1.0 PURPOSE / INTRODUCTION:

Introduction:

Culture media are nutrient substances (solid or liquid) that is used to cultivate micro-organisms. As the media undergoes reconstitution, heating and at times supplementation with additives in the laboratory, therefore it is essential to have control over these processes for quality and reliable results.

Purpose:

To give guidance in performing the quality control of fresh prepared culture media.

2.0 SCOPE / RESPONSIBILITY:

This sop is applicable to all trained technologists working in the microbiology laboratory.

The Principal Investigator (through the study coordinator when applicable) retains the overall responsibility of implementation of these standard procedures.

The Study Laboratory Coordinator is responsible for answering questions you may have about the content of this SOP and any other relevant study documentation. Please contact the Study Laboratory Coordinator through your site coordinator.

3.0 SAFETY/RISK ASSESSMENT:

3.1 Always wear personal protective equipment such as gloves and lab coat when handling control strains and cultures.
3.2 Wear face mask when measuring out media in powdered form
3.3 Wear safety glasses when handling liquid media
3.4 Use the BSC when handling Salmonella, Shigella or Vibrio species.

4.0 DEFINITIONS:

4.1 BA- Blood agar
4.2 XLD -Xylose lysine deoxycholate agar.
4.3 SS -Salmonella/Shigella
4.4 MAC -MacConkey agar.
4.5 CAMPY -Campylobacter agar..
4.6 TSI -Triple Sugar Iron agar.
4.7 MIO -Motility Indole Ornithine Decarboxylase agar.
4.8 CIT -Citrate agar.
4.9 UREA -Urea agar.
4.10 SFB -Selenite-F broth.
4.11 MHA -Mueller Hinton agar
4.12 MAC GENT-MacConkey Agar with 8% Gentamicin
4.13 FM-Freezing Mixture
4.14 ATCC- American Type culture collection

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INTERNAL QUALITY CONTROL FOR CULTURE MEDIA SOP (MASTER)

4.15 NCTC - National collection of type cultures
4.16 SCH - Salmonella cholerasuis
4.17 ECO - Escherichia coli
4.18 CJE - Campylobacter jejuni

4.19 KPN - Klebsiella pneumoniae
4.20 STY - Salmonella typhi
4.21 NLF - Non lactose fermenter
4.22 H₂S - Hydrogen sulphide
4.23 SSO - Shigella sonnei
4.24 LF - Lactose fermenters
4.24 SF - Sucrose fermenters OR Sorbitol fermenters
4.25 CO₂ - Carbon dioxide
4.26 S/C - Sub culture

4.27 CLED - Cystine Lysine Electrolyte Deficient agar
4.28 CHOC - Chocolate agar

5.0 SPECIMEN
5.1 ATCC control strain.
5.2 NCTC control strain

6.0 EQUIPMENT / MATERIALS / REAGENTS:

6.1 Equipments:
6.1.1 Fridge.
6.1.3 –80°C freezer.
6.1.4 Aerobic and CO₂ incubators.

6.2 Materials:
6.2.1 Gloves.
6.2.2 Media on petri-dish
6.2.3 Universal bottles with caps.
6.2.4 1.0µl loop.
6.2.5 Straight wire.
6.2.6 Bunsen burner.
6.2.7 Lighter.
6.2.8 Anaerobic jar.
6.2.9 1000µl Pipettes
6.2.10 Sterile Sarstedt tubes

6.3 Reagents:
6.3.1 Campylobacter gas mixture.
6.3.2 Kovacs reagent.
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6.3.3 0.85% sterile normal saline.

7.0 METHODOLOGY:

7.1 Principle:
N/A

7.2 Procedure:

7.2.1 Select randomly a pair of the newly prepared batch of media from media preparation room and place them on the working bench.

7.2.2 Check on the physical appearance of the media and ascertain that it is of the right depth, colour, contains no air bubbles, has a smooth surface, gelling of the media and if it is not contaminated.

7.2.3 Depending on the type of prepared media, retrieve the appropriate ATCC/NCTC control strain from a metal rack placed on the floor of the media fridge/CO₂ incubator and at times from –80°C freezer.

7.2.4 Label one plate with a positive and a second with a negative ATCC/NCTC strain code number or uninoculated and date.

7.2.5 Place 4 sarstedt tubes on a rack and label them 1-4 in ascending order.

7.2.6 Dispense 900ul of Normal saline into all the tubes.
7.2.7 Prepare 0.5MC Farland of the control organism in normal saline.

7.2.8 Add 100ul of the 0.5MC Farland suspension into tube No. 1 and mix by vortexing for five seconds.

7.2.9 Pick 100ul from tube 1 and add into tube No. 2 and mix by vortexing for five seconds. Repeat the serial dilution up to tube No. 3.

7.2.10 Using a pre-flamed 1.0ul wire loop, inoculate across the labeled media then streak severally and perpendicularly against the initial inoculum.

7.2.11 Incubate the media at the desired growth conditions, overnight or up to 48 hrs and 72 hrs depending on the type of control organism.

7.2.12 For TSI, CITRATE, UREA and MIO media, do not make serial dilutions but pick one colony using a pre-flamed straight wire and inoculate onto the respective media with the ATCC/NCTC control organism. (Refer to appendix 8.1)

7.3 Results interpretation:

7.3.1 After its desired incubation time, remove the inoculated media and place them on the bench for interpretation. (Refer to appendix8.2)
7.3.2 Examine the plates carefully for expected growth characteristics and count colonies which should not be less than 100 colonies.

7.3.2 Report as PASSED when the intended characteristics are achieved/attained or FAILED when the intended characteristics are not achieved (refer to appendix 8.2)

7.3.3 If the Media fails, it is discarded and the details are documented in the corrective action record file and another batch of media is prepared.

7.3.4 Log in this information into an internal media quality control chart

8.0 APPENDICES:

8.1 Media type and the inoculated ATCC control strain:

<table>
<thead>
<tr>
<th>MEDIA</th>
<th>POSITIVE CONTROL</th>
<th>NEGATIVE CONTROL</th>
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<tbody>
<tr>
<td>BA</td>
<td>S. aureus ATCC 25923</td>
<td>Uninoculated</td>
</tr>
<tr>
<td>XLD</td>
<td>SCH ATCC 13076</td>
<td>E. coli ATCC 25922</td>
</tr>
<tr>
<td>SS</td>
<td>SCH ATCC 13076</td>
<td>E. faecalis ATCC 29212</td>
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<tr>
<td>CAMPY</td>
<td>C. jejuni NCTC 11322</td>
<td>E. coli ATCC 25922</td>
</tr>
<tr>
<td>TSI</td>
<td>E. coli ATCC 25922</td>
<td>P. aeruginosa ATCC 27853</td>
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<tr>
<td>MIO</td>
<td>E. coli ATCC 25922</td>
<td>P. aeruginosa ATCC 27853</td>
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<tr>
<td>CITRATE</td>
<td>K. pneumoniae ATCC 13883</td>
<td>E. coli ATCC 25922</td>
</tr>
<tr>
<td>UREA</td>
<td>P. vulgaris ATCC 13315</td>
<td>E. coli ATCC 25922</td>
</tr>
<tr>
<td>SFB</td>
<td>SCH ATCC 13076</td>
<td>E. coli ATCC 25922</td>
</tr>
<tr>
<td>MAC</td>
<td>E. coli ATCC 25922</td>
<td>S. sonnei</td>
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<tr>
<td>MHA</td>
<td>E. coli ATCC 25922</td>
<td>Uninoculated</td>
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<td>MAC-GENT</td>
<td>KPN NEQAS 1057</td>
<td>ECO ATCC 25922</td>
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8.2. MEDIA QC RESULTS INTERPRETATION CHART
<table>
<thead>
<tr>
<th>CULTURE MEDIA</th>
<th>POSITIVE CONTROL</th>
<th>RESULT</th>
<th>NEGATIVE CONTROL</th>
<th>RESULT</th>
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<tbody>
<tr>
<td>Blood Agar</td>
<td>SAU ATCC 25923</td>
<td>BETA HAEMOLYTIC WHITISH GROWTH</td>
<td>UNINOCULATED MEDIUM</td>
<td>NO GROWTH</td>
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<td>XLD Agar</td>
<td>SCH ATCC 13076</td>
<td>NON LACTOSE FERMENTER</td>
<td>ECO ATCC 25922</td>
<td>LACTOSE FERMENTER</td>
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<tr>
<td>Salmonella Shigella Agar</td>
<td>SCH ATCC 13076</td>
<td>NON LACTOSE-FERMENTER</td>
<td>EFA ATCC 29212</td>
<td>NO GROWTH</td>
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<tr>
<td>Triple Sugar Iron Agar</td>
<td>ECO ATCC 25922</td>
<td>BUTT SLOPE GAS H2S ACID +VE -Ve</td>
<td>PAE ATCC 27853</td>
<td>BUTT SLOPE GAS H2S Alk Alk -Ve -Ve</td>
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<tr>
<td>Motility-Indole-Ornithine Agar</td>
<td>ECO ATCC 25922</td>
<td>MOTILITY INDOLE +Ve +Ve ORNITHINE +Ve</td>
<td>KPN ATCC 13883</td>
<td>MOTILITY INDOLE -Ve -Ve ORNITHINE -Ve</td>
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<td>Citrate</td>
<td>KPN ATCC 13883</td>
<td>BLUE COLOUR</td>
<td>ECO ATCC 25922</td>
<td>GREEN COLOUR</td>
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<td>Urea Agar</td>
<td>PVU ATCC 13315</td>
<td>PINK COLOUR</td>
<td>ECO ATCC 25922</td>
<td>YELLOW COLOUR</td>
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<tr>
<td>Selenite -F Broth</td>
<td>SCH ATCC 13076</td>
<td>SCH ATCC 13076</td>
<td>ECO ATCC 25922</td>
<td>NO TURBIDITY</td>
</tr>
<tr>
<td>Selenite -F S/C</td>
<td>SCH ATCC 13076</td>
<td>NON LACTOSE FERMENTER ON XLD(PINK)</td>
<td>ECO ATCC 25922</td>
<td>NO GROWTH ON MAC</td>
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<tr>
<td>MacConkey</td>
<td>ECO ATCC 25922</td>
<td>LACTOSE FERMENTER (PINK)</td>
<td>SSO ATCC 25931</td>
<td>NON LACTOSE FERMENTER (PALE)</td>
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<tr>
<td>Campylobacter Agar</td>
<td>Campylobacter Agar</td>
<td>WATER-LIKE DROPLETS GROWTH</td>
<td>WATER-LIKE DROPLETS GROWTH</td>
<td>NO GROWTH</td>
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<tr>
<td>MHA</td>
<td>SAU ATCC 25923</td>
<td>CREAM COLOURED COLONIES</td>
<td>UNINOCULATED</td>
<td>NO GROWTH</td>
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<tr>
<td>MAC-GENT</td>
<td>KPN NEQAS 1057</td>
<td>LACTOSE FERMENTERS</td>
<td>ECO ATCC 25922</td>
<td>NO GROWTH</td>
</tr>
<tr>
<td>Freezing Mixture</td>
<td>Freezing Mixture</td>
<td>GROWTH AFTER S/C ON CHOC</td>
<td>UNINOCULATED</td>
<td>NO GROWTH</td>
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NB: All uninoculated plates should have no growth
9.0 REFERENCES:


Document history

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<td>Joseph Waichungo</td>
<td>Caroline Tigoi</td>
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Site training record

All sites are required to maintain a master copy of this SOP that documents the site staff that have been trained on this SOP.

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