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Viral Transport Medium produced for Covid-19 swab samples collection in Nigeria

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Abstract

Accuracy in viral diagnosis depends both on the technology employed for the Assay and the medium used for preservation during transportation to the laboratory. SARS CoV-2 virus causes coronal virus disease (COVID-19) and it spreads from an infected person's mouth or nose in small liquid particles when they cough, sneeze, speak, sing or breathe. Hence, swabs collected from nostril and throat are used for its definite diagnosis, by using molecular technique. The challenge to prepare in-country viral transport medium (VTM) to preserve samples collected for COVID-19 testing starred Nigeria Scientists at the face, when the imported VTMs were exhausted shortly after the pronounced global lock-down in the second quarter of 2020. As a way of responding to this challenge, VTM was produced and validated using amino acid substrates, fungizone, gentamycin and phosphate buffer saline. The products were subjected to sterility test using Chocolate agar and Sabouraud dextrose agar. The following bacteria viz: *E. coli, Pseudomonas sp., Bacillus sp., Staphylococcus sp. and Klebsiella sp*, were also inoculated into the VTM vials and incubated for 34 consecutive days from which the wire-loop filled inoculums were sub-cultured on Chocolate agar and Sabouraud dextrose agar at 24 hours interval. No growth was recorded in both situations. Two thousand five hundred vials of the VTM produced were tested in parallel in the NCDC accredited laboratories in Oyo, Lagos, Imo, Ekiti and Osun States; alongside the few imported VTMs in circulation and the performance was 100% the same. This interventional study clearly showed in comparison with imported brands of VTMs, the possibility of using cheap readily sourced materials in low-income setting for the production on in-house VTMs, giving engagement of skillful personnel for the purpose.

Keywords: VTM, Covid-19, Alternative nutrient sources, Intervention.

Introduction

It has been established that COVID-19 is the third highly pathogenic human coronavirus disease to date. Although, not as deadly as SARS and MERS, the rapid spread of the causative agent, SARS-CoV-2 makes the highly contagious disease the severest threat to global health in this century (Tang *et al.,* 2020). Early diagnosis is crucial for controlling the spread of COVID-19 and the molecular detection of SARS-CoV-2 nucleic acid is the gold standard (Zhou *et al.,* 2020; Konrad *et al.,* 2020). SARS-CoV-2 has been detected from a variety of respiratory sources, including throat swabs, posterior oropharyngeal saliva, nasopharyngeal swabs, sputum and bronchial fluid. The viral load is higher in lower respiratory tract samples (Zhou *et al.,* 2020; Han *et al.,* 2020). A good VTM must sustain viral integrity and suppress contaminating microbial agents which may interfere with diagnosis (Druce *et al.,* 2012). Sabouraud Dextrose Agar (SDA) is a selective medium used for the isolation of dermatophytes, yeasts, and filamentous bacteria such as *Nocardia.* The acidic pH of this medium (pH about 5.0) ordinarily inhibits the growth of bacteria but permits the growth of yeasts and most filamentous fungi (Das *et al.,* 2010).

Chocolate agar on the other hand, is so enriched a culture medium that it can be used without further supplementation for specimens obtained from sites or materials that would normally be expected to be sterile (Thankur and Dillon, 2018). The team, as part of her interventions produced some vials of viral transport medium (VTM) and presented same as in-vitro material to the Medical Laboratory Science Council of Nigeria for validation, as well as registering a trade name with the Ministry of Commerce, for a brand produced by our team.

Purpose

The purpose of this procedure is to describe the process for producing Viral Transport Medium for transporting of specimen for viral testing.

Scope

This protocol is adaptable to any facility with intention to produce VTM for molecular diagnosis of COVID-19.

Responsibility

It is the responsibility of the professional members with expertise in in-vitro procedures, to ensure adherence of the protocol and to ensure that no step is compromised in the process.

Equipment and Materials

A. Laminar flow hood or Biosafety Cabinet Level 2 is preferred.
B. Autoclave and Autoclave tape
C. Pipettes and pipette Tips (sterilized)
D. Needles and syringes.
E. Sterile screw-capped tubes
F. Autoclave bottles
G. Labels
H. Hot-air oven
I. Incubator
J. Reagents:
   - Amino-acid based substrate (ABS)
   - Isotonic solution
   - Gentamycin sulfate (40mg/ml sterile solution)
   - Fungizone (200mg/ml sterile solution)
   - Quality control plates (chocolate and sabouraud dextrose agar)
   - Disinfectant (methylated spirit).

Safety Precautions

Strict observance of Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP) is vital to ensure the effective use of this transport medium.

Procedure

Preparation of ingredients for the Transport Medium

a) Amino-acid substrate base: The Amino-acid substrate is prepared from commercially prepared base powder following manufacturer's instruction strictly. The stipulated quantity of the powder is weighed and dissolved in the corresponding distilled water quantity and then sterilized by autoclaving at 121^0C for 15mins.
b) Antibiotic/Fungizone preparation: Prepare a dilution of Gentamycin Sulphate with sterile phosphate buffer saline to give 50mg/ml.

Make a dilution of the fungizone with sterile normal saline also to give 250µg/ml.

Add 50ml of the fungizone to the Gentamycin. Store at 2-8^0C.

Label appropriately with lot information and preparation details and record in the laboratory-controlled notebook.

Preparation of Transport Medium

a) Disinfect work surface with disinfectant.
b) Arrange already sterilized substrate based on the bench to cool.
c) With the aid of a sterile pipette, add 2ml of the gentamycin/fungizone mixture to 500ml of the ABS. This results in final concentrations of 100µg/ml for gentamycin and 0.5µg/ml for fungizone.
d) Cap the bottle securely and mix by inverting the bottle severally but carefully.
e) Withdraw 2ml of the medium for QC sample. *(Refer to Sterility Check section below).*
f) Label the bottle with the date, additives and expiration date as follows:
   i. 2.5% ABS
   ii. 100µg/ml Gentamycin
   iii. 0.5 µg/ml Fungizone

iv. *Manufacturing Date (Insert current Date)*
v. *Expiry Date (1year after Manufacturing Date)*

This medium is to be dispensed the same day or shortly after preparation.

g) Store at 2-8^{0}C until dispensed.
h) Aliquot 3ml of medium into sterile 5 or 10ml screw-capped tubes and keep lids tightly closed after medium is dispensed.
i) Label each tube with the following information:
   i. VIRAL TRANSPORT MEDIUM
   ii. **For transport of specimen only**
   iii. **Not to be taken internally**
   iv. Store at 2-8^{0}C. Do Not Freeze.
   v. Composition: Amino-acid enriched broth, Gentamycin, Fungizone
   vi. Manufacturing Date (Current Date)
   vii. Expiry Date (1 year after manufacturing date)
j) Store tubes and any medium remaining in the bottle at 2-8^{0}C.

Sterility Check

Sterility check is performed as follows:

a) Dry the already prepared blood or chocolate agar and Sabouraud dextrose agar (SDA) quality-assured plates.
b) Using a sterile syringe or pipette, withdraw 1ml of medium and apply to surface of the control.
c) Carefully and aseptically spread the medium over the plate.
d) Incubate the plate for 48hrs at 37^{O}C+2^{O}C.
e) Check plates daily for growth.
f) Record results of sterility check (Growth or no growth) and lot specific information in laboratory-controlled record.
g) Should bacteria or fungal growth be encountered, take appropriate action to remove the bottle(s) of medium from service and dispose appropriately.
h) Store medium at 2-8^{O}C.

Validation Done

A total of two thousand five hundred vials of the VTM produced were tested in parallel in the NCDC accredited laboratories in Oyo, Lagos, Imo, Ekiti and Osun States; alongside the few imported VTMs in circulation and the performance was 100% the same.

Conclusion

The outbreak of COVID-19 pandemic in Nigeria was faced with a lot of challenges. Containing this viral infection requires carrying out a significant number of laboratory testing. As Medical Laboratory Scientists in the frontline of this fight against this SARS COV-2 virus, saddled with various assignments ranging from sample collection, transportation and testing, we identified a gap in the availability of viral transport medium nationwide which could potentially be an impediment in the accuracy of test results. Our team then came up with this formulation which turned out to be very effective for the purpose in order to limit the country's dependence on imported product even in the face of the world lockdown. The conservative preservation of SARS-CoV-2 in patient samples to enable molecular diagnosis of the virus is a global obligation. We have demonstrated a simple method of VTM preparation which is critical to preserving the capacity of the virus to replicate and support nucleic acid-based diagnosis.

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Conflict of Interest

The authors declare that there was no conflict of interest.

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