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| Viral Media, Reagents and Antibiotics Quality Control | | | | | |
| **Purpose** | This procedure provides instructions for the storage, preparation and quality control of the media, reagents and antibiotics used in virology. | | | | |
| **Policy Statements** | * This procedure applies to all virology technical staff. * This procedure is in compliance with CAP checklist items MIC .61140, .61160, and .61330. * Corrective action is taken when QC results are not acceptable | | | | |
| **Principle and Clinical Significance** | Media and reagents used to maintain cell culture must be sterile and meet product specifications. All purchased media and reagents undergo QC by the manufacturers. Media and reagent sterility is verified by virology concurrent with use .PH is verified where applicable. Each lot of fetal bovine serum (FBS) is checked for cytotoxicity prior to use. | | | | |
| **Sample** | New lot/shipment of media or reagent | | | | |
| **Materials** | * Sheep blood agar (BAP) * Sabouraud dextrose agar (SAB) * Thioglycollate (THIO) * 1 ml sterile pipet * 1-3ml syringe * Blunt needle * Disposable sterile loops | | | | |
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| **Procedure** | To perform sterility testing:  1) Inoculate a BAP and SAB plate with 0.1 of media/reagent.  2) Inoculate THIO with 1 ml media/reagent.  3) Incubate in non- CO2 incubator.  4) Read BAP, SAB and THIO for any growth at 7 days. Record on QC log.  5) Hold SAB for additional 7 days. Read and record on QC log.  If any growth occurs, assess cell culture where media/reagent has been used. Quarantine media/reagent .Repeat testing as necessary. Notify Lead MLS. | | | | |
| VIRAL MEDIA: REAGENTS AND SOLUTIONS | A. COMPONENTS PURCHASED COMMERCIALLY (Gibco Laboratories)  1. Antibiotics  a. Gentamicin Reagent Solution (10 mg/ml), liquid 10 ml  Spectrum: gram positive and gram negative  bacteria and mycoplasma  Storage: 15° to 30°C  Shelf life: expiration date stated on label  b. Gentamicin Reagent Solution (50 mg/ml), liquid 10 ml  Spectrum: gram positive and gram negative  bacteria and mycoplasma  Storage: 15°C to 30°C  Shelf life: expiration date stated on label  c. Amphotericin B (250 mcg/ml) 20 ml  Spectrum: fungi and yeast  Storage after rehydration: -20°C or below  Shelf life: expiration date stated on label  d. Pen-Strep (10,000 units/ml Penicillin G Sodium and 20ml  10,000ug/ml Streptomycin Sulfate)  Spectrum: gram positive and gram negative bacteria  Storage: -20°C or below  Shelf life: expiration date stated on label  Quality Control: Gibco evaluates all antibiotic mixtures for their ability to inhibit and/or eliminate the growth of microbial strains. The products undergo a cytotoxicity evaluation and are tested for the absence of bacteria, fungi, pH, and osmolality levels.    2. Enzymes  a. Trypsin, 0.25% (1X), liquid 100 ml  2.5g Trypsin (1:250)/L in Hanks’ Balanced Salt Solution without Ca++ and Mg++.  Source: porcine  Storage: Aliquot 5 ml in sterile 50-ml tubes.  Store at - 20°C or below.  Shelf life: 6 months  Quality Control: All enzyme preparations are tested by Gibco for the absence of bacterial and fungal contamination, pH, and osmolality and screened for mycoplasma. Trypsin solutions are tested for their ability to remove adherent cell lines while preserving cellular integrity and screened for porcine parvovirus**. Sterility testing is performed by virology before trypsin is put into use.**  3. Balanced Salt Solutions/Media  a. Hanks’ Balanced Salt Solution (HBSS) 1X, liquid 500 ml  Storage: 15°C- 30°C  Shelf life: expiration date stated on label  PH: 6.7 to 7.8  b. Hanks’ Balanced Salt Solution (1X), liquid 100 ml  w/o Ca++ and Mg++  Storage: 15°C-30°C  Shelf life: expiration date stated on label  PH: 6.7 to 7.  c. Minimum Essential Medium (MEM), 1X, liquid 500ml  with Earle’s salts, with L-glutamine.  Storage: 2- 8° C, protect from light  Shelf life: expiration date stated on label  PH: 7.0 to 7.4  d. Minimum Essential Medium (MEM), 10X, liquid 500 ml  with Earle’s salts, without L-glutamine and sodium bicarbonate  Storage: 2-8°C, protect from light  Shelf life: expiration date stated on label  Each use: pH is adjusted to 2.0 after dilution  e. Minimum Essential Medium (MEM) 1x, liquid 500 ml  with Earle’s salts, without L-glutamine  Storage: 2-8°C, protect from light  Shelf life: expiration date stated on label  PH: 7.0 to 7.4  f. Minimum Essential Medium (MEM) 1X, liquid 500 ml  with Earle’s salts, and 25mM HEPES buffer  without L-glutamine  Storage: 2°C-8°C, protect from light  Shelf life: expiration date stated on label  PH: 7.0 to 7.4  g. Leibovitz’s L-15 Medium (1X), liquid 500 ml  with L-glutamine  Storage: 2°C-8°C, protect from light  Shelf life: expiration date stated on label  PH: 7.1 to 7.8    h. R-Mix Refeed Medium (1X), liquid 500 ml  Storage: 2°C-8°C, protect from light  Shelf life: expiration date stated on label  PH: 6.8 to 7.2  Quality Control: All balanced salt solutions and media are screened by Gibco or DHI for the absence of bacterial and fungal contaminants, mycoplasma, growth promotion, and cytotoxicity. PH and osmolality are tested to meet product specifications. **Sterility testing is performed by virology and pH is verified concurrent with use.**  4. Sera  a. Fetal Bovine Serum, Qualified, heat inactivated 500 ml  Storage: Aliquot 5 ml in 15 ml sterile Falcon tubes.  Store at -20°C or below.  Shelf life: 6 months  Quality Control: Testing is done by Serum Source International, Inc for performance, bacterial, fungal and mycoplasma contamination, viral contamination, pH and osmalality. **Sterility testing is performed by virology prior to serum being put into use. Cytotoxicity is checked by inoculating 3 fibroblast (MRC-5, SF) cell culture tubes with 0.2ml of FBS each and incubating for 7 days. Examine daily for toxicity. Record results at 7 days. Do not put FBS into use if excessive toxicity is noted and notify manufacturer.**   1. Additional Reagents   a. Alsever’s Solution, liquid 100ml  4.2g NaCl,8.0g sodium citrate and  20.5g glucose/L  Storage: room temperature  Shelf life: expiration date stated on label  Quality Control: Alsever’s solution is cell culture tested, sterile filtered and endotoxin tested by Sigma. **Sterility testing is performed by virology concurrent with use.**   1. Sodium Bicarbonate,7.5% , liquid 100ml   Storage: 15-30 °C  Shelf life: expiration date stated on label  Disposal: dispose in alkaline waste container in Core Lab  B. PREPARED VIRAL ANTIBIOTIC MIXTURES (VAM/VTM) FOR TREATMENT OF CULTURE SPECIMENS    1. VAM for Urine Specimens 40 tubes  Gentamicin (50 mg/ml) 4 ml  Amphotericin B (250 ug/ml) 10 ml  Dispense 0.3 ml aliquots into 15 ml sterile centrifuge tubes and store at -20°C or below until needed. To each tube of thawed mixture, add 5-10 ml of urine.  Shelf life: 4 months after preparation  2. VTM for Stool Specimens  MEM 1X with Earle’s salts, without L-glutamine ,with bicarbonate 500ml  Gentamicin (10mg/ml) 7.5 ml  Amphotericin B 250 ug/ml 15.0 ml  Dispense 9-ml aliquots into 50ml sterile centrifuge tubes containing 3-5 sterile glass beads.  Shelf life: 2 months after preparation  C. CELL CULTURE MEDIA FOR REFEEDING  1. Antibiotic Mixture for Maintenance Medium in 50 ml aliquots.  Gentamicin (50 mg/ml) 2 ml  Amphotericin B (250 mcg/ml) 20 ml  Pen-Strep (10,000 units/10,000ug/ml) 20 ml    Mix well and dispense 1.0 ml aliquots into sterile 50 ml tubes and freeze  at -20°C or below. **Sterility testing is performed on each batch prepared.**  2. Growth Medium (10% Fetal bovine serum)  Thaw aliquot of antibiotic mixture for 50 ml of maintenance media.  Add: Fetal bovine serum 5 ml  MEM with Earle’s salts and L-glutamine – Bring final  volume to 50 ml  Mix well and store at 4°C.  Expires after 7 days.  MAINTENANCE MEDIA MADE DAILY:  3. Maintenance Medium for Primary Monkey Kidney (PMK) cells (serum free)  Thaw aliquot of antibiotic mixture for 50 ml of maintenance media.  Add: MEM with HEPES without L-glutamine – Bring final volume to 50 ml.    4. Maintenance Medium for HEp-2 Cells (0.5% FBS L-15)  Thaw aliquot of antibiotic mixture for 50 ml of maintenance media.  Add: Fetal bovine serum 0.25 ml  Leibovitz’s L-15 medium – Bring final  volume to 50 ml.    5. Maintenance Medium for SF Cells (2% FBS.)  Thaw aliquot of antibiotic mixture for 50 ml of maintenance media  Add: Fetal bovine serum 1.0 ml  MEM with Earle’s salts and L-glutamine – Bring final  volume to 50 ml.   1. Maintenance Medium for MRC-5 Cells (5% FBS)   Thaw aliquot of antibiotic mixture for 50ml of maintenance media  Add: Fetal bovine serum 2.5ml  MEM with Earle’s salts and L-glutamine – bring final  Volume to 50ml.      E. PHOSPHATE BUFFERED SALINE (PBS) pH 7.0-7.6  contains 0.15 M NaC1 and 0.1 NaH2PO4.  Reconstitution: Add vial contents to 1.0 liter of Type 1 water. Stir until  Contents are dissolved. Give sample to Chemistry to pH.  Storage of powder: 2-8° C  Storage after reconstitution: 4-22°C  Expiration date: 2 months 2-8°C  1 month at RT  F. TRYPSIN, 0.25% working solution  Trypsin (thawed) 5 ml  HBSS without Ca++ and Mg++ 45 ml    Mix well and store at 4°C. Stable for 1 month. | | | | |
| **Result Reporting** | Problems with commercial reagents should be reported promptly to the vendor if the product is not performing to specifications. | | | | |
| **References** | 1. Finegold, S.M., and Baron, E.J., Bailey and Scott’s Diagnostic Microbiology, 7th edition, 1986, pg. 898-899. 2. Virology, Laboratory Procedure Manual, 1986, Mayo Clinic, Rochester, MN. 3. GIBCO Invitrogen Corp, Carlsbad CA. Product Certificate of Analysis, 2011. 4. Sigma-Aldrich St.Louis MO, Cell Culture Product Information, 2003. 5. Leber, A (Ed), Clinical Microbiology Procedures Handbook, 4th edition, Section 10, American Society for Microbiology, Washington D.C., 2016. | | | | |
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| **Training Plan/ Competency Assessment** | **Training Plan** | | | **Initial Competency Assessment** | |
| 1. Employee must read the procedure 2. Employee will observe trainer performing the procedure. 3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer. | | | 1. Direct Observation 2. Complete Virology Desk3 New Employee Training Checklist | |
| **Historical Record** |  |  |  | |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | | **Summary of Revisions** |
| 1 | Pat Ackerman | 1987 | | Initial Version |
| 1.4 | Helen Stefan | 11/5/06 | | Shelf life added to Viral antibiotic mixtures |
| 1.5 | Helen Stefan | 7/31/08 | | R-Mix Refeed added |
|  | 1.6 | Helen Stefan | 10/28/10 | | Sodium Bicarb added, SF refeed changed to 1% FBS |  |  |
| 1.7 | Helen Stefan | 6/26/11 | | Pen-Strep added, FBS supplier name changed to Serum Source International |
| 1.8 | Helen Stefan | 6/24/17 | | refeed changed to 2% FBS, Fungizone changed to AmphtericinB |
| 9 | Helen Stefan | 8/16/17 | | Reformatted for Sharepoint online manual, version changed to whole numbers |
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| **Archived by:** |  | **Archived Date:** | |  |