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| **Document Number:** | **SASoM/METHOD/055.v5** |
| **Title:** | **Mycoplasma Detection using Minerva Biolabs 'Venor GeM One Step' PCR kit.** |
| **Version:** | **v5** |
| **Author:** | **Peter Mullen** |

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| Effective from: | 05/10/2021 |
| Valid to: | 05/10/2023 |

| **SOP History** | | |
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| Number | Date | Reason for Change |
| v1 | 17/12/2013 | Original |
| V2 | 10/11/2015 | Update |
| V3 | 10/11/2017 | Update and change of author |
| V4 | 03/04/2020 | Update |
| V5 | 05/10/2021 | Minor ammendments |
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**1.0 Purpose –**
This SOP describes the current procedure for Detection of Mycoplasma using a Venor GeM One Step PCR kit (Minerva Biolabs) for use in Laboratory 248 at the St Andrews School of Medicine (SASoM).

**2.0 Scope –**
This SOP applies to all staff in the SASoM carrying out Mycoplasma detection using the Minerva Biolabs 'Venor GeM One Step' PCR kit.

**3.0 Responsibilities –**
All staff involved in Mycoplasma testing of cell culture supernatants are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

**4.0 Procedure –**

Mycoplasma detection is performed using the Venor GeM One Step Mycoplasma Detection Kit (Minerva Biolabs: #11-8100). Kit reagents consist of (i) One Step Mix, (ii) Rehydration Buffer, (iii) Positive Control DNA and (iv) PCR-grade water. All kit components can be stored between +2°C and +8°C until rehydrated; hydrated components should be stored at < -18°C (ideally -40°C).

**Preparation of Reagents:**

0.5M EDTA (pH 8.0) - Add 46.525g of EDTA to a large glass beaker and add 150ml of deionized water. Mix with a magnetic stirrer before adjusting to pH8 by adding concentrated NaOH until the solution becomes clear. Adjust the final volume to 250mL with deionized water. (NB Make sure that you have safety glasses on when using concentrated NaOH).

TBE Buffer (x5) - Dissolve 54g of Tris base (mw = 121.14) and 27.5g of boric acid (mw = 61.83) in approximately 800mL of deionized water. Add 20mL of 0.5 M EDTA (pH 8.0) and adjust final volume to 1L. Once prepared, dilute 1:10 (0.5X concentration) prior to use. TBE is stable at 4°C for 3 months.

**Preparation of Media Samples:**

Cell supernatant samples should be derived from cultures which are 90-100% confluence. I ml cell of supernatant should be spun down at max speed in a small bench top centrifuge for 3 mins to remove any cellular debris. Transfer 100µl of this supernatant to a fresh sterile micro centrifuge tube. Ensure it is sealed tightly and then put on a heat block at 95°C for 10 minutes. Briefly spin down to sediment any cellular debris and then store in a fridge for up to 6 days. Samples should be stored at -20°C if not being used immediately.

**Assay Method:**

Spin down all lyophilized components for 5 sec at max speed in a mini centrifuge. Working inside an open-fronted flow hood which has been thoroughly wiped down with DNAse wipe, add 600µl Rehydration Buffer (Blue Cap) to the One Step Mix (Red cap). Leave at room temperature for 5 mins, gently vortex and then briefly spin down for 5 seconds. Use the One Step Mix immediately, or aliquot into 2 x 200µl aliquots and store in -20 degrees freezer. Keep on ice at all times.

Working in the designated area (which has been wiped clean with DNase wipe), add 300µl of PCR-grade water (White Cap) to Positive Control DNA (Green Cap). Leave at room temperature for 5 mins, gently vortex and then briefly spin down for 5 seconds. Aliquot Positive Control DNA into 10µl aliquots and store at -20 degrees or less. Repeated freezing and thawing should be avoided.

Equilibrate all reagents and samples to between +2 to and +8°C prior to use. Briefly spin down the One Step Mix if necessary.

Working inside open fronted flow hood (which has been wiped down with a DNAse wipe) prepare PCR tubes – one tube required for each sample + one Negative Control tube (N/C) and one Positive Control tube (P/C). Clearly label each tube. Test samples should all be allocated a number for ease.

Add 23µl of One Step Mix into each PCR tube. Add 2µl of PCR-grade water (White Cap) to the Negative Control Tube and immediately close the tube lid.

Add 2µl of previously prepared cell supernatant to each test sample tube. Close each tube once the sample has been added.

Prepare the Positive Control tube last in order to avoid cross-contamination. Add 2µl of positive control to P/C tube. Briefly spin down all PCR tubes and load into the 'Mycycler' PCR instrument (SHG bench). This instrument is already pre-programmed with the Venor Gem One Step protocol. Ensure the following options are selected – No Hot start, Algorithmic, 25µl sample volume. Start the programme.

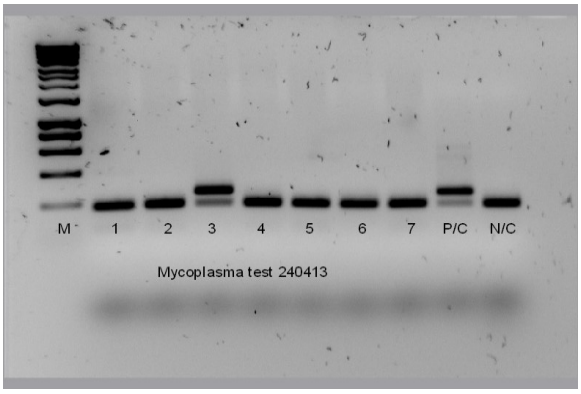
**Agarose Gel Electrophoresis:**

Assemble the Gel Electrophoresis apparatus as required.

Prepare an agarose gel (1.5%) by adding 1.5g agarose in 100mL of 0.5X TBE buffer (or 2.25g in 150mL). Heat in the microwave on 'low setting' until dissolved and then add DNA stain (SYBR® Safe DNA Gel Stain) at 10µL/100mL (15uL/150mL) agarose. Carefully pour the gel with an appropriate comb(s) in situ.

Samples are prepared using 16µl of PCR product + 4µl of 5x DNA loading buffer (Bioline; #BIO-33056). This is best achieved by placing a drop of Loading Dye (4µl) onto a piece of parafilm to which the PCR product (16µl) is added. Each sample can then be mixed on the parafilm using a pipette loading tip then loaded directly into a gel well. 5µl of DNA 100bp Hyperladder (Bioline; #810-37045) is separately loaded into the first well of the gel followed by samples and controls. Run the gel @ 110V for ~45mins or until bands are sufficiently separated. The Gel can then be visualised on BioRad 'Gel Analyzer'.

The following image illustrates the double band indicating Mycoplasma infection in both the Positive Control (P/C) and a Test Sample (#3). All other test samples and the Negative control (N/C) showed a single band and were Mycoplasma free.



Save the data on a memory stick and computer file for future documentation

**5.0 Personal protection -**
A Howie laboratory coat and lab gloves must be worn at all times.

**6.0 Spillages -**
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard. Spillages should be mopped up with white tissue, then disinfected with 70% ethanol.

**7.0 Training -**
All staff should be trained in PCR preparation techniques before starting any PCR work.

**8.0 Related documents –**

8.1 Risk assessments:
CHARM_RA20918_Mycoplasma Testing

## 9.0 Approval and sign off –

**Author:**

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 05/10/2021

**Management Approval:**

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 05/10/2021

**QA release by:**

Name: John O' Connor

Position: QA Manager

Signature:  Date: 05/10/2021

# STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

| NAME | POSITION HELD | SIGNATURE | DATE |
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