

OptiGene *Campylobacter* Detection from Boot Swab (Liquid Reaction Mix)

Standard Operating Procedure

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NOTE

The reaction mix contains a light sensitive DNA-intercalating dye that will photobleach when exposed to long periods of ambient light. As such, please shield the reaction mix from light when not in use i.e. store in the supplied box at -20°C. The reaction mix may degrade with repeated freeze-thaw cycles.

SAFETY

This test involves the use of Potassium Hydroxide which is caustic and should not be allowed to come into contact with skin or eyes. Safety glasses and protective disposable gloves should be worn at all times. Refer to the supplied MSDS for further details.

1. Preparation

- i) Set the HotBlock temperature to 80°C and the heating time to 5 minutes.
- ii) With the run mode at 'Wait at target' press the start button.
- iii) Place the Lysis Tube(s) and Control Buffer into the Setup Rack.
- iv) Remove the cap(s) from the Lysis Tube(s).

2. Process Boot Swab

- i) Unscrew the lid from Sample Pot and remove the ball bearing.
- v) Add water to the Sample Pot up to the level of the black line (~100 ml).
- vi) Put the Boot Swab in the Sample Pot.
- vii) Place the ball bearing on top of the Boot Swab in the Sample Pot.
- viii) Screw the lid securely back on the Sample Pot.
- ix) Holding the pot with both hands shake vigorously for 15 seconds.

3. Collect Extract

- i) Remove the luer cap from the Sample Pot lid.
- ii) Connect the Luer Syringe in its place.
- iii) Withdraw at least 3 ml of fluid into the Syringe.
- iv) Disconnect the Syringe and dispense 1 ml directly into the 2 ml Lysis Tube. Cap the Lysis Tube.
- v) Discard the Syringe to waste.
- vi) Replace the luer cap back on the Sample Pot lid and discard the Pot to waste.
- vii) Shake the Lysis tube vigorously for a few seconds to mix.

4. Lysis and Genie Strip preparation

- i) Place the Lysis Tube in the HotBlock (now at 80°C) and press the start button.
- ii) After 5 minutes the HotBlock will beep.
- iii) Transfer the Lysis Tube(s) back to the Setup Rack and leave to cool for 5 minutes.

WHILE THE LYSIS TUBE(S) IS COOLING:-

- iv) Transfer 20 µl of the *Campylobacter* reaction mix into wells 1 - 7 of the Genie Strip (single pipette tip).
- v) Transfer 20 µl of the *D.ra* positive control reaction mix into well 8 of the Genie Strip.
- vi) Transfer 5 µl of Control Buffer into tubes 7 and 8 of the Genie Strip (single pipette tip) and close caps.

5. DNA Amplification

- i) Once the 5 minute cooling period is over, transfer 5µl from the clearest liquid at the top of each Lysis Tube(s) into one of wells 1 – 7 of the Genie Strip and cap the tube.
- ii) Cap the Lysis Tube and dispose to waste.
- iii) Once all samples have been transferred to the Genie Strip make sure that all 8 tubes are securely capped.
- iv) 'Flick' the strip rapidly to make sure that the contents of each tube is at the base.
- v) Place the Genie Strip into the Genie Instrument making sure that the two pins pass through the two slots of the strip.
- vi) Close the door of the Genie Instrument.
- vii) Select the 'Campylobacter' button from the touch screen menu.
- viii) Genie II: select block A or block B to run the test
Genie III: press the Start button to run the test

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Kit Requirements

Equipment

1. Genie II or Genie III instrument with mains power adaptor
2. Genie HotBlock or Genie Mini HotBlock instrument with mains power adaptor
3. Pipette, 20µl
4. Pipette, 5µl
5. Setup rack
6. Disposable gloves
7. Safety glasses
8. Standard Operating Procedure (SOP)
9. Materials Data Safety Sheet for Potassium Hydroxide

Consumables

1. Boot swab Sample Pot
2. Plastic tongs
3. Luer syringe 5 ml
4. Genie Strip
5. *Campylobacter* reaction mix
6. *D.ra* positive control reaction mix
7. Control Buffer (KOH 0.3M)
8. Lysis Tube (KOH dried)
9. Pipette tips P20/2000