SpeeDx

ResistancePlus® MG FleXible

Internal technical training

December 2023

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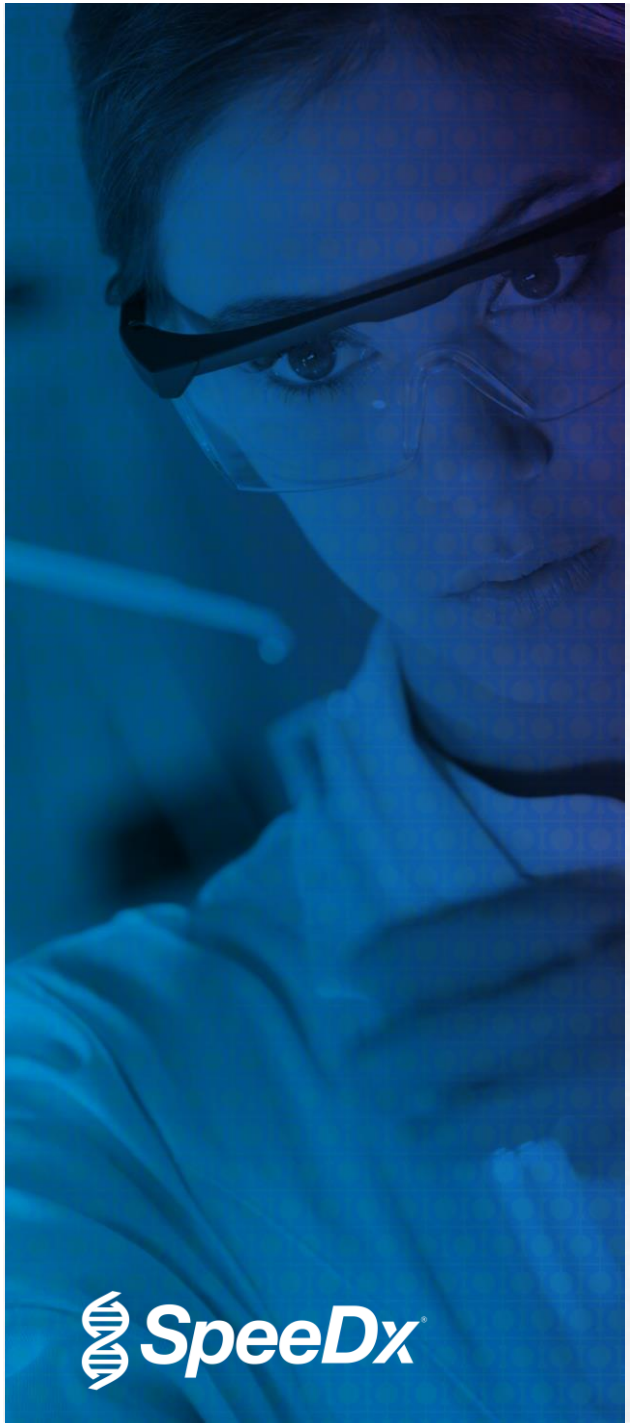
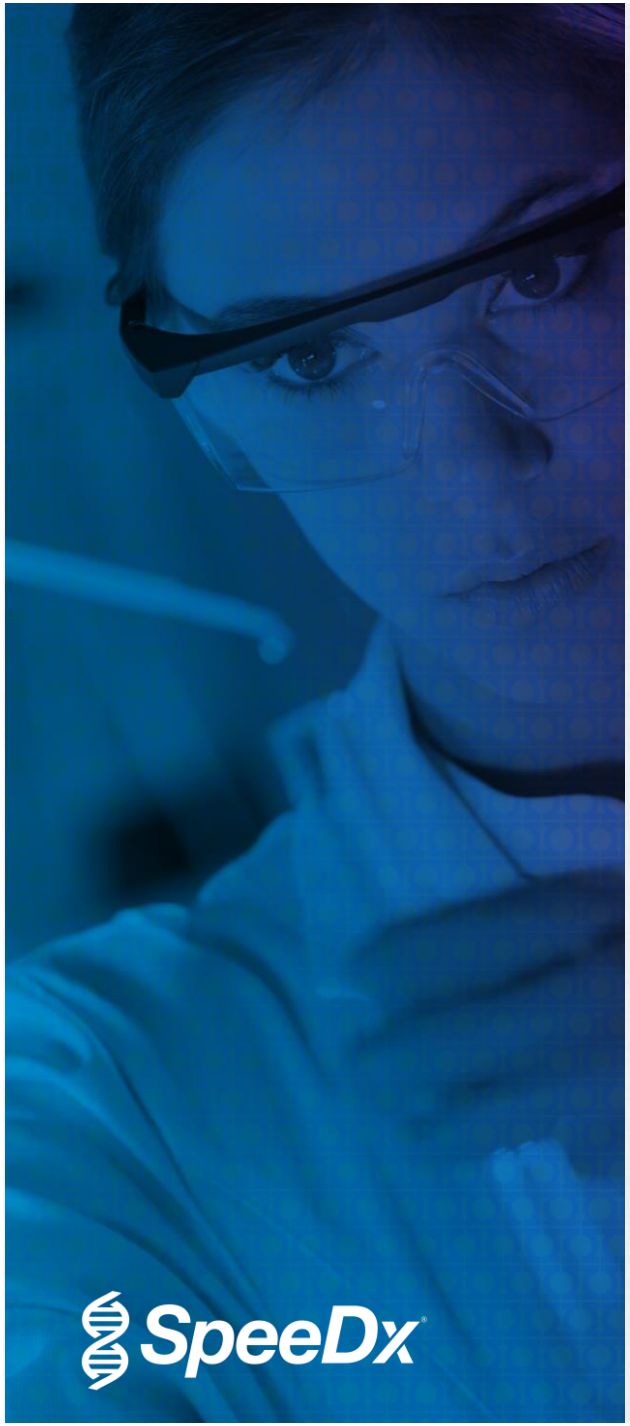
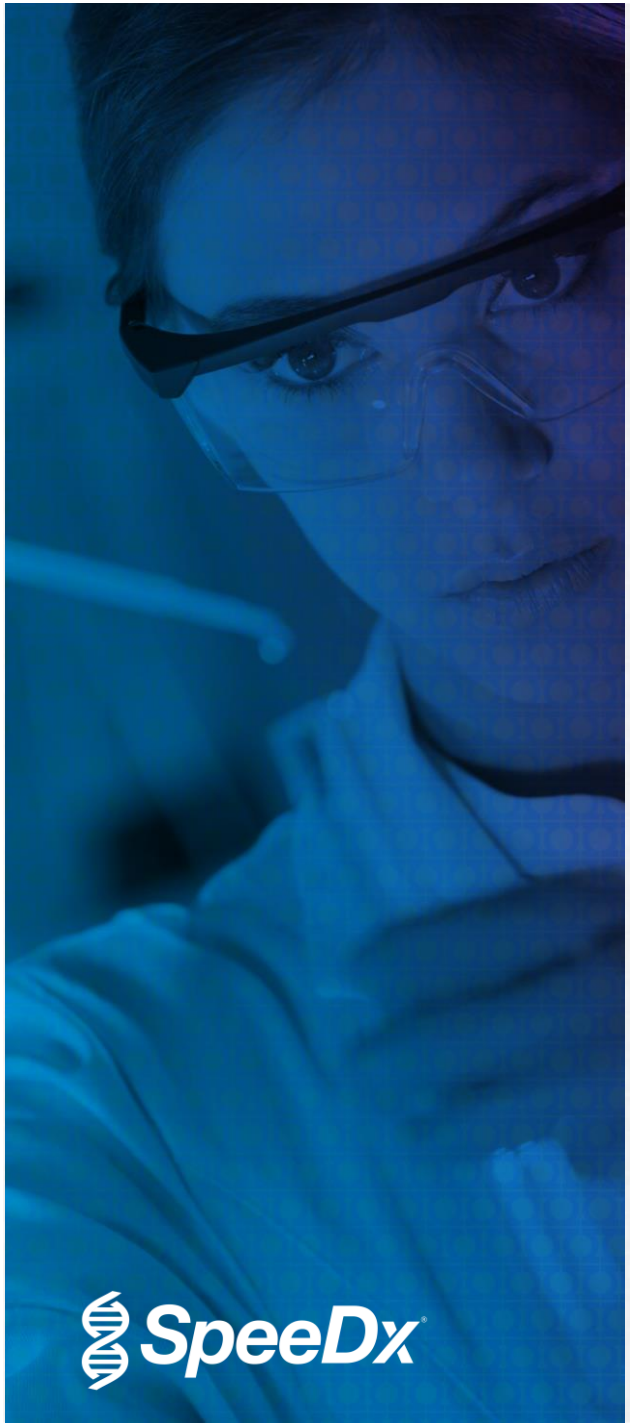


- **Part 1: Background**
  - *Mycoplasma genitalium* (MG) & antibiotic resistance
  - MG current testing situation & guidelines
- **Part 2: Technology**
  - ***Plex****PCR*® & ***Resistance****Plus*®



- **Part 3: Test information**
  - ***Resistance****Plus*® MG FleXible – The solution
  - Intended use
  - Sample collection, storage & transport
  - Kit components & storage
  - GeneXpert® & ***Resistance****Plus*® MG FleXible Cartridge



- **Part 4: Running *Resistance*Plus® MG FleXible**
  - Test preparation
  - ADF
  - Cartridge loading
- **Part 5: Results**
  - Viewing results
  - Result examples
- **Part 6: Performance data**
  - Analytical & Clinical performance studies

Part 1

Background

Mycoplasma genitalium (MG)

- Bacterial sexually transmitted infection
- Clinical associations:
  - Men – Non-Gonococcal Urethritis
  - Women – Cervicitis, Pelvic Inflammatory Disease
- Antimicrobial resistance
  - 1st line treatment = Azithromycin (macrolide antibiotic)
  - Macrolide resistance associated with 23S rRNA mutations
  - A2058G, A2059G, A2058T, A2058C, A2059C (*E. coli* numbering)

Greater awareness in the news

BBC NEWS

Emerging sex disease MG 'could become next superbug'

The Telegraph

Rare STI could turn into superbug, doctors warn

CNN health

New UK guidelines aimed at stopping potential sexually transmitted superbug

Unemo, Jensen. Nat Rev Urol. 2017 Mar;14(3):139-152

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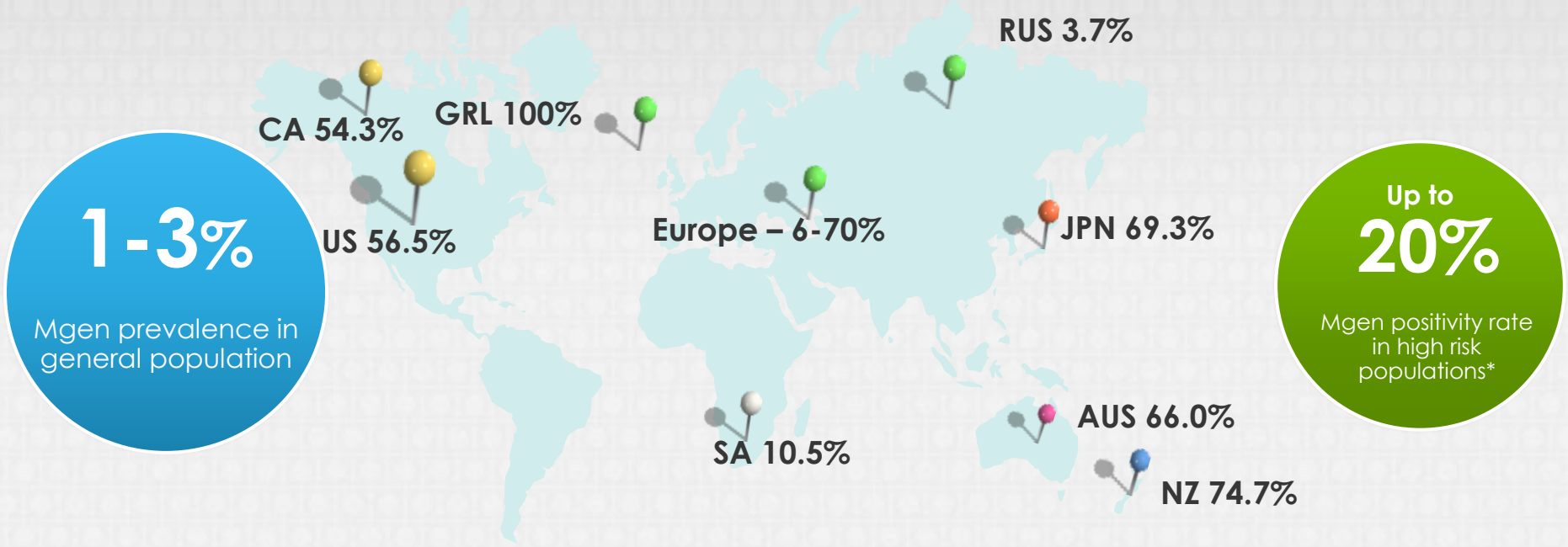
M. genitalium Azithromycin Resistance rates | Global



1-3%
Mgen prevalence in general population

Up to 20%
Mgen positivity rate in high risk populations*

Syndromic management and empiric treatment with Azithromycin is driving resistance rates.

***High risk populations include men who have sex with men (MSM), sex workers, and people with multiple sex partners**

Baumann et al. Sex Transm Infect. 2018 Jun; 94(4): 255–262
Machalek et al. Lancet Infect Dis. 2020 Jul 2;S1473-3099(20)30154-7

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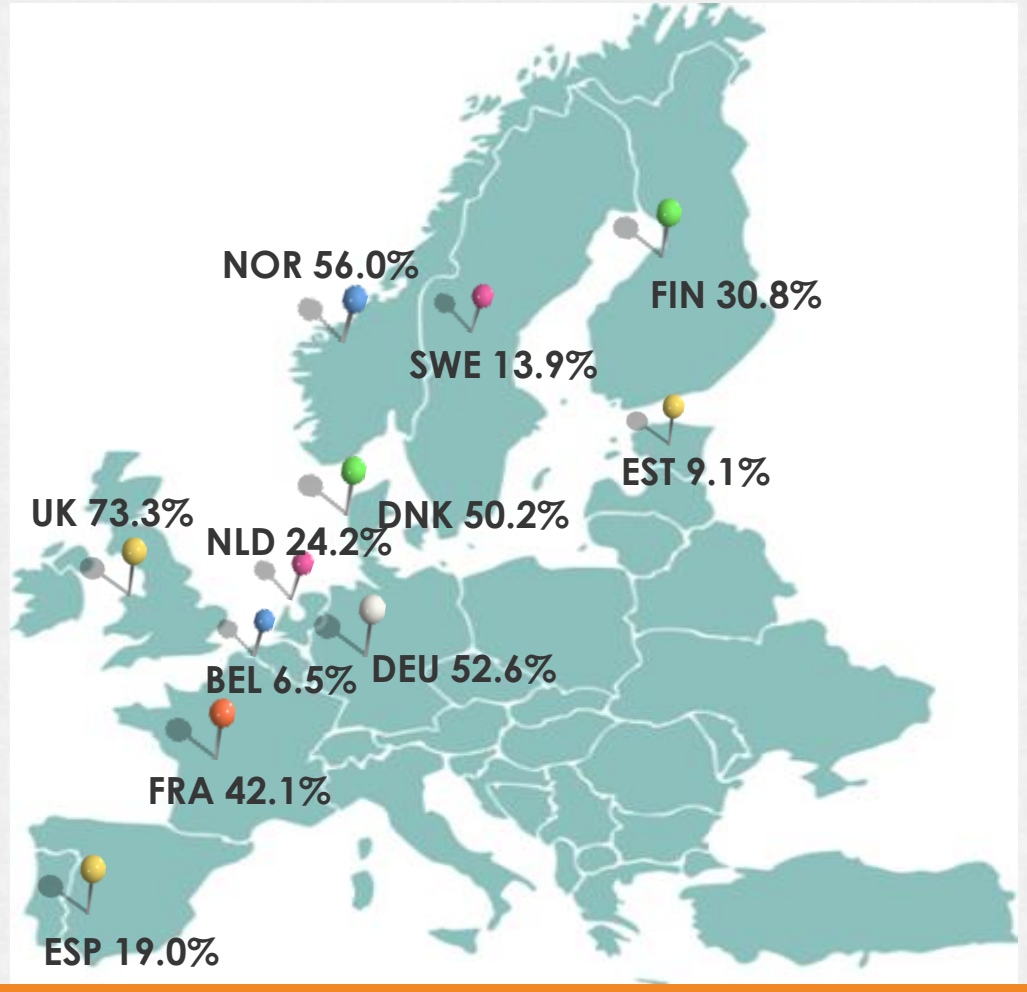
M. genitalium Azithromycin Resistance Rates | Europe



Prevalence studies and surveillance programs are continually ongoing.

Refer to your country's national reference centre for the most up-to-date information.

Machalek et al. Lancet Infect Dis. 2020 Jul 2;S1473-3099(20)30154-7
Pereyre et al. Sex Transm Infect. 2023 Jun;99(4):254-260

Syndromic management and empiric treatment with Azithromycin is driving resistance rates.

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M. genitalium testing
Current situation

- *M. genitalium* is fastidious to culture
  - 6 months to grow a single inoculum – impractical for diagnostics
- Molecular detection is available
  - In house qPCR tests and recently available CE marked tests
- Methods for macrolide resistance mutation detection
  - Sequencing – Costly and generally not convenient for routine diagnostics
  - High resolution melt analysis – Separate assay to MG detection, not easy to analyse
  - Fluorescence resonance energy transfer (FRET) – Lacking in sensitivity

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Global *M. genitalium* Guidelines

Test only symptomatic patients and their contacts

IUSTI	Europe	Australia
Mgen: With the widespread macrolide resistance in Europe, it is strongly recommended that all positive tests be followed up with an assay capable of detecting macrolide resistance mediating mutations[1]	**France:** As far as possible, associate that of its sensitivity to macrolides (azithromycin) to guide treatment in case of positivity[3]	Pre-treating *M. genitalium* infections with doxycycline 100mg bd for one week and then treating susceptible infections with azithromycin and macrolide-resistant infections with a fluoroquinolone eradicated >90% of infections[5]
NGU: Testing male patients with urethritis for *M. genitalium*, preferably with screening for macrolide resistance, is highly likely to improve clinical outcomes[2]	**UK:** All *M. genitalium*-positive specimens should be tested for macrolide resistance mediating mutations[4]	 **Meets guideline requirements** CE IVD In Vitro Diagnostic Medical Device

1. Jensen et al. 2016 European guideline on Mycoplasma genitalium infections. J Eur Acad Dermatol Venereol. 2016 Oct;30(10):1650-1656
2. Horner et al 2016 European guideline on the management of non-gonococcal urethritis. Int J STD AIDS. 2016 Oct;27(11):928-37.
3. https://www.sfdermato.org/site/groupe-infectiologie-dermatologique-et-infections-sexuellement-transmissibles.html
4. 2018 BASHH UK national guideline for the management of infection with Mycoplasma genitalium. Available online at: https://www.bashhguidelines.org/media/1198/mg-2018.pdf
5. Australian STI Management Guidelines – Mycoplasma genitalium 2018. http://www.sti.guidelines.org.au/sexually-transmissible- infections/mycoplasma-genitalium

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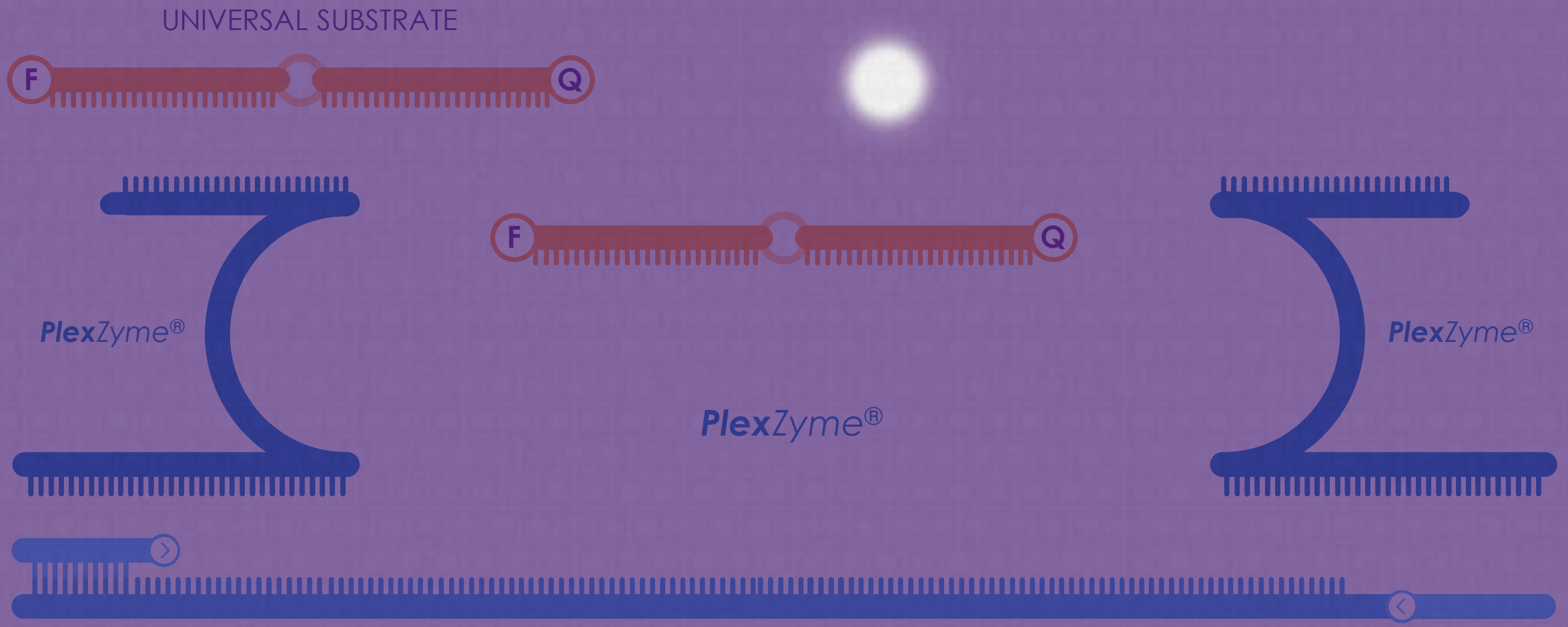
Part 2 – SpeeDx Technology

Plex*PCR*®
Resistance*Plus*®

PlexPCR®



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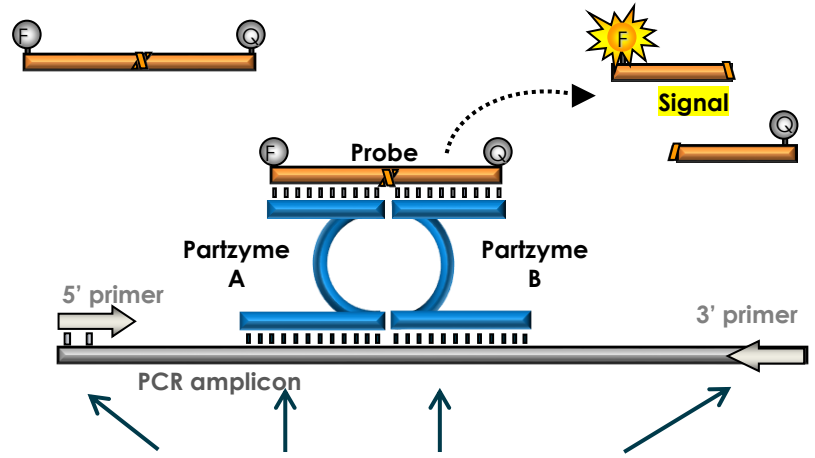
*Plex*PCR® Advantages

*Plex*Zyme® qPCR

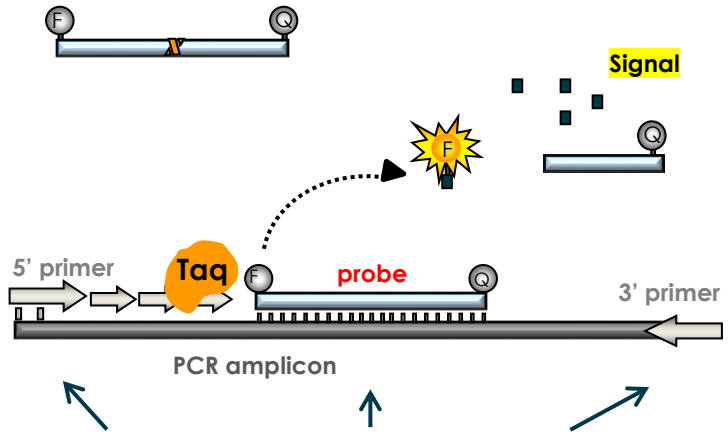
Universal Fluorescent Probes are for any target



High specificity requires 4 binding events
(2 primers, 2 partzymes)

TaqMan qPCR

Fluorescent Probes are target specific



Specificity requires 3 binding events
(2 primers, 1 probe)

***Plex*Zymes® have equivalent sensitivity to TaqMan probes (≤10 copies of target)**

***Plex*Zymes® have superior capacity to multiplex due to the use of universal probes**

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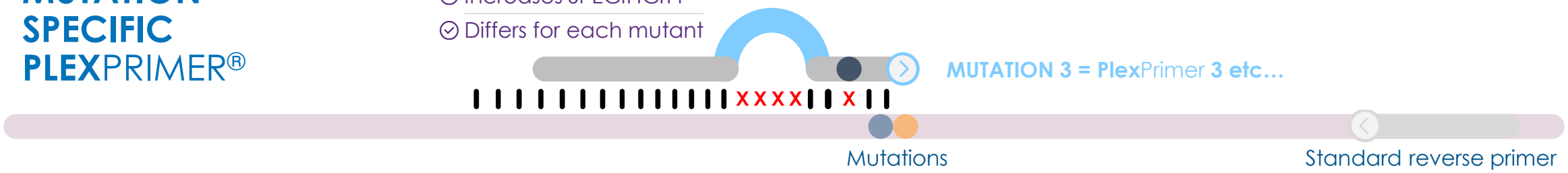
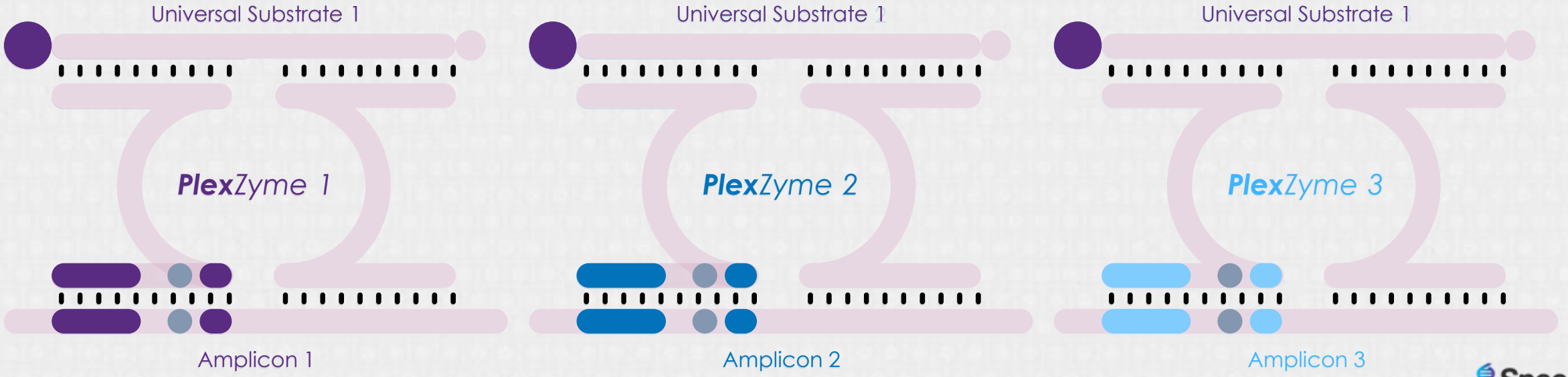
ResistancePlus®

MUTATION SPECIFIC PLEXPRIMER®

- Increases SPECIFICITY
- Differs for each mutant



CHANNEL 1 - Mutation specific amplification AND mutation specific detection



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Part 3

Test Information

ResistancePlus® **MG FleXible** | The solution

- The first test in Cepheids FleXible cartridge program, designed to be run on the GeneXpert® system
- Simultaneous detection of MG and associated macrolide resistance
- On-board controls for each individual sample
  - Probe Check Control (PCC)
  - Specimen Processing Control (SPC)
- Results available in approximately 120 minutes
- Closed cartridge system minimizes risk of contamination
- On-demand results
- Random access

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Intended Use

- Qualitative multiplexed *in vitro* diagnostic real-time PCR test
- Identification of *M. genitalium* and detection of mutations in the 23S rRNA gene (A2058G, A2059G, A2058T, A2058C, *E. coli* numbering), associated with resistance to azithromycin (macrolide antibiotic).

Channel	Target
1	*M. genitalium* (MgPa)
2	23S rRNA mutations (A2058T, A2058C, A2058G, A2059G)
3	Internal Control

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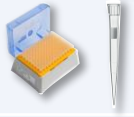
Associated products and consumables

The following materials are **Essential** for laboratories to run the ***Resistance****Plus*® MG FleXible test

Laboratory Equipment		
Freezer (between - 25°C to - 15°C)		Storage of ***Resistance****Plus*® MG FleXible reagents Please note: a freezer set to a temperature below -30°C cannot be used as this will adversely affect the enzyme
Vortex Mixer		Mix contents of reagent tubes prior to use
Benchtop centrifuge for 1.5 mL tubes		Spin down contents of reagent tubes prior to use
Micropipettors Covering the range of 10 - 100 µL		Preparation and addition of *Plex* MasterMix and Internal Control cells to the FleXible cartridge
Laboratory Consumables		
Gloves		Good laboratory practice for technician safety and to minimize risk of contamination
Clean lab coats		
Sterile aerosol-resistant, DNAse/RNAse free, pipette tips		Preparation and addition of *Plex* MasterMix and Internal Control cells to the FleXible cartridge
Sterile transfer pipettes capable of transferring at least 1mL volume		Transfer of specimen to the FleXible cartridge Preparation of positive control and transfer to the FleXible cartridge

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Associated products and consumables

The following materials are **Essential** for laboratories to run the ***Resistance****Plus*® MG FleXible test

GeneXpert® Instrument		
6-color GeneXpert® instrument		Required to run the ***Resistance****Plus*® MG FleXible test
Computer with GeneXpert® Software Version 4.7b or higher		
Barcode Scanner		
OR		
GeneXpert® Infinity-48s Xpertise software version 6.4b or higher		Required to run the ***Resistance****Plus*® MG FleXible test
OR		
GeneXpert® Infinity-80 Xpertise software version 6.4b or higher		Required to run the ***Resistance****Plus*® MG FleXible test

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Materials required but not provided

- Customers **Must** also have a dedicated space for preparation of PCR reagents within their laboratory
- Refer to the example across:

Note: a standard laboratory workbench may also be used if a PCR set-up hood is not available

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Sample collection, storage & transport

- The following specimen types have been validated for use with the test:

Male	Female
Urine	Urine
Urethral swabs	Urethral swabs
Rectal swabs	Cervical swabs
	Vaginal swabs
	Rectal swabs

- The following specimen collection devices are validated for use:
  - Xpert® Vaginal/Endocervical Specimen Collection kit (Cepheid, Cat no. SWAB/A-50)
  - Xpert® Swab Specimen Collection Kit (Cepheid, Cat no. SWAB/G-50)
  - Xpert® Urine Specimen Collection Kit (Cepheid, Cat no. URINE/A-50)
  - Sterile urine collection cup
  - Regular FLOQSwab™ in 3 mL of UTM™ media (Copan, Cat no. 306C)
  - Cobas® PCR media (Roche, Cat no. 06466281190)
  - Dry swab, resuspended in 3 mL of PBS

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Sample collection, storage & transport

Known incompatible collection devices

- The following collection kits are known to be **incompatible:**
  - **Aptima® media (Hologic)**
  - **Multi-collect specimen collection kit for the Alinity m (Abbott)**
- Please confirm that your customers will **not** use these devices

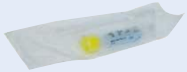
Sample collection, storage & transport

Specimen types	Collection Device	Image	Manufacturer Cat No.	Unity Qty	Transport & Storage Temp (°C)*	Storage time*
Male & Female: Urine	Neat urine in sterile collection cup	N/A	N/A	N/A	4 °C#	35 days#
	cobas® PCR media		Roche 06466281190	100	2 - 8 °C^	≤90 days^
					15 - 30 °C^	≤90 days^
	Xpert® Urine Specimen Collection kit		Cepheid URINE/A-50	50	Female Urine: 2 - 15 °C	Female Urine: ≤45 days
					Female Urine 2 - 30 °C	Female Urine: ≤3 days
					Male Urine 2 - 30 °C	Male Urine: ≤45 days

* Recommended by the manufacturer according to their instructions for use
# Neat urine storage from ***Resistance****Plus*® MG Neat urine stability Technical Bulletin. Transport neat urine specimens according to standard laboratory techniques
^ Transport and storage conditions recommended in the cobas® 6800 MG/TV assay
≠ Store and transport dry swab specimens according to standard laboratory techniques

Confidential

Sample collection, storage & transport

Specimen types	Collection Device	Image	Manufacturer Cat No.	Unity Qty	Transport & Storage Temp (°C)*	Storage time*
Female: Vaginal swab Cervical swab	Xpert® Vaginal/Endocervical Specimen Collection kit		Cepheid SWAB/A-50	50	2 - 30 °C	≤60 days
Male & Female: Rectal swab	Xpert® Swab Specimen Collection kit		Cepheid SWAB/G-50	50	2 - 30 °C	≤60 days
Female: Vaginal swab Cervical swab Male & Female: Urethral swab Rectal swab	FLOQSwab™ in 3 mL of UTM™ media		Copan 306C	50	2 - 25 °C	≤48 hours
					≤ - 70 °C	≥48 hours
	Dry swab added to cobas® PCR media		Roche 06466281190	100	2 - 8 °C^	≤90 days^
					15 - 30 °C^	≤90 days^
	Dry swab, resuspended in 3 mL of PBS	N/A	N/A	N/A	≠	≠

* Recommended by the manufacturer according to their instructions for use
# Neat urine storage from ***Resistance****Plus*® MG Neat urine stability Technical Bulletin. Transport neat urine specimens according to standard laboratory techniques
^ Transport and storage conditions recommended in the cobas® 6800 MG/TV assay
≠ Store and transport dry swab specimens according to standard laboratory techniques

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Sample collection, storage & transport

Xpert® Urine Specimen collection kit

Urine Specimen Collection (First Catch)

1. Direct patient to provide first-catch urine (20-50mL) into a urine collection cup.

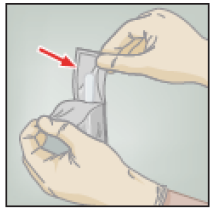
   Note: The patient should not have urinated for at least 1 hour prior. Patient should not cleanse the genital area prior to collecting specimen.

2. The Xpert® Urine Specimen Collection kit contains

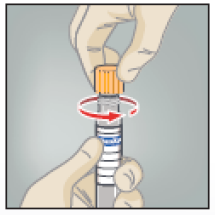
   Ⓐ Large transfer pipette

   Ⓑ Urine Transport Reagent tube

3. Open the package of disposable transfer pipette provided in the kit.

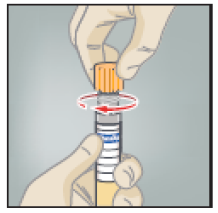


4. Remove the yellow cap from the transport tube.

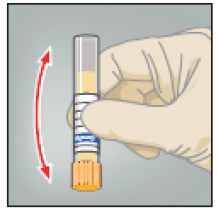


5. Transfer approximately 7mL of urine from the bottom of the collection cup into the transport tube, using the disposable transfer pipette. The correct volume is marked by the black dashed line on the transport reagent tube label.

6. Replace the yellow cap on the transport tube and tighten securely.

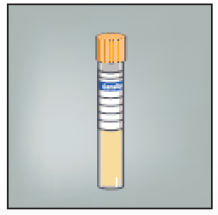


7. Invert the transport tube 3-4 times to ensure that the specimen and reagent are well mixed.



8. Return the tube as instructed by your doctor, nurse or care-provider.

   Note: Health care provider should label the transport tube with the sample identification information, including date of the collection, as required.



Cepheid
URINE/A-50

301-2888, Rev. A
March, 201520

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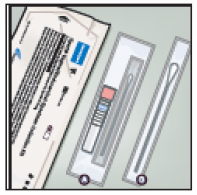
Sample collection, storage & transport

Xpert® Vaginal/Endocervical Specimen collection kit

Patient-Collected Vaginal Swab Specimen Collection

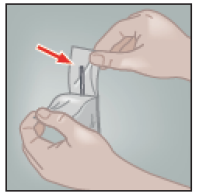
1 Wash hands before starting and undress from the waist down. Open the individual collection package Ⓐ that contains the pink-capped Xpert® Swab Transport Reagent tube and individually wrapped collection swab. Set the tube aside before beginning to collect sample.

Discard the larger swab Ⓑ.

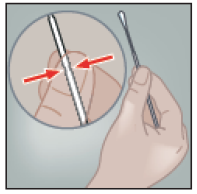


2 Open the collection swab wrapper by peeling open the top of the wrapper.

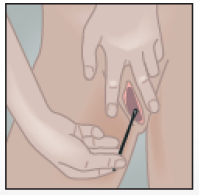
Remove the swab, taking care not to touch the tip or lay it down. If the soft tip is touched, the swab is laid down, or the swab is dropped, request a new collection kit.



3 Hold the swab in your hand, placing your thumb and forefinger in the middle of the swab shaft across the scoreline.

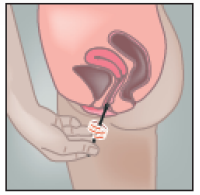


4 Carefully insert the swab into your vagina about 5 cm (two inches) inside the opening of the vagina.



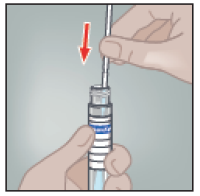
5 Gently rotate the swab for 10 – 30 seconds. Ensure the swab touches the walls of the vagina so that moisture is absorbed by the swab.

Withdraw the swab and continue to hold it in your hand.

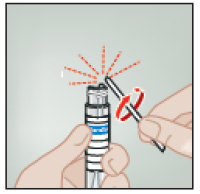


6 While holding the swab in the same hand, unscrew the cap from the Xpert Swab Transport Reagent tube. Do not spill the contents of the tube. If the contents of the tube are spilled, request a new collection kit. Immediately place the collection swab into the transport reagent tube.

⚠ WARNING: If the contents of the tube are spilled on your skin, wash the affected area with soap and water. If the contents of the tube are splashed in your eyes, immediately flush your eyes with water. Notify your doctor, nurse or care-provider if irritation develops. If the contents of the tube are spilled, your test result may be invalidated. Do not take internally.



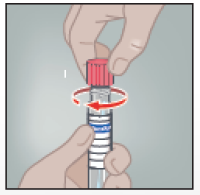
7 Identifying the scoreline on the collection swab shaft, carefully break the swab shaft against the side of the tube at the scoreline. If needed, gently rotate the swab shaft to complete the breakage. Discard the top portion of the swab shaft. Avoid splashing contents on the skin. Wash with soap and water if exposed.



8 Re-cap the transport tube and tighten the cap securely.

Return the tube as instructed by your doctor, nurse or care-provider.

Note: Health care provider should invert or gently shake the tube 3-4 times to elute material from the swab. Avoid foaming. Label the transport tube with the sample identification information, including date of the collection, as required.



Cepheid
SWAB/A-50

301-1827, Rev. E
February, 2019

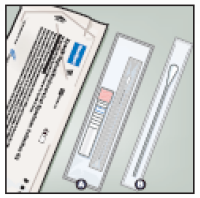
SpeeDx

Sample collection, storage & transport

Xpert® Vaginal/Endocervical Specimen collection kit

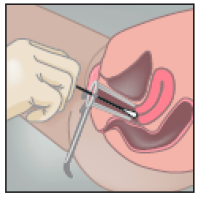
Endocervical Specimen Collection

1 The Xpert Vaginal/Endocervical Specimen Collection kit contains
- Ⓐ Individual Collection Kit
- Ⓑ Cleaning Swab

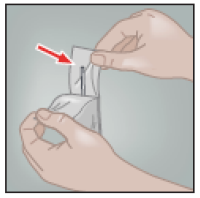


2 Partially peel open the cleaning swab wrapper and remove the swab.

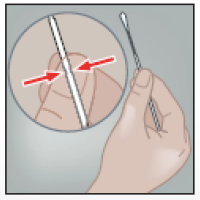
Remove excess mucus from the cervical os and surrounding mucosa using the large individually wrapped cleaning swab Ⓑ.
Discard the swab.



3 Open package Ⓐ that contains the pink-capped Xpert Swab Transport Reagent tube and the individually wrapped collection swab. Set the tube aside before beginning to collect sample.
Open the collection swab wrapper by peeling open the top of the wrapper. Remove the swab, taking care not to touch the tip or lay it down.
If the soft tip is touched, the swab is laid down, or the swab is dropped, use a new Xpert Vaginal/Endocervical Specimen Collection Kit.

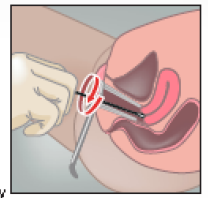


4 Hold the swab in your hand, placing your thumb and forefinger in the middle of the swab shaft.



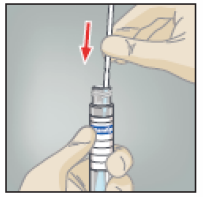
5 Insert the collection swab into the endocervical canal. Gently rotate the swab clockwise for 10-30 seconds in the endocervical canal.

Withdraw the swab carefully.



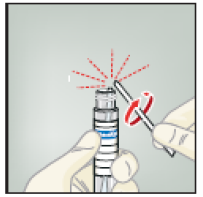
6 While holding the swab in the same hand, unscrew the cap from the Xpert Swab Transport Reagent tube. Do not spill the contents of the tube. If the contents of the tube are spilled, use a new collection kit. Immediately place the collection swab into the transport reagent tube.

⚠ WARNING: If the contents of the tube are spilled on your skin, wash the affected area with soap and water. If the contents of the tube are splashed in your eyes, immediately flush your eyes with water. Notify your doctor, nurse or care-provider if irritation develops. If the contents of the tube are spilled, your test result may be invalidated. Do not take internally.



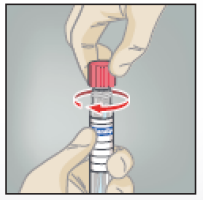
7 Identify the scoreline on the collection swab shaft. Carefully break the swab shaft against the side of the tube at the scoreline. If needed, gently rotate the swab shaft to complete the breakage. Discard the top portion of the swab shaft.

Use care to avoid splashing the contents. Wash with soap and water if exposed.



8 Re-cap the swab transport reagent tube and tighten the cap securely. Invert or gently shake the tube 3-4 times to elute material from the swab. Avoid foaming.

Label the transport tube with the sample identification information, including date of the collection, as required.



Cepheid
SWAB/A-50

301-1826 Rev. C
April , 2017

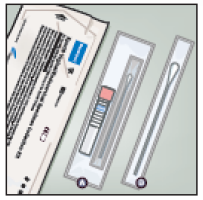
SpeeDx

Sample collection, storage & transport

Xpert® Swab collection kit

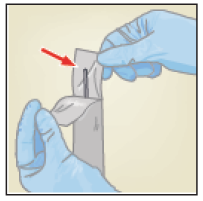
Clinician-Collected Rectal Swab Specimen Collection
For use with Xpert® Swab Specimen Collection Kit – Catalog #SWAB/G-50

1. Wash hands before starting. Open the individual collection package (A) that contains the pink-capped Xpert Swab Transport Reagent tube and individually wrapped collection swab. Set the tube aside before beginning to collect sample. Discard the larger swab (B).

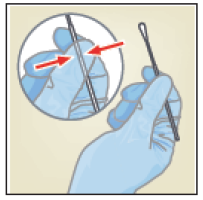


2. Open the collection swab wrapper by peeling open the top of the wrapper. Remove the swab, taking care not to touch the tip or lay it down.

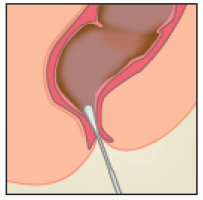
   If the soft tip is touched, the swab is laid down, or the swab is dropped, use a new collection kit.



3. Hold the swab in your hand, placing your thumb and forefinger in the middle of the swab shaft across the scoreline.

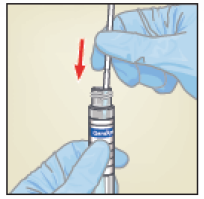


4. Carefully insert the swab approximately 1 cm beyond the anal sphincter (so that the fiber tips are no longer visible), and rotate gently.

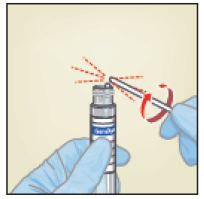


5. While holding the swab in the same hand, unscrew the cap from the Xpert Swab Transport Reagent tube. Do not spill the contents of the tube. If the contents of the tube are spilled, use a new collection kit. Immediately place the collection swab into the transport reagent tube.

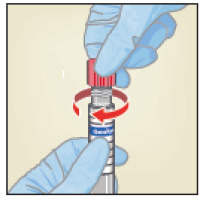
   WARNING: If the contents of the tube are spilled on your skin, wash the affected area with soap and water. If the contents of the tube are splashed in your eyes, immediately flush your eyes with water.



6. Identifying the scoreline on the collection swab shaft, carefully break the swab shaft against the side of the tube at the scoreline and discard the top portion of the swab shaft. If needed, gently rotate the swab shaft to complete the breakage. Avoid splashing contents on the skin. Wash with soap and water if exposed.



7. Re-cap the transport tube and tighten the cap securely.



8. Invert or gently shake the tube 3-4 times to elute material from the swab. Avoid foaming. Label the transport tube with the sample identification information, including date of the collection, as required.

   Specimen should be transported at 2-30 °C.

   Prior to testing, specimen may be stored for up to 60 days at 2-30 °C.

Cepheid
SWAB/G-50

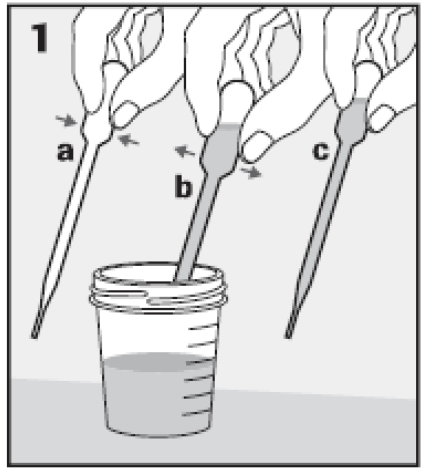
301-1790, Rev. A
December, 2012

SpeeDx

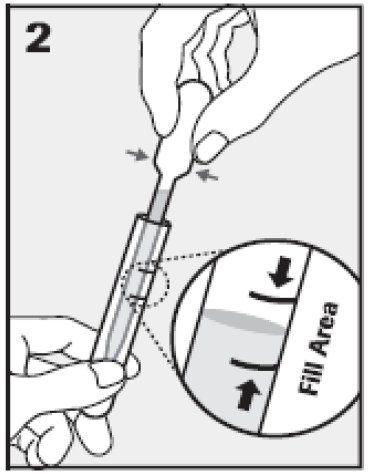
Sample collection, storage & transport

cobas® PCR media (urine)

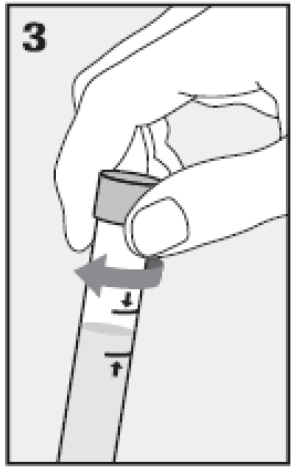
SPECIMEN COLLECTION



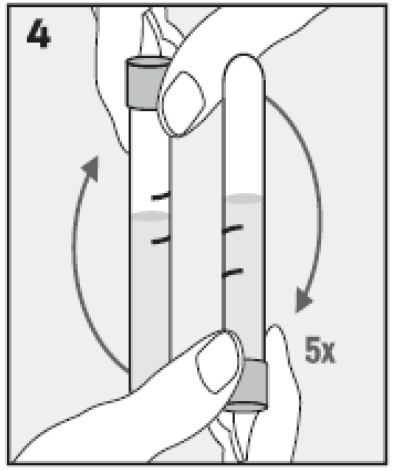
1. **PIPETTE:** Mix and transfer the urine into the **cobas®** PCR Media tube using a disposable pipette (not provided).
NOTE: Urine can be stored at 2˚C to 30˚C for up to 24 hours prior to transferring into the **cobas®** PCR Media tube.



2. **TRANSFER:** The correct volume of urine has been added when the fluid level is between the two black lines on the tube label.



3. **CAP:** Tightly re-cap the **cobas®** PCR Media tube.



4. **MIX:** Invert the tube 5 times to mix. The specimen is now ready for transport and testing.

Roche
06466281190

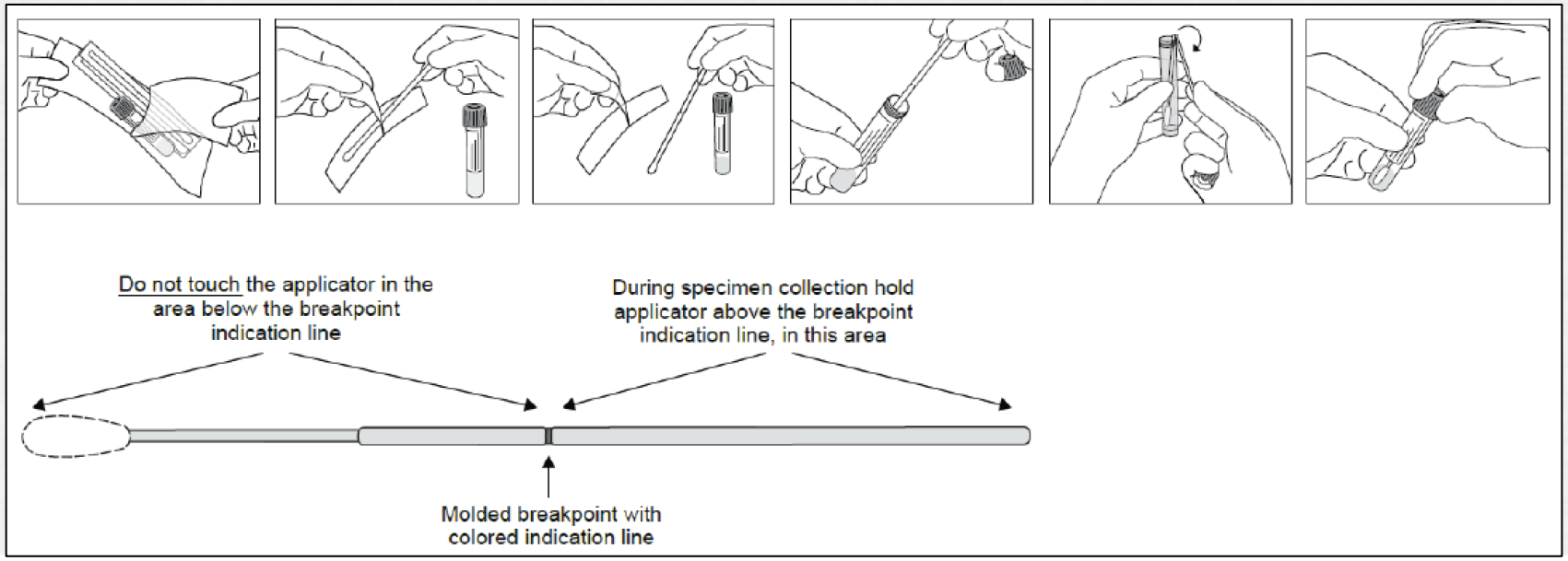
Doc Rev. 5.0

SpeeDx

Sample collection, storage & transport

FLOQSwab™ in 3 mL of UTM™ media

1. Open the UTM kit package and remove the medium test tube and the internal bag containing the sterile swab.
2. Take the sterile swab out of its bag and collect the clinical specimen; to prevent the risk of contamination, make sure that the swab tip comes into contact with the collection site only.
3. After collecting the specimen, unscrew and remove the cap from the test tube taking care not to spill the medium.
4. Insert the swab into the test tube until the breakpoint is level with the test tube opening.



Copan
306C

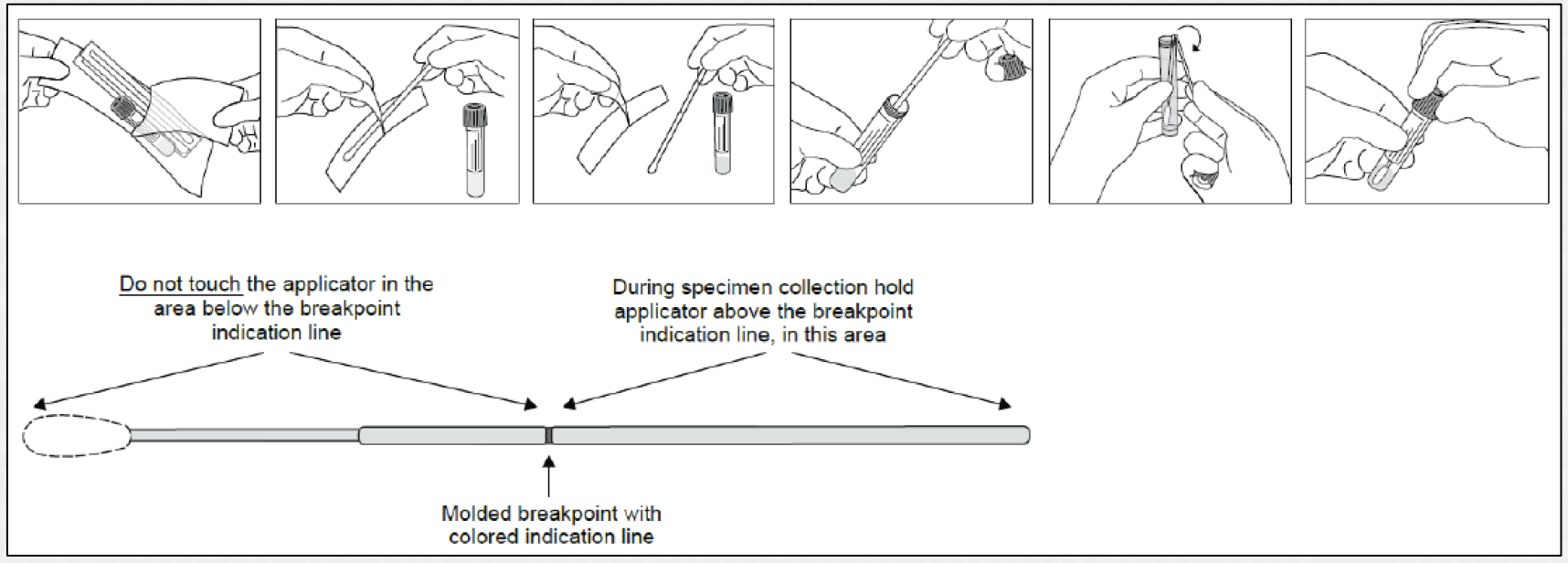
44C Rev.03 Date 2019.02

SpeeDx

Sample collection, storage & transport

FLOQSwab™ in 3 mL of UTM™ media

5. Bend and break the swab at the breakpoint holding the test tube away from your face and discard the excess part.
6. Screw the cap back onto the test tube and hermetically seal it.
7. Process the specimen contained in the UTM within 48 hours from collection storing the test tube at 2-25°C.
8. Before processing, vortex for 20 seconds in order to encourage specimen release from the swab and homogenize the medium.



Copan
306C

44C Rev.03 Date 2019.02

SpeeDx

ResistancePlus® MG FleXible Kit Contents

Overview

- The ***ResistancePlus***® MG FleXible kit will consist of 2 boxes that will be shipped together

Box #	Components	Units	Shipping Conditions	Storage Conditions
1	Assay reagents Cartridge labels MG FleXible mix label (optional)	10	Ice gel packs	- 25°C to - 15 °C
2	Cartridges	10	Room temp	2 - 28 °C

- When stored under the recommended conditions and handled correctly, activity of the kit is retained until the expiry date stated on the label (~12 months from date of production)

ResistancePlus® MG FleXible Kit Contents

Box 1 Contents

Box #	Cap Colour	Contents (10 reactions)	Description	Quantity
1	**Blue**	***Plex*** Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, DNA polymerase and buffer	1 x 440 µL
	Brown	MG+23S (GX) Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of *M. genitalium*, 23S rRNA mutations and internal control	1 x 50 µL
	Red	Internal Control Cells#	Internal control cells containing internal control DNA template to monitor extraction and amplification efficiency	1 x 100 µL
	N/A	***Resistance****Plus*® MG FleXible Labels*	Cartridge labels containing Lot-specific barcode, Master Lot number, expiry date and ADF information	10 units
	N/A	MG FleXible Mix Label	Label to identify combined MG FleXible Reaction Mix (optional use)	1 label

^ Oligonucleotides are PCR primer pairs (including ***Plex****Primer*® primers), ***Plex****Zyme*® enzymes and fluorescent probes
# Store template tubes separately from oligo mixes, i.e. template or nucleic acid handling room
* **Do not dispose of cartridge labels**

Store Box 1 between -25°C to -15°C .

SpeeDx

Stability and Storage of Box 1 reagents

- The contents of Box 1 should be stored between **-25°C -15°C**
- The tube of Internal Control cells (**Red cap**) has been validated to withstand up to **8 freeze-thaw cycles**
- The remaining tubes will be used to prepare the combined reaction mix which is described in Section 4a.

SpeeDx

ResistancePlus® MG FleXible Kit Contents

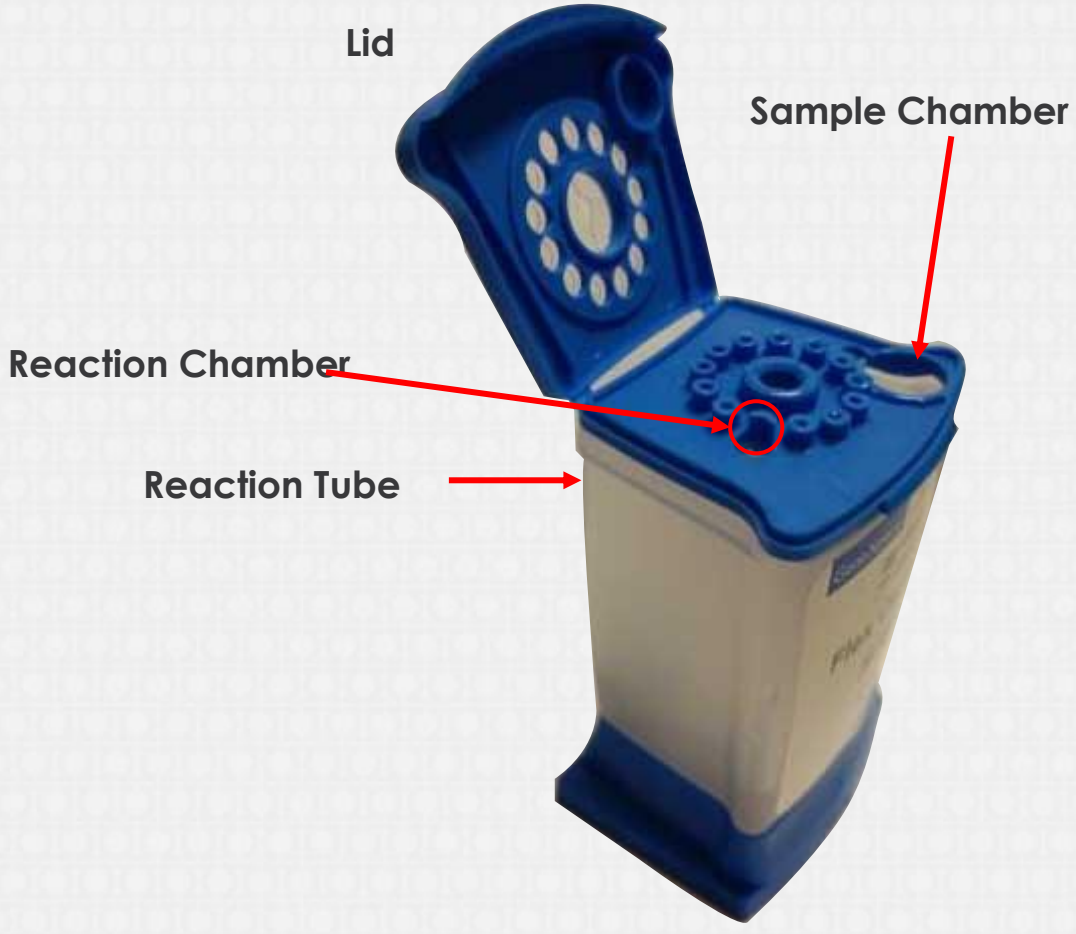
Box 2 Contents

Box #	Cap Colour	Contents	Description	Quantity
2	N/A	Cartridges	Single-use cartridge for sample processing, nucleic acid amplification and detection	10 units

Store Box 2 between 2 - 28°C
Cartridges should be appropriately disposed as clinical waste after use

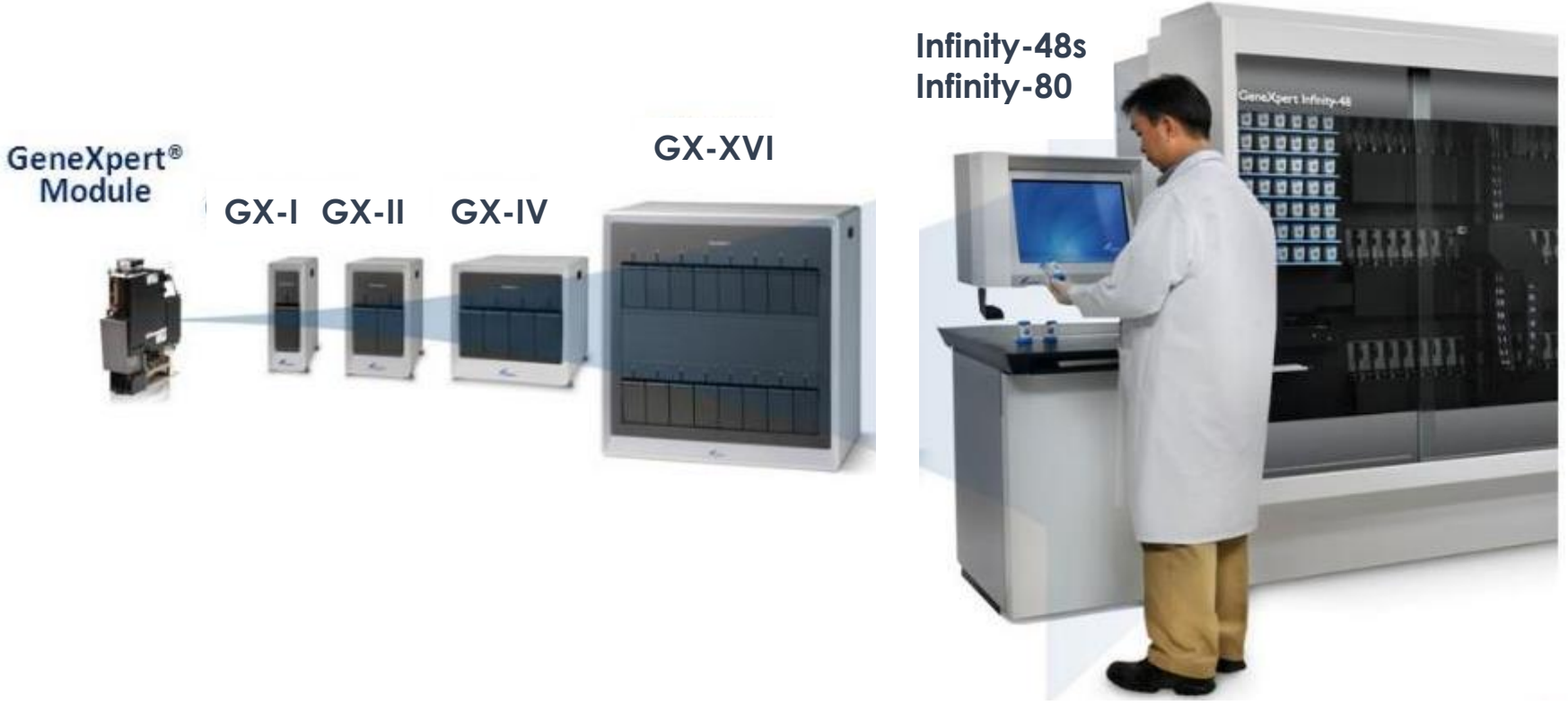
SpeeDx

ResistancePlus® MG FleXible Cartridge



Store between 2°C-28°C

GeneXpert® Instruments



The test can be run on the full GeneXpert® modules:
GX-I, GX-II, GX-IV, GX-XVI, Infinity-48s and Infinity-80

SpeeDx

Test Comparison

Xpert® CT/NG vs *ResistancePlus*® MG FleXible

Feature	Xpert® CT/NG Assay	*ResistancePlus*® MG Flexible
Assay reagents and Mastermix	Lyophilized; already inside cartridge	• Liquid reagents • Combined by user to form MG FleXible Reaction Mix • MG FleXible Reaction Mix is added to reaction chamber opening by user
SPC target	Lyophilized; already inside cartridge	• Liquid reagent (Internal Control Cells) • **Must be added to sample in cartridge by user or Invalid results may occur**
SPC assay	Lyophilized; already inside cartridge	In assay reagents (Internal Control assay)
Barcode	Already on cartridge	• **User must add barcode label to front of cartridge (Cartridge label)** • **Label must have same Master Lot # as cartridge and assay reagents being used**
Sample adequacy control	Lyophilized; already inside cartridge	Not included
Cartridge	One opening for addition of sample	Two openings: • One for Reaction Mix addition (Left) • One for sample addition (Right - as standard)

SpeeDx

Part 4

Running ***Resistance****Plus*® MG FleXible

4a

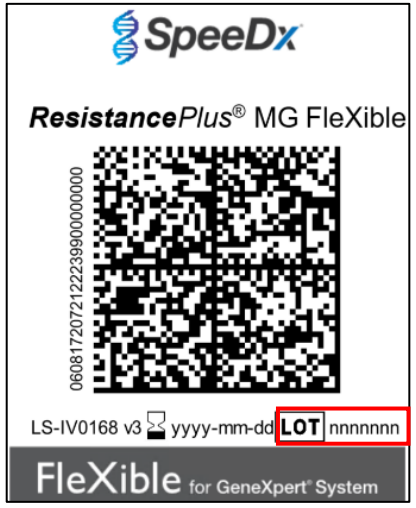
Test Preparation

ResistancePlus® MG FleXible Procedure

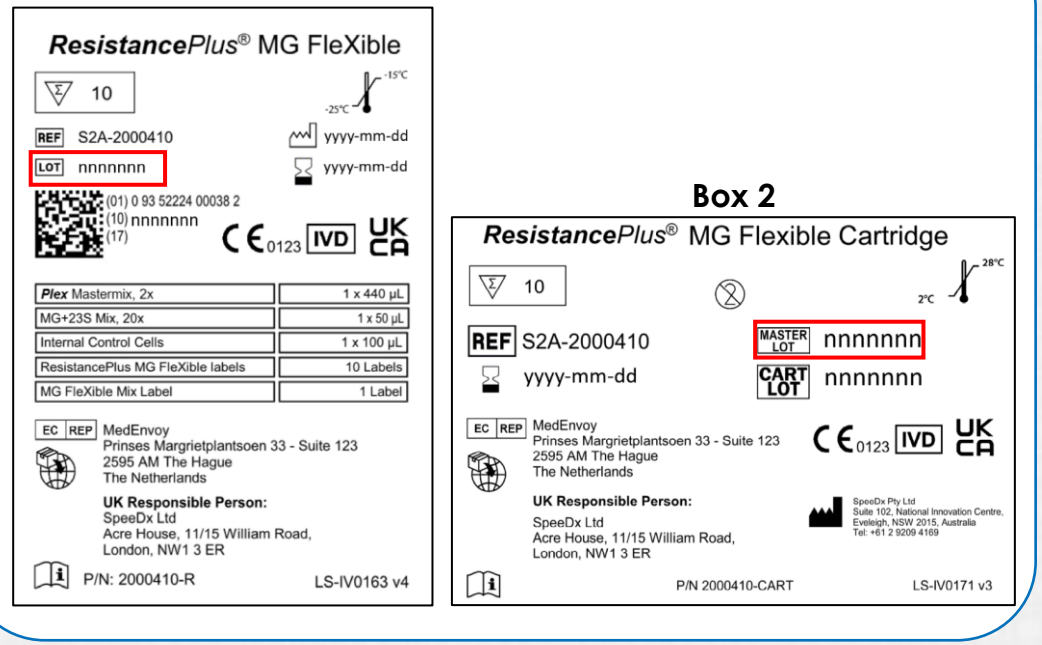
1. Obtain cartridge labels from Box 1.

Check the MASTER LOT number matches between the cartridge labels, Box 1 (reagents) and Box 2 (cartridges).

Cartridge labels



Box 1



Box 2

SpeeDx

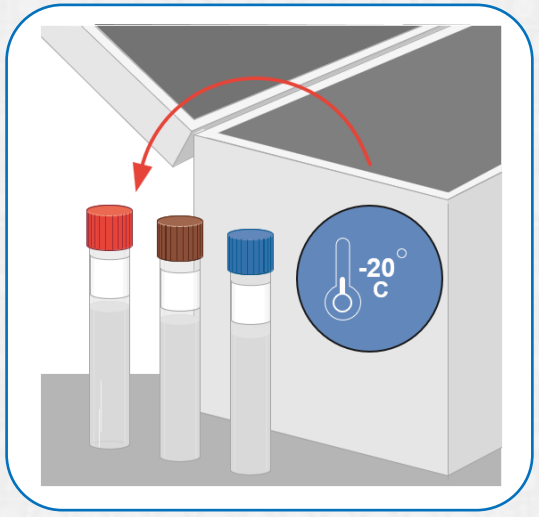
ResistancePlus® MG FleXible Procedure

2. Affix cartridge label to the front of the cartridge.

Note: *Ensure label on cartridge is straight.*

3. Take out and Thaw reagents including the internal control.

Note: *Reagents should be completely thawed before use*



SpeeDx

ResistancePlus® MG FleXible Procedure

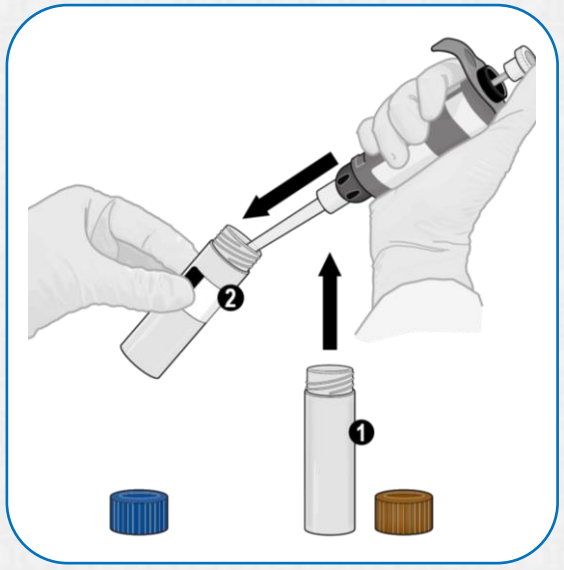
4. Vortex tubes for 5 - 10 seconds to mix contents and centrifuge for 5 - 10 seconds at a low speed to collect liquid in the bottom of the tube.

ResistancePlus® MG FleXible Procedure

5. Pipette 44 µL of MG+23S(GX) mix (**Brown lid**) into ***Plex*** Mastermix tube (**Blue lid**).

Return and tighten lid of the Plex Mastermix tube (**Blue lid**). This is now your combined **Reaction** mix.

*Discard the empty MG+23S (GX) tube (**Brown lid**)*
After transferring *contents*



SpeeDx

ResistancePlus® MG FleXible Procedure

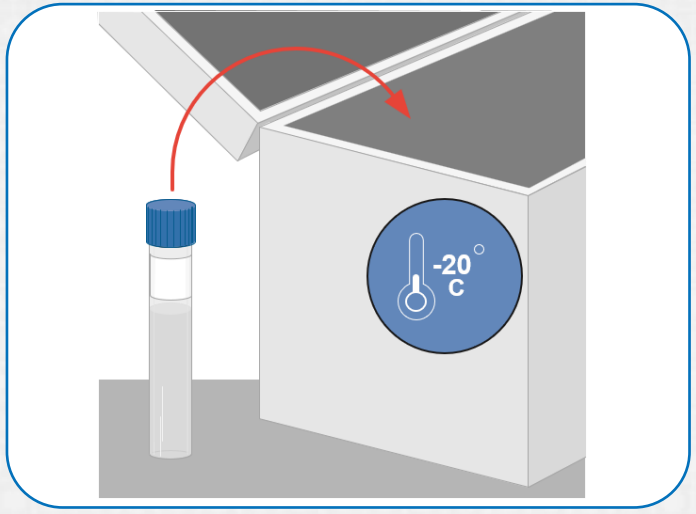
6. Vortex the combined reaction mix (**Blue lid**) for 5 - 10 seconds.

Centrifuge for 5 - 10 seconds at low speed to collect liquid in the bottom of the tube.

7. The combined Reaction mix is now sufficient for 10 reactions

Note: *Combined Reaction mix can be stored between - 25°C to - 15°C for up to 8 weeks or no more than 8 freeze-thaw cycles*

Note: *Do not prepare aliquots*



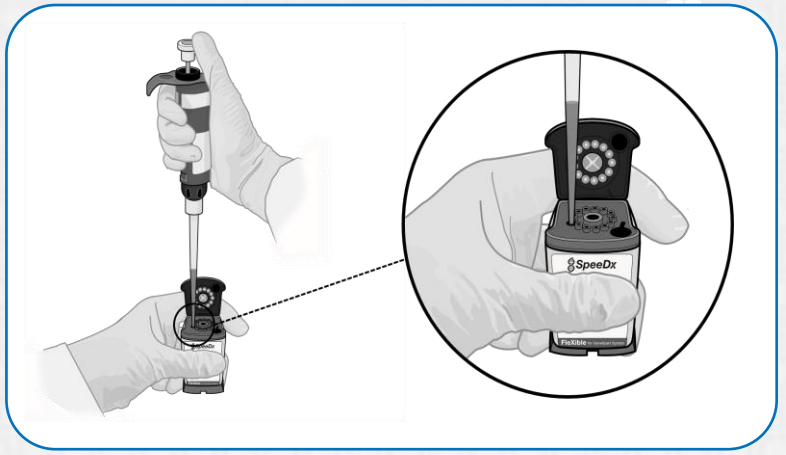
SpeeDx

*Resistance*Plus® MG FleXible Procedure

8. Open cartridge lid and pipette 44 µL of combined Reaction Mix (**Blue lid**) into Reaction Chamber (Left).

Insert tip vertically as far as it will go inside chamber before expelling liquid.

9. Gently tap bottom of cart on bench to settle liquid and prevent any air bubbles.



Confidential

SpeeDx

ResistancePlus® MG FleXible Procedure

10. Open the sample tube lid, **slowly** compress the bulb of the transfer pipette provided, insert the pipette into the sample tube and **slowly** release the bulb to fill the transfer pipette **above the 1 mL mark** on the pipette shaft.

The aspirated sample should not contain air bubbles.

SpeeDx

ResistancePlus® MG FleXible Procedure

11. Slowly compress the bulb to dispense the sample from the transfer pipette into the Sample Chamber of the cartridge (right).

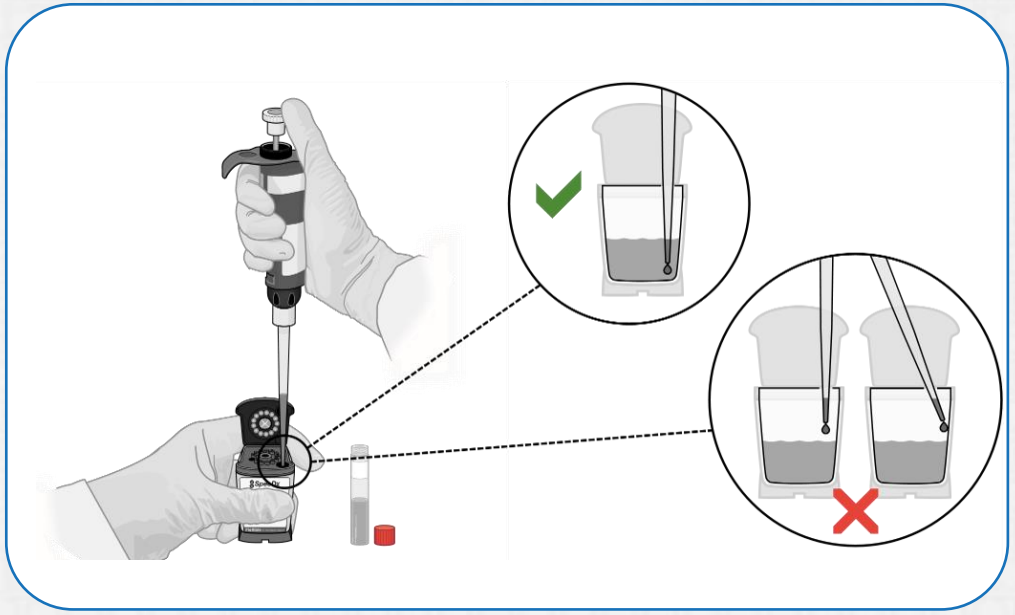
Note: *Excessive force can create bubbles.*

Gently pipette to avoid unnecessary bubbles

SpeeDx

ResistancePlus® MG FleXible Procedure

12. Pipette 10 µL of Internal Control Cells (**Red lid**) into Sample Chamber (Right).



Note: *Ensure pipette tip is **correctly immersed in the sample** before expelling the liquid*

Note: *The Internal Control Cells can be stored between -25°C to -15°C and undergo no more than 8 freeze-thaw cycles*

SpeeDx

ResistancePlus® MG FleXible Procedure

13. Close the cartridge lid. **Do not mix or shake cartridge**

Note: *The cartridge should be loaded within 30 minutes of preparation*

14. Place in the GeneXpert® instrument.

    Start test

Note: *The cartridge should be loaded within 30 minutes of preparation*

SpeeDx

Centrifugation steps

Centrifugation steps are required to collect liquid at the bottom of the tube before use. These can be performed on a small benchtop centrifuge which commonly used by labs for PCR/molecular tests and are designed to fit tubes between 1-2 mL in volume.

Examples are shown below with their maximum speeds:

Microcentrifuges – Max speeds of approx. 15,000 rpm (21,000 x g)

Minicentrifuges – These are much simpler and usually don't have programmable speeds, but reach a maximum speed of approx. 12,500 x g

SpeeDx

Storage of Reaction Mix

- Reaction Mix should **Always** be made for **10 reactions at a time**
  (10 reactions per kit)
- Residual volumes of Reaction Mix **should not be pooled** into another tube
- To store residual combined MG FleXible Reaction Mix, contents can remain in the ***Plex*** Mastermix tube (**Blue**), and the tube can be relabelled using the MG FleXible Mix Label (Box 1). Record the date of preparation in the space provided on the label.
- The combined MG FleXible Reaction Mix can be **stored between - 25°C to - 15°C for up to 8 weeks**. It is recommended that **freeze/thaw cycles be limited to less than 8**.

SpeeDx

ResistancePlus® MG Flexible

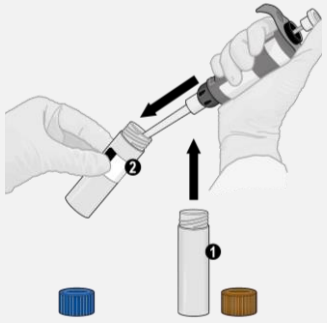
Work flow Summary

Hands on time for 1 sample : ~5 minutes

Run time: 2 hours, 11 minutes

Label the cartridge

Mix kit reagents and add to cartridge



Transfer sample to cartridge

Add Internal control

Load and run cartridge

Sample to Result: ~2 hours, 15 minutes

SpeeDx

*Resistance*Plus® MG S2A Positive Control Kit

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

Shipping and storage conditions

Box #	Components	Units	Shipping Conditions	Storage Conditions
1	***Resistance****Plus*® MG S2A Positive Control Kit	2 each control	Ice gel packs	-25°C to -15 °C

SpeeDx

*Resistance*Plus® MG S2A Positive Control Kit

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

SpeeDx

ResistancePlus® MG FleXible

Positive Control Procedure

Positive control material may be prepared using either a micropipettor or transfer pipette.

Micropipettor

1. Pipette 1mL Dilution Buffer (NEUTRAL) into a Positive Control tube (e.g. MG, 23S rRNA wild type (WHITE)).

2. Return and tighten lid. Vortex and centrifuge for 5 - 10 seconds each.

ResistancePlus® MG FleXible

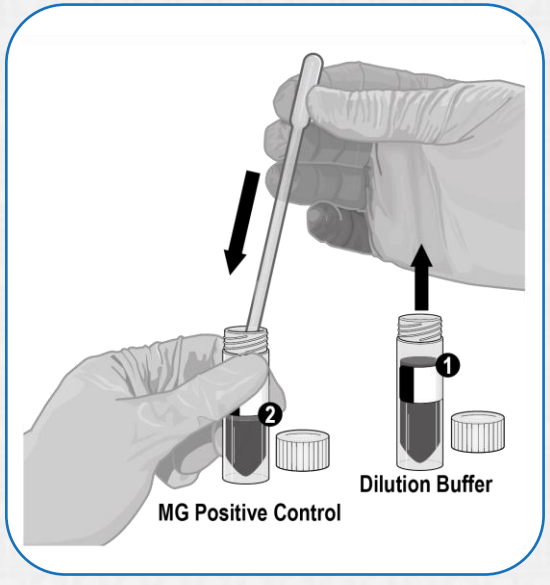
Positive Control Procedure

Transfer pipette

1. Open the Dilution Buffer (NEUTRAL) tube lid. Compress the bulb of the transfer pipette and slowly insert the tip into the Dilution Buffer tube to about a quarter from the bottom.

2. Gently release the pressure on the bulb to fill the transfer pipette while slowly moving the tip to the bottom of the tube. Ensure the transfer pipette has filled approximately up to the 1 mL mark.

3. Insert the transfer pipette into the Positive Control tube so that it touches the interior wall, and gently release the Dilution Buffer from the transfer pipette. Remove the transfer pipette from the tube.

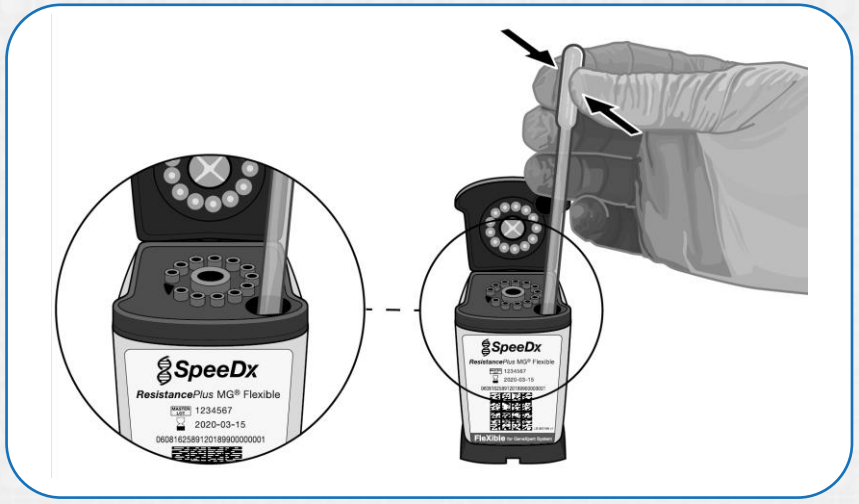


SpeeDx

*Resistance*Plus® MG FleXible

Positive Control Procedure

4. Using a transfer pipette, add the diluted positive control to the sample chamber of the cartridge (right).



5. Run the diluted Positive Control following the same procedure as a clinical sample with the ***Resistance****Plus*® MG FleXible test.

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Warnings and Precautions

Inspect cartridge before use and handle with care!

- Do **NOT** use a cartridge that:
  - Appears damaged
  - Has a damaged reaction tube
  - Has been dropped or shaken
  - Displays signs of reagent leakage or crystallisation
- Do not open the cartridge lid except when adding reaction mix and sample
- Do not place the sample ID label on the cartridge lid or on the barcode label
- Do not reuse cartridges
- Do not dispose of cartridge labels

SpeeDx

4b

ADF

Assay Definition File (ADF)

- The ADF contains the instructions required to run the assay on the GeneXpert® instrument

- The ADF contains:
  - The extraction protocol
  - A QC check (probe check)
  - The thermocycling profile
  - The result interpretation settings

SpeeDx

Probe Check

- Before the reaction commences, the starting fluorescence is measured for each target and compared to the validated Lot Specific Parameter (LSP) range
  - **PASS:** Fluorescence falls within validated LSP range > reaction proceeds
  - **FAIL:** Fluorescence falls outside of validated LSP range > reaction aborted

- Probe Check failure could indicate the following:
  - Incorrect mix preparation or loading;
  - Incorrect reaction-tube filling;
  - Probe integrity/dye stability issues

SpeeDx

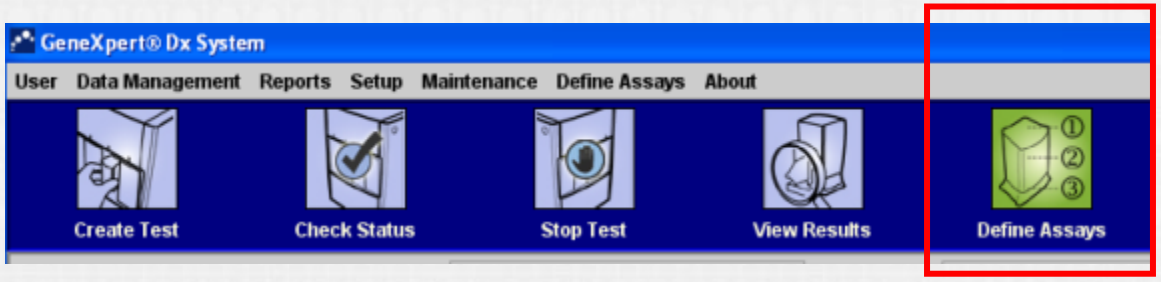
4c

Cartridge loading on the GeneXpert®

Importing the ADF

GeneXpert® DX software

- Select Define Assays from main menu of GeneXpert® Dx software



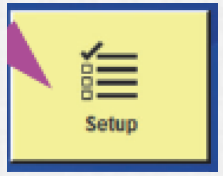
- Browse to the location of the ADF, then click the Open button on the Import Assay dialogue box

SpeeDx

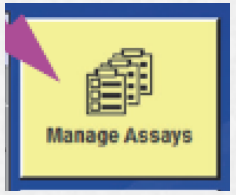
Importing the ADF

Infinity Xpertise software

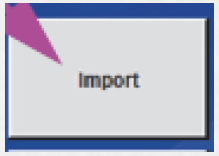
- Select the **Home** icon to display the Xpertise Software Home workspace
- Select the **Setup** button



- In the Setup menu. select **Manage Assays**



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



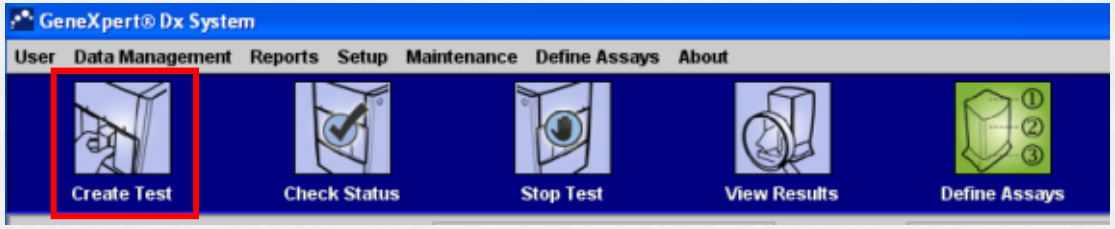
- Browse to the location of the ADF, then click the Open button on the Import Assay dialogue box

SpeeDx

Starting the run

GeneXpert® Dx software

1. Select **Create Test** from main menu



2. Scan/ enter **Patient ID** and **Sample ID**

3. **Scan Cartridge Barcode**. Barcode scan uploads:

- ADF from assay menu
- Min-max values used for checking mix integrity (Probe Check)
- Lot-specific parameters (LSP) used for normalization

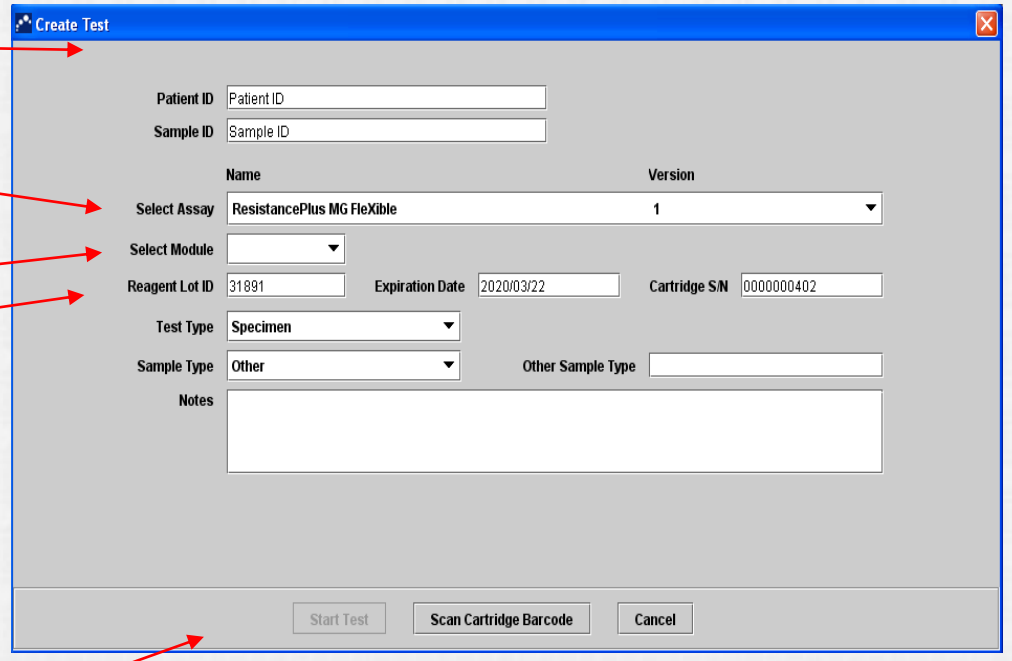
SpeeDx

Starting the run

GeneXpert® Dx software

4. Verify Patient & Sample ID
5. Verify correct ADF is loaded
6. Select the reaction Module
   populated from barcode
7. Load the cartridge

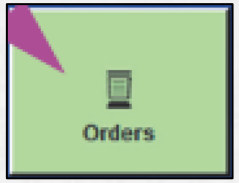
8. Select **Start Test**, close module door



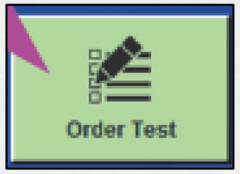
Starting the run

Infinity Xpertise software

1. In the main menu, select **Orders**



2. Select **Order Test**



3. Scan/ enter **Patient ID** and **Sample ID**

4. **Scan Cartridge Barcode**. Barcode scan uploads:
    - ADF from assay menu
    - Min-max values used for checking mix integrity (Probe Check)
    - Lot-specific parameters (LSP) used for normalization

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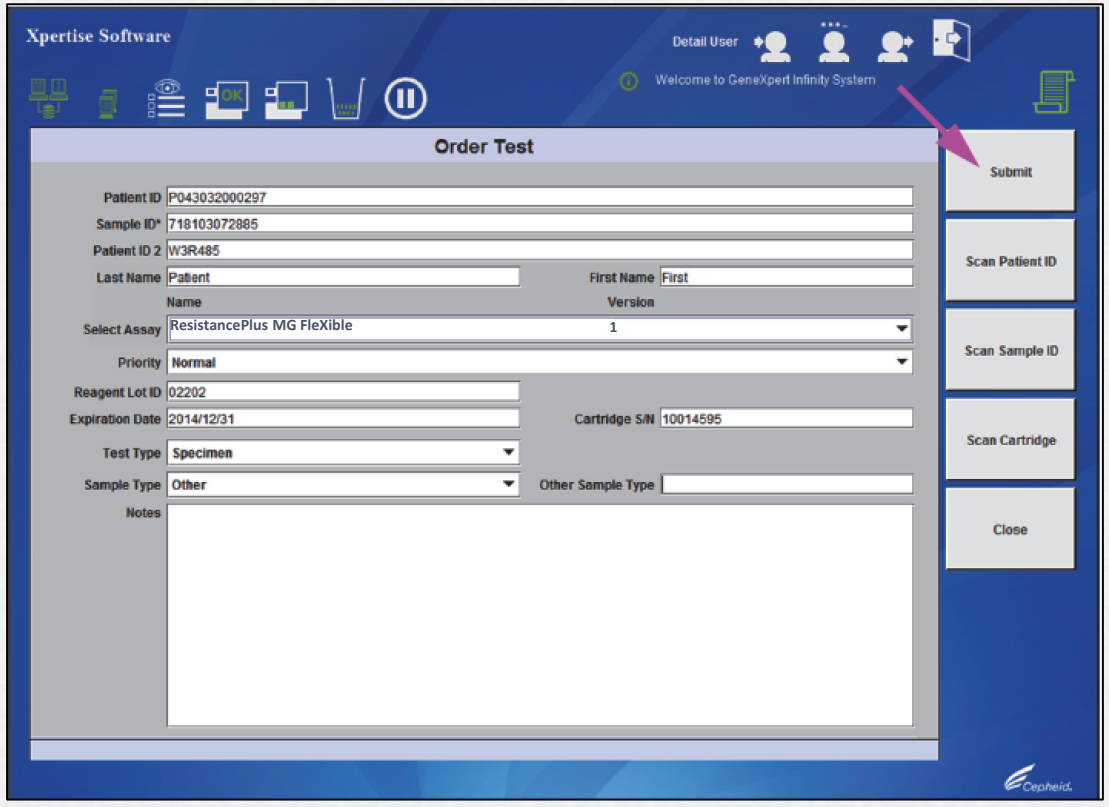
Starting the run

Infinity Xpertise software

1. In the order test workspace:
    - Verify Patient and Sample ID
    - Verify correct ADF is loaded
2. Select **Submit**
   (enter password, if required)
3. Place the cartridge on the conveyor belt
4. The Infinity instrument will automatically load the cartridge and run the test



Part 5

Results

Viewing Test

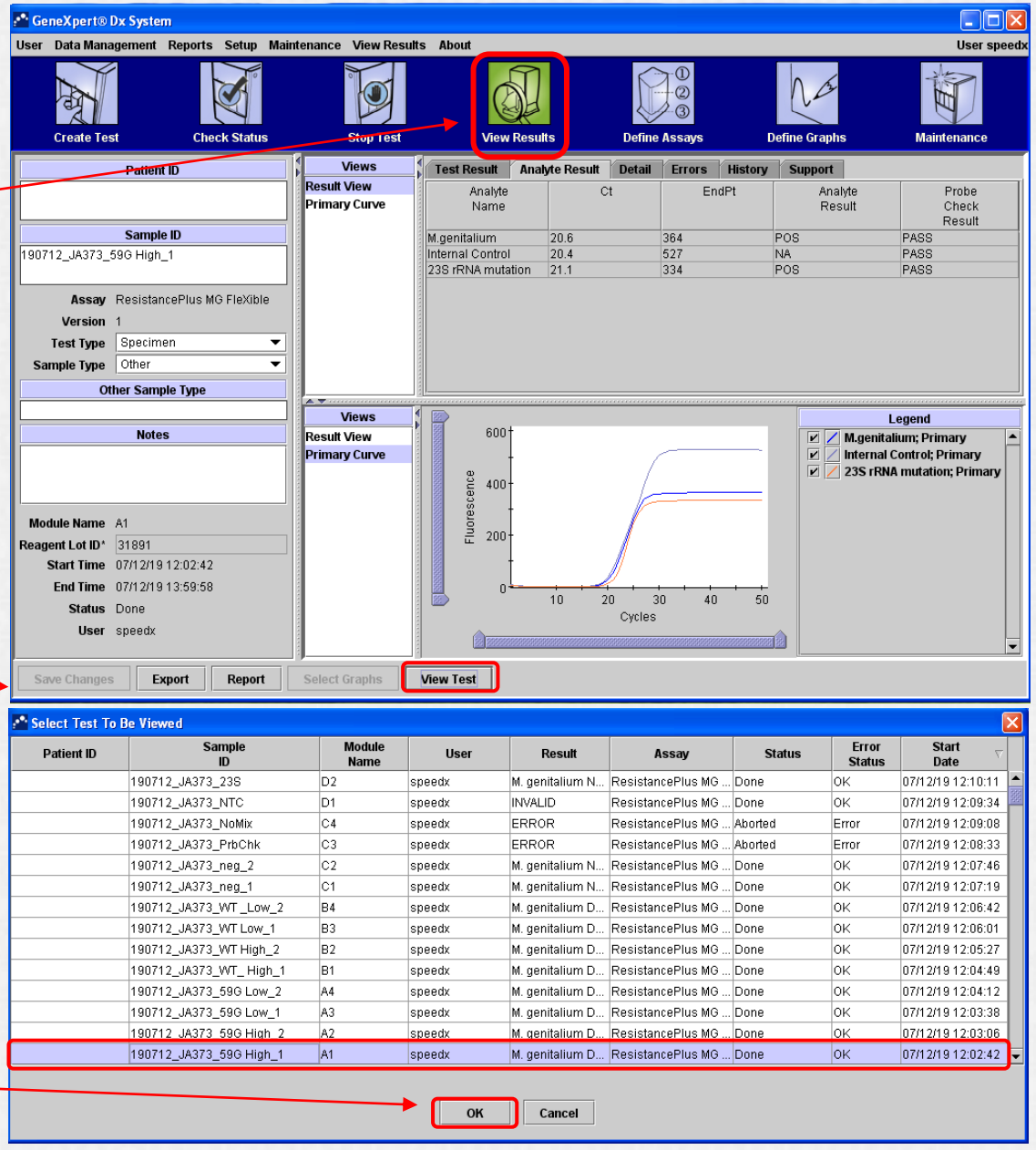
Results

1. Click **View Results**

2. Click **View Test**

3. Select the test to be viewed

4. Click **OK**



Result Example 1:

M. genitalium, 23S rRNA mutant sample

Test Result | Analyte Result | Detail | Errors | History | Support

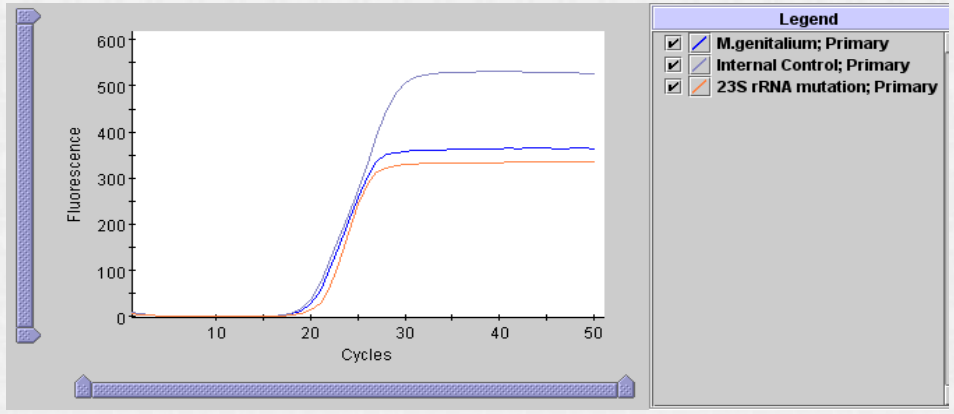
Assay Name ResistancePlus MG FleXible **Version** 1

Test Result M. genitalium DETECTED; 23S rRNA mutation DETECTED

For In Vitro Diagnostic Use Only.

Test Result | Analyte Result | Detail | Errors | History | Support

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



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

SpeeDx

Result Example 2:

M. genitalium, 23S rRNA WT sample

Test Result | Analyte Result | Detail | Errors | History | Support

Assay Name ResistancePlus MG FleXible **Version** 1

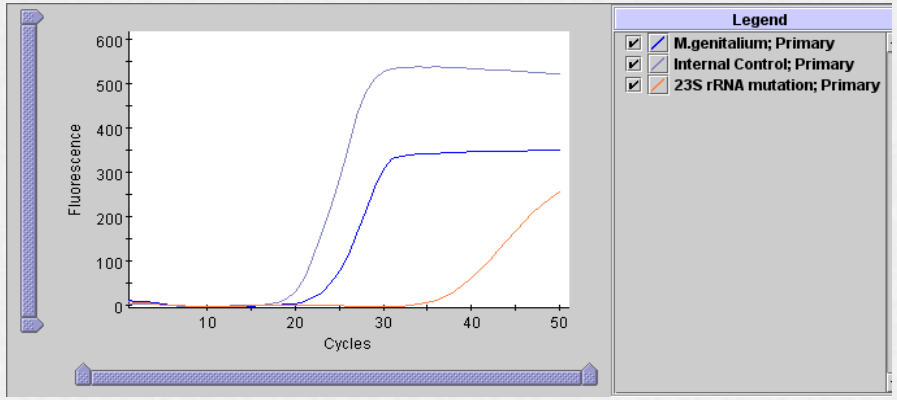
Test Result

M. genitalium DETECTED;
23S rRNA mutation NOT DETECTED

For In Vitro Diagnostic Use Only.

Test Result | Analyte Result | Detail | Errors | History | Support

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
M.genitalium	23.7	351	POS	PASS
Internal Control	20.6	523	NA	PASS
23S rRNA mutation	37.8	257	POS	PASS



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

SpeeDx

Result Example 3:

Negative sample

Test Result | Analyte Result | Detail | Errors | History | Support

Assay Name ResistancePlus MG FleXible **Version** 1

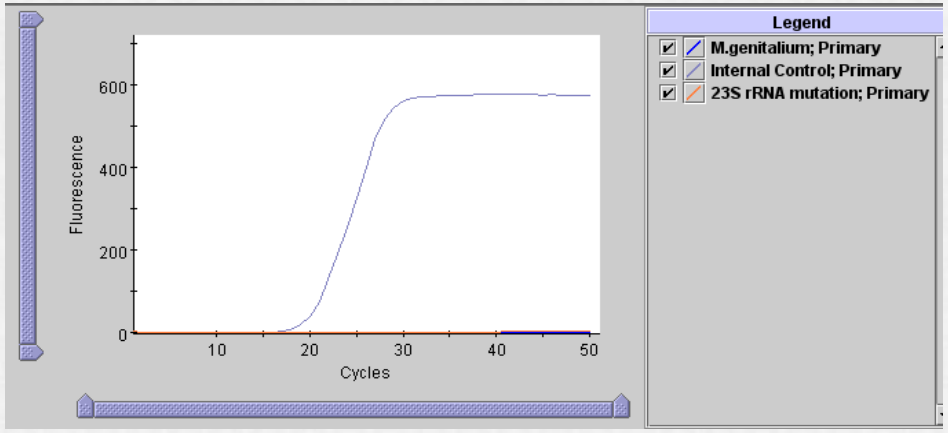
Test Result

M. genitalium NOT DETECTED;
23S rRNA mutation NOT DETECTED

For In Vitro Diagnostic Use Only.

Test Result | Analyte Result | Detail | Errors | History | Support

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



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

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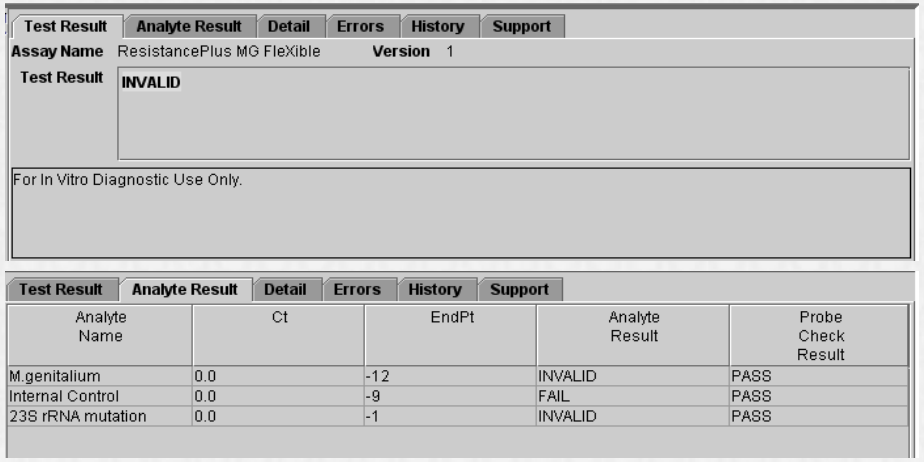
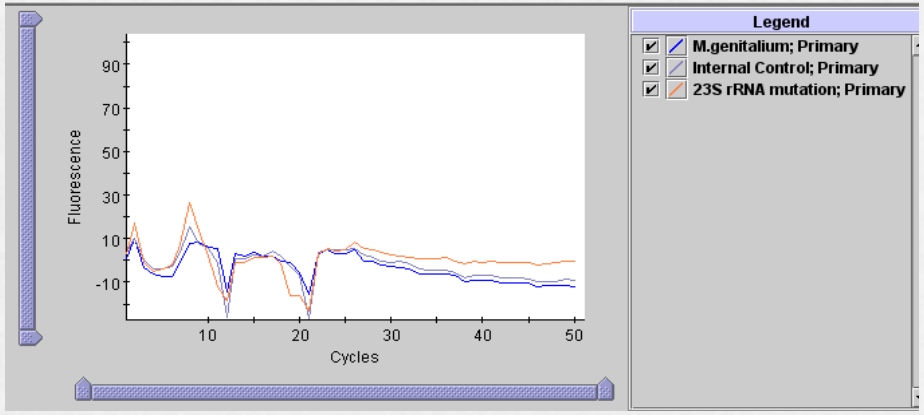
Result Example 4:
Invalid sample





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

Result Example 5:

Error

Test Result | Analyte Result | Detail | Errors | History | Support

Assay Name ResistancePlus MG FleXible **Version** 1

Test Result ERROR

For In Vitro Diagnostic Use Only.

Test Result | Analyte Result | Detail | Errors | History | Support

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

<No Data Available>

Result	Interpretation
ERROR	Presence or absence of *M. genitalium* and 23S rRNA mutation target DNA cannot be determined. **Repeat the test.** If the repeat test does not produce a valid result, collect a new sample to re-test. • Internal control: NO RESULT • Probe check: FAIL*; all or one of the probe check results fail. The PCC may have failed because the reaction mix was made incorrectly, the reaction chamber was filled improperly, or a mix integrity problem was detected.

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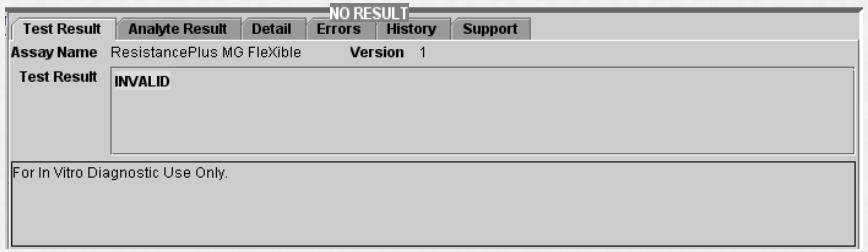
Result Example 6:

No Result



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

Re-test procedure

- A sample re-test will be required when the following results are observed:
  - INVALID
  - ERROR
  - NO RESULT
- The re-test procedure will involve:
  1. Repeat the test using the original sample, if sufficient sample volume (1 mL) is available.
  2. If a valid result is still not produced or if sufficient volume is not available, collect a new sample to re-test.

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All possible results

M.Genitalium	23S rRNA MUTATION	Internal Control	Within ΔCq cutoff?	TEST RESULT	
POS	POS	N/A	YES	**M. genitalium DETECTED**	**23S rRNA mutation DETECTED**
POS	POS	N/A	NO	**M. genitalium DETECTED**	**23S rRNA mutation NOT DETECTED**
POS	NEG	N/A	N/A	**M. genitalium DETECTED**	**23S rRNA mutation NOT DETECTED**
POS^	INVALID^	N/A	N/A	**M. genitalium DETECTED**	**23S rRNA mutation NOT DETECTED**
NEG	POS	N/A	N/A	**M. genitalium NOT DETECTED**	**23S rRNA mutation NOT DETECTED**
NEG	NEG	PASS	N/A	**M. genitalium NOT DETECTED**	**23S rRNA mutation NOT DETECTED**
NEG	INVALID	N/A	N/A	**M. genitalium NOT DETECTED**	**23S rRNA mutation NOT DETECTED**
NEG	NEG	FAIL	N/A	**INVALID**	
INVALID	POS	N/A	N/A	**INVALID**	
INVALID	NEG	N/A	N/A	**INVALID**	
INVALID	INVALID	N/A	N/A	**INVALID**	
INVALID	INVALID	N/A	N/A	**INVALID**	

^If the results output indicate that the MgPa is positive and 23S rRNA is invalid, these samples must be re-tested on an alternative module. Refer to ***Resistance****Plus*® MG FleXible Technical Bulletin (R-1187) for more information

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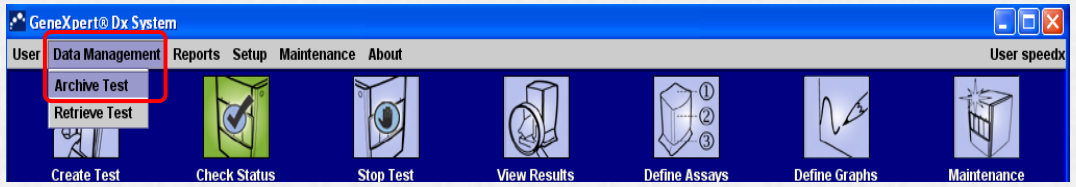
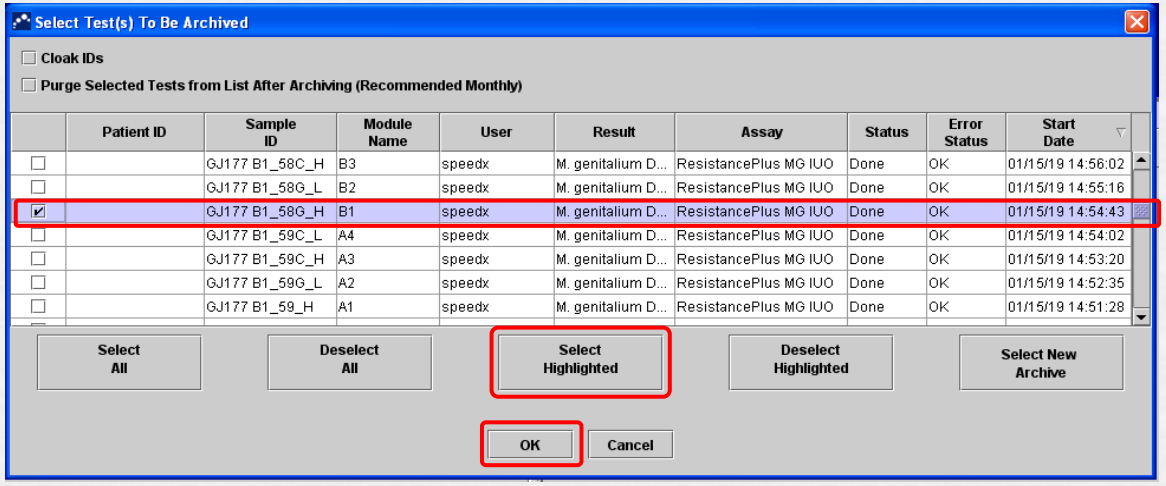
Exporting Test Results – GeneXpert file (.gxx)

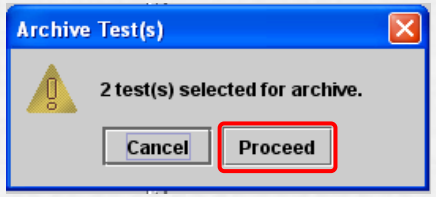
1. From **Data Management** menu, choose **Archive Test**



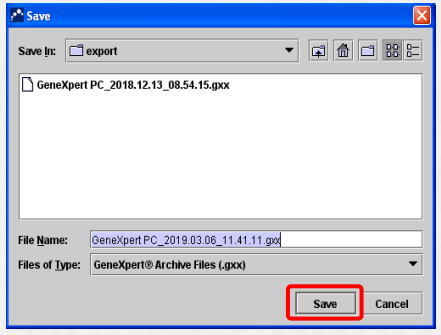
2. Select the run



3. Select **Proceed**



4. Save to desired location



* Depending on instrument user settings some user types may or may not have rights to export.

SpeeDx

Part 6

Performance data

Analytical performance studies

Full details are available in the IFU

<table>
<tr><th>Analytical Study</th><th>Summary</th></tr>
<tr><td>Reproducibility</td><td>100 % result agreement</td></tr>
<tr><td>Analytical sensitivity</td><td>
<table>
<tr><th>M. genitalium 23S rRNA type</th><th>Strain</th><th>Urine - LOD (genomes per sample)</th><th>Vaginal swab - LOD (genomes per sample)</th></tr>
<tr><td>Wild type</td><td>G37</td><td>157</td><td>157</td></tr>
<tr><td>A2058C</td><td>M6302</td><td>317</td><td>317</td></tr>
<tr><td>A2059G</td><td>M6593</td><td>147</td><td>220</td></tr>
<tr><td>A2058G</td><td>M6604</td><td>387</td><td>387</td></tr>
<tr><td>A2058T</td><td>M6926</td><td>151</td><td>151</td></tr>
</table>
</td></tr>
<tr><td>Inclusivity</td><td>8 different M. genitalium strains from geographically diverse locations were all correctly identified</td></tr>
<tr><td>Analytical specificity</td><td>A panel of 42 microorganisms tested. None produced false positive results in the M. genitalium negative urine matrix.</td></tr>
<tr><td>Cross-reactivity to other 23S rRNA mutations</td><td>ResistancePlus® MG FleXible test cross-reacts to the M. genitalium, A2059C 23S rRNA target (5000 copies/ml) at a > 90% hit rate</td></tr>
<tr><td>Interfering substances</td><td>No interference from substances tested other than:
- Blood at a concentration greater than 0.4% v/v
- Bilirubin at a concentration greater than 0.18 mg/mL
- Vagisil intimate powder at a concentration greater than 0.1% w/v</td></tr>
<tr><td>Carry-over contamination</td><td>No carry-over contamination observed</td></tr>
</table>

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Clinical performance

University of Alabama, USA

- 76 specimens
  - 38 male urine (collected in Xpert® Urine Specimen Collection Kit)
  - 38 vaginal swabs (collected in Xpert® Vaginal/Endocervical Specimen Collection kit).

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

SpeeDx

Thank you!

For further enquiries:

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