# Aim

To describe the preparation and quality control of media in routine use in the microbiology laboratory.

# Principle

A variety of culture media are required to identify bacteria of medical importance. Many are non-selective and support the growth of a wide range of bacteria (e.g. sheep blood agar), whilst others are selective (e.g. Ashdown and CNA-blood agars) and/or contain indicator substances to aid identification (e.g. MacConkey and Brilliance UTI Clarity agars). Enrichment broths (e.g. Brain Heart Infusion + SPS blood culture broth and selenite broth), enable the culture of even a small number of target organisms. Chocolate agar is a solid media with additional nutrients (from lysed red blood cells) enabling fastidious organisms such as *Haemophilus influenzae* and *Neisseria gonorrhoeae* to grow.

# General procedures

Use distilled water for all media preparation.

Use a clean labelled 1L Duran bottle (blue screw top lid).

Weigh out the appropriate medium powder carefully into a disposable weighing boat.

Place a magnetic stirring bar into the bottle.

Place the bottle on the stirring hot plate set at 100°C.

Heat and stir the liquid until the powder has dissolved and the solution is clear.

Do not over tighten the bottle lid: it should be loose.

Place a strip of autoclave tape over the bottle lid.

Unless the specific media method states a specific autoclave cycle, autoclave the bottle at 121°C for 15 minutes.

After the autoclave has finished carefully remove the bottle, mix and then place in 50°C waterbath to allow it to cool.

Whilst the bottle is cooling, label sterile petri dishes according to the medium. Write the medium, date and batch number if more than one bottle is prepared.

When cool enough to hold by hand, any supplements required can be aseptically added (e.g. antibiotics, blood).

Mix the medium thoroughly.

Pour 20ml for each plate aseptically within the Class II biosafety cabinet. For slopes, dispense 5ml for each tube and allow to set at an angle.

When agar has been dispensed leave the plates in the cabinet overnight.

The next morning:

* Perform QC and sterility testing.
* Pack the plates into plastic bags and put in the media fridge (store plates upside down: plate lid at the bottom).

# Quality control of media

Every batch of agar or broth must be quality controlled for both sterility and the ability to support growth of target organisms (and suppression of non-target organisms in certain cases).

After the plates/tubes are poured, a number should be sent for QC testing (Table 1). The other plates/tubes from that batch are put into plastic bags and stored at the top of the fridge. When the batch passes QC, the agar can be moved to the in-use position in the fridge. If the batch fails QC, all plates/tubes should be discarded and a new batch prepared: consideration should be given to the source of failure (e.g. incorrect autoclave cycle, omission of supplement).

# Storage of media

Media should generally be stored at 2-8°C out of direct sunlight.

Plates should be stored inverted (lid bottom) in sealed plastic bags.

Fresh media performs better than older stock. For agar plates, stock should be kept for no longer than 4-6 weeks (for some media this may be shorter: e.g. Brilliance UTI Clarity (Chromogenic) should be used within two weeks of preparation). Agar slopes and broth tubes may be kept for longer (up to 3-6 months in some cases).

**Table 1.** Media QC requirements (in addition to sterility plate)

| **Medium**  **(atmosphere)** | **QC organisms** | **Expected result** |
| --- | --- | --- |
| Ashdown (ASH)  (O2) | *B pseudomallei* (in-house)  *P. aeruginosa* ATCC 27853 | Growth  No growth |
| Bile aesculin  (O2) | *E. faecalis* ATCC 29212  *S. pyogenes* (in-house) | Black colonies  No growth |
| Blood culture bottles  (O2) | *H. influenzae* ATCC 49247  *S. pneumoniae* ATCC 49619 | Growth  Growth |
| Brilliance UTI  Chromogenic  (O2) | *E. coli* ATCC 25922  *E. faecalis* ATCC 29212  *P. aeruginosa* ATCC 27853 | Growth (pink)  Growth (blue / turquoise)  Growth (green / brown) |
| Chocolate  (CO2) | *H. influenzae* ATCC 49247  *N. gonorrhoeae* ATCC 43069 | Growth  Growth |
| CNA  (CO2) | *S. pneumoniae* ATCC 49619  *S. aureus* ATCC 25923  *E. coli* ATCC 25922 | Growth + alpha haemolysis  Growth  No / scanty growth |
| Columbia  (O2) | *S. aureus* ATCC 25923  *E. coli* ATCC 25922 | Growth  Growth |
| DNase  (O2) | *S aureus* ATCC 25923  *S. epidermidis* (in-house) | Positive  Negative |
| HTM  (CO2) | *H. influenzae* ATCC 49247 | Growth |
| MacConkey  (O2) | *P. aeruginosa* ATCC 27853  *E. coli* ATCC 25922  *E. faecalis* ATCC 29212 | Growth (colourless colonies)  Growth (pink colonies)  Some inhibition |
| Mannitol salt  (O2) | *S aureus* ATCC 25923  *S. epidermidis* (in-house)  *E. coli* ATCC 25922 | Yellow colonies at 48h  Pink colonies at 48h  No / scanty growth |
| MIL  (O2) | *E. coli* ATCC 25922 | M +; I +; L + |
| Mueller Hinton  (O2) | *E. coli* ATCC 25922  *S. aureus* ATCC 25923 | Growth  Growth |
| Mueller Hinton  + sheep blood (CO2) | *S. aureus* ATCC 25923  *S. pneumoniae* ATCC 49619 | Growth  Growth |
| Sabouraud  (O2) | *C. albicans* (in-house) | Growth |
| Selective broth (SB)  (O2) | *E. coli* ATCC 25922  *B. pseudomallei* (in-house) | No growth on ASH  Small purple colonies on ASH |
| Selenite broth  (O2) | *S.* Typhi (in-house)  *S. dysenteriae* (in-house) | Black colonies on XLD  Clear pink colonies on XLD |
| Sheep blood  (CO2) | *S. aureus* ATCC 25923  *E. coli* ATCC 25922  *S. pneumoniae* ATCC 49619  *S. pyogenes* ATCC19615 | Growth  Growth  Growth + alpha haemolysis  Growth + beta haemolysis |
| Sheep blood + 5mg/L gentamicin  (CO2) | *S. pneumoniae* ATCC 49619  *S. aureus* ATCC 25923  *E. coli* ATCC 25922 | Growth + alpha haemolysis  No / scanty growth  No / scanty growth |
| Simmons citrate  (O2) | *K. pneumoniae* (in-house)  *E. coli* ATCC 25922 | Growth (blue colour change)  No growth (no colour change) |
| STGG  (CO2) | *S. pneumoniae* ATCC 49619 | Growth and recovery after freezing at -80°C for 48h |
| TSB-glycerol broth  (O2) | *E. coli* ATCC 25922 | Growth and recovery after freezing at -80°C for 48h |
| Triple sugar iron  (O2) | *E. coli* ATCC 25922  *S.* Typhi (in-house) | A/A; gas +; H2S –  K/A; gas –; H2S + |
| Urea  (O2) | *Proteus mirabilis* (in-house)  *E. coli* ATCC 25922 | Growth (pink colour change)  Growth (no colour change) |
| VCNT  (CO2) | *Neisseria gonorrhoeae* (in-house)  *S. aureus* ATCC 25923 | Good growth  No / scanty growth |
| XLD  (O2) | *S.* Typhi (in-house)  *S. dysenteriae* (in-house) | Black colonies  Clear pink colonies |

# Ashdown agar (ASH)

## Intended use

A selective medium developed by Ashdown for the isolation of *B. pseudomallei* from contaminated samples. This medium comprises tryptone soya agar base supplemented with glycerol, crystal violet, neutral red and gentamicin. The incorporation of glycerol produces characteristic wrinkled colonies of *B. pseudomallei* which develop a deep pink colour due to the absorption of the neutral red dye. Species of *Pseudomonas* which may be confused with *B. pseudomallei* due to their oxidase reaction do not absorb the dye. The addition of crystal violet inhibits the growth of Gram-positive organisms, while the addition of gentamicin inhibits most other aerobic and facultative Gram-negative bacilli.

## Method

Mix all of the following ingredients in a 1L Duran bottle and autoclave at 121°C for 15 minutes without prior boiling:

Tryptone soya broth (Oxoid CM0129) 10 g

Agar No 1 (Oxoid LP0011) 15 g

Glycerol 40 ml

Crystal Violet 0.1% (0.1g/100ml)\* 5 ml

Neutral red 1% (1g/100ml) 5 ml

Distilled water 950 ml

\*New stock needs to be incubated at 37°C for 2 weeks before use.

Put in a 50°C waterbath to cool.

When the medium has cooled, add gentamicin for injection (80mg/2ml) to give the desired concentration: 125μl (5mg/l) for clinical use.

Pour 20ml for each plate aseptically within the Class II biosafety cabinet.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# Bile aesculin agar slopes (Oxoid CM0888)

## Intended use

A differential medium to help identify enterococci.

## Method

Dissolve 44.5g powder in 1L of distilled water.

Follow the preparation procedure outlined in section 3.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared slopes at 2-8°C.

# Blood culture bottles (Brain Heart Infusion, Oxoid CM0225)

## Intended use

Brain Heart Infusion is a highly nutritious infusion medium used for cultivating a variety of fastidious organisms including streptococci, pneumococci and meningococci. Because of its nutritive qualities, Brain Heart Infusion is suitable for use in in-house blood culture bottles.

## Method

Dissolve 37g Brain Heart infusion broth and 0.25g sodium polyanethol sulfonate (SPS) in 1L of distilled water.

Mix well and distribute 20ml into clean glass bottles.

Apply a rubber stopper and aluminium cap (use the capping tool to fit the aluminium cap securely).

Sterilise by autoclaving at 121°C for 15 minutes.

## Quality assurance

Follow guidance in Appendix 1.

## Storage

Store the prepared bottles protected from sunlight at room temperature (15-30°C) for up to three months.

# Brilliance UTI Clarity agar (Oxoid CM1106)

## Intended use

A medium for differentiation and presumptive identification of common urinary tract infection isolates.

## Method

Dissolve 37g powder in 1L of distilled water.

Follow the preparation procedure outlined in section 3.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# Chocolate agar (Modified Thayer Martin agar, Oxoid CM0367)

## Intended use

A medium for the growth of fastidious organisms. This recipe was devised by AHC staff and Vannaporn Wuthiekanun (MORU) and shown to be acceptable for culture of *H. influenzae* and other fastidious organisms.

## Method

Dissolve 36g of GC Agar Base in 470ml of distilled water:

Bring gently to the boil on the hotplate to dissolve the agar.

Sterilise by autoclaving at 121°C for 15 minutes.

Add 10g of Soluble Haemoglobin Powder (Oxoid LP0053) to 500ml warm distilled water (2% solution):

Autoclave at 121°C for 15 minutes.

When agar has cooled to 50°C aseptically add 10ml of Vitox (Oxoid SR0090) to GC agar base.

Aseptically add the haemoglobin solution to the GC agar base.

Mix, avoiding producing air bubbles in the agar, and pour aseptically into agar plates within the Class II biosafety cabinet.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# CNA-sheep blood agar (Oxoid CM0331 + SR0070)

## Intended use

A medium for selective culture of Gram positive organisms.

## Method

Dissolve 19.5g of Columbia blood agar base in 500ml of distilled water.

Mix well and boil to dissolve the medium on the hotplate.

Sterilise by autoclaving at 121°C for 15 minutes.

Cool the medium to 50°C in the waterbath.

Reconstitute one vial of CNA supplement (Oxoid SR0070) in 5ml of 95% ethanol; aseptically add this to the agar.

Add 25ml of sterile citrated sheep blood to the agar.

Pour 20ml for each plate aseptically within the Class II biosafety cabinet.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# Columbia agar (Oxoid CM0331)

## Intended use

A general purpose medium for culture of non-fastidious isolates.

## Method

Dissolve 39g powder in 1L of distilled water.

Follow the preparation procedure outlined in section 3.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# DNase agar (Oxoid CM0321)

## Intended use

A medium for differentiation of *Staphylococcus aureus* from coagulase negative staphylococci.

## Method

Dissolve 19.5g powder in 500ml of distilled water.

Follow the preparation procedure outlined in section 3.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# Haemophilus test medium, HTM (Oxoid CM0898 and SR0158)

## Intended use

A medium for antimicrobial susceptibility testing of *Haemophilus* spp.

## Method

Dissolve 21.5g powder in 500ml of distilled water.

Bring to the boil on the hotplate to dissolve.

Sterilise by autoclaving at 121°C for 15 minutes.

Cool to 50°C in the waterbath.

Add 2ml sterile distilled water to one vial of HTM supplement (Oxoid SR0158) and mix.

Add one vial of the HTM supplement to the cooled agar.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# MacConkey agar (Oxoid CM0007)

## Intended use

A differential medium for the isolation of coliforms and intestinal pathogens.

## Method

Dissolve 52g powder in 1L of distilled water.

Follow the preparation procedure outlined in section 3.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# Mannitol salt agar (Oxoid CM0085)

## Intended use

A selective medium for the isolation of presumptive pathogenic staphylococci.

## Method

Dissolve 111g powder in 1L of distilled water.

Follow the preparation procedure outlined in section 3.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# MIL medium slopes (BD Difco 218041)

## Intended use

A medium for differentiating *Enterobacteriaceae* based on motility, lysine decarboxylation, lysine deamination and indole production.

## Method

Dissolve 36.5g powder in 1L of distilled water.

Follow the preparation procedure outlined in section 3.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared slopes at 2-8°C.

# Mueller-Hinton agar +/- sheep blood (Oxoid CM0337)

## Intended use

Mueller-Hinton agar is the international standard media for antimicrobial susceptibility testing. 5% sheep blood is added for susceptibility testing of more fastidious organisms such as *Streptococcus pneumoniae*.

## Method

Dissolve 38g powder in 1L of distilled water.

Follow the preparation procedure outlined in section 3.

If Mueller-Hinton blood agar is required, add 50ml sterile sheep blood to 1L of the cool Mueller-Hinton agar (as per plain sheep blood agar).

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# Sabouraud dextrose agar (Oxoid CM0041)

## Intended use

A medium for the isolation of fungi.

## Method

Dissolve 65g powder in 1L of distilled water.

Follow the preparation procedure outlined in section 3.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# Selective broth (SB)

## Intended use

A selective enrichment broth containing colistin and crystal violet for the isolation of *B. pseudomallei* from clinical and environmental samples

## Method

### Preparing Stock Solution

Add 1.6 mL of distilled water to one red-capped glass vial of Forest Laboratories Colomycin (containing 1 million units; equal to 80 mg colistimethate).

Mix until the colistimethate (white powder) has fully dissolved. This creates a 50 mg/mL colistimethate stock solution.

### Preparing Medium

Mix the following ingredients in a 1L Duran bottle and autoclave at 121°C for 15 minutes without prior boiling:

Tryptone soya broth (Oxoid CM 0129) 10 g

Glycerol 40 mL

Crystal Violet 0.1%\* 5 mL

Distilled water 950 mL

\*New stock needs to be incubated at 37°C for 2 weeks before use.

Allow the medium to cool to 50°C in a water bath.

Add 1 mL colistimethate stock solution (50 mg/mL) – final concentration 50 mg/L.

In the Class II biosafety cabinet, aseptically aliquot 10 mL volumes of the broth into sterile glass tubes.

Re-cap and store at 2-8°C for up to 6 months, after which the medium should be discarded and a fresh batch made.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the tubes at 2-8°C.

# Selenite broth (Oxoid CM0395 and LP0121)

Note: Sodium biselenite is toxic by inhalation and if swallowed (with danger of cumulative effects). To minimize any possible risk to laboratory workers, the sodium biselenite must be added as a solution to this medium. Miscarriages and possible teratogenic effects on pregnant laboratory assistants have been described which may have been caused by ingested sodium biselenite. Although no further reports have been received sodium biselenite is now considered to be very toxic and should be handled with great care.

## Intended use

A selective enrichment medium for the isolation of *Salmonella* spp.

## Method

Dissolve 4g of sodium biselenite (Oxoid LP0121) in 1L of distilled water and then add 19 grams of Selenite agar (CM0395).

Warm on heat plate to dissolve, mix well and fill out into clean tubes to a depth of 5cm.

Sterilise in a boiling water bath, or in free flowing steam, for 10 minutes (do not autoclave).

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared tubes at 2-8°C.

# Sheep blood agar (Oxoid CM0331)

## Intended use

A general purpose non-selective culture medium.

## Method

Dissolve 39g powder in 1L of distilled water.

Follow the preparation procedure outlined in section 3.

When the media has cooled to 50°C add 50ml sterile sheep blood using a sterile syringe (50ml blood / 1L agar = 5%).

Pour 20ml for each plate aseptically within the Class II biosafety cabinet.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# Sheep blood + 5mg/L gentamicin agar (Oxoid CM0331)

## Intended use

A selective medium for isolation of *Streptococcus pneumoniae*. Internal QC has shown that pharmacy gentamicin for injection has similar characteristics (and notably does not inhibit *S. pneumoniae*) as in-house prepared solutions.

## Method

### Preparing 5mg/ml stock gentamicin solution

Add 2ml pharmacy gentamicin (1 vial = 80mg/2ml) to 14 ml of distilled and autoclaved water.

Alternatively, prepare using gentamicin sulphate powder (Sigma G3632; potency ≥590µg/mg):

* Weight of gentamicin sulphate powder required (mg) =
  + (volume (ml) x concentration required (µg/ml)) / potency (µg/mg)
* To make 50ml of 5mg/ml:
  + (50 x 5,000) / 590 = 250,000 / 590 = 423.7mg (0.43g) gentamicin sulphate powder

Filter sterilise (0.2µm filter) and dispense 1ml aliquots into sterile 1.5ml Eppendorf tubes.

Store at -80°C.

### Preparing Medium

Dissolve 38g powder in 1L of distilled water.

Follow the preparation procedure outlined in section 3.

When the media has cooled to 50°C add 50ml sterile sheep blood using a sterile syringe (50ml blood / 1L agar = 5%) and 1ml of thawed gentamicin stock solution (5ml/ml).

Pour 20ml for each plate aseptically within the Class II biosafety cabinet.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# Simmons citrate slopes (Oxoid CM0155)

## Intended use

A medium for the differentiation of Enterobacteriaceae based on the utilisation of citrate as the sole source of carbon.

## Method

Dissolve 23g powder in 1L of distilled water.

Follow the preparation procedure outlined in section 3.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared slopes at 2-8°C.

# STGG medium (Oxoid CM0129 and LP0021)

## Intended use

A transport and storage medium for Streptococcus pneumoniae. Studies have also shown that STGG can be used as a general purpose low temperature storage medium (-80°C).

## Method

Mix the following ingredients in a 500ml Duran bottle:

Skim milk powder (Oxoid LP0031) 2g

Tryptone Soya Broth (Oxoid CM0129) 3g

Glucose 0.5g

Glycerol 10ml

Distilled water 100ml

Dispense in 1.0 ml amounts in screw-capped 2ml cryotubes.

Loosen the screw-cap tops and autoclave at 121°C for 10 minutes.

Tighten caps after autoclaving.

## Quality assurance

Select two random cryotubes from each box from the batch.

***Tube 1***

Vortex well.

Plate 100μl onto a blood agar plate and incubate overnight at 37°C in 5% CO2: there should be NO growth on the plate.

***Tube 2***

Vortex well.

Inoculate with *Streptococcus pneumoniae* ATCC 49619.

Freeze at -80°C for 48h.

Thaw out at room temperature and vortex well.

Subculture 100μl onto a blood agar plate and incubate overnight at 37°C in 5% CO2: there should be GOOD growth on the plate.

## Storage

Store the prepared tubes at 2-8°C for up to six months.

# TSB-glycerol medium (Oxoid CM0129)

## Intended use

A storage medium for faecal samples.

## Method

Mix the following ingredients in a 500ml Duran bottle:

Tryptone Soya Broth (Oxoid CM0129) 3g

Glycerol 10ml

Distilled water 100ml

Dispense in 1.5 ml amounts in screw-capped 2ml cryotubes.

Loosen the screw-cap tops and autoclave at 121°C for 15 minutes.

Tighten caps after autoclaving.

## Quality assurance

Select two random cryotubes from each box from the batch.

***Tube 1***

Vortex well.

Plate 100μl onto a blood agar plate and incubate overnight at 37°C in air: there should be NO growth on the plate.

***Tube 2***

Vortex well.

Inoculate with *Escherichia coli* ATCC 25922.

Freeze at -80°C for 48h.

Thaw out at room temperature and vortex well.

Subculture 100μl onto a blood agar plate and incubate overnight at 37°C in air: there should be GOOD growth on the plate.

## Storage

Store the prepared tubes at 2-8°C for up to six months.

# Triple sugar iron slopes (Oxoid CM0277)

## Intended use

A medium for the differentiation of Enterobacteriaceae by three sugar fermentations and hydrogen sulphide production as part of the biochemical reactions.

## Method

Dissolve 65g powder in 1L of distilled water.

Follow the preparation procedure outlined in section 3.

Pour the slopes ensuring a sufficiently deep butt.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared slopes at 2-8°C.

# Urea slopes (Oxoid CM0053 and SR0020)

## Intended use

A medium to detect rapid urease activity of the Proteae and non-rapid urease activity of some Enterobacteriaceae

## Method

Suspend 2.4g in 95ml of distilled water.

Bring to the boil with frequent agitation to dissolve the agar.

Autoclave at 115°C for 20 minutes.

Allow the medium to cool to 50°C in a water bath.

Aseptically add 5ml of 40% urea solution (Oxoid SR0020) to the media and mix well.

Pour the media aseptically within the Class II biosafety cabinet (approximately 5ml per tube).

Allow the tubes to set on a slope.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared slopes at 2-8°C.

# VCNT, Thayer Martin selective agar (Oxoid CM0367)

## Intended use

A medium used for the selective growth of *Neisseria* species. It contains vancomycin, colistin, nystatin and trimethoprim.

## Method

Dissolve 18g of GC Agar Base in 240ml of distilled water:

Bring gently to the boil on the hotplate to dissolve the agar.

Sterilise by autoclaving at 121°C for 15 minutes.

Add 5g of Soluble Haemoglobin Powder (Oxoid LP0053) to 250ml warm distilled water (2% solution):

Autoclave at 121°C for 15 minutes.

When agar has cooled to 50°C:

Aseptically add 10ml of Vitox (Oxoid SR0090) to GC agar base.

Dissolve the contents of a vial of VCNT Antibiotic Supplement SR0091 in 2ml of distilled water and add aseptically to the GC agar base.

Aseptically add the haemoglobin solution to the GC agar base.

Mix, avoiding producing air bubbles in the agar, and pour aseptically into agar plates within the Class II biosafety cabinet.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# XLD agar (Oxoid CM0469)

## Intended use

A selective medium for the isolation of salmonellae and shigellae.

## Method

Dissolve 53g powder in 1L of distilled water.

It is important not to overheat the medium when dissolving the powder.

Follow the preparation procedure outlined in section 3.

## Quality assurance

Follow guidance in section 4.

## Storage

Store the prepared plates at 2-8°C.

# References

1. Cheesbrough, M. District Laboratory Practice in Tropical Countries, Part 2. 2nd Edition Update (2006). Cambridge University Press.
2. Oxoid website: <http://www.oxoid.com/uk/blue/index.asp>.
3. Standard Operating Procedures from LOMWRU, SMRU and AHC.

# Synopsis / Bench aids

Not applicable

# Risk assessment

|  |  |
| --- | --- |
| **COSHH risk assessment - University of Oxford COSHH Assessment Form** | |
| **Description of procedure**  Preparation and QC of bacterial culture and storage media | **Substances used**  Powdered culture media  Various supplements (Vitox, VCNT, 40% Urea, Selenite F, HTM / CNA supplement, Gentamicin) |
| **Quantities of chemicals used**  <150g agar powder  5-10ml supplements | **Frequency of SOP use**  Weekly |
| **Hazards identified**  1. Autoclaved liquids  2. Potentially pathogenic QC organisms  3. Media powder can be a respiratory irritant  4. Sodium biselenite is teratogenic | **Could a less hazardous substance be used instead?**  No |
| **What measures have you taken to control risk?**  1. Training in good laboratory practices (GLP)  2. Appropriate PPE (lab coat, gloves, eye protection, N95 mask)  3. Laboratory is well ventilated  4. Pregnant laboratory staff do not handle sodium biselenite | |
| **Checks on control measures**  Observation and supervision by senior staff | |
| **Is health surveillance required?**  No | **Training requirements:**  GLP |
| **Emergency procedures**:  1. Report all incidents to Safety Adviser  2. In event of any solution coming in contact with the eyes, immediately flush with running water for at least 15 minutes  3. If contact with skin, remove contaminated clothing and wash skin thoroughly  4. If any substance inhaled, move to fresh air and seek medical attention if any respiratory symptoms. | **Waste disposal procedures**:  All inoculated media is autoclaved prior to disposal |

# Appendix 1. Blood culture media QC protocols

