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 that 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

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 H_2S- Hydrogen sulphide
4.23 SSO- *Shigella sonnei*
4.24 LF- Lactose fermenters
4.24 SF- Sucrose fermenters OR Sorbitol fermenters
4.25 CO_2-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 CO_2 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.

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/ CO_2 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:

MEDIA	POSITIVE CONTROL	NEGATIVE CONTROL
BA	S. aureus ATCC 25923	Uninoculated
XLD	SCH ATCC 13076	E. coli ATCC 25922
SS	SCH ATCC 13076	E. faecalis ATCC 29212
CAMPY	C. jejuni NCTC 11322	E. coli ATCC 25922
TSI	E. coli ATCC 25922	P. aeruginosa ATCC 27853
MIO	E. coli ATCC 25922	P. aeruginosa ATCC 27853
CITRATE	K. pneumoniae ATCC 13883	E. coli ATCC 25922
UREA	P. vulgaris ATCC 13315	E. coli ATCC 25922
SFB	SCH ATCC 13076	E. coli ATCC 25922
MAC	E. coli ATCC 25922	S. sonnei
MHA	E. coli ATCC 25922	Uninoculated
MAC-GENT	KPN NEQAS 1057	ECO ATCC 25922

8.2. MEDIA QC RESULTS INTERPRETATION CHART

INTERNAL QUALITY CONTROL FOR CULTURE MEDIA SOP (MASTER)

CULTURE MEDIA	POSITIVE CONTROL	RESULT	NEGATIVE CONTROL	RESULT
Blood Agar	SAU ATCC 25923	BETA HAEMOLYTIC WHITISH GROWTH	UNINOCULATED MEDIUM	NO GROWTH
XLD Agar	SCH ATCC 13076	NON LACTOSE FERMENTER	ECO ATCC 25922	LACTOSE FERMENTER
Salmonella Shigella Agar	SCH ATCC 13076	NON LACTOSE-FERMENTER	EFA ATCC 29212	NO GROWTH
Triple Sugar Iron Agar	ECO ATCC 25922	BUTT SLOPE GAS H2S ACID ACID +VE -VE	PAE ATCC 27853	BUTT SLOPE GAS H2S Alk Alk -Ve -Ve
Motility-Indole-Ornithine Agar	ECO ATCC 25922	MOTILITY INDOLE +Ve +Ve ORNITHINE +Ve	KPN ATCC 13883	MOTILITY INDOLE -Ve -Ve ORNITHINE -Ve
Citrate	KPN ATCC 13883	BLUE COLOUR	ECO ATCC 25922	GREEN COLOUR
Urea Agar	PVU ATCC 13315	PINK COLOUR	ECO ATCC 25922	YELLOW COLOUR
Selenite -F Broth	SCH ATCC 13076	SCH ATCC 13076	ECO ATCC 25922	NO TURBIDITY
Selenite -F S/C	SCH ATCC 13076	NON LACTOSE FERMENTER ON XLD(PINK)	ECO ATCC 25922	NO GROWTH ON MAC
MacConkey	ECO ATCC 25922	LACTOSE FERMENTER (PINK)	SSO ATCC 25931	NON LACTOSE FERMENTER (PALE
Campylobacter Agar	Campylobacter Agar	WATER-LIKE DROPLETS GROWTH	WATER-LIKE DROPLETS GROWTH	NO GROWTH
MHA	SAU ATCC 25923	CREAM COLOURED COLONIES	UNINOCULATED	NO GROWTH
MAC-GENT	KPN NEQAS 1057	LACTOSE FERMENTERS	ECO ATCC 25922	NO GROWTH
Freezing Mixture	Freezing Mixture	GROWTH AFTER S/C ON CHOC	UNINOCULATED	NO GROWTH
				NO GROWTH AFTER 1ML S/C ON BA

NB: All uninoculated plates should have no growth

9.0 REFERENCES:

9.1 Practical Medical Microbiology 13th Edition by Mackie and McCartney, 1989.

9.2 Medical Laboratory Manual for Tropical countries Vol. II by Monica Cheesbrough, 1984.

9.3 Oxoid Media Manual 9th Edition, 2006

Document history

Version	Author	Approved by	Dated
1.01 **(MASTER): INTERNAL QUALITY CONTROL FOR CULTURE MEDIA - CHN57**	Joseph Waichungo	Caroline Tigoi	10/10/2016
1.02			

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.

Document History				
Version No.	**Trained staff initials**	**Signature of trained staff**	**Date**	**Trainer's Initials**
1.01	KDT	Example row	1st Jan 2016	DM