



Protocol

MGH/UUP/TV Diagnostic GPack

(REF: MUT/GPACK/100)

PRINCIPLE OF METHOD

GeneProof PCR kits offer our innovative One Workflow solution allowing the detection of multiple parameters in one run using our Universal PCR Profile for majority of the GeneProof PCR kits (RNA and DNA pathogens).

ASSAY PROCEDURE

1. Use the required sample volume in accordance with the extraction manual.
2. Thaw the Universal Internal Control (purple cap) in the refrigerator (2 - 8 °C) for about 15 minutes. Vortex and briefly centrifuge the thawed solution before use. Add Universal Internal Control at 10% of the elution volume to each sample.
3. Perform sample extraction according to the appropriate protocol.
4. Thaw the PCR reagents in a refrigerator (2 - 8 °C) for 15 to 30 min. Vortex and centrifuge briefly before use:

GeneProof *Mycoplasma genitalium/* *hominis* PCR Kit

- MGH Master Mix (blue cap)
- MGH Positive Control (white cap)
- Negative Control (NFW* – not included)

GeneProof *Ureaplasma* PCR Kit

- UUP Master Mix (blue cap)
- UUP Positive Control (white cap)
- Negative Control (NFW* – not included)

GeneProof *Trichomonas vaginalis* PCR Kit

- TV Master Mix (blue cap)
- TV Positive Control (white cap)
- Negative Control (NFW* – not included)

*NFW= Nuclease-free water

5. **PCR preparation** (total reaction volume is 40 µl), pipette:

- a) A1: 30 µl MGH Master Mix + 10 µl MGH Positive Control
- b) B1: 30 µl MGH Master Mix + 10 µl Negative Control
- c) C1: 30 µl MGH Master Mix + 10 µl extracted DNA
- d) A2: 30 µl UUP Master Mix + 10 µl UUP Positive Control
- e) B2: 30 µl UUP Master Mix + 10 µl Negative Control
- f) C2: 30 µl UUP Master Mix + 10 µl extracted DNA
- g) A3: 30 µl TV Master Mix + 10 µl TV Positive Control
- h) B3: 30 µl TV Master Mix + 10 µl Negative Control
- i) C3: 30 µl TV Master Mix + 10 µl extracted DNA

PCR Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	MGH PC	UUP PC	TV PC									
B	MGH NC	UUP NC	TV NC									
C	MGH S1	UUP S1	TV S1									
D	MGH S2	UUP S2	TV S2									
E									
F												
G												
H												

MGH = *M. genitalium*/*M. hominis*; UUP = *Ureaplasma*; TV = *Trichomonas vaginalis*; PC = Positive Control;
NC = Negative Control; S1 = unknown sample 1; S2 = unknown sample 2

MGH/UUP/TV Diagnostic GPack

- Centrifuge the PCR plate briefly.
- Set up the Universal PCR Profile on the PCR cyclor according to the GeneProof Mycoplasma genitalium/hominis PCR Kit.

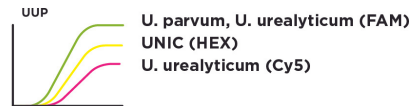
Step	Process	Temperature °C	Time
1	UNG decontamination/ Reverse Transcription	42	15 mins
2	Initial denaturation	95	10 mins
3	Denaturation	95	5 s
	Annealing	60	40 s
	Extension	72	20 s

EVALUATION

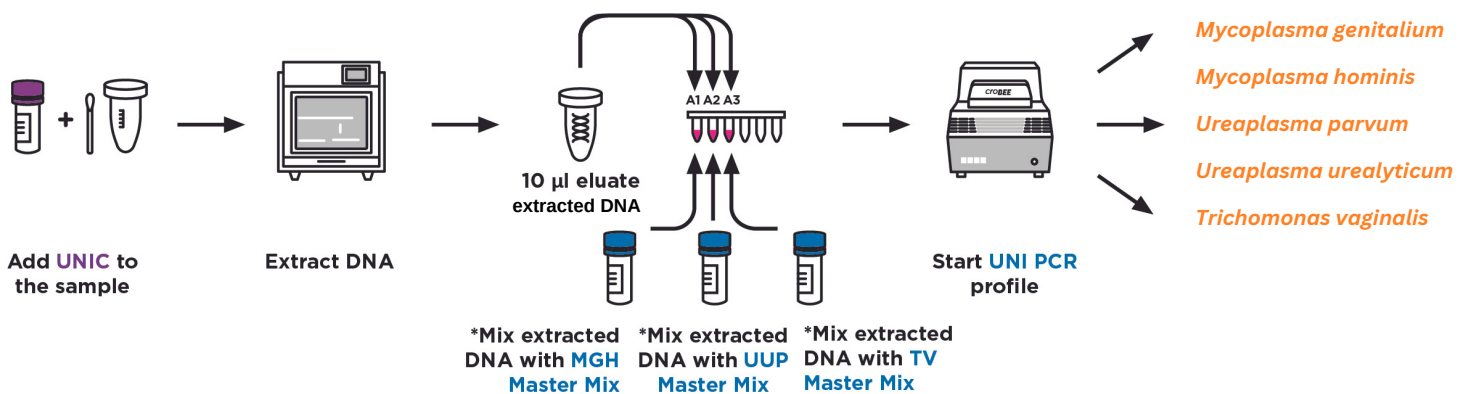
Column 1 - *M. hominis*, *M. genitalium*

Column 2 - *U. parvum*, *U. urealyticum*

Column 3 - *T. vaginalis*



MGH/UUP/TV Diagnostic GPack Workflow



*Add appropriate number of Positive and Negative Controls according to the IFU

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For the results that matter most.

1. Urethritis GPack: detection of 4 pathogens

Chlamydia trachomatis, *Neisseria gonorrhoeae*,
Mycoplasma genitalium, *Trichomonas vaginalis*

2. Urethritis Plus GPack: detection of 7 pathogens

Chlamydia trachomatis, *Neisseria gonorrhoeae*,
Mycoplasma genitalium, *Trichomonas vaginalis*,
Mycoplasma hominis,
Ureaplasma urealyticum/parvum

3. Genital Ulcer GPack: detection of 3 pathogens

Herpes Simplex Virus 1, *Herpes Simplex Virus 2*,
Treponema pallidum

4. CT/NG Screening GPack: detection of 2 pathogens

Chlamydia trachomatis, *Neisseria gonorrhoeae*

5. MGH/UUP/TV Diagnostic GPack: detection of 5 pathogens

Mycoplasma genitalium, *Trichomonas vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum/parvum*