Molecular detection of *Herpes Simplex Virus Type 1 and 2* from skin-mucosal lesion swab samples

HGHSVR

Instructions for Use

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CE

1. Kit Contents

HG HSV 1&2 Combo 30-test kits contain:

Component	Description
HG HSV 1&2 Reaction Strips **Part # HSV-RX**	3 pouches, each containing 10 x 2-tube reaction strips with desiccant sachets. Reaction strips contain complete lyophilized reaction mixes for *Herpes Simplex Virus Types 1 and 2* and Extraction Control.
HG Herpes Simplex Virus 1&2 Dilution Buffer **Part # HSV-DB**	30 x tubes, each containing 0.35ml of HSV 1&2 Dilution Buffer

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 28 days of initial opening of the pouch.

3. Accessories

Supplied by HiberGene:

- **HG Swift Strip Carriers** are supplied with the HG Swift instrument and are used for handling, loading and unloading of HG Herpes Simplex Virus 1&2 reaction strips. These are also available to order separately (Part # HGCAR).
- **HG Swift Set-up Racks** are supplied with the HG Swift instrument and are used for loading samples into HG Herpes Simplex Virus 1&2 reaction strips. These are also available to order separately (Part # HGRACK).
- **HG Herpes Simplex Virus 1&2 Control** Kit (Part # HGHSVC) is a positive control available as a separate kit from HiberGene. This provides a control for the performance of both Targets and Extraction Control reaction mixes.

Required but not provided:

- Dry bath/Heat block – the selected thermal block must be capable of holding 2.0ml tubes and maintaining temperature of 105°C +/-1°C.
- Centrifuge – the selected centrifuge must be capable of holding 2.0ml microcentrifuge tubes and spinning at 2680 rcf
- Calibrated micropipettes.
- Micropipette tips with filters, certified nuclease-free.
- Vortex mixer.

4. Intended Use

HG Herpes Simplex Virus 1 & 2 is a LAMP-based molecular diagnostic test for the detection of Herpes Simplex Virus Type 1 and Type 2 in skin-mucosal lesions swabs from the genital area.

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

5. Herpes Simplex Virus Type 1 and 2 – Clinical Significance

Two types of herpes simplex virus exist: herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). Both viruses consist of a capsid and lipid bilayer which contain a relatively long double-stranded DNA genome (approximately 150,000 base pairs).

HSV-1 is mainly transmitted by oral contact and can cause both oral herpes (cold sores) and genital herpes [1] HSV-2 is a sexually transmitted infection that causes genital herpes. Infections with both HSV1 and HSV2 are lifelong [2]. An estimated 67% of people under age 50 have HSV-1 infection globally, while 11% of people worldwide aged 15-49 have HSV-2 infection [3], [4]. Symptoms of herpes include painful blisters or ulcers at the site of infection; infections are most contagious when symptoms are present, but the viruses can also be transmitted when symptoms are absent [5].

Neonatal herpes can occur when an infant is exposed to the virus in the genital tract during delivery. Although rare (approximately 0.01% of births globally), this can lead to lasting neurologic disability or death [6]. The risk for neonatal herpes is greatest when a mother acquires HSV infection for the first time in late pregnancy.

In addition, HSV-2 infection has been shown to increase the risk of acquiring a new HIV infection by approximately three-fold, while coinfection can increase the risk of HIV transmission to others. Accordingly, HSV-2 is amongst the most common infections in people living with HIV, occurring in up to 90% of cases [7] [8].

Although classically diagnosed by the presence of typical papular lesions and associated ulcer formation, the features of genital HSV infection can vary widely between patients, with many infected individuals suffering from atypical lesions which can easily be confused with other conditions. Relying upon clinical diagnosis alone should therefore be avoided. HSV DNA detection is now considered the gold standard for diagnosis, being more sensitive and specific than cell culture. HSV typing into HSV-1 and HSV-2 is recommended in all patients with first-episode genital herpes to guide counselling and management [9].

6. Principle of the Assay

The HG HSV 1&2 assay utilizes isothermal LAMP technology to deliver a rapid and sensitive testing solution for *Herpes Simplex Virus Type-1 and Type-2*, with an integrated sample handling control. HiberGene's embodiment of LAMP technology (loop-mediated isothermal amplification [10] [11] employs novel primer design, a highly efficient strand-displacing polymerase and fluorescent probes to facilitate specific fluorescent detection at constant temperature.

LAMP assays targeting highly conserved gene regions of both HSV-1 and HSV-2 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 the sample.

The reaction mixes contain specific fluorescent probes which allow the amplification of both species to be monitored. Binding of HSV-1 probe results in a quenching of fluorescence in the instrument's blue channel, while binding of HSV-2 probe to its specific target sequence results in an increase in fluorescence in the yellow channel of the HG Swift. These changes in fluorescence are monitored in real-time on board the HG Swift instrument, which also maintains precise temperature control for the duration of the run.

7. Precautions

General Precautions

- The HG Herpes Simplex Virus 1&2 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 establish safety procedures.
- Never mouth-pipette, eat or drink in the laboratory.
- To avoid burns, exercise caution when removing samples from 105°C incubation.
- Take every care to avoid cross-contamination between samples during extraction and assay set-up.
- Only HG Herpes Simplex Virus 1&2 Reaction Strips and Dilution Buffer from the same kit lot should be used together.
- 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.

Preventing amplicon contamination

Escape of amplified material from the reaction strips after LAMP amplification can result in laboratory contamination which could impact on subsequent test results. HG reaction strips are specifically designed to resist accidental reopening, but the following specific precautions must always be followed:

- After sample is added 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 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

8. Performing the Test

The flowchart below summarizes the workflow to be followed:

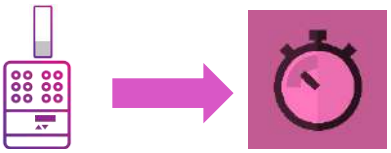
1. **Collect swab with validated swab type and transport media**

2. **Mix by vortexing for 10secs**

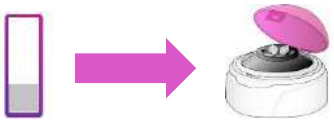
3. **Transfer 50µl of the transport media to a vial of Dilution Buffer**

4. **Mix diluted sample by vortexing for 10secs**

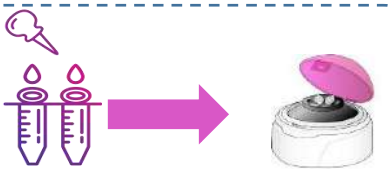
5. **Lyse sample at 105°C for 10min and equilibrate to room temperature for 5min**



6. **Centrifuge the sample for 10secs @ 2700-3300 rcf**



7. **Add 25µl of lysed samples to target and EC wells and centrifuge for 10 secs @2700-3300 rcf**



8. **Run on HG Swift**

8.1. Sample Collection & Handling

1. Collect genital lesion swabs per applicable clinical guidelines using one of the following validated swab and transport media combinations:

    - 3ml VCM transport medium + standard Sigma Swab (Medical Wire Cat. No. MW918S)
    - 3ml Copan UTM + Copan Flocked Swab (Copan, Cat. No. 306C)
    - 3ml Puritan UniTranz + Flocked Swab PurFlock Ultra (Puritan Medical Products Cat. No. UT-306)

    Swab samples in transport media should be stored at 2-8^{o}C in the short-term (up to 7 days), or frozen for longer-term storage (up to 4 weeks).

2. Close the cap of the vial containing the transport medium and swab and vortex for 10 secs.
3. Carefully open the tube not to spill any liquid and with a calibrated micropipette transfer 50µl of swab sample eluate into a vial of HG HSV Dilution Buffer (HSV-DB – purple/white screwcap). For each sample to be tested, use an individual vial of HSV Dilution Buffer.
4. Close the cap of the dilution buffer vial and mix the diluted sample by vortexing for 10 secs.

Note: **Alternative swab types must be validated locally prior to use in clinical diagnosis.**

Note: *Whole blood has been found to be inhibitory to the assay performance, therefore avoid testing swabs which are visually blood-stained.*

8.2. Lysis

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

1. Place each diluted swab eluate in a dry bath incubator at 105°C for 10 minutes.
2. Remove and leave to equilibrate the sample to room temperature for 5 minutes.
3. Following the equilibration step, centrifuge the sample tube at 2700-3300 rcf[1] 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.

***Take every care to avoid cross-contamination when opening tubes after 105°C incubation, due to the possible presence of aerosol. It is recommended to change gloves after handling*.**

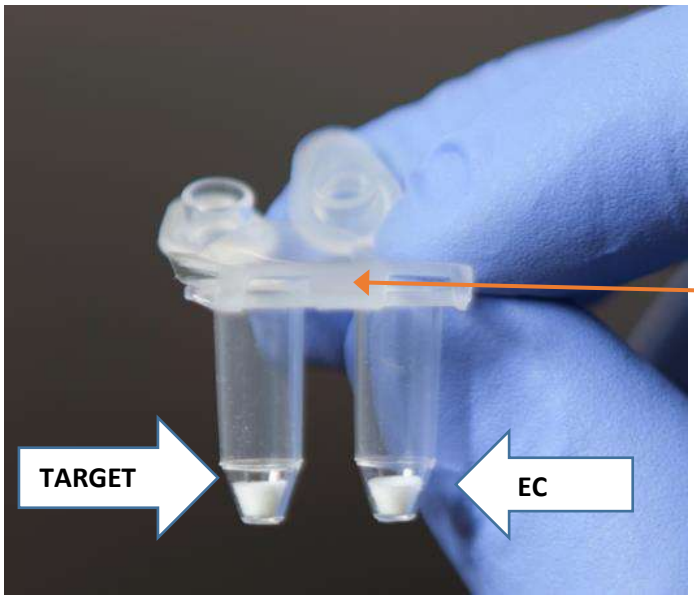
8.3. Assay set up

1. Open the pouch containing the HG Herpes Simplex Virus 1&2 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 the HSV 1&2 Target reaction mix and the **right-hand** tube contains EC reaction mix, as shown below:

[1]RCF = Relative Centrifugal Force. This is calculated based on the radius (r) of the rotor (in mm) and the centrifuge speed in revolutions per minute (rpm) as follows: $RCF = 1.12 \times r \times \left(\frac{rpm}{1000}\right)^2$



Use this space to label your samples, if needed. **DO NOT** write on the reaction tube or lid.

4. If lyophilized pellets are sticking to the sealing film, tap the strip lightly until they fall to the bottom of the tubes and place the strip(s) in the HG Swift Set-up Rack in the correct orientation.

5. Peel off the plastic seal on the tubes carefully, taking care not to disrupt the lyophilized pellet and add 25µl of lysed 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

7. Reconstitute the reaction mixes by briefly vortexing for 5 seconds. Centrifuge the tubes for 10 secs at 2700-2300 rcf to ensure that the reconstituted reaction mix is returned to the bottom of the well, as shown below:

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

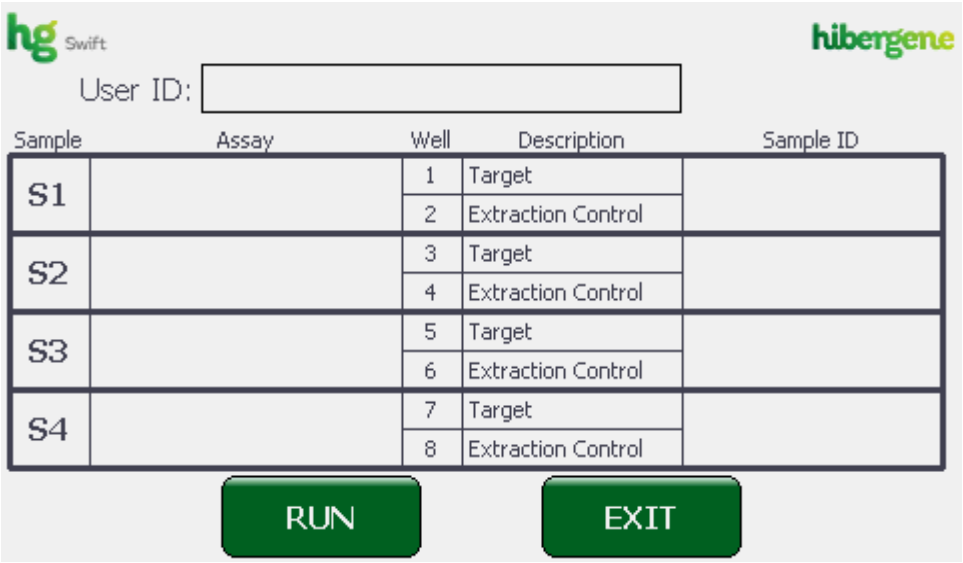
Final reaction mixtures must be loaded and run on the HG Swift as soon as possible after reconstitution. Do not allow to stand for any longer than 10 minutes before starting the run.

8.4. Run set up

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

2. Select START NEW RUN:

The Run Table will then be displayed:

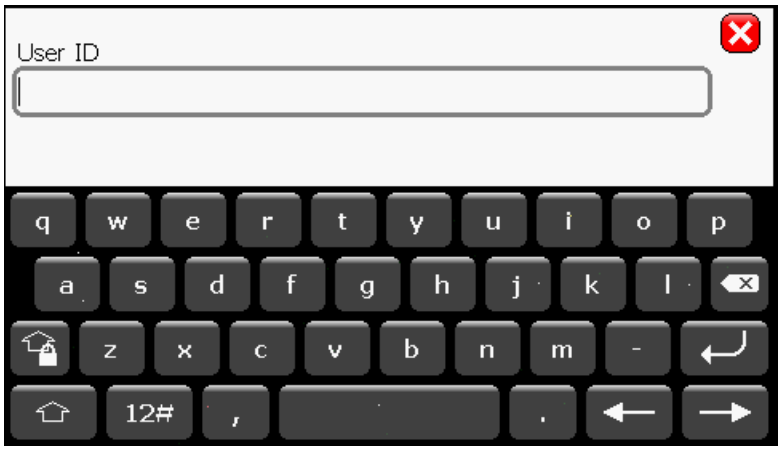


3. Load the reaction strip(s) onto the block of the HG Swift using a Strip Carrier, as shown below:

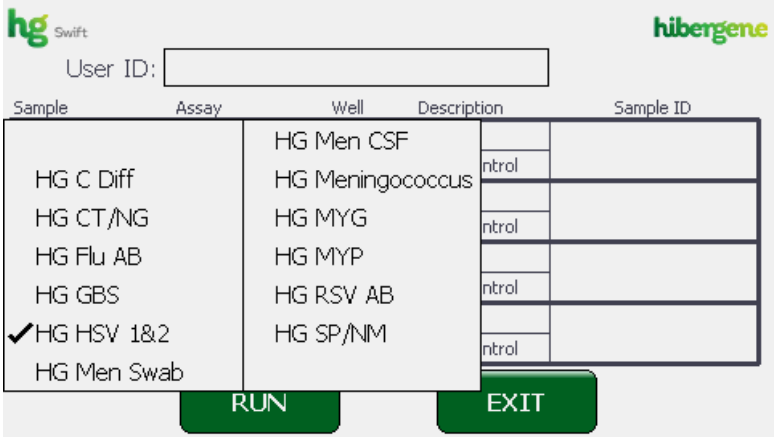
The reaction strips must be loaded IN THE CORRECT ORIENTATION as shown above, with hinges towards the rear of the instrument.

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

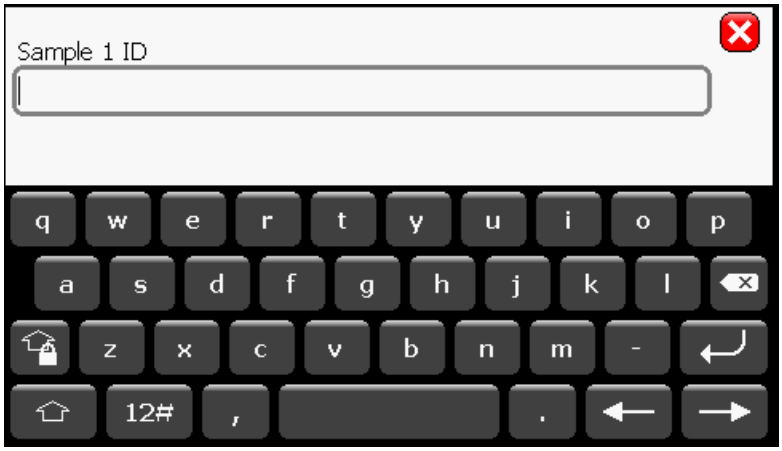
4. Enter the User ID using either the on-screen keyboard or a barcode reader attached to the HG Swift USB port:



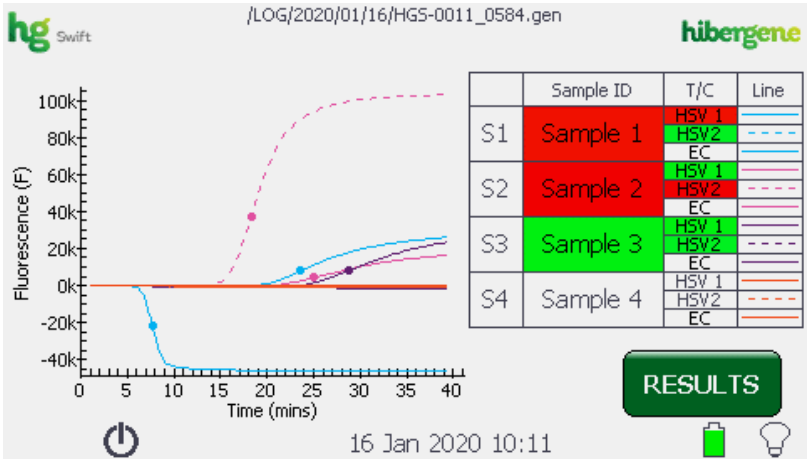
5. For each sample to be tested, select HG HSV1&2 from the dropdown assay menu:



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

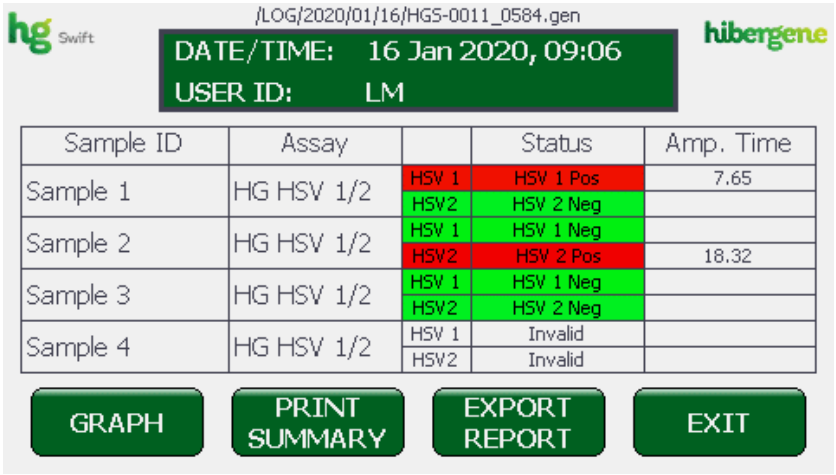


7. Press RUN. The HG Herpes Simplex Virus 1&2 run will take 40 minutes to complete. During the run the fluorescence of all samples will be displayed (see example below). The run may be aborted by pressing the STOP button:



9. Assay Results

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



The sample status which is displayed can be interpreted as follows (**Table 2**):

Displayed	Description
HSV 1 Pos	HSV-1 detected in the sample
HSV 2 Pos	HSV-2 detected in the sample
HSV 1 Neg	HSV-1 not detected in the sample.
HSV 2 Neg	HSV-2 not detected in the sample
Invalid	Extraction Control did not amplify. This indicates issues with sample treatment or the presence of inhibitors. No sample result will be returned, and the sample should be re-tested.

10. Result printing & storage

When a label printer is attached via the HG Swift 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 HGHSVC IFU will indicate acceptable performance.

If required, a No Target Control (NTC) may be included in the run by adding 50µl of negative transport medium (excluding patient swab) to a vial of dilution buffer and adding this to both tubes of a strip – this should return an HSV-1&2-Negative result and only EC amplification should be visible in the EC well during the run.

11. Performance Characteristics

11.1. Analytical Performance

The analytical sensitivity of the HG HSV 1&2 reaction mix (HSV-RX) was determined for both assay targets in swab samples. Specificity was determined by testing a panel of potential cross-reactive organisms.

11.1.1. Analytical Sensitivity

The Limit of Detection (LOD), defined as the lowest concentration of viral DNA that is detected in at least 95% of replicates, was determined using quantified cellular material (HSV-1: MacIntyre, ATCC VR-539; HSV-2: Strain G, ATCC VR-734). Samples were prepared by spiking specimen transport buffer from negative simulated swabs collected using VCM transport media.

Samples were prepared at a range of concentrations and treated per the standard protocol described above. 21 replicates of each virus at each concentration were tested across 3 batches of HG HSV 1&2 product. The LOD as determined by Probit analysis was as follows:

Target Material	LOD- 95% Probability of Detection
HSV-1 Strain MacIntyre (ATCC VR-539)	**6.2 $TCID_{50}$/ml**[2] (95% CI: 3.5-17.6 $TCID_{50}$/ml)
HSV-2 Strain G (ATCC VR-734)	**4.8 $TCID_{50}$/ml**[2] (95% CI: 3.1- 17.7 $TCID_{50}$/ml)

[2] *$TCID_{50}$ = Median tissue culture infectious dose. This is the quantity of virus which produces a cytopathic effect in 50% of inoculated cultures.*

11.2. 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 anogenital swabs across multiple lots of reaction mix:

Chlamydia trachomatis	*Candida glabrata*	*Lactobacillus crispatus*
Clostridium difficile	*Acinetobacter lwoffii*	*Enterococcus faecalis*
Pseudomonas aeruginosa	*Neisseria gonorrhoea*	*Clostridium perfringens*
Klebsiella pneumoniae	*Lactobacillus acidophilus*	*Mycoplasma hominis*
Enterobacter cloacae	*Corynebacterium genitalium*	*Bacteroides fragilis*
Human Herpes 6Virus	*Human Papillomavirus*	*Candida tropicalis*
Proteus mirabilis	*Mycoplasma orale*	*Coxsackievirus*
Candida albicans	*Staphylococcus epidermidis*	*Proteus vulgaris*
Escherichia coli	*Enterococcus faecium*	*Varicella Zoster Virus*
Ureaplasma urealyticum	*Gardnerella vaginalis*	*Trichomonas vaginalis*

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.

11.3. Relative Sensitivity and Specificity

A panel of 90 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 swab samples previously tested using a commercial diagnostic assay at an independent hospital laboratory.

- Artificial positive panels constructed by spiking the following strains of quantified HSV1 or HSV2 viral particles at a range of low doses in simulated negative donor samples.

Organism	Strain	Vendor and Cat. No.
HSV 1	MacIntyre Strain	ATCC VR-539
HSV 1	HF Strain	ATCC VR-260
HSV 2	G Strain	ATCC VR-734
HSV 2	MS Strain	ATCC VR-540

- Artificial negative panels consisting of simulated negative donor samples.

Below is a summary of the results:

Target Material	Relative Sensitivity (%)	Relative Specificity (%)
HSV 1	100%	100%
HSV 2	100%	100%

12. Interferents

The impact of the following potentially inhibitory substances at the concentrations listed below was assessed by testing multiple replicates of HSV positive and negative simulated swab samples in the presence of the substances.

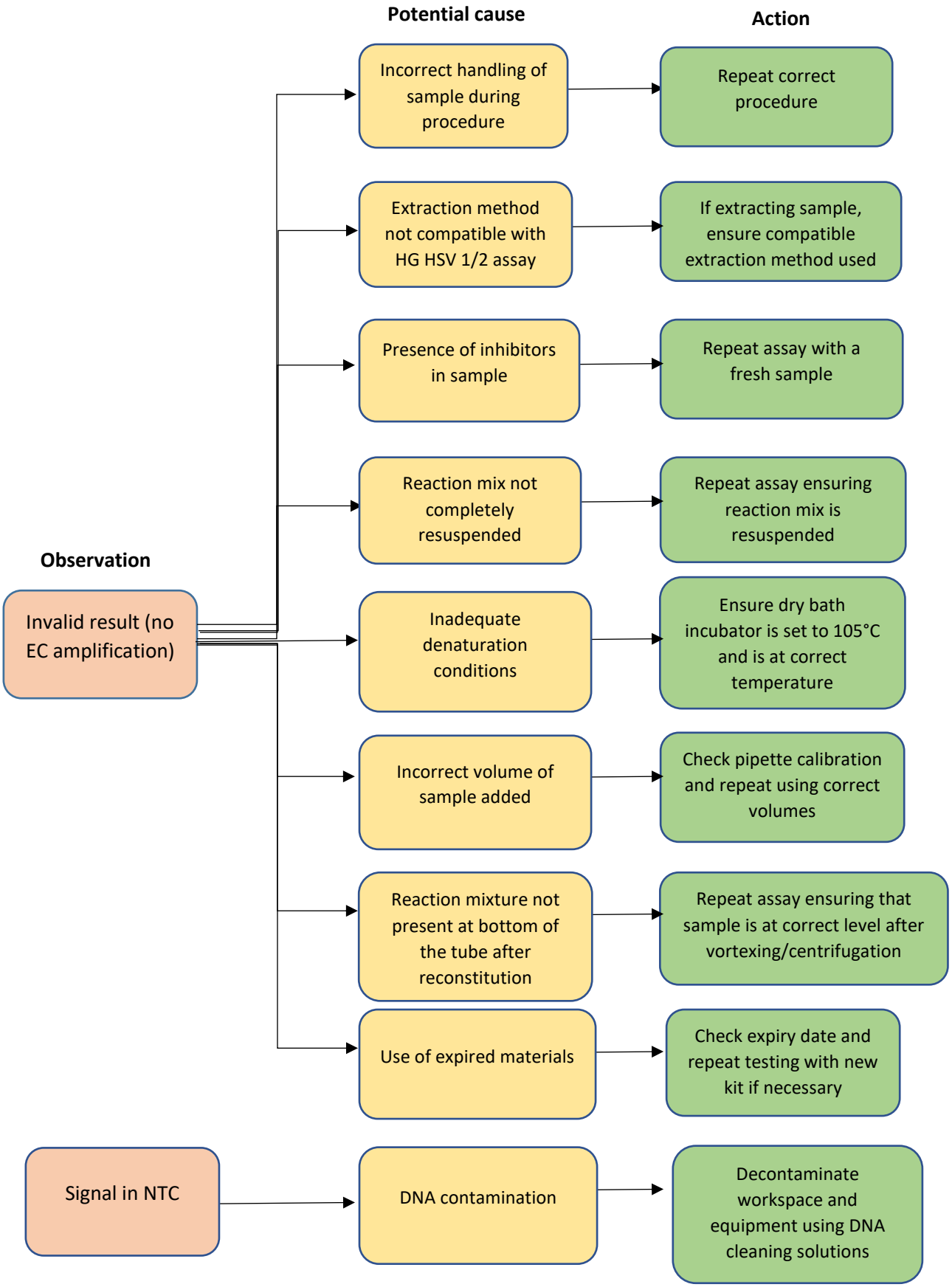
Potential inhibitor	Concentration
Whole Blood	0.1% v/v
Vagisil Wash	0.1%v/v
Genomic DNA	9ng/µl
Faeces	0.1%v/v
Urine	7% v/v
Seminal Fluid	0.5% v/v
Acyclovir (Zovirax)	40 mg/mL
Albumin	10 mg/mL
Buffy Coat (Leukocytes)	7% v/v
Casein	10 mg/mL
K-Y Jelly	7% w/v
Mucin	60 µg/ml
Preparation H	7% w/v
Tioconazole	7% w/v

The data indicates that the whole blood, Vagisil wash and seminal fluid at the concentrations listed above may be inhibitory to the HG HSV 1&2 assay. Care should be taken to avoid testing samples contaminated with these substances.

13. Limitations of Use

- Any diagnosis of HSV 1 or HSV 2 disease must be made in conjunction with the entire clinical profile of the patient, including results of any other clinical laboratory tests which are available.
- Results do not provide information on antimicrobial susceptibility.
- The assay may not perform correctly if these instructions are deviated from, or if inhibitory substances (e.g. whole blood, seminal fluid or Vagisil Wash) are present in clinical samples.

14. Troubleshooting



15. References

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[13] Rolfe KJ, "An internally controlled, one-step, real-time RT-PCR assay for norovirus detection and genogrouping," *Journal of Clinical Virology,* pp. Aug;39(4):318-21, 2007.

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16. Interpretation of Symbols

	In Vitro Diagnostic Medical Device
	Catalogue number
	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

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