



HG *Clostridium difficile* Control

HGCDIFFC

Instructions for Use

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1. Kit Contents

HG *C. difficile* Control kit contains sufficient material for 18 control runs:

	Description
HG <i>C. difficile</i> Positive Control	1 x 1.1 mL vial
Part # CDIFF-PC	Buffered plasmid solution containing preservative

2. Shelf life & Storage

- Kits must be stored at 2-8°C and used before the expiry date on the kit label.
- Opened vials of Positive Control must be sealed tightly before storage at 2-8°C and used before the expiry date on the vial label.

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 *C. difficile* 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 *C. difficile* reaction strips. These are also available to order separately (Part # HGRACK)
- **HG *C. difficile* 30-Test Kit (Part # HGCDIFFR)** is a molecular diagnostic assay for the detection of *C. difficile* in human clinical samples.

Required but not provided:

- Calibrated micropipettes.
- Micropipette tips with filters, certified nuclease-free.
- Centrifuge.
- Vortex mixer.

4. Intended Use

The HG *C. difficile* Control Kit contains positive control material for use with the HG *C. difficile* assay. The positive control contains a nucleic acid sequence which is detected by both the Target and Extraction Control reaction mixes of the HG *C. difficile* kit, and is intended to be used for Quality Control purposes to detect deterioration in reagent performance, operator-driven variation, and the impact of environmental factors.

Laboratory usage of control material may depend on local regulations and practice, accreditation requirements, and staff proficiency. It's recommended that the control is run, at a minimum, upon receipt of a new lot or new shipment of HG *C. difficile* assay kits.

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. Precautions

General Precautions

- The HG *C. difficile* Control kit is for *in vitro* diagnostic use only.
- Training on the test protocol must be carried out before use of the test.
- Surplus kit components should be disposed of in accordance with establish safety procedures.
- Never mouth-pipette, eat or drink in the laboratory.
- 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. Recommended cleaning solution: Invitrogen™, DNAZap™ PCR DNA Degradation Solutions or similar.
- 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

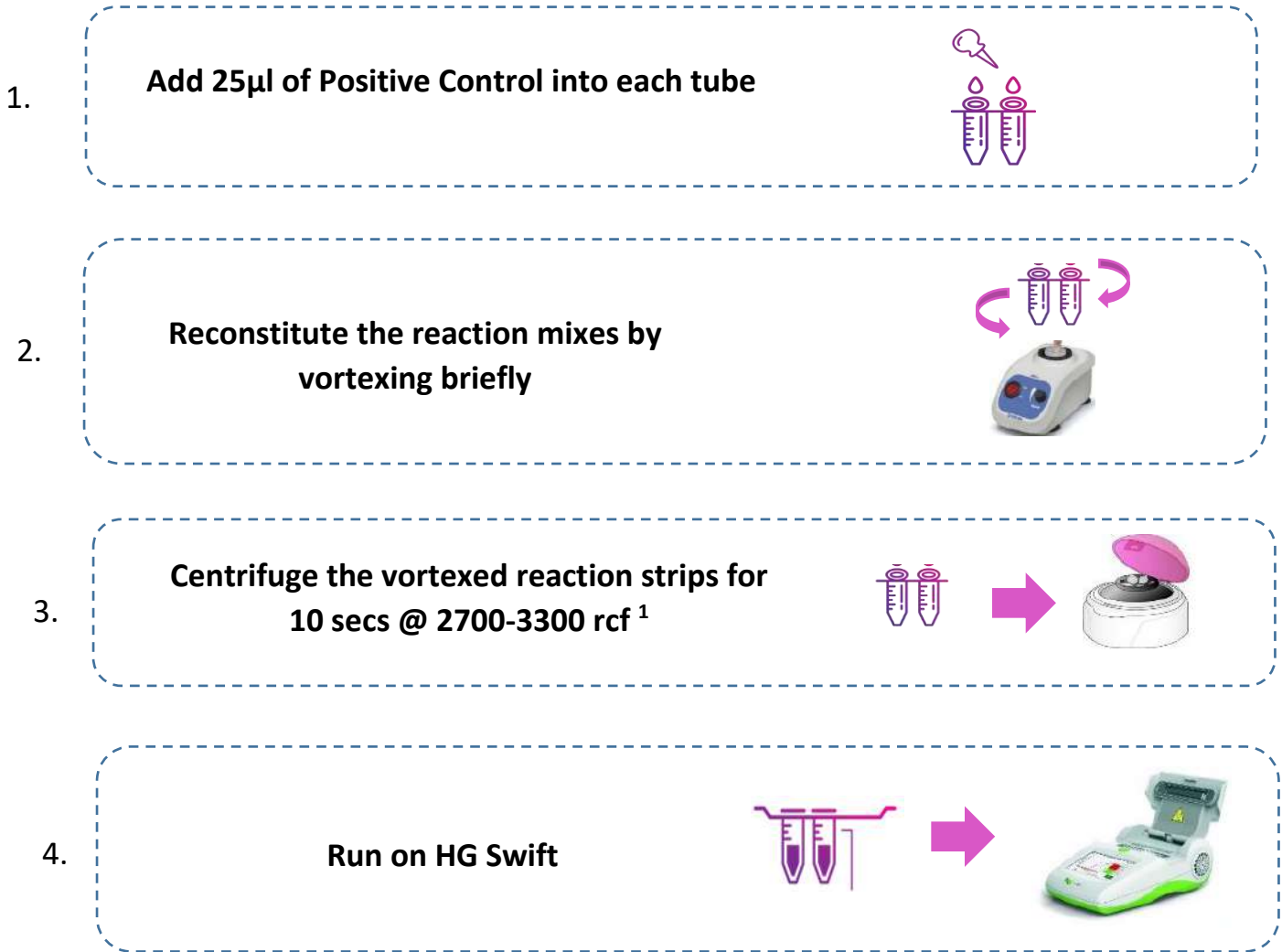
Escape of amplified material from the reaction strips after LAMP amplification can result in laboratory contamination which could impact on subsequent test results. HG *C. difficile* reaction strips are specifically designed to resist accidental reopening, but the following specific precautions **must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



6. Using the Control

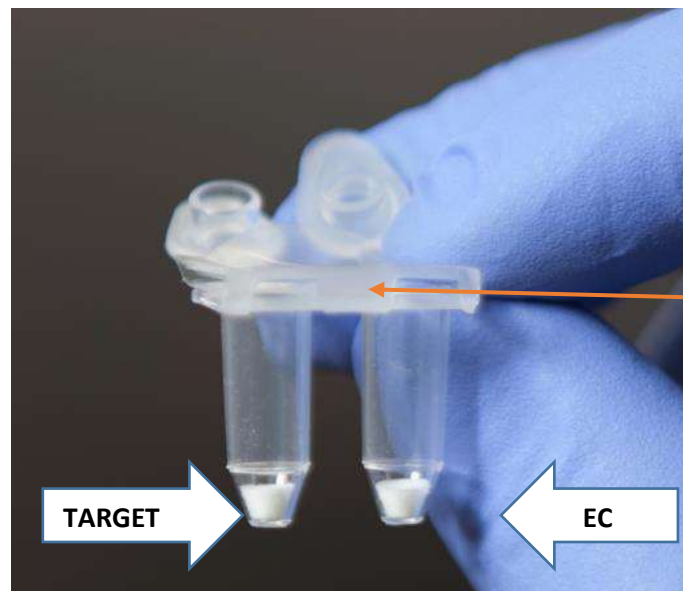
The flowchart below summarizes the workflow to be followed:



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: $RCF = 1.12 \times r \times \left(\frac{rpm}{1000}\right)^2$

6.1 Assay set up

1. Open the pouch containing the HG *C. difficile* reaction strips, tearing across from the notches on the pouch.
2. Remove the reaction strips, one strip per Positive Control to be tested.
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 *C. difficile* Target reaction mix and the **right-hand tube** contains EC reaction mix, as shown below:



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

4. Peel off the plastic seal on the tubes carefully, taking care not to disrupt the lyophilized 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 Set-up Rack in the correct orientation and add 25µl of HG *C. difficile* Positive Control 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. Press lids down very firmly until a click or closure is evident.



7. Reconstitute the reaction mixes by vortexing briefly – minimise foaming of the solution. The vortexed reaction strips should then be briefly centrifuged (e.g. 10secs at 2700-3300 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.

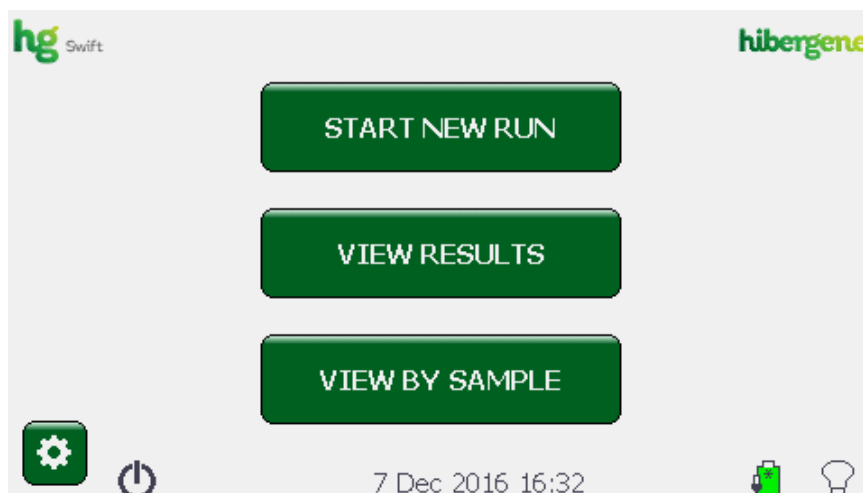
Final reaction mixtures must be loaded and run on the HG Swift as soon as possible after reconstitution. Do not allow to stand for any longer than 10 minutes before starting the run.



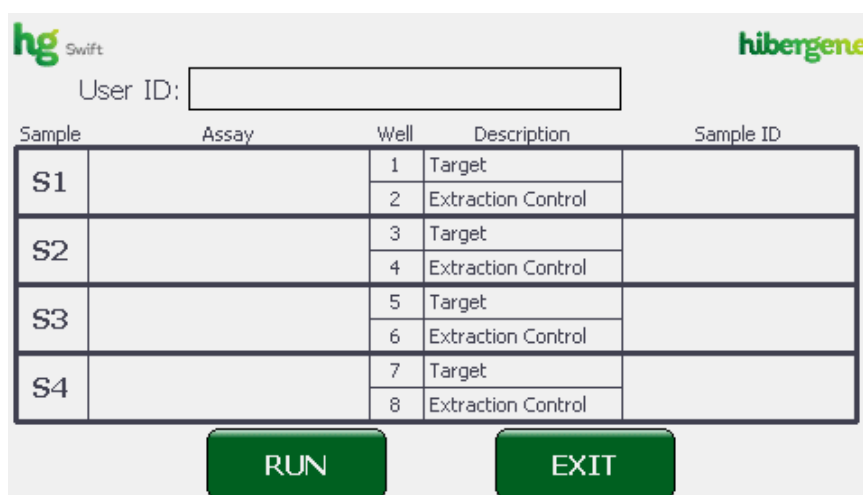
After loading the strips on the instrument, press lids down again very firmly to ensure they are closed.

6.2 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:

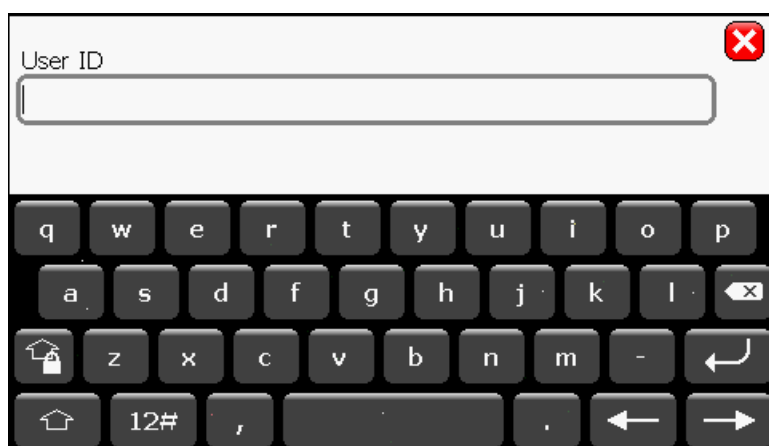


The reaction strips must be loaded IN THE CORRECT ORIENTATION as shown above, with hinges towards the rear of the instrument.

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 control to be tested, select HG C Diff from the dropdown assay menu:

The screenshot shows the 'hg Swift' interface. At the top left is the 'hg Swift' logo, and at the top right is the 'hibergene' logo. Below the logos is a 'User ID:' label followed by an empty text input field. Below this is a table with the following columns: 'Sample', 'Assay', 'Well', 'Description', and 'Sample ID'. The table contains the following data:

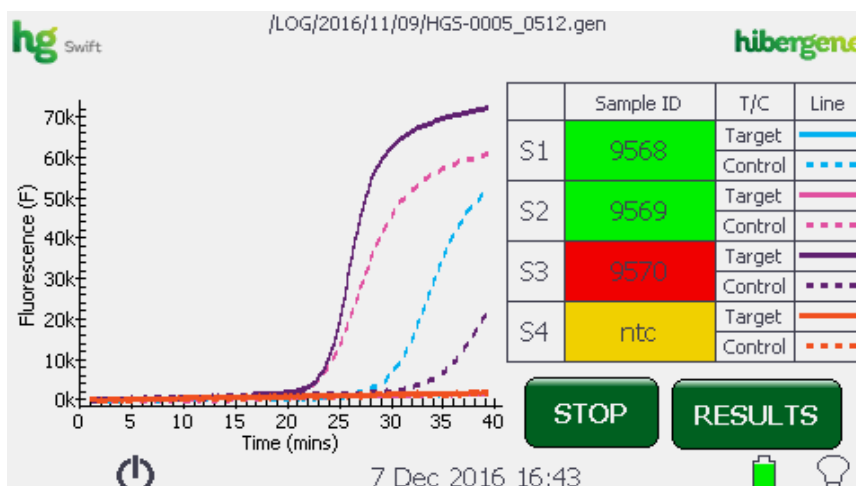
Sample	Assay	Well	Description	Sample ID
S1	HG C Diff	1	Target	
		2	Extraction Control	
S2	HG Meningococcus	3	Target	
		4	Extraction Control	
S3	HG GBS	5	Target	
		6	Extraction Control	
S4	HG Meningo Swab	7	Target	
		8	Extraction Control	

Below the table are two green buttons: 'RUN' and 'EXIT'. There is also a checkmark in the 'Assay' column for the first row (S1).

6. Enter an identifiable sample ID (e.g. "HG C Diff Control") for the positive control using the on-screen keyboard:

The screenshot shows a close-up of the 'Sample 1 ID' input field. The field is empty and has a red 'X' icon in the top right corner. Below the input field is an on-screen keyboard with the following keys: q, w, e, r, t, y, u, i, o, p; a, s, d, f, g, h, j, k, l, [backspace]; [home], z, x, c, v, b, n, m, [enter]; [home], 12#, [,], ., [left arrow], [right arrow].

- Press RUN. The HG *C. difficile* 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:



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

Displayed	Description
<i>C. difficile</i> Pos	<i>C. difficile</i> detected in the sample
<i>C. difficile</i> Neg	<i>C. difficile</i> not detected in the sample.
Invalid	Endogenous 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.

7. Validity Criteria

In order for the run to be considered valid, the following criteria must be met:

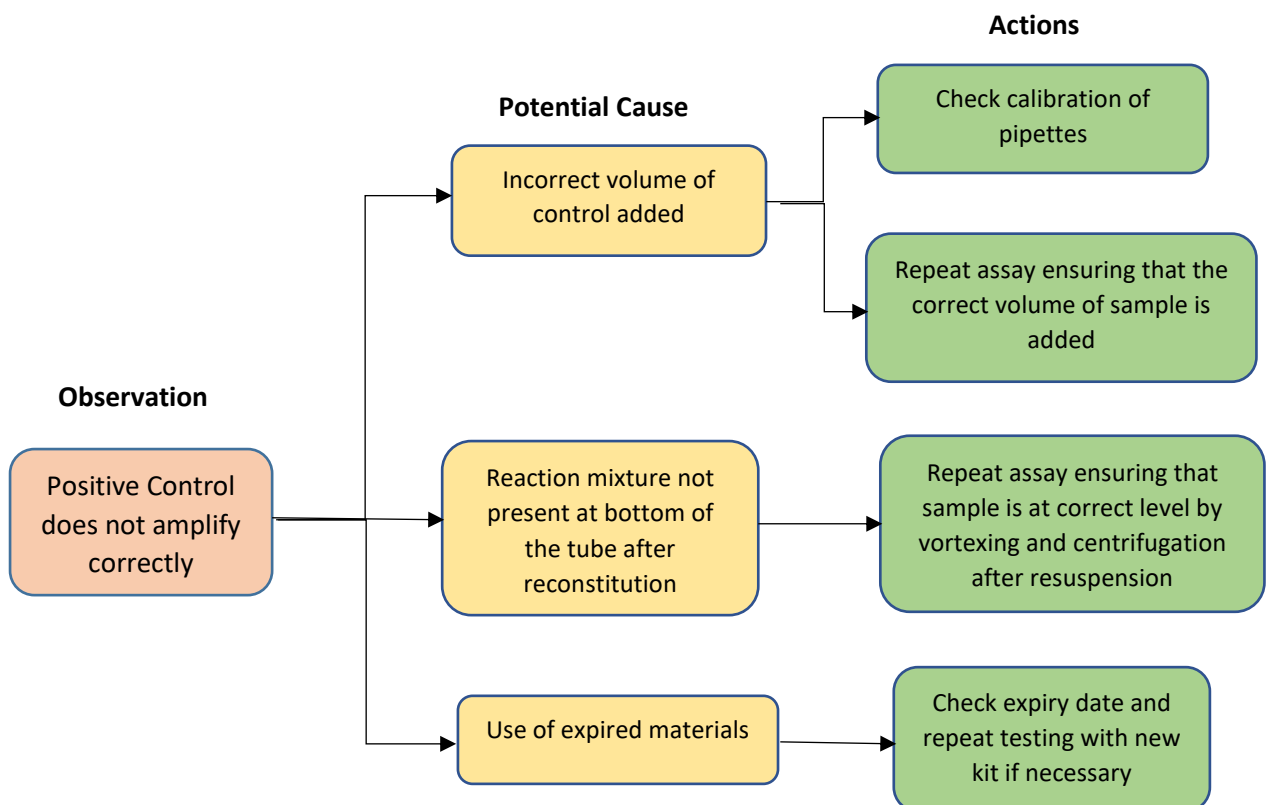
- **HG *C. difficile* Control is identified as a valid Positive sample**
- **The amplification time for HG *C. difficile* Control is ≤ 29.78 minutes.**

If the Positive Control does not meet these criteria, check the Troubleshooting guide below and repeat the testing. If invalid results continue to be generated, contact your local distributor.











8. Limitations of Use

- The control may not perform correctly if these instructions are deviated from.

9. Troubleshooting



10. 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

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