AusDiagnostics

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URINOGENITAL AND RESISTANCE 12-WELL
REF 87123 VER 01

CONSISTING OF THE IVD COMPONENTS:
STEP 1 TUBES FOR URINOGENITAL AND RESISTANCE 12 WELL REF 87123S
STEP 2 PLATES FOR URINOGENITAL AND RESISTANCE 12 WELL REF 87123P

FOR THE HIGH-PLEX 24 SYSTEM

INSTRUCTIONS FOR USE

IVD ARTG

IVD CE 0123

These Instructions for Use (IFU) must be read in conjunction with the High-Plex 24 System IFU.

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1. WARNINGS AND LIMITATIONS

- **IMPORTANT**: IVD performance claims are only applicable when the instructions provided are followed.
- **IMPORTANT**: Do not use if product or packaging is compromised in any way.
- **IMPORTANT**: Do not use Step 1 or Step 2 panel components with different catalogue and/or version numbers.
- **IMPORTANT**: Do not use expired products.
- Always handle and dispose of specimens potentially containing human pathogens according to relevant safety procedures.
- This panel must only be used with the High-Plex 24 System.
- Good laboratory practice is essential for the intended performance of this panel. For further safety information, please consult the relevant Safety Data Sheets (SDS). **Note:** No AusDiagnostics reagents contain hazardous substances, as listed in Regulation (EC) No 1272/2008[1] and according to the Globally Harmonised System (GHS) classification. AusDiagnostics SDS's can be accessed online at http://www.ausdiagnostics.com/regulatory.html
- This panel is designed to measure specific nucleic acid sequences. Therefore, a negative result does not exclude the possibility that an unusual sequence variant is present. The results obtained with this product should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. A negative result cannot be relied upon for a definitive diagnosis. Failure or delay in treatment of an infected patient may lead to death, with immunocompromised patients being at highest risk.

2. FURTHER INSTRUCTIONS REQUIRED

The Instructions for Use (IFU) for Urinogenital and Resistance 12 well includes panel-specific information not provided in other IFUs. The document ID of the IFU for this product is provided on the outer box label next to the IFU symbol and in the footer of this document. The IFU for this product can be downloaded from the URL printed on the outer box label. Additionally, a paper copy is available upon request by contacting customer service via phone, email, or mail (see **Section 12.** Technical Enquiries).

These Instructions for Use must be read in conjunction with the IFU for:

- High-Plex 24 System (REF 91501)
- Reagent Cassette (REF 40001)

and if applicable:

- Synthetic Positive Controls (REF 91001)
- Swab Elution Tubes (buffer) (REF 90200)

3. NAME AND INTENDED USE

Urinogenital and Resistance (12-well) is intended for *in vitro* diagnostic (IVD) use by suitably trained personnel in qualified laboratories using the High-Plex 24 System (REF 91501).

These tests utilise a multiplex-tandem polymerase chain reaction (MT-PCR)[2] for the amplification of targeted DNA or RNA. For the full description of the principle of the method, see the High-Plex 24 System IFU, **Section 5. Principle of Method**.

Urinogenital and Resistance 12-well is intended as a semi-automated IVD test for the identification of pathogens in nucleic acid extracts from appropriate specimen types. For specimen and sample types that may be used, please see **Section 6. Specimen Requirements**. The Urinogenital and Resistance 12-well panel is for diagnostics purposes only, this product is not suitable for use as a screening test.

The pathogens targeted in this panel are listed and described in **Section 4. Summary and Explanation of the Test.**

4. SUMMARY AND EXPLANATION OF THE TEST

URINOGENITAL AND RESISTANCE 12-WELL REF 87123 VER 01

IVD IVD ARTG CE 0123

ARTG Identifier: 236377; CE Notified Body Number: 0123

Assay	Target
Chlamydia	*Chlamydia trachomatis* (includes all strains)
LGV	Lymphogranuloma venereum (includes all strains)
Gonorrhoeae *	*Neisseria gonorrhoeae* (opa J + opa H gene) (includes all strains)
RUO: Ceftriaxone R	Mosaic penA from *Neisseria gonorrhoeae* (strains containing A311V mutation)
T.vaginalis	*Trichomonas vaginalis* (excludes strain G3)
U.urealyticum	*Ureaplasma urealyticum* (serovars 2, 4, 5 and 7-13)
M.hom, U.parvum	*Mycoplasma hominis* (includes all isolates), *Ureaplasma parvum* (serovars 1, 3, 6, 14)
M.genitalium	*Mycoplasma genitalium* (includes all isolates)
Fluoroquinolone R	*Mycoplasma genitalium* fluoroquinolone resistance
Mgen 23S	*Mycoplasma genitalium* macrolide resistance
Human DNA	Human DNA reference gene for sample adequacy control
SPIKE	Artificial sequence for assay control

***IMPORTANT:** The *Gonorrhoeae* assays detect the opa J and opa H genes. If only one is present a *N.gonorrhoeae* confirmation assay is needed. Please refer to local guidelines for *N.gonorrhoeae* confirmation procedures.

IMPORTANT: *U.urealyticum*, *U.parvum,* and *M.hominis* are conditionally pathogenic. Positive results should always be interpreted whilst considering clinical symptoms.

This product, excluding the RUO marked assay, complies with the regulatory requirements for IVD medical devices of the competent authorities in Australia (ARTG Identifier 236377) and the European Union (CE-marked).

These tests utilise a multiplex-tandem polymerase chain reaction (MT-PCR)[2] for the amplification of targeted DNA or RNA. For the full description of the principle of the method, see the High-Plex 24 System IFU, **Section 5. Summary and Explanation of the Test.**

The Urinogenital and Resistance 12-well panel is intended to detect the following bacteria, parasites, and resistance markers that have been associated with urinogenital infections.

4.1 TARGET DESCRIPTIONS: BACTERIA

Chlamydia trachomatis

Chlamydia trachomatis is the most common bacterial cause of STIs[3]. Different serovars are associated with different disease manifestations: serovars A, B, Ba and C causes trachoma; serovars D-K cause urogenital diseases; and serovars L1, L2 and L3 are responsible for lymphogranuloma venereum (LGV)[4, 5]. The *Chlamydia* and LGV assays are designed to detect all serovars of *C. trachomatis*.

Neisseria gonorrhoeae

Neisseria gonorrhoeae is the second most prevalent sexually transmitted bacterium after C.*trachomatis*. Accurate diagnosis of both symptomatic and asymptomatic infection is critical to prevent transmission and more serious clinical manifestations such as pelvic inflammatory disease (PID) and infertility[3]. The *Gonorrhoea* assay is designed to detect all strains of *N. gonorrhoeae* carrying the opaj and opaH genes.
IMPORTANT: The *Gonorrhoeae* assays detect the opaJ and opaH genes, and if only one is present, a *N.gonorrhoea* confirmation assay is needed (see Smith, *et al.,* (2005) paper on guidelines for Gonorrhoea detection in Australia)[6]. Please refer to local guidelines for *N.gonorrhoea* confirmation procedures.

Mycoplasma hominis

Mycoplasma hominis is of the Mollicute class of bacteria, which are opportunistic pathogens frequently seen in asymptomatic, healthy people. The colonisation of *Mycoplasma* is associated with an increased risk of developing pregnancy complications and other pathogenic conditions including urethritis and pelvic inflammatory disease (PID)[7]. The *M.hominis* assay is designed to detect all known isolates of *M.hominis*.

Ureaplasma urealyticum and ***Ureaplasma parvum***

Ureaplasma urealyticum and Ureaplasma parvum are Mollicutes found in the lower urogenital tract of men and women[8]. The colonisation of *Ureaplasma* is associated with an increased risk of pregnancy complications and neonatal infections[8]. These assays will detect *U.urealyticum* (including serovars 2, 4, 5 and 7 to 13) and *U.parvum* (including serovars 1, 3, 6 and 14) respectively.

Mycoplasma genitalium

Mycoplasma genitalium belongs to the Mollicute class. It is a sexually transmitted infection that has been associated with a range of genitourinary tract infections in both men and women. It has also been identified as a cofactor in HIV transmission[9]. This assay is designed to detect all isolated of *M.genitalium*.

4.2 TARGET DESCRIPTIONS: RESISTANCE GENES

Ceftriaxone-resistance

Ceftriaxone-resistance Neisseria gonorrhoeae FC428 clone was first observed in Japan in 2015, and in 2017, it was documented in Denmark, Canada, and Australia. The FC428 clone harbors a mosaic penA-allele, designated as PenA-60.001 by results of N.gonorrhoeae sequence typing and encodes alterations including A311V and T483S that have previously been associated with N. gonorrhoeae ceftriaxone resistance. The assays for detection of this clone uses primers designed against the PenA-60.001 allele and the A311V mutation. Resistance status is predicted by comparing the concentration of the FC428-ceftriaxone marker (calculated by the MT-PCR Results software) with the specific Neisseria gonorrhoeae markers opaJ and opaH[10].

Macrolide and ***fluoroquinolone resistance***

Macrolide and fluoroquinolone resistance in Mycoplasma genitalium are designed to amplify wild-type (i.e. unmutated) sequences (23S gene for macrolide resistance, or parC gene for fluoroquinolone resistance). Strains containing resistance-associated mutations in these regions (A2058 or A2059 for 23S, S83 or D87 for parC) will be amplified with lower efficiency. Comparison of the concentrations calculated by the MT-PCR Results software for each of these targets with a marker elsewhere in the Mycoplasma genitalium genome allows the specific identification of mutant strains and therefore prediction of resistance status.[11]

4.3 TARGET DESCRIPTIONS: PARASITES

Trichomonas vaginalis

Trichomonas vaginalis is a single-celled protozoan parasite that is the most common non-viral cause of

STIs. In 2008, there was an estimated 276.4 million new adult cases of *T.vaginalis* worldwide[3]. Approximately 80% of *T.vaginalis* detections in women are asymptomatic. The *Trichomonas* assay detects *T.vaginalis* strains and some *T.tenax* strains. The *T.vaginalis* assay is designed to detect all *T.vaginalis* strains excluding G3.

5. PANEL COMPONENTS: MATERIALS AND STORAGE

Note: No AusDiagnostics reagents contain hazardous substances, as listed in Regulation (EC) No 1272/2008[1] and according to the Globally Harmonised System (GHS) classification.

Product Name	Component products	REF	GTIN
Urinogenital and Resistance 12 well	Step 1 Tubes: Urinogenital and Resistance 12-well	87123S	9343044002861
	Step 2 Plates: Urinogenital and Resistance 12-well	87123P	9343044002854
To be used with:	Low DNA Reagent Cassette	40231DNA	9343044003080

5.1 STEP 1 TUBES

Step 1 Tubes for Urinogenital and Resistance 12-well contain tubes for 96 samples.

Materials	Label	Description	Function	Qty.
Step 1 Tubes	**STEP 1 TUBES**	1 x 96-slot plastic frame containing 12 x 8-well tube strips with dried oligonucleotides.	Receptacle for the Step 1 reaction	96

STORAGE AND HANDLING INSTRUCTIONS

The Step 1 Tubes may be stored between 14°C - 29°C.

Expiration of product is 6 months from manufacture and expiration date is provided on the label.

5.2 STEP 2 PLATES.

The Step 2 Plates box for Urinogenital and Resistance 12-well contains materials for 288 samples.

Materials	Label	Description	Function	Qty.
Step 2 Plates box	**STEP 2 PLATES**	Outer box	Outer packaging	1
Step 2 Plate	**STEP 2 PLATE**	Sealed bag containing 384-well plate with dried oligonucleotides and two desiccant pouches	Receptacle for the Step 2 PCR reaction	12
Dilution plate	(D)	Empty, 96-well plate	Houses the dilution for Step 2	6

STORAGE AND HANDLING INSTRUCTIONS

The Step 2 Plates box may be stored between 14°C - 29°C.
IMPORTANT: Ensure desiccant is intact before removing product from bag; do not use product if desiccant is compromised or missing
Expiration of product is 6 months from manufacture and expiration date is provided on the label.

5.3 REAGENT CASSETTE*

Outer box label	Materials	Description
LOW DNA REAGENT CASSETTE REF 40231DNA	Step 1 DNA Mastermix	Enzymes in buffer for the Step 1 reaction
	Step 2 DNA Mastermix	Enzymes in buffer for the Step 2 reaction
	Water	Used to dilute mastermix and samples
	Oil	To prevent evaporation of the Step 1 reaction

STORAGE AND HANDLING INSTRUCTIONS

WARNING: Reagent cassettes may arrive thawed. This will not affect the performance of the reagent cassettes. Upon arrival, immediately store the reagent cassettes below -20°C.

WARNING: A run must be started within 30 minutes of thawing the reagent cassette. Reagent cassettes are single-use only. Do not re-freeze reagent cassettes.

Note: Take care to fully defrost reagent cassettes before **gently** inverting and spinning down as frozen reagents may pierce the foil seal.

Note: Do not re-use reagent cassettes. Dispose of them according to appropriate safety procedures. Expiration of product is 6 months from manufacture and expiration date is provided on the label.

5.4 MATERIALS REQUIRED BUT NOT PROVIDED

Required reagents and equipment that are not provided by AusDiagnostics are:

- Personal protective equipment (PPE)
- Bleach with 0.4% available chlorine (4 mL required per run)
- Nuclease-free and adjustable pipettes
- Nuclease-free and sterile filtered tips

5.5 ADDITIONAL CONSUMABLES

- Robot tips, ZTF-100-R-S, Carton (50 racks of 96) (REF 93250 VER 01)
- Robot tips, ZTF-100-R-S, Carton (10 racks of 96) (REF 93210 VER 01)
- Tip disposal bags (100) for High-Plex (REF 91502 VER 01)
- Bleach tubes (60) for High-Plex (REF 91503 VER 01)
- Pack of 6 Dilution Plates, Foil Sealed (REF 90020 VER 03)

Please contact AusDiagnostics to purchase these consumables (see **Section 12. Technical Enquiries**).

6. SPECIMEN REQUIREMENTS

6.1 SPECIMEN TYPES AND VOLUME

A nucleic acid extract that is suitable for PCR should be used with this product. Acceptable specimen types include genital swabs, vaginal swab, urethral swab, endocervical swab, rectal swab, mouth swab, lesion swab, eye swabs, skin swab, urine, and semen.

M.hominis, U.urealyticum, U.parvum, Chlamydia, and T.vaginalis may be detected directly, without nucleic acid extraction, from eluates of vaginal, endocervical and urethral swabs. (See **Section 6.3 Swab Preparation**).

Note: please store your nucleic acid extract in tubes free from PCR inhibitors and nucleases.

IMPORTANT: Always handle and dispose of specimens potentially containing human pathogens according to relevant safety procedures.

Volume of sample to be added to Step 1 tube must be 10 µL.

6.2 SUITABLE NUCLEIC ACID EXTRACTION METHODS

Manual extraction and pipetting of nucleic acid extracts into Step 1 Tubes is best performed in a biological safety cabinet or PCR setup area.

The following manual and automated nucleic acid extraction methods have been validated by AusDiagnostics customers, and have been deemed suitable to produce nucleic acid extracts compatible with the Urinogenital and Resistance (12-well) panel.

Extraction System	Protocol Name	Type
AusDiagnostics MT-Prep Extractor	MT-Prep Virus/Pathogen Extraction Kit	Automated
Abbott 2000	mSample Preparation System DNA	Automated
bioMerieux NucliSENS easyMAG	NUCLISENS magnetic silica particles	Automated
bioMerieux EMAG	EMAG magnetic silica particles	Automated
PerkinElmer	chemagic Prepito-D	Automated
PerkinElmer JANUS	Chemagic DNA Kit	Automated
Qiagen	QIAcube	Automated
Qiagen EZ1	EZ1 DSP Virus Kit	Automated
Qiagen QIAsymphony	DSP/Virus/Pathogen Mini Kit	Automated
Roche Cobas 4800	Cobas 4800	Automated
Roche High Pure series	High Pure Viral Nucleic Acid Kit	Manual
Roche MagNA Pure series	High Pure Viral Nucleic Acid Kit	Automated
Roche MagNA Pure LC 2.0	LC Total NA panel LV Kit	Automated
Roche MagNA Pure 96	Pathogen Universal 200	Automated

6.3 SWAB PREPARATION

As clinically appropriate, this panel can be used directly with eluates from swabs, either dry or in viral transport medium, combined with Swab Elution Tube (0.5 mL buffer) (REF 90220). See **Section 6.1 Specimen Types and Volume** for suitable swab types.

Swab preparation using Swab Elution Tube (0.5 mL buffer) (REF 90220) has been validated for use with Copan and Ditch transport medium swabs.

Please consult the Swab Elution Tube (0.5 mL buffer) IFU before use.

7. FURTHER MT ASSAY SET UP OPTIONS

This section is intended to be read in conjunction with the High-Plex 24 System IFU (**Section 7.3 Run the Processor)**.

Additional MT Assay Setup software options are as follows:

Sampling: The table below explains the options provided for robotic sampling:

Sampling options	Applicable situation
Manual pipetting into tube strip	Nucleic acid extracts have been manually transferred to the Step 1 Tubes prior to starting the run (therefore no robotic sampling required).
Robot sampling from 5 mL tubes	Specimens eluted into Swab Elution Tube (0.5 mL buffer) (REF 90220) are loaded on the Autosampling block for robotic sampling. Minimum volume 500 µL.
Robot sampling from 2 mL tubes	Nucleic acid extracts stored in 2 mL tubes are loaded on the Autosampling block for robotic sampling. Minimum volume 40 µL.
Robot sampling from 1.5 mL flip-cap tubes	Nucleic acid extracts stored in 1.5 mL flip-cap tubes are loaded on the Autosampling block for robotic sampling.
Robot sampling from 96 well plate samples 1-24	Nucleic acid extracts stored in a 96-well plate are placed on the Autosampling block section for robotic sampling of wells 1 - 24. Minimum volume 40 µL.
Robot sampling from 96 well plate samples 25-48	Nucleic acid extracts stored in a 96-well plate are placed on the Autosampling block section for robotic sampling of wells 25 - 48. Minimum volume 40 µL.
Robot sampling from 96 well plate samples 49-72	Nucleic acid extracts stored in a 96-well plate are placed on the Autosampling block section for robotic sampling of wells 49 - 72. Minimum volume 40 µL.
Robot sampling from 96 well plate samples 73-96	Nucleic acid extracts stored in a 96-well plate are placed on the Autosampling block section for robotic sampling of wells 73 - 96. Minimum volume 40 µL.

8. RESULTS

WARNING: IVD performance claims do not extend to any changes made by the user to a result (i.e. "Reject" or "Confirm" a result). Any user changes will be clearly indicated in the Analysis Report.

The cycling curves and the melt curves of a run are displayed in the MT Analysis Software. Based on predefined parameters, the software will call the target as 'Present', 'Check' or blank (not detected). Note that multiple infections are possible. Molecular target concentrations, expressed as arbitrary units, are calculated relative to the internal control SPIKE, which amplifies a known amount of target molecules. As the concentration of SPIKE is not measured these values are in arbitrary units. Furthermore, in some panels the relative concentration of each target may be inferred from the normalised percent (displayed in the parentheses after the 'Present' call.

For targets LGV, Ceftriaxone Resistance, Fluoroquinolone resistance, and Macrolide Resistance, the results software identifies predicted phenotype based on the relative concentrations of these targets to other markers in the genome of the relevant organism (Chlamydia for LGV, Neisseria gonorrhoeae for ceftriaxone resistance, and Mycoplasma genitalium for fluoroquinolone and macrolide resistance). Results for these targets are displayed in the Diagnosis window, under the Results table, and are independent of 'present' calls.

For further details on analysis of results, please refer to the *High*-Plex System IFU (**Section 11. Interpreting Software Results**).

IMPORTANT: Please report results to state or local public health departments, if applicable.

9. CONTROLS

9.1 POSITIVE CONTROL

It is recommended that positive controls be included in every run. Please refer to individual laboratory procedures. The failure of a positive control should lead to the reassessment of any negative result obtained since the last control was run.

The Synthetic Positive Controls for STDs and Herpes (REF 91021) contains all targets for Urinogenital and Resistance 12 well.

The Synthetic Positive Controls IFU must be read before using this product.

9.2 NEGATIVE CONTROL

It is recommended that a negative control be run according to the individual laboratory procedures. Amplification of the negative control indicates contamination from the environment (e.g. from handling during set up or spillages of sample material on the MT Processor deck). In this case, the surface of the MT Processor deck (including the thermal cycler cover) should be wiped with a non-corrosive nucleic acid denaturing reagent (e.g. DNA-OFF™) and then UV treated. The relevant samples should be retested.
DO NOT USE BLEACH TO CLEAN THE INSTRUMENT.

9.3 SAMPLE ADEQUACY AND HUMAN DNA CONTROL

The Human DNA control assay targets a human DNA reference gene, to indicate the presence of human DNA in the nucleic acid extract or direct sampling specimen. If no target is detected and the Human DNA control assay does not amplify, the results cannot be relied upon. In this circumstance, AusDiagnostics recommends re-testing on a new extract or sample.

9.4 SUITABLE NUCLEIC ACID CONTROL

It is the user's responsibility to ensure a suitable nucleic acid extraction procedure is in place. It is recommended a known positive control be included per extraction run.

If the nucleic acid extraction control is not detected, the negative results cannot be relied upon. It is recommended that any sample with a negative nucleic acid extraction control should be re-collected and re- extracted if appropriate, and analysis repeated

9.5 INSTRUMENT FUNCTION AND SAMPLE INHIBITION CONTROL

SPIKE is a completely artificial sequence that is present in Step 1 Tubes to monitor sample inhibition and instrument performance. SPIKE has been designed to have no cross-reactivity with diagnostic targets or assays. If SPIKE is shown to be inhibited, then this suggests that the sample contained inhibitory substances, or that the reaction conditions are suboptimal. In this case a negative result cannot be relied upon and it is recommended that the sample should be re-extracted if appropriate, and analysis repeated. For further details on analysis of SPIKE, please refer to the High-Plex 24 System IFU (**Section 9.2 Verification of Instrument Function and Sample Inhibition**).

10. PERFORMANCE CHARACTERISTICS

10.1 REPRODUCIBILITY AND REPEATABILITY

The reproducibility of the assays on the High-Plex 24 System was assessed by testing five samples on three batches across three days (with each batch tested once per day) and three systems with three operators. The coefficient of variation (c_V) for the resulting mean cycle take-off values (Ct values) for each of the samples were calculated. It was found that the Ct values for all samples at all concentrations were highly reproducible with c_V values ranging from 2.08% to 4.86%, averaging 3.40%.

The repeatability of the assays on the High-Plex 24 System was assessed by testing five samples on three batches with one batch tested three times each day on one system. It was found that the Ct values for all samples at all concentrations were highly repeatable with c_V values ranging from 2.26% to 5.11%, averaging 3.84%.

The low c_V from these studies provides evidence that the High-Plex 24 System is suitably precise for IVD use.

10.2 INTERFERING SUBSTANCES

A range of exogenous and endogenous substances including those expected to be found on skin or in blood were tested for potential PCR interference. Minimal or no interference was seen due to the presence of any one of the substances tested.

The presence of the internal control, SPIKE, in all AusDiagnostics panels controls for possible PCR interference in each sample.

For further details on interfering substances, please refer to the High-Plex 24 System IFU (**Section 13.1 Interfering Substances**).

10.3 ANALYTICAL SPECIFICITY

The cross-reactivity of the primers with non-targeted pathogens was assessed as part of the clinical validations of the targets.

Macrolide and fluoroquinolone resistance is assessed by comparing calculated concentrations for these targets relative to the specific Mycoplasma genitalium marker. No cross-reaction of these resistance markers was seen in samples containing high concentrations of other targets on the panel (*Neisseria gonorrheae*, *Chlamydia trachomatis*, *Ureaplasma parvum*, *U.urealyticum*, *Mycoplasma hominis*, *Trichomonas vaginalis*) or in negative samples containing normal vaginal flora.

10.4 ANALYTICAL SENSITIVITY

The limit of detection (LoD) was determined using serial dilutions of plasmids, with a multiplexed Step 1 amplification and 16 replicates per dilution tested. The LoD was determined to be the lowest concentration that showed 100% target amplification. Ranges are used when samples with higher concentrations were not detected, contrary to 100% amplification of samples with lower concentrations. The LoD calculation is given as either copies per 10 µL sample or copies per mL of the original sample. The sample copies/mL calculation is based on 100% efficient nucleic acid extraction that concentrates a 200 µL sample into a 50 µL eluate.

Assay	**LoD (copies/ 10 µL)**	**LoD (copies/ mL)**
Chlamydia momp	16-41	400-1025
LGV	2-3	50-75
Gonorrhoeae (opaJ) *	6-16	150-400
Gonorrhoeae (opaH) *	8-13	200-325
RUO: FC428 Ceftriaxone R	91-114	2275-2850
Trichomonas	22	550
U.urealyticum	11-28	275-700
M.hominis	2-33	50-825
U.parvum	12-30	300-750
M.genitalium	38	950
Fluoroquinolone R	104-130	2600-3250
Mgn 23S	2-31	50-775

*The LoD of these targets have been determined separately (i.e. not as a combined assay).

10.5 CLINICAL PERFORMANCE FOR NUCLEIC ACID EXTRACTED SPECIMENS

The clinical performance for the targets used in this product were assessed by multiple clinical laboratories in Australia and Europe. Each clinical laboratory's alternative method was compared to the assays offered in this product.

Assay	Sensitivity % (95% CI)	Specificity % (95% CI)
Chlamydia	96.5 (92.8-98.3)	100.0 (99.2-100.0)
LGV	96.0 (85.1-99.3)	100.0 (92.7-100.0)
Gonorrhoeae (opaJ+opaH)	97.2 (92.4-99.1)	99.7 (98.9-100)
Gonorrhoeae (opaJ)	96.5 (91.7-98.7)	99.9 (99.1-100.0)
Gonorrhoeae (opaH)	91.8 (84.6-95.9)	99.7 (98.1-100.0)
RUO: FC428 CeftriaxoneR *	**Research Use Only**	
Trichomonas	100.0 (85.0-100.0)	100.0 (94.7-100.0)
M.hominis	91.4 (80.2-96.8)	98.7 (96.5-99.5)
U.parvum	97.6 (93.6-99.2)	96.4 (92.5-98.4)
U.urealyticum	94.2 (85.1-98.1)	97.2 (94.1-98.8)
M.genitalium	100.0 (95.2-100.0)	100.0 (97.3-100.0)
Fluoroquinolone resistance associated mutations	100.0 (89.9-100.0)	100.0 (79.1-100.0)
Macrolide resistance associated mutations **	94.2 (85.1-97.6)	97.2 (83.8-99.9)

* These assays have been validated with <20 confirmed positive samples. During primer design for each assay, bioinformatics analysis is conducted whereby the target sequence of the primers is analysed in all publicly available sequences. This analysis is based on published research and in-house studies on the importance of any mismatches in primer sequence on the efficiency of qPCR. This provides evidence that AusDiagnostics assays should detect all specified targets, and therefore assays validated with a low number of confirmed samples can be relied upon.

** The false negatives for this assay were also missed by the comparative CE-marked method. They were determined by sequencing.

The FC428 CeftriaxoneR assay is for Research Use Only (RUO).

10.6 CLINICAL PERFORMANCE FOR DIRECT SAMPLING OF URINE AND SWABS

The clinical performance for the targets used in this product for directly testing eluates from swab specimens (see **Section 6. Specimens Requirements**) was assessed by multiple clinical laboratories in Australia and Europe. Each clinical laboratories' alternative method was compared to the assays in this product.

Assay	Sensitivity % (95% CI)	Specificity % (95% CI)
Chlamydia	88.5 (75.9-95.2)	100.0 (82.2-100.0)
Gonorrhoeae (opaJ)*	100.0 (From 2 positive samples)	100.0 (88.3-100.0)
Trichomonas*	100.0 (From 4 positive samples)	100.0 (87.7-100.0)
M.hominis*	100.0 (From 15 positive samples)	100.0 (90.8-100.0)
U.parvum	100.0 (85.4-100.0)	100.0 (85.4-100.0)
U.urealyticum*	100.0 (From 7 positive samples)	100.0 (86.7-100.0)

* These assays have been validated with <20 confirmed positive samples. During primer design for each assay, bioinformatics analysis is conducted whereby the target sequence of the primers is analysed in all publicly available sequences. This analysis is based on published research and in-house studies on the importance of any mismatches in primer sequence on the efficiency of qPCR. This provides evidence that AusDiagnostics assays should detect all specified targets, and therefore assays validated with a low number of confirmed samples can be relied upon.

11. LIMITATIONS OF THE PROCEDURE

- This product is only for use by suitably trained personnel in qualified laboratories.
- Urinogenital and Resistance 12-well Panel performances has only be established on High-Plex 24 systems.
- The assays in this product do not provide a quantitative value for the pathogen(s) in the sample.
- The performance of the test has been evaluated for use with human specimen material only.
- The performance of this test has only been validated with nucleic acid extracts from the following specimens types: genital swabs, vaginal swab, urethral swab, endocervical swab, rectal swab, mouth swab, lesion swab, eye swabs, skin swab, urine, and semen. *M.hominis, M.genitalium, U.urealyticum, U.parvum, and Chlamydia* may be detected directly, without nucleic acid extraction, from eluates of vaginal, endocervical and urethral swabs. It has not been validated for use with other sample types.
- The performance of this test has not been established for patients without symptoms of Genital Infectious Agents.
- The performance of this test has not been established for immunocompromised individuals.
- The Urinogenital and Resistance 12-well panel is designed to measure specific nucleic acid sequences. Therefore, a negative result does not exclude the possibility that an unusual sequence variant is present. The results obtained with this product should be used in conjunction with information available form clinical evaluations and other diagnostics procedures. A negative result cannot be relied upon for a definitive diagnosis. Failure or delay in treatment of an infected patient may lead to death, with immunocompromised patients being at highest risk.

- The detection of nucleic acid is dependent upon proper specimen collection, handling, transportation, storage, and preparation (using manufacturer of specimen collection device instructions). Failure to follow proper procedures can lead to incorrect results.
- The *Gonorrhoeae* assays detect the opa J and opa H genes, and if only one is present a *N.gonorrhoeae* confirmation assay is needed (see Smith, *et al.,* (2005) paper on guidelines for *Gonorrhoeae* detection in Australia)[6]. Please refer to local guidelines for *N.gonorrhoeae* confirmation procedures.
- **IMPORTANT:** *U.urealyticum*, *U.parvum,* and *M.hominis* are conditionally pathogenic. Positive results should always be interpreted whilst considering clinical symptoms.
- **WARNING:** The FC428 Ceftriaxone R assay is Research Use Only (RUO) until sufficient clinical performance data has been obtained.

12. TECHNICAL ENQUIRIES

Further instructions and troubleshooting can be found in the High-Plex 24 System IFU (**Section 14. Troubleshooting**).
For assistance or if any issues recur, please contact AusDiagnostics.
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13. ACKNOWLEDGEMENTS

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14. GLOSSARY

The following symbols can be found on AusDiagnostics Urinogenital and Resistance 12-well panel components or throughout these Instructions for Use (IFU). Use the definitions below as a guideline to interpret the symbols.

14.1 SYMBOLS

Where relevant, symbols are taken from ISO 15233-1:2016 Medical devices - Symbols to be used with medical device labels, labelling and information to be supplied.

	Manufacturer
	Authorised representative in the European Union
 or REF	Catalogue Number
 or Ver	Version number
LOT	Lot/Batch code
GTIN	Global Trade Identification Number
	Indicates that the relevant product is intended for *in vitro* diagnostic use in Australia and is included on the Australian Register of Therapeutic Goods (ARTG).
	Indicates that the relevant product is intended for *in vitro* diagnostic use in the European Economic Area and is compliant with the European IVD Directive 98/79/EC as certified by TÜV SÜD Product Service GmbH, Notified Body with identification no. 0123.
	WARNING. Please read the indicated section carefully.
	Please consult the identified instructions for use before use
	Do not re-use
	Storage temperature range (upper and lower limit)
	Storage temperature range (upper limit only)
	Positive Control
	Expiry date (yyyy-mm-dd)

14.2 DEFINITIONS

Panel of Assays: A set of panel components that are intended to be used together to detect a specific group ("panel") of targets. Panel components include Step 1 tubes, Step 2 plates, Mastermix, and template.

Panel-specific IFU: Instructions for Use (IFU) which contain information specific to a panel.

Operator: The individual who is interacting with the High-Plex 24 System.

Primers: Short synthetic oligonucleotides specifically designed to bind to and amplify specific gene sequences under conditions provided during PCR.

A Run: All steps from starting the MT Assay Setup Software (see **Section 7.3 Run the processor**) to generation of the MT Analysis file considered "a run".

RUO: ASSAY FOR RESEARCH USE ONLY (RUO).

PSO:ASSAY FOR PERFORMANCE STUDY ONLY (PSO).

Target: A gene or sequence that primers are designed to hybridise with.

WARNING: Indicates a statement that alerts users about a situations that, if not avoided, could result in hazards or other serious adverse consequences from the use of the device.

IMPORTANT: Indicates a statement that alerts the user to special care or special activities necessary for the safe and effectives use of the device.

Note: Indicates additional information.

15. DISCLAIMER

AusDiagnostics does not warrant or guarantee that its products are merchantable or satisfactory for any particular purpose, and there are no warranties, express or implied, to such effect. AusDiagnostics will not be liable for any incidental, consequential or contingent damages involving the use of its products. AusDiagnostics' responsibility is limited to replacement of items ordered only.

AusDiagnostics reserves the right to discontinue or change specifications, products, services, or models at any time without incurring obligations.

In no event shall AusDiagnostics be responsible for failures, errors, or other liabilities resulting from customers' noncompliance with the procedures and precautions outlined herein or as a result of pathogen variants which were not sequenced at the time of assay design.

16. REFERENCES

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17. PUBLISHED PAPERS

Published papers using AusDiagnostics products can be found on our website under the COMPANY tab, Publications: https://www.ausdiagnostics.com/publications.html.

18. DOCUMENT HISTORY

Document ID of IFU	Date of Change	Changes
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