Molecular detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* from urine and vaginal swab samples

HGCTNGR

Instructions for Use

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CE 2797

1. Kit Contents

HG CT/NG Combo 30-test kits contain:

	Description
HG CT/NG Combo Reaction Strips **Part # CTNG-RX**	3 pouches, each containing 10 x 2-tube reaction strips with desiccant sachets Reaction strips contain complete lyophilized reaction mixes for CT/NG and Extraction Control Component 1, Reaction Strips: Contains: oligonucleotides, fluorescent dye, nucleoside triphosphates, TE buffer solution polymerase enzyme, excipients Component 2, Extraction control: Contains: nucleic acid, buffer solution, enzyme Contains no active ingredients Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.
HG CT/NG Resuspension Buffer Tubes **Part # CTNG-RB**	30 x tubes, each containing 1.4 ml of Extraction Control in buffered solution Component 3, Resuspension buffer: Contains: nucleic acid, buffer solution, Proclin 950 Contains no active ingredients Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

2. Shelf life & Storage

- Kits must be stored at 2-8°C and used before the expiry date on the kit label
- Reaction strip pouches must be re-sealed fully after use and returned to storage at 2-8°C
- All 10 reaction strips from an individual pouch must be used within 3 weeks of initial opening of the pouch

3. Accessories

Supplied by HiberGene:

- **HG Swift/Swift Plus Strip Carriers** are supplied with the HG Swift/Swift Plus instrument and are used for handling, loading and unloading of HG CT/NG reaction strips. These are also available to order separately (Part # HGCAR).
- **HG Swift/Swift Plus Set-up Racks** are supplied with the HG Swift/Swift instrument and are used for loading samples into HG CT/NG reaction strips. These are also available to order separately (Part # HGRACK).
- **HG CT/NG Control Kit (Part # HGCTNGC)** is a separate positive control kit available from HiberGene. This kit provides controls for the performance of both Target and Extraction Control reaction mixes.

Required but not provided:

- Dry bath/Heat block– the selected thermal block must be capable of holding 2ml tubes and maintaining a temperature of 105ºC +/- 1ºC.
- Centrifuge – must be capable of holding 2ml microcentrifuge tubes and spinning at 2700-3300 rcf.
- Calibrated micropipettes.
- 2ml microcentrifuge tubes, certified nuclease-free.
- Micropipette tips with filters, certified nuclease-free.

4. Intended Use

HG CT/NG Combo is a LAMP-based molecular diagnostic test for the detection and differentiation of clinically relevant strains of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine and vaginal swabs.

The intended end user is a trained laboratory/health professional. Users must have received training from the distributor prior to using the device.

5. C. trachomatis & N. gonorrhoeae - Clinical Significance

Chlamydia trachomatis (CT) is a bacterium which causes a common sexually-transmitted infection (STI); with WHO estimates of 131 million new cases per year [1]. The infection affects both men and women, across all age groups.

CT infection is asymptomatic in 70% of women and 50% of men. Where present, symptoms may include painful urination, lower abdominal pain and discharge from the penis / vagina. If untreated, the infection may persist for up to 4 years [2]. Complications of infection in men include epididymitis and reduced fertility. In women, untreated CT infection may result in pelvic inflammatory disease (PID); the risk of infertility associated with PID may be as high as 18% [3]. In pregnant women, CT infection can increase the risk of ectopic pregnancy and preterm delivery. Vertical transmission from infected mothers to neonates can give rise to complications including *C. trachomatis* conjunctivitis [4].

C. trachomatis is a gram-negative bacterium; 15 separate serovars of the organism have been classified, based on variations in the outer membrane protein [5]. Recommended sample types for diagnosis of lower genital tract CT infection are first void urine and vaginal swabs, and nucleic acid amplification testing (NAAT) is regarded as the gold standard method [6].

Neisseria gonorrhoeae (NG) is the causative agent of gonorrhoea, an STI which infects more than 100 million people each year [7], making it the second most common bacterial STI after *C. trachomatis*.

As with *C. trachomatis*, the majority of cases of gonorrhoea are asymptomatic [8] – especially in women. Undetected or untreated infection can give rise to severe complications including PID and infertility. Rates of HIV acquisition are also increased for individuals with gonorrhoea [9]. Vertical transmission can also cause life-threatening conditions, including sepsis, for newborns [10].

Neisseria gonorrhoeae are gram-negative cocci, and more than 50 serovars have been classified based on the structure of the porin protein [11]. NAAT is the test of choice for diagnosis of NG infection, with urine and genital swabs the recommended sample type for men and women, respectively [12].

6. Principle of the Assay

The HG CT/NG combo assay utilizes isothermal LAMP technology to deliver a rapid and sensitive testing solution for *Chlamydia trachomatis* and *Neisseria gonorrhoeae,* with an integrated control for sample handling and assay set-up. HiberGene's embodiment of LAMP technology (loop-mediated isothermal amplification [13] [14]) employs novel primer design, a highly efficient strand-displacing polymerase and specific fluorophore-labelled probes to detect and differentiate the nucleic acid of the pathogens at constant temperature.

LAMP assays targeting conserved genes of both CT and NG form the basis of the assay and are found in the "Target" tube. Also present in the reaction strip are primers for an Extraction Control (EC) sequence, which controls for the absence of inhibitors and for correct assay set-up.

Both Target and EC reaction mixes are presented in a ready-to use lyophilized format, containing the primers, enzyme, cofactor and buffering components required to allow LAMP amplification when reconstituted with sample.

The reaction mixes contain specific fluorescent probes which allow the amplification of both species to be monitored. Binding of CT probe to its specific target sequence results in an increase in fluorescence in the yellow channel of the HG Swift, while binding of NG probe results in a quenching of fluorescence in the instrument's blue channel. These

changes in fluorescence are monitored in real-time on board the HG Swift instrument.

7. Precautions

General Precautions

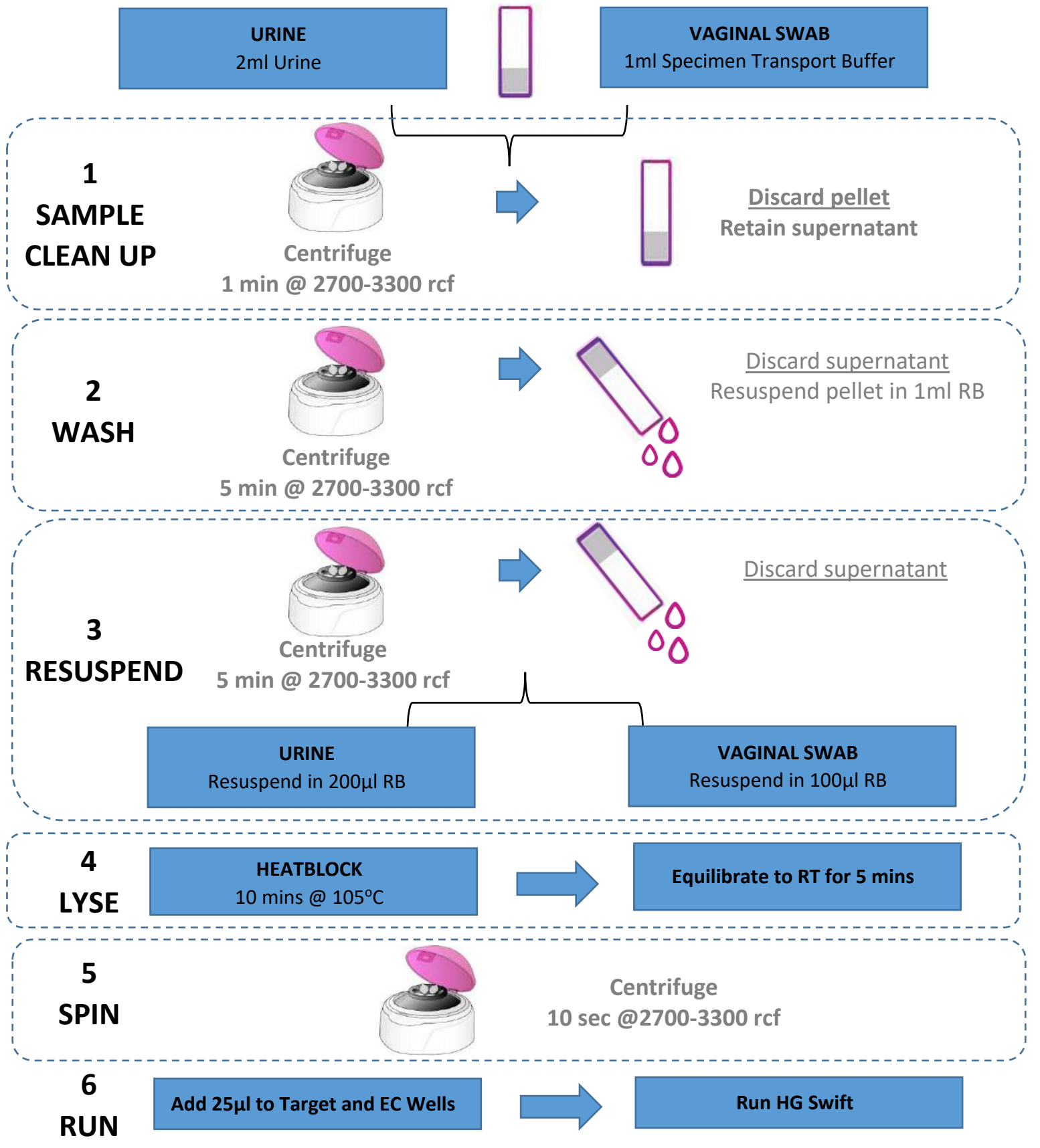
- The HG CT/NG Combo kit is for *in vitro* diagnostic use only.
- Training on the test protocol must be carried out before use of the test.
- Appropriate personal protective equipment should be worn when handling clinical specimens. Gloves should be worn at all times during sample handling and assay-set-up.
- Specimens should be handled and disposed of per Good Laboratory Practice, and all specimens should be dealt with as if infectious. Hands should be washed thoroughly after handling clinical specimens.
- Surplus kit components should be disposed of in accordance with established safety procedures.
- Never mouth-pipette, eat or drink in the laboratory.
- To avoid burns, exercise caution when removing samples from 105^{o}C heating.
- Take every care to avoid cross-contamination between samples.
- Pipette tips used should include filters and be certified nuclease-free. Micropipettes used should be calibrated in accordance with applicable guidelines.
- Clean down all work surfaces after assay runs with a disinfectant solution with proven efficacy in DNA removal.
- The instrument should not be used in an area with a high or low magnetic field.
- Kits with damaged packaging or opened pouches should not be used.
- Any serious incidents that occur in relation to HGCTNGR must be reported to the manufacturer. This reporting may be completed via the relevant distributor/supplier, or directly to the manufacturer (HiberGene) using the contact details in Section 15 below.

Preventing amplicon contamination

- Escape of amplified material from reaction strips after LAMP amplification can result in laboratory contamination which could impact on subsequent test results. HG CT/NG Combo reaction strips are specifically designed to resist accidental reopening, but the following specific precautions must always be followed:
  - After adding sample into tubes, close caps **firmly and completely**.
  - **Never** re-open the caps of the reaction strips after closing.
  - After the run, remove the reaction tube strip from the HG Swift/Swift Plus lifting by the handles of the Strip Carrier.
  - Dispose of the used strips firstly into a small sealable plastic bag and then into a bin. Empty the bin regularly and do not allow large amounts of waste to build up on top of bags containing used reaction strips.
  - Work areas must be regularly cleaned with appropriate DNA decontamination solutions.
  - It is recommended to run NTCs periodically to check for contamination (See section 9).

8. Performing the Test

The flowchart below illustrates the workflow to be followed for both urine and vaginal swab in Specimen Transport Buffer:



8.1. Sample Collection & Handling - Urine

Collect first void urine samples per applicable clinical guidelines in a suitable sterile container. Urine may be stored at 2-8ºC for up to 7 days before testing.

Using a calibrated micropipette, transfer 2ml of urine into a nuclease-free 2ml microcentrifuge tube and process as follows:

1. **Sample clean-up**
   Centrifuge for 1 min at 2700-3300 rcf. Retain supernatant by pouring into a separate nuclease-free 2ml microcentrifuge tube. Discard the pellet.

2. **Wash**
   Centrifuge the retained supernatant for 5 min at 2700-3300 rcf. Decant the supernatant by pouring off the liquid and then gently tapping the inverted tubes on a paper towel in order to remove any final drops. Resuspend the pellet in 1ml of CT/NG Resuspension Buffer by adding buffer to the tube and pipetting up and down several times in the location of the pellet.

3. **Resuspend**
   Centrifuge the resuspended pellet for 5 min at 2700-3300 rcf. Decant and discard the supernatant as above and resuspend the pellet by pipetting with 200 µl of CT/NG Resuspension Buffer.

Note: *During centrifugation steps, it may be useful to align the microcentrifuge tubes in the same direction, noted by the position of the tabs/hinges on the tube lids. Following centrifugation, the cell pellet will be located at the lower region of the tube wall furthest away from the centre-point of the rotor. Aligning the tubes in uniform manner will allow the location of the pellet to be identified in cases where it is not visible*

8.2. Sample Collection & Handling – Vaginal Swabs

Collect vaginal swabs per applicable clinical guidelines using one of the following validated swab and transport media combinations:

- 3ml Copan UTM + Copan Flocked Swab (Copan, Cat. No. 306C)
- 3ml Puritan UniTranz + Flocked Swab PurFlock Ultra (Puritan Medical Products Cat. No. UT-306)
- Abbott multi-Collect Specimen Collection Kit (1.2ml transport media + swab, Abbott, US, Cat. No. 9K12-02)

Note: Consult manufacturer's guidelines for sample stability data.

Using a calibrated micropipette, transfer 1ml of Specimen Transport Buffer into a nuclease-free 2ml microcentrifuge tube and process as follows:

1. **Sample clean-up**
   Centrifuge for 1 min at 2700-3300 rcf. Retain supernatant by pouring into a separate nuclease-free 2ml microcentrifuge tube. Discard the pellet.

2. **Wash**
   Centrifuge the retained supernatant for 5 min at 2700-3300 rcf. Decant the supernatant by pouring off the liquid and then gently tapping the inverted tubes on a paper towel in order to remove any final drops. Resuspend the pellet in 1ml of CT/NG Resuspension Buffer by adding buffer to the tube and pipetting up and down several times in the location of the pellet.

3. **Resuspend**
   Centrifuge the resuspended pellet for 5 min at 2700-3300 rcf. Decant and discard the supernatant as above and resuspend the pellet by pipetting with 100 µl of CT/NG Resuspension Buffer.

Note 1: *During centrifugation steps, it may be useful to align the microcentrifuge tubes in the same direction, noted by the position of the tabs/hinges on the tube lids. Following centrifugation, the cell pellet will be located at the lower region of the tube wall furthest away from the*

centre-point of the rotor. Aligning the tubes in uniform manner will allow the location of the pellet to be identified in cases where it is not visible

Note 2: Adjustment of sample volume for swab specimens
It is possible to reduce the volume of swab medium used, provided:

a) That the resuspension buffer used at the final step is also reduced pro rata, such that the ratio of initial sample to resuspended volume remains 10:1.

b) That the final resuspension volume is sufficient to allow 2 x 25µl dispenses into the Target and EC wells of the reaction strip, with a dead volume of at least 10µl – i.e. minimum final volume of 60µl

8.3. Lysis

Note: *Positive Controls do not require heat-treatment*

1. Place each resuspended sample in a dry bath incubator/heat block at 105°C for 10 minutes.

2. Remove and leave to equilibrate to Room Temperature for 5 minutes.

3. Following the equilibration step, centrifuge the sample tube at 2700-3300 rcf for 10 seconds to remove condensate from the lid and top of the tube.

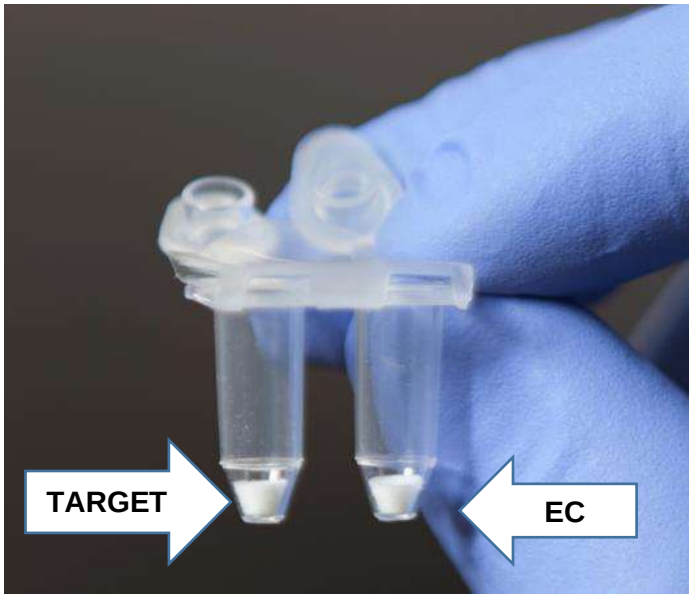
4. Add the lysed diluted sample to the reaction mix as soon as possible after the centrifugation step.

8.4. Assay set up

1. Open the pouch containing the HG CT/NG Combo reaction strips, tearing across from the notches on the pouch.

2. Remove the reaction strips, one strip per test to be conducted.

3. **It is vital to orient the reaction tubes in the correct direction.** When lids are facing away from the user, the **left-hand tube** in the strip contains **Target** reaction mix and the **right-hand tube** contains EC reaction mix, as shown below:



4. Peel off the plastic seal on the tubes carefully, taking care not to disrupt the lyophilised pellet. (If lyophilized pellets are sticking to the sealing film, tap the strip lightly until they fall to the bottom of the tubes.)

5. Place the strip(s) in the HG Swift/Swift Plus Set-up Rack in the correct orientation and add 25µL of sample to both the target (T) and extraction control (EC) tubes.

6. Close the lid on each tube tightly by pressing firmly on the lids.

It is critically important to ensure that lids are fully closed before commencing the run.

7. Reconstitute the reaction mixes by vortexing briefly – minimise foaming of the solution. The vortexed reaction strips should then be briefly centrifuged (e.g. 10 sec at 3300 rcf) to ensure that all liquid is returned to the bottom of the tubes, as shown below:

Visually examine the reaction mix pellet after mixing to ensure lyophilized reaction mixture pellets are fully dissolved.

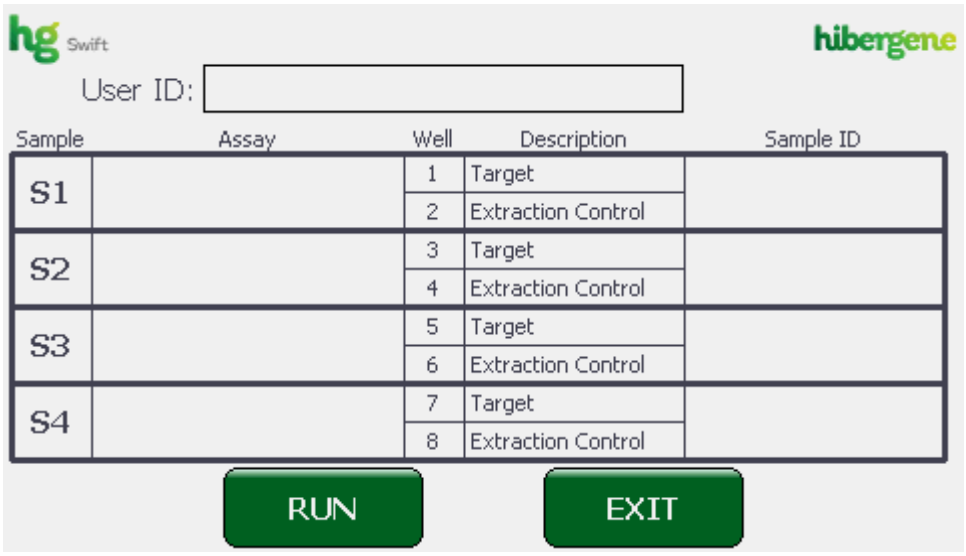
Final reaction mixtures must be loaded and run on the HG Swift/HG Swift Plus as soon as possible after reconstitution. Do not allow to stand for any longer than 10 minutes before starting the run.

8.5. Run set up

1. Turn on the HG Swift/Swift Plus using the power switch located at the back of the instrument.

2. Select START NEW RUN:

The Run Table will then be displayed:

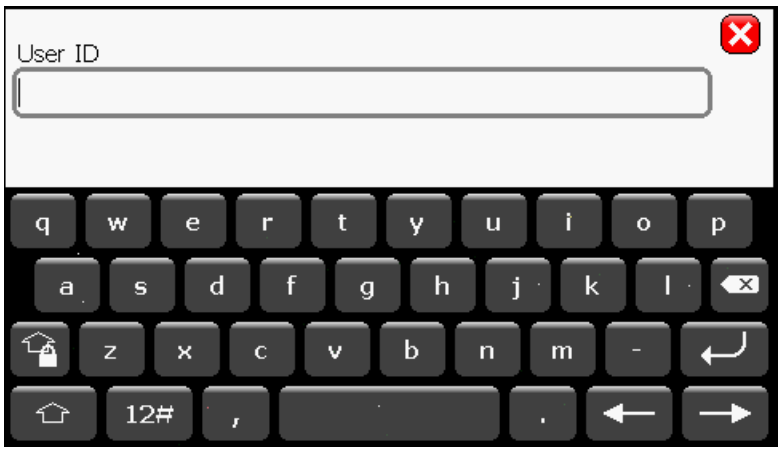


3. Load the reaction strip(s) onto the block of the HG Swift/Swift Plus using a Strip Carrier, as shown below:

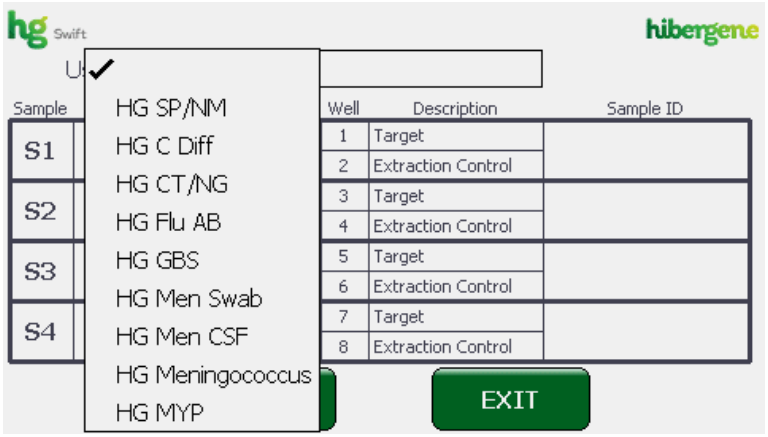
The reaction strips must be loaded IN THE CORRECT ORIENTATION as shown above, with hinges towards the rear of the instrument.

Once reaction strips are loaded into the instrument, press down firmly on the lids a final time to ensure they are closed

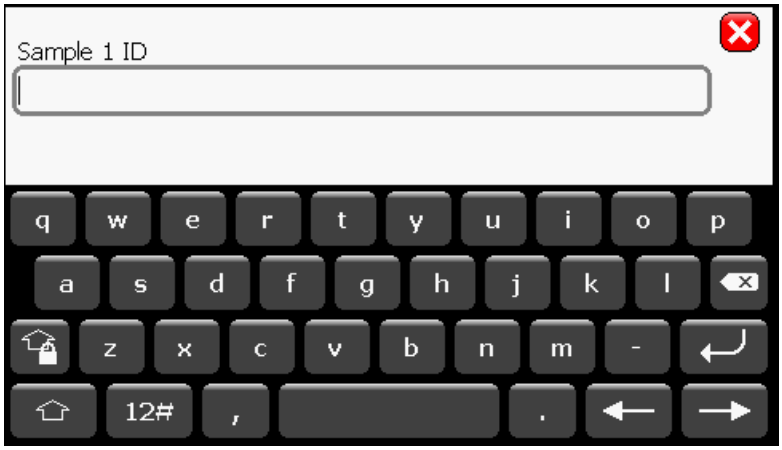
4. Enter the User ID using either the on-screen keyboard or a barcode reader attached to the HG Swift/Swift Plus USB port:



5. For each sample to be tested, select HG CT/NG from the dropdown assay menu:

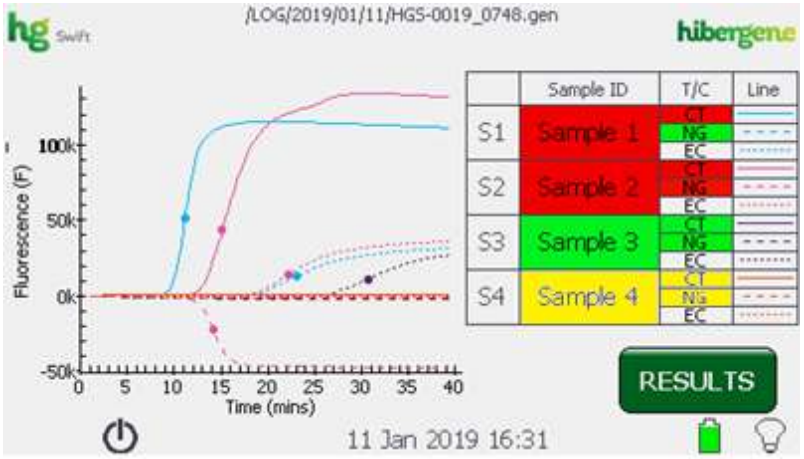


6. For each sample to be tested, enter the sample ID using either the on-screen keyboard or a barcode scanner:



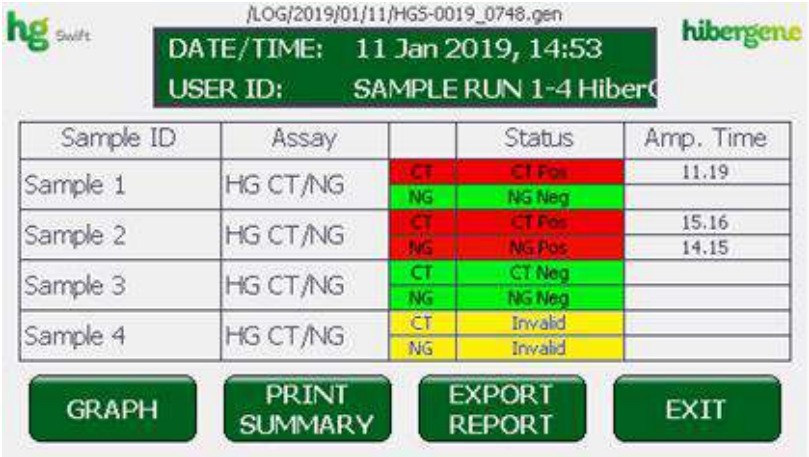
7. Press RUN.

8. The HG CT/NG Combo run will take 40 mins to complete. During the run, the fluorescence of all samples will be displayed (see example below). The run may be aborted by pressing the STOP button:



9. Assay Results

The HG Swift/Swift Plus software interprets the fluorescent signals in Target and Control wells and returns the status of samples as in the following example:



The sample status which is displayed can be interpreted as follows.

Displayed	Description
CT Pos	*Chlamydia trachomatis* detected in the sample
NG Pos	*Neisseria gonorrhoeae* detected in the sample
CT Neg	*Chlamydia trachomatis* not detected in the sample
NG Neg	*Neisseria gonorrhoeae* not detected in the sample
INVALID	Extraction Control did not amplify. If no positive result is returned for either target, this indicates issues with sample treatment or the presence of inhibitors. No sample result will be returned, and the sample should be re-tested.

For positive samples, the amplification time is displayed. This provides semi-quantitative information: earlier Amp Times reflect higher levels of target sequence.

9.1. Result printing & storage

When a label printer is attached via the HG Swift/Swift Plus USB port, a printed summary including User ID, Date and status of all samples can be obtained by pressing the PRINT SUMMARY button.

Pressing the EXPORT REPORT button generates a more detailed report which includes the amplification graphs. This report can be retrieved from the Reports folder on the HG Swift when a PC is attached (see HG Swift Instrument Manual).

If PCs are run as samples, amplification times per the HGCTNGC IFU will indicate acceptable performance.

If required, a No Target Control (NTC) may be included in the run by adding Resuspension Buffer to both tubes of a strip – this should return a valid Negative result and no amplification should be visible in the target tube during the run.

10. Performance Characteristics

10.1. Analytical Performance

The analytical sensitivity of the HG CT/NG Combo reaction mix (CTNG-RX) was determined for both assay targets in both specimen matrices. Specificity was determined by testing a panel of potential cross-reactive organisms.

10.1.1. Analytical Sensitivity – *Chlamydia trachomatis*

For CT LOD testing in urine, samples were prepared by spiking negative donor urine with quantified *Chlamydia trachomatis* Serovar E cells (Cat # VR348B, ATCC).

For CT LOD testing in vaginal swabs, samples were prepared by spiking Specimen Transport Buffer from negative vaginal swabs collected using Abbott multi-Collect Specimen Collection Kit with quantified *Chlamydia trachomatis* Serovar E cells (Cat # VR348B, ATCC).

Samples were prepared at a range of concentrations, and treated per the standard protocol with centrifugation and resuspension. Treated samples were tested across 3 Validation batches (7 reps per dose per batch - a total of 21 replicates per dose).

The observed detection rates were used for Probit analysis; the Limit of Detection (LOD) is quoted below as that concentration with a 95% probability of detection:

Target Material	Sample Matrix	LOD - 95% Probability of Detection (95% CI)
Chlamydia trachomatis Serovar E	Urine	17.5 CFU/ml (13.4 – 27.8 CFU/ml)
	Swab	20.5 CFU/ml (16.3 – 43.2 CFU/ml)

10.1.2. Analytical Sensitivity – *Neisseria gonorrhoeae*

For NG LOD testing in urine, samples were prepared by spiking negative donor urine with quantified *Neisseria gonorrhoeae* F-18 cells (Cat # 49226, ATCC).

For NG LOD testing in vaginal swabs, samples were prepared by spiking Specimen Transport Buffer from negative vaginal swabs collected using Abbott multi-Collect Specimen Collection Kit with quantified *Neisseria gonorrhoeae* F-18 cells (Cat # 49226, ATCC).

Samples were prepared at a range of concentrations, and treated per the standard protocol with centrifugation and resuspension. Treated samples were tested across 3 Validation batches (7 reps per dose per batch - a total of 21 replicates per dose).

The observed detection rates were analysed, and the Limit of Detection (LOD) is quoted below as the lowest concentration of NG for which 100% of replicates were amplified:

Target Material	Sample Matrix	LOD – Lowest Dose with 100% Detection
Neisseria gonorrhoeae F-18	Urine	1.0×10^{-4} CFU/ml
	Swab	1.0×10^{-4} CFU/ml

10.1.3. Analytical Specificity

Analytical specificity of the reaction mix was determined by testing a panel of 30 potentially cross-reacting organisms, including common human pathogens encountered in the urethra and vagina.

The following organisms were tested:

Neisseria meningitidis	*Citrobacter freundii*	*Lactobacillus crispatus*
Neisseria sicca	*Acinetobacter lwoffii*	*Enterococcus faecalis*
Pseudomonas aeroginosa	*Neisseria flavescens*	*Mycoplasma genitalium*
Klebsiella pneumoniae	*Neisseria lactamica*	*Mycoplasma hominis*
Enterobacter aerogenes	*Neisseria cinerea*	*Bacterioides fragilis*
Serratia marcescens	*Neisseria polysaccharea*	*Candida tropicalis*
Acinetobacter baumannii	*Haemophilus influenza*	*Listeria monocytogenes*
Candida albicans	*Staphylococcus epidermidis*	*Streptococcus mutans*
Escherichia coli	*Enterococcus faecium*	*Streptococcus pyogenes*
Enterobacter cloacae	*Gardnerella vaginalis*	*Morganella morganii*

No non-specific amplification was observed for any of these organisms.

In addition, BLAST analysis with the assay primers did not identify any significant homology with the sequence of any databased organism which is likely to lead to non-specific amplification.

10.2. Clinical Sample Testing

A panel of 185 samples was tested across multiple validation batches of product to demonstrate the clinical sensitivity and specificity of the assay. The panel comprised the following:

- Characterised positive and negative urine/swab samples, previously tested using a commercial PCR assay at an independent hospital laboratory.

- Artificial positive panels constructed by spiking characterised negative specimens with quantified *Chlamydia trachomatis* or *Neisseria gonorrhoeae* cells.

30 characterised CT/NG-negative first void urine (FVU) and 30 characterised CT- and NG-negative Specimen Transport Buffer samples were tested across 3 Validation batches.

10.2.1. Clinical Sample Testing – *Chlamydia trachomatis*

5 characterised CT-positive FVU clinical samples were tested alongside a panel of 25 spiked positive urine samples. The spiked samples were prepared by adding a range of concentrations of *Chlamydia trachomatis* cells (serovars D, E, F & G) to individual characterised negative FVU samples. Samples were tested across three batches of product.

30 characterised CT-positive vaginal swab samples in Specimen Transport Buffer were tested across three batches of product.

Including the data for CT-negative samples, the following results were obtained:

CT in Urine		PCR Result	
		Pos	**Neg**
HG CT/NG Result	**Pos**	30	0
	Neg	0	63
% Relative Sensitivity		**100%**	
% Relative Specificity			**100%**

CT in Swabs		PCR Result	
		Pos	**Neg**
HG CT/NG Result	**Pos**	29	1
	Neg	1	61
% Relative Sensitivity		**96.7%**	
% Relative Specificity			**98.4%**

The overall performance for *Chlamydia trachomatis* in all sample matrices was as follows:

CT Detection (Urine & Swabs)		PCR Result	
		Pos	**Neg**
HG CT/NG Result	**Pos**	59	1
	Neg	1	124
% Relative Sensitivity		**98.3%**	
% Relative Specificity			**99.2%**

10.2.2. Clinical Sample Testing – *Neisseria gonorrhoeae*

10 characterised NG-positive FVU clinical samples were tested alongside a panel of 23 spiked positive urine samples. The spiked samples were prepared by adding a range of concentrations of *Neisseria gonorrhoeae* F-18 cells to individual characterised negative FVU samples. Samples were tested across three batches of product.

32 characterised NG-positive vaginal swab samples in Specimen Transport Buffer were tested across three batches of product.

Including the data for NG-negative samples, the following results were obtained:

NG in Urine		PCR Result	
		Pos	**Neg**
HG CT/NG Result	**Pos**	33	0
	Neg	0	60
% Relative Sensitivity		**100%**	
% Relative Specificity			**100%**

NG in Swabs		PCR Result	
		Pos	**Neg**
HG CT/NG Result	**Pos**	30	0
	Neg	2	60
% Relative Sensitivity		**93.8%**	
% Relative Specificity			**100%**

The overall performance for *Neisseria gonorrhoeae* in all sample matrices was as follows:

NG Detection (Urine & Swabs)		PCR Result	
		Pos	**Neg**
HG CT/NG Result	**Pos**	61	0
	Neg	2	120
% Relative Sensitivity		**96.8%**	
% Relative Specificity			**100%**

11. Inhibition

The impact of eight likely inhibitory components of clinical specimens was assessed by spiking negative urine samples with *Neisseria gonorrhoeae* F-18 cells and the inhibitors listed below, before processing and testing per the standard urine protocol:

Potential inhibitor	Final concentration
Whole Blood	1% v/v
Genomic DNA	0.05µg/µl
Acidic Urine	pH 4 (100% v/v)
Alkaline Urine	pH 9 (100% v/v)
Mucin	0.2% w/v
BSA	10mg/ml
Miconazole	0.25% w/w
Glucose	10mg/ml

No inhibition was observed with any of the substances tested at the quoted concentrations.

12. Limitations of Use

- Any diagnosis of *Chlamydia trachomatis* or *Neisseria gonorrhoeae* infection must be made in conjunction with the entire clinical profile of the patient, including results of any other clinical laboratory tests which are available.

- The assay may not perform correctly if these instructions are deviated from, or if inhibitory substances are present in clinical samples.

13. Troubleshooting

Observation	Potential Causes	Actions
EC or PC do not amplify correctly.	Incorrect handling of the sample (EC)	Repeat sample preparation
	Presence of inhibitors in sample (EC)	
	Inadequate lysis conditions or omission of lysis step (EC)	Ensure dry bath incubator is set to 105°C and has reached temperature
		Repeat lysis of sample at 105°C for 10min
	Incorrect volume of sample added (EC, PC)	Check calibration of pipettes
		Repeat assay ensuring the correct volume of sample is added
	Reaction mixture not present at bottom of tube after reconstitution (EC, PC)	Repeat assay ensuring that sample is at correct level by tapping after mixing
	Use of expired materials (EC, PC)	Check expiry date and repeat testing with new kit if necessary
Signal in NTC	DNA contamination	Decontaminate workspace and equipment using DNA cleaning solutions and UV irradiation.

14. References

[1] C. M. O'Connell and M. E. Ferone, "Chlamydia trachomatis Genital Infections," *Microb Cell,* vol. 3, no. 9, pp. 390-403, 2016.

[2] M. Molano, C. J. Meijer, E. Weiderpass, A. Arslan, H. Posso, S. Francheschi, M. Ronderos, N. Munoz and A. J. van den Brule, "The natural course of Chlamydia trachomatis infection in asymptomatic Colombian women: a 5-year follow-up study," *J Infect Dis,* vol. 191, no. 6, p. 907–916, 2005.

[3] R. B. Ness, K. J. Smith, C. C. Chang, E. F. Schisterman and D. C. Bass, "Prediction of pelvic inflammatory disease among young, single, sexually active women," *Sex Transm Dis,* vol. 33, no. 3, p. 137–142, 2006.

[4] G. J. Rours, M. R. Hammerschlag, A. Ott, T. J. De Faber, H. A. Verbrugh, R. de Groot and R. P. Verkooyen, "Chlamydia trachomatis as a cause of neonatal conjunctivitis in Dutch infants," *Pediatrics,* vol. 121, no. 2, p. e321–e326, 2008.

[5] R. S. Stephens, R. Sanchez-Pescador, E. A. Wagar, C. Inouye and M. S. Urdea, "Diversity of Chlamydia trachomatis major outer membrane protein genes," *J Bacteriol,* vol. 169, no. 9, p. 3879–3885, 1987.

[6] T. Meyer, "Diagnostic Procedures to Detect Chlamydia trachomatis Infections," *Microorganisms,* vol. 4, no. 3, p. 25, 2016.

[7] "World Health Organization (WHO) Global Action Plan to Control the Spread and Impact of Antimicrobial Resistance in Neisseria gonorrhoeae," World Health Organisation, Geneva, Switzerland, 2012.

[8] T. Peterman, L. Tian, C. A. Metcalf, C. L. Satterwhite, C. K. Malotte, N. DeAugustine , S. M. Paul, H. Cross, C. A. Rietmeijer, J. M. Douglas and RESPECT-2 Study Group., "High incidence of new sexually transmitted infections in the year following a sexually transmitted infection: a case for rescreening.," *Ann Intern Med,* vol. 145, no. 8, pp. 564-72, 2006.

[9] D. Fleming and J. Wasserheit, " From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection," *Sex Transm Dis,* vol. 75, no. 1, p. 3–17 , 1999.

[10] H. Thadepalli, K. Rambhatla, J. Maidman, J. J. Arce and E. C. J. Davidson, "Gonococcal sepsis secondary to fetal monitoring," *Am J Obstet Gynecol,* vol. 126, no. 4, p. 510–512, 1976.

[11] J. S. Knapp, M. R. Tam, R. C. Nowinski, K. K. Holmes and E. G. Sandstrom, "Serological classification of Neisseria gonorrhoeae with use of monoclonal antibodies to gonococcal outer membrane protein I," *J Infect Dis,* vol. 50, p. 44–48, 1984.

[12] C. Bignell, M. Unemo and European STI Guidelines Editorial Board., "2012 European guideline on the diagnosis and treatment of gonorrhoea in adults," *Int J STD AIDS,* vol. 24, no. 2, pp. 85-92, 2013.

[13] T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino and T. Hase, "Loop-mediated isothermal amplification of DNA," *Nucleic Acids Research,* vol. 28, no. 12, p. e63, 2000.

[14] K. Nagamine, T. Hase and T. Notomi, "Accelerated reaction by loop-mediated isothermal amplification using loop primers," *Molecular and Cellular Probes,* vol. 16, pp. 223-229, 2002.

15. Interpretation of Symbols

IVD	In Vitro Diagnostic Medical Device
REF	Catalogue number
LOT	Batch number
	Use-by date
	Temperature limitation
	Do not reuse
	Manufacturer
	Contains sufficient for <n> tests
	Consult instructions for use (at www.hibergene.com)
	IFU can be requested by phone if not accessible online

Hibergene Diagnostics Ltd.
Block 2,
Bracken Business Park,
Sandyford,
Dublin 18, Ireland.
Tel: +353 1 905 3160
Email: mdx@hibergene.com
www.hibergene.com

16. Changes from Previous Revision

IFU Section	Change(s)
3. Accessories	References to the HG Swift Plus have been added
4. Intended Use	Statement has been added to clarify the intended end user and training requirements
7. Precautions	Precaution added in relation to the reporting of serious incidents to the manufacturer. Reference to the HG Swift Plus added to 'Preventing amplicon contamination'.
8. Performing the Test	References to the HG Swift Plus have been added throughout this section
9. Assay Results	References to the HG Swift Plus have been added throughout this section