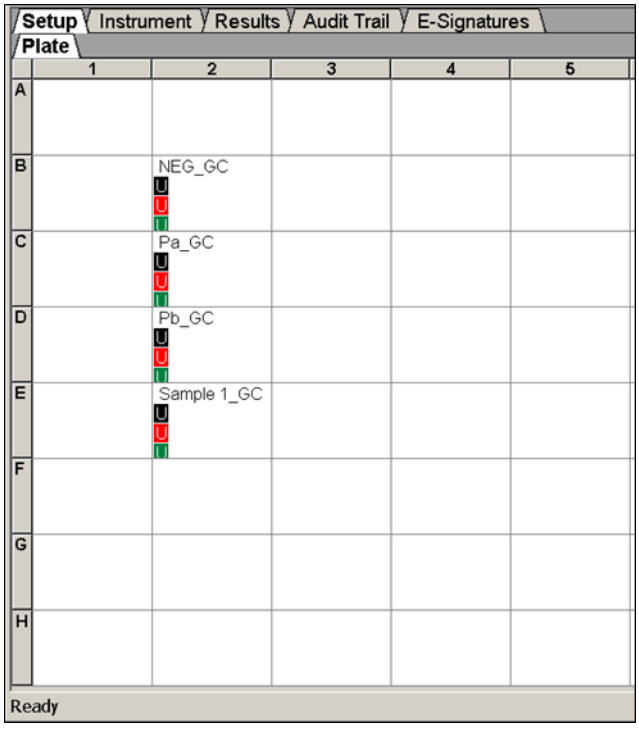
Samples are labelled as *Prefix_Suffix* (**Table 64** and **Figure 41**)

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

Table 64. Sample nametags for analysis software

Sample Type	Prefix_ (in analysis software)	Suffix_ (in analysis software)	Sample Name (in 7500 Fast Dx)
Regular sample	Sample	_GC	Sample_GC
Negative Control	NEG	_GC	NEG_GC
Positive Control (MT, GC gyrA S91F) (Pa)	Pa	_GC	Pa_GC
Positive Control (WT, GC gyrA S91) (Pb)	Pb	_GC	Pb_GC

Figure 41. Setup plate view – Assigning nametags to wells



Select **Next**

In **Instrument** tab

In **Settings** box

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

Create the following Thermal Cycler Protocol (**Table 65** and **Figure 42** and **Figure 43**)

Table 65. Thermal Cycler Protocol				
Program Name	**Cycles**	**Target °C**	**Hold**	**Ramp≠**
Polymerase activation	1	95°C	2 min	100%
Touch down cycling: Step down -0.5°C/cycleδ	10	95°C	5 s	100%
		61°C – 56.5°Cδ	30 s	100%
Quantification cycling+: Acquisition/Detection	50	95°C	5 s	100%
		52°C+	40 s	100%

≠ Default ramp rate

δ Enable AutoDelta: -0.5°C/cycle

+ Collect data on hold

Figure 42. Thermal Cycler Protocol (30 µl reaction) – Thermal Profile

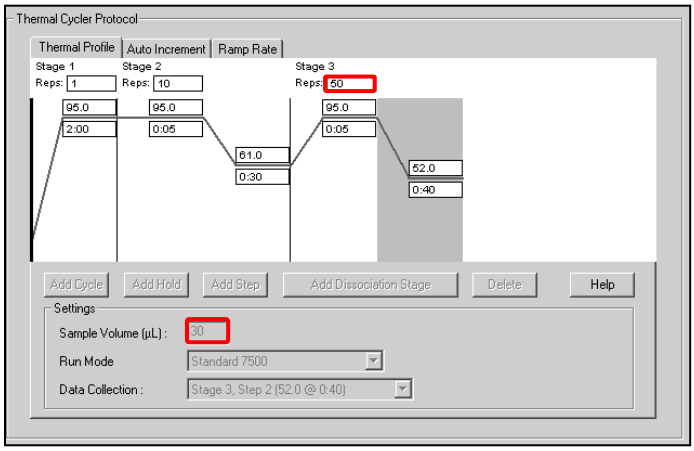
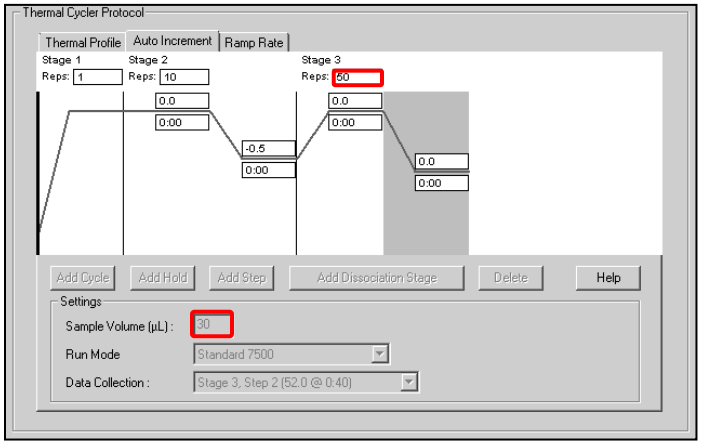


Figure 43. Thermal Cycler Protocol (30 µl reaction) – Auto Increment



26.2 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 29** for instructions on using the ***ResistancePlus***® GC (7500) analysis software.

27 Appendix 8: Bio-Rad CFX96™ IVD & 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.

27.1 Programming the CFX96™ IVD 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 44**)

Set **Sample Volume** > 20 µl

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

For Touch down cycling, select Step 3 and select **Step options** > Increment: -0.5°C/cycle (shown in more detail in **Figure** 45).

Table 66. Thermocycling Program

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

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

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

Figure 44. Thermocycling Protocol – Graphical view

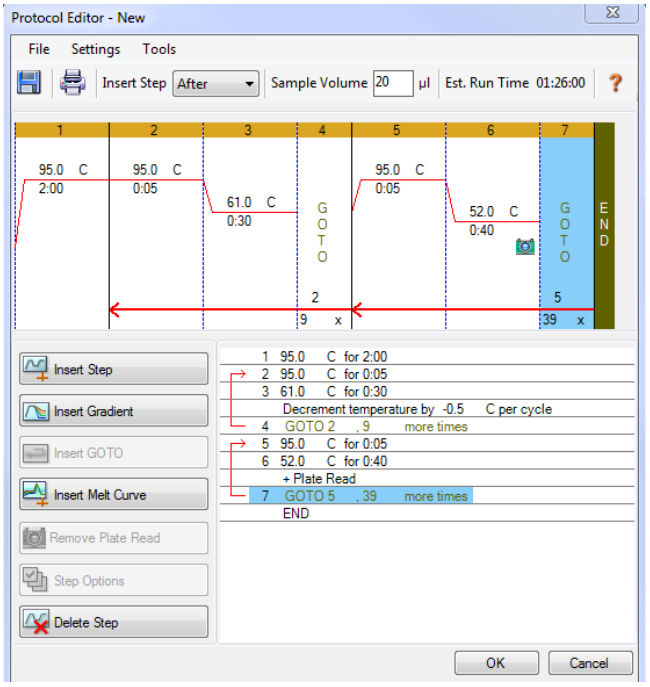
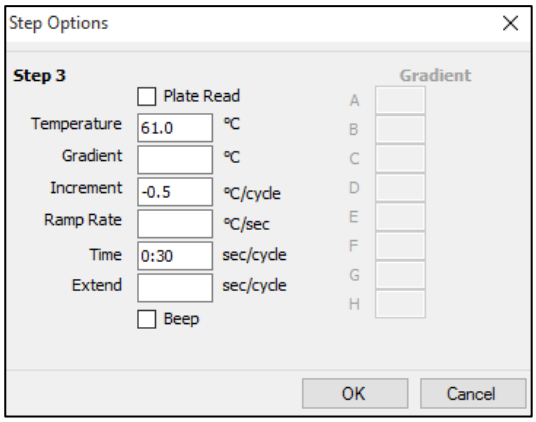


Figure 45. Step Options



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 67**)

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

Save plate

Table 67. Channels for *ResistancePlus*® GC$_{(675)}$ targets

***N. gonorrhoeae* *gyrA* S91 (wild type)**	***N. gonorrhoeae* *porA***	***N. gonorrhoeae* *gyrA* S91F (mutant)**	**Internal Control**	***N. gonorrhoeae* *opa***
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, assign nametags to the wells on the plate

Open the **Plate Setup** module

Select well

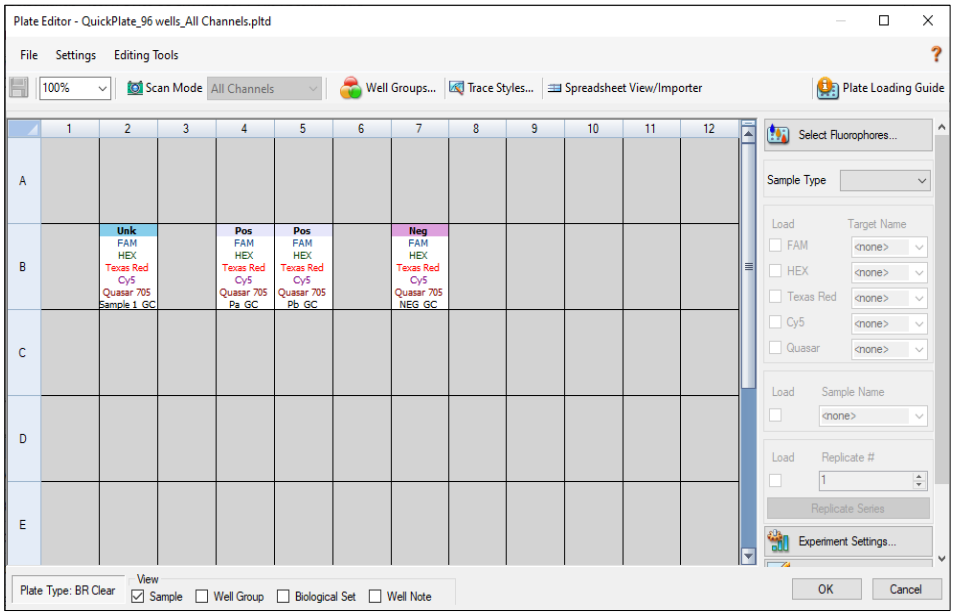
Edit **Sample Name** to match nametag defined in Assays module of the analysis software (**Section 29.4**)

Samples are labelled as *Prefix_Suffix* (as shown in **Table 68** and **Figure 46**)

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

Table 68. Sample nametags for analysis software			
Sample Type	**Prefix_ (in analysis software)**	**_Suffix (in analysis software)**	**Sample Name (in CFX96)**
Regular sample	Sample	_GC	Sample_GC
Negative Control	NEG	_GC	NEG_GC
Positive Control (GC gyrA S91F mutant) (Pa)	Pa	_GC	Pa_GC
Positive Control (GC gyrA S91 wild type) (Pb)	Pb	_GC	Pb_GC

Figure 46. Sample Editor – Assigning nametags to wells



27.2 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 29** for instructions for using the ***ResistancePlus***® GC (CFX) analysis software.

28 Appendix 7: Bio-Rad CFX96™ IVD & 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.

28.1 Programming the CFX96™ IVD 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 47**)**:**

Set **Sample Volume** > 30 µl

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

For Touch down cycling, select Step 3 and select **Step options** > Increment: -0.5°C/cycle (shown in more detail in **Figure 48**).

Table 69. 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 47. Thermocycling Protocol – Graphical view

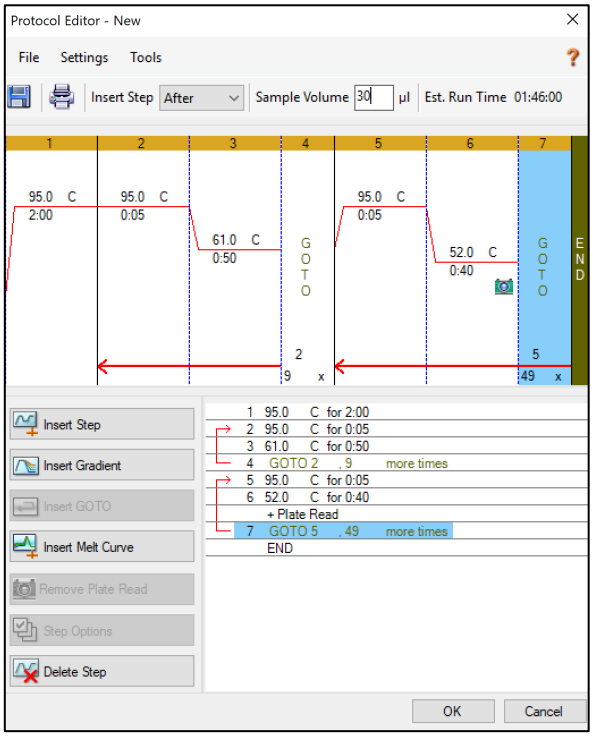
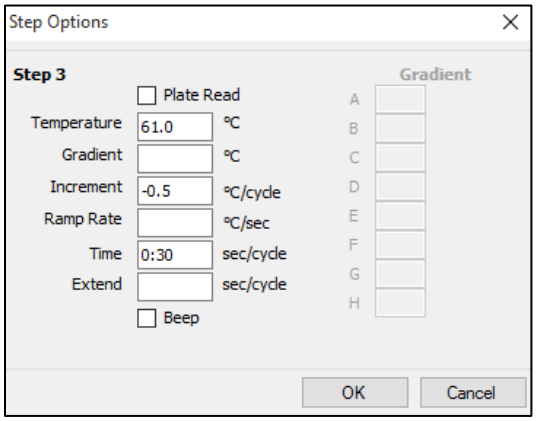


Figure 48. Step Options



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 70**)

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

Save plate

Table 70. Channels for *ResistancePlus*® GC(675) targets

***N. gonorrhoeae* *gyrA* S91 (wild type)**	***N. gonorrhoeae* *porA***	***N. gonorrhoeae* *gyrA* S91F (mutant)**	**Internal Control**	***N. gonorrhoeae* *opa***
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, assign nametags to the wells on the plate

Open the **Plate Setup** module

Select well

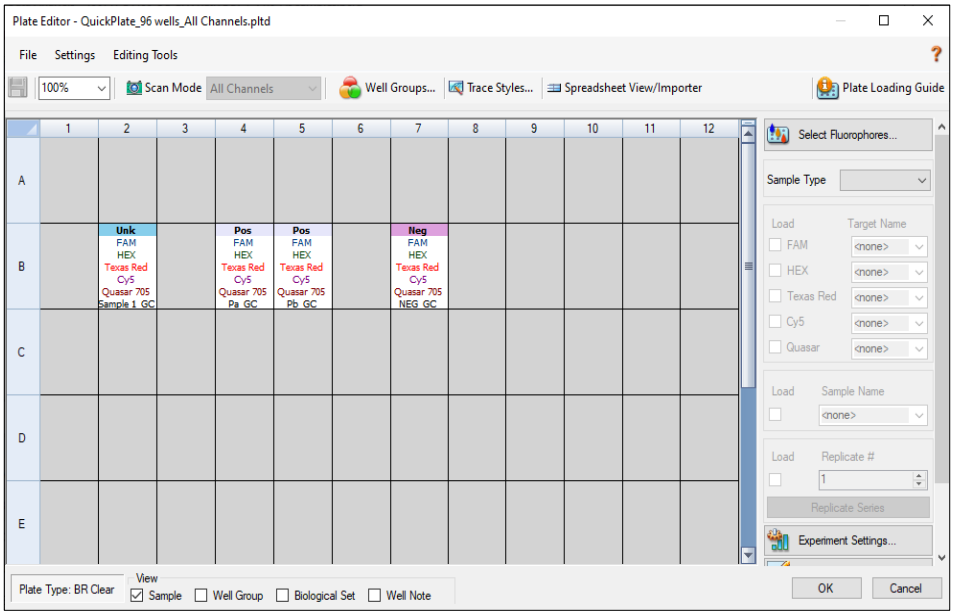
Edit **Sample Name** to match nametag defined in Assays module of the analysis software (**Section 29.4**)

Samples are labelled as *Prefix_Suffix* (as shown in **Table 71** and **Figure 49**)

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

Table 71. Sample nametags for analysis software			
Sample Type	**Prefix_ (in analysis software)**	**_Suffix (in analysis software)**	**Sample Name (in CFX96)**
Regular sample	Sample	_GC	Sample_GC
Negative Control	NEG	_GC	NEG_GC
Positive Control (GC gyrA S91F mutant) (Pa)	Pa	_GC	Pa_GC
Positive Control (GC gyrA S91 wild type) (Pb)	Pb	_GC	Pb_GC

Figure 49. Sample Editor – Assigning nametags to wells



28.2 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 29** for instructions for using the ***ResistancePlus***® GC *(CFX)* analysis software.

29 Appendix A: Result interpretation

Data interpretation requires the ***Resistance****Plus*® GC analysis software. See **Table 72** 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 72. *ResistancePlus*® GC analysis software

Cat no	Analysis software*	Real-time PCR instrument
99010	***Resistance****Plus*® GC (LC480)	LC480 II
99019	***Resistance****Plus*® GC (z480)	z 480
99009	***Resistance****Plus*® GC (7500)	7500 Fast and 7500 Fast Dx
99015	***Resistance****Plus*® GC (CFX)	CFX96 IVD 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.

29.1 FastFinder platform – Minimum IT requirements

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

Hardware requirements

PC (Mac computers are not supported)

Processor: 2 GHz, 2 GB RAM

Disk space: 10Gb

Internet Connection Cable or DSL, proxy not supported

Min. screen resolution: 1366x768 pixels

Supported client operating system

Operating system	Supported editions
Windows 10	32-bit and 64-bit
Windows 8.1	32-bit, 64-bit, and ARM
Windows 8	32-bit, 64-bit, and ARM
Windows 7 SP1	32-bit and 64-bit
Windows Vista SP2	32-bit and 64-bit

Supported browsers

FastFinder Administrator account users require one of the following:

• Internet Explorer 11 or newer

• Microsoft Edge 25 or newer

• Firefox 45 or newer

• Google Chrome 47 or newer.

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

Software requirements

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

Antivirus settings

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

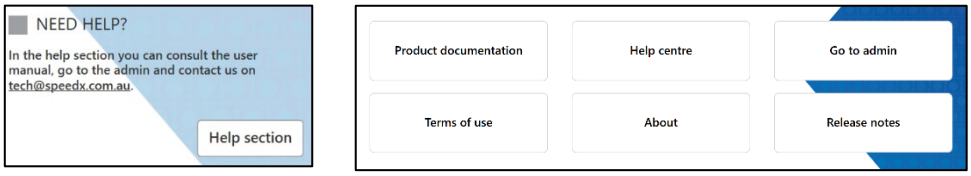
Firewall requirements

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

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

To access the Help menu

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



29.2 Device set up (new user or device)

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

Open **FastFinder**

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

29.2.1 Colour Compensation

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

- Select the LC480 II or z 480 device
  - > In the **Colour Compensation** section, select
  - > Select the colour compensation file for the device from the directory

NOTE: See **Section 20.2** and **Section 22.2** for more information on Colour Compensation

- To change the Current directory
  > Select **Browse** and select the folder containing relevant files
- Select **Next**
- Select **ResistancePlus GC (LC480)** or **ResistancePlus GC (z480)** from the list to link to this assay
- Select **Save**

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

In the device colour compensation section

- Next to the file name, select 
- Select 

to activate or deactivate a colour compensation file for an assay
- Select **Save**

29.3 Assay plug-in (new user)

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

Open **FastFinder**

- Select **Assays** from the workflow bar
- Select **Add**
  > For LC480 II > Select **ResistancePlus GC (LC480)** from the list
  > For z 480 > Select **ResistancePlus GC (z480)** from the list
  > For 7500 Fast and 7500 Fast Dx > Select **ResistancePlus GC (7500)** from the list
  > For CFX96 IVD and CFX96 Touch > Select **ResistancePlus GC (CFX)** from the list
- Select **Add**

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

> In General assay information
> Select  Versions
> Select 

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

29.4 Sample naming

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

Select **Assays** from the workflow bar

- In the Sample type nametags (prefix), select 
  > Select  to add a nametag to define sample type nametags (Negative control, Positive control/s and Regular sample)
  > Add desired word, acronym or letter to text box
  > Select **Save**
- In the Mix definition nametags (suffix), select 

> Select  to add a nametag to define the mix name

> Add desired word, acronym or letter to text box

> Select **Save**

- In the instrument software (before or after run is completed) assign the same nametag to appropriate wells

> For **LC480 II** see **Section 20** and **Section 21** for instructions on programming sample nametags in the run file

> For **z 480** see **Section 22** for instructions on programming sample nametags in the run file

> For **7500 Fast** see **Section 23** and **Section 24** for instructions on programming sample nametags in the run file

> For **7500 Fast Dx** see **Section 25** and **Section 26** for instructions on programming sample nametags in the run file

> For **CFX96 IVD** and **CFX96 Touch** see **Section 27** and **Section 28** 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.

29.5 Adding mix lot numbers

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

- Select **Assays** from the workflow bar

> In the **Assay Lot:** select  to add a new lot or select  to edit an existing lot

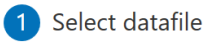
> Once added, lot numbers will become available in the analysis module

> Select 

to show all lot numbers or only active lot numbers

29.6 Analysis

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



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

- To change the **Current directory**

> Select **Browse** and select the folder containing relevant files

- Select run (data) file from the list

> Select **Next step**

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

- For **LC480 II** > Select **ResistancePlus GC (LC480)**
- For **z480** > Select **ResistancePlus GC (z480)**
- For **7500 Fast** and **7500 Fast Dx** > Select **ResistancePlus GC (7500)**
- For **CFX96 IVD** 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)

- Select **Next step**

To save the plate layout as a template for future use

- Select wells and assign types of samples
  > Select  to Save template
- Specify template name for future use
  > Select **Save**

To load a previously saved plate template

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

3 Configure assay(s)

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

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

- For **7500 Fast** and **7500 Fast Dx** > Select **ResistancePlus GC (7500)**
  > Select **Assay Lot** from the drop down menu
  > Select **Analyse**

- For **CFX96 IVD** and **CFX96 Touch** > Select **ResistancePlus GC (CFX)**
  > Select **Assay Lot** from the drop down menu
  > Select **Analyse**

29.7 Results

See **Table 73** for a summary of possible reported sample results.

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

To resolve any uncertain results

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

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

To finalise analysis and prevent further user edits

- > Select **Authorise Analysis**
- > Select **Yes** to confirm

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

29.8 Reference curve

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

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

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

29.9 Overview of results

Table 73. Example results for interpretation of the *ResistancePlus*® GC analysis software

	Well	Name	Assay	Result	Cq Values^	Overall results
	A1	Sample 1	ResistancePlus GC	Negative	CHANNEL D: 25.31	Sample 1 - Negative N. gonorrhoeae not detected, IC valid.
	A2	Sample 2	ResistancePlus GC	Positive	CHANNEL A: 19.60 CHANNEL B: 18.89 CHANNEL C: 28.49 CHANNEL D: 28.04 CHANNEL E: 17.14	Sample 2 - Positive N. gonorrhoeae detected, gyrA mutation not detected.

	A3	Sample 3	ResistancePlus GC	Positive	CHANNEL A: 31.34 CHANNEL B: 27.02 CHANNEL D: 27.91 CHANNEL E: 25.18	**Sample 3 - Positive** N. gonorrhoeae detected, gyrA mutation not detected.
	A4	Sample 4	ResistancePlus GC	Positive	CHANNEL A: 33.51 CHANNEL B: 20.92 CHANNEL C: 24.40 CHANNEL D: 27.62 CHANNEL E: 22.90	**Sample 4 - Positive** N. gonorrhoeae detected, gyrA mutation detected.
	A5	Sample 5	ResistancePlus GC	Positive	CHANNEL B: 26.60 CHANNEL C: 30.76 CHANNEL D: 28.20 CHANNEL E: 27.81	**Sample 5 - Positive** N. gonorrhoeae detected, gyrA mutation detected.
	A6	Sample 6	ResistancePlus GC	Invalid	CHANNEL D: 36.71	**Sample 6 - Invalid** IC invalid, repeat test.[1]
	A7	Sample 7	ResistancePlus GC	Positive	CHANNEL D: 27.65 CHANNEL E: 32.35	**Sample 7 - Positive** N. gonorrhoeae detected, gyrA indeterminate.
ⓘ	C1	Sample 8 (Flagged to resolve)	ResistancePlus GC	Positive	CHANNEL A: 38.99[2] CHANNEL B: 20.40 CHANNEL D: 26.80 CHANNEL E: 21.50	**Sample 8 - Positive** N. gonorrhoeae detected, gyrA mutation not detected.
⚑	C1	Sample 8 (Resolve to Inconclusive)	ResistancePlus GC	Invalid	CHANNEL A: 38.99[3] CHANNEL B: 20.40 CHANNEL D: 26.80 CHANNEL E: 21.50	**Sample 8 - Invalid** Inconclusive result, repeat test.
	B1	Pa (Mutant type Positive control)	ResistancePlus GC	Positive	CHANNEL A: 30.43 CHANNEL B: 17.38 CHANNEL C: 21.49 CHANNEL E: 17.14	**Pa - Positive** Positive control valid.
	B2	Pb (Wild type Positive control)	ResistancePlus GC	Positive	CHANNEL A: 18.62 CHANNEL B: 15.50 CHANNEL C: 32.52 CHANNEL E: 14.22	**Pb - Positive** Positive control valid.
	B3	N (Negative control)	ResistancePlus GC	Negative		**N - Negative** Negative control valid.

^ Refer to instrument **Table 7** for channel/target information

[1] For IC invalid and inconclusive samples, re-extract and re-test

[2] A sample with an uncertain Cq will be flagged for resolution with ⓘ

[3] A sample resolved to be Inconclusive will be flagged with ⚑

29.10 Exporting results

To export results

> Select **Exports** in the workflow bar

> Export one or more of the following report types: **Cq values list (CSV)**, **Results (CSV)**, **Generic Amplification CSV** or the appropriate LIS-integration file.

> Select **Exports**

To download exports

> Select **Reports** in the workflow bar

> Select files and save

Alternatively export a customised report

> Export **Amplification Curve Analysis (PDF)**

> Select desired included information (graphs, audit trail, results overview)

> Select desired report settings to customise sample order

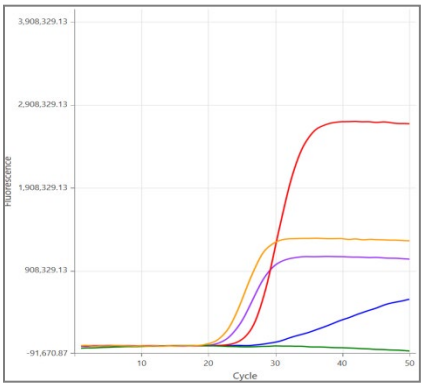
> Select **Exports**

> Open in **Report Viewer** to view, save and print

29.11 Control example graphs

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

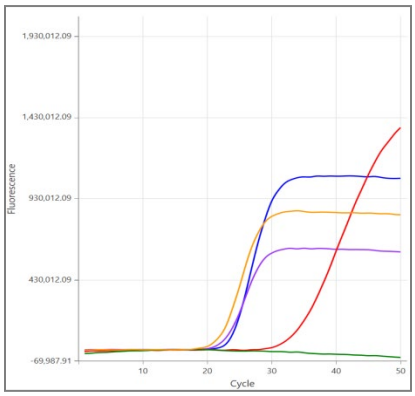
29.11.1 Positive Control (GC gyrA S91F mutant) (Pa)



CHANNEL A **CHANNEL B** **CHANNEL C** **CHANNEL D** **CHANNEL E**

Well	Name	Assay	Result	Cq Values	Overall results
H10	Pa	ResistancePlus GC (7500)	Positive	CHANNEL A: 29.97 CHANNEL B: 22.98 CHANNEL C: 26.60 CHANNEL E: 22.04	**Pa - Positive** Positive control valid.

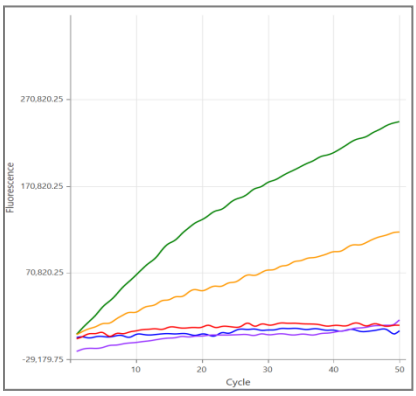
29.11.2 Positive Control (GC gyrA S91 wild type) (Pb)



CHANNEL A **CHANNEL B** **CHANNEL C** **CHANNEL D** **CHANNEL E**

Well	Name	Assay	Result	Cq Values	Overall results
H11	Pb	ResistancePlus GC (7500)	Positive	CHANNEL A: 23.58 CHANNEL B: 22.45 CHANNEL C: 33.96 CHANNEL E: 21.92	**Pb - Positive** Positive control valid.

29.11.3 Negative Control (N)

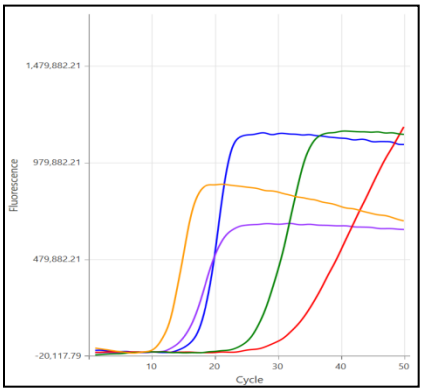


CHANNEL A **CHANNEL B** **CHANNEL C** **CHANNEL D** **CHANNEL E**

Well	Name	Assay	Result	Cq Values	Overall results
H12	N	ResistancePlus GC (7500)	Negative		**N - Negative** Negative control valid.

29.12 Examples

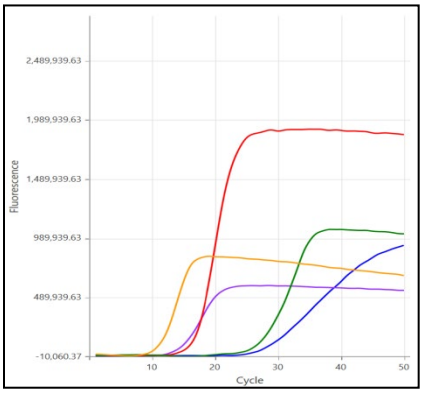
29.12.1 High copy *N. gonorrhoeae* positive, *gyrA* wild type



CHANNEL A CHANNEL B CHANNEL C CHANNEL D CHANNEL E

Well	Name	Assay	Result	Cq Values	Overall results
A1	Sample 11	ResistancePlus GC (7500)	Positive	CHANNEL A: 17.48 CHANNEL B: 14.92 CHANNEL C: 32.36 CHANNEL D: 26.80 CHANNEL E: 11.77	**Sample 11 - Positive** N. gonorrhoeae detected, gyrA mutation not detected.

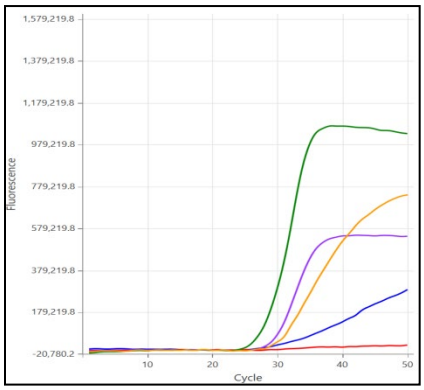
29.12.2 High copy *N. gonorrhoeae* positive, *gyrA* mutant



CHANNEL A CHANNEL B CHANNEL C CHANNEL D CHANNEL E

Well	Name	Assay	Result	Cq Values	Overall results
G7	Sample 12	ResistancePlus GC (7500)	Positive	CHANNEL A: 28.98 CHANNEL B: 14.94 CHANNEL C: 17.64 CHANNEL D: 26.74 CHANNEL E: 12.59	**Sample 12 - Positive** N. gonorrhoeae detected, gyrA mutation detected.

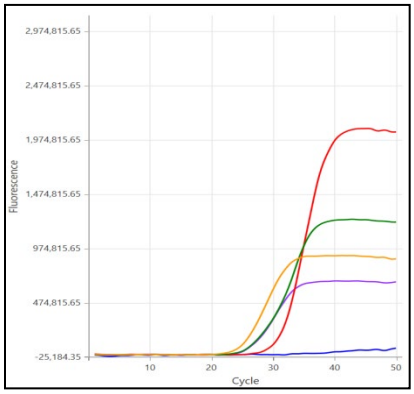
29.12.3 Low copy *N. gonorrhoeae* positive, *gyrA* wild type



CHANNEL A **CHANNEL B** **CHANNEL C** **CHANNEL D** **CHANNEL E**

Well	Name	Assay	Result	Cq Values	Overall results
B2	Sample 13	ResistancePlus GC (7500)	Positive	CHANNEL A: 32.86 CHANNEL B: 29.29 CHANNEL D: 28.28 CHANNEL E: 29.90	**Sample 13 - Positive** N. gonorrhoeae detected, gyrA mutation not detected.

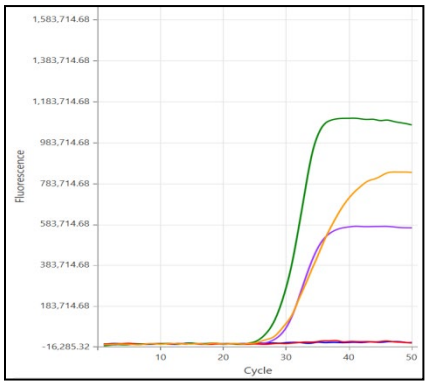
29.12.4 Low copy *N. gonorrhoeae* positive, *gyr*A mutant



CHANNEL A **CHANNEL B** **CHANNEL C** **CHANNEL D** **CHANNEL E**

Well	Name	Assay	Result	Cq Values	Overall results
C5	Sample 14	ResistancePlus GC (7500)	Positive	CHANNEL B: 26.33 CHANNEL C: 31.28 CHANNEL D: 28.30 CHANNEL E: 24.74	**Sample 14 - Positive** N. gonorrhoeae detected, gyrA mutation detected.

29.12.5 *N. gonorrhoeae* positive, *gyrA* indeterminate

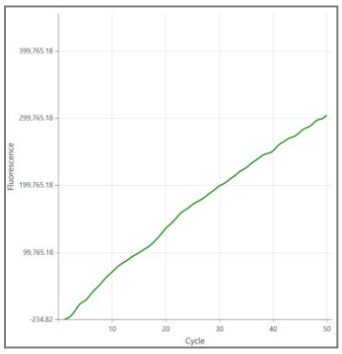


CHANNEL A **CHANNEL B** **CHANNEL C** **CHANNEL D** **CHANNEL E**

Well	Name	Assay	Result	Cq Values	Overall results
A7	Sample 15	ResistancePlus GC (7500)	Positive	CHANNEL B: 29.27 CHANNEL D: 28.55 CHANNEL E: 28.97	**Sample 15 - Positive** N. gonorrhoeae detected, gyrA indeterminate.

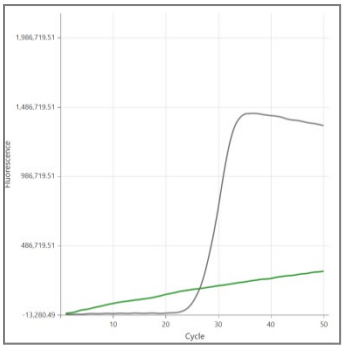
29.12.6 Sample to resolve – confirm negative

In this example, the CHANNEL D was flagged for resolution, with the software suggesting the signal is Negative.



CHANNEL D

Select Ref to plot a reference curve for the channel. After inspection of the amplification curves (shown below) it can be seen that there is no amplification in the channel, where the **green** signal is the signal flagged and the **grey** the reference curve.



CHANNEL D ☑

The negative result is confirmed by selecting the icon, and the change is saved. The resolved result is shown below.

Resolved result as shown in the resolve menu

	Target	Channel	Cq	Curve result	
	gyrA-S91	465-510	23.29	Positive	
	porA	533-580	19.95	Positive	
	gyrA-S91F	533-610	34.49	Positive	
	IC	533-640	—	Negative	
	opa	618-660	16.98	Positive	

Final sample interpretation as seen in the ResistancePlus GC (7500) software (Result Overview)

Well	Name	Assay	Result	Cq Values	Overall results
A1	Sample x	ResistancePlus GC (7500)	Positive	CHANNEL A: 23.29 CHANNEL B: 19.95 CHANNEL C: 34.49 CHANNEL E: 16.98	**Sample x - Positive** N. gonorrhoeae detected, gyrA mutation not detected.

30 Appendix B: Instructions for testing *ResistancePlus*® GC at 30 µl reaction volume

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

Refer to **Section 11.5** for instructions for testing at a final reaction volume of 20 µl.

30.1 Preparation of PCR

30.1.1 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 µl reaction volume, 20 µl of Master Mix and 10 µl sample is required. Prepare Master Mix as outlined in **Table 74**.

- 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 74. Master Mix - 30 µl reaction

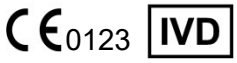
Reagent	Concentration	Volume per 30 µl reaction (µl)
Nuclease Free Water	N/A	2.0
Plex Mastermix (**BLUE**)	2x	15.0
GC+*gyrA* Mix (**BROWN**)	20x	1.5
Control Mix≠ (**WHITE**)	20x	1.5
Total volume (µl)		20.0
Add **10 µl** sample for a final volume of 30 µl		

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

30.1.2 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.

31 Glossary



European Conformity
For *In Vitro* Diagnostic Use

Catalogue number

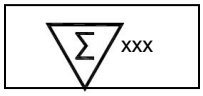
Batch code

Authorised Representative
In the European Community

Manufacturer

Date of manufacture

Temperature limitation



Contains sufficient for
xxx determinations

Use by Date

European Importer

United Kingdom Conformity
Assessment Mark