**UW Medicine - Pathology**

400-08-01-06

Culture and Lab Sterility / Contamination Procedure

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| Adopted Date: 08/1991  Review Date: 09/2005, 05/03/11, 04/04/13, 8/26/13  Revision Date: 05/03/11 |

PURPOSE

To test for media sterility and avoid culture contamination.

PROCEDURE

1. **Check for Sterility**
   1. **Media and culture reagents**: All media, media additives and tissue culture reagents are checked for sterility. Each lot of fetal bovine serum should be tested for cloning efficiency before using. Media should be prepared weekly for amniotic fluid and bone marrow cultures; and no less frequently than monthly for blood cultures, skin cultures and tumor cultures. All freshly prepared media should have an aliquot incubated 48 hr and visually inspected to ensure sterility. Two different lots of media are used for each case. All media additives are checked by incubation in media for 48 hr. All completed media are also tested for 48 hr before use. Contamination is recorded on the QC summary sheet along with actions taken to correct the problem. See corrective action.
   2. **Distilled Water**: Distilled H2O from laboratory stills must be used to change waterbaths. Distilled H2O should be stored in chemically clean and inert plastic or Pyrex containers, which are protected from outside contamination. Sterile distilled H2O is purchased from Baxter (Travenol) to be used in lab-made reagents and solutions calling for dH2O. Sterile distilled H2O is also made by autoclaving, lab-distilled H2O for use in wet incubators. Purity/sterility checks of the distilled, or deionized H2O are made semiannually by culture, fluorescence or conductance to meet the requirements of use by the Medical Center Microbiology Lab. If the results show inadequate purity, the Hospital Epidemiologist is consulted for further tests. The results of all tests must be recorded and placed into Media/QC book in AA108E. Grey tap water is collected 2x/year and sent to Micro: test mnemonic “CH2OC”. Contamination is recorded on the QC summary sheet along with actions taken to correct the problem. See corrective action.
   3. **Object sterilization-Autoclave:** Glassware, instruments, distilled H2O and other objects used in tissue culture should be sterilized using the appropriate cycle by the K-319 autoclave. Duo-Spore autoclave test strips (Propper Mfg. NY, 11101) are run weekly and sent to UWMC Microbiology lab. If a result shows a sterility failure, any recently sterilized objects are quarantined until testing and corrective action is taken. See corrective action. The failure is recorded on the QC summary sheet along with actions taken to correct the problem.
      1. **Contamination Control**
2. **Contamination protocol:** If bacterial contamination occurs, Gentamycin (broad spectrum antibiotic) can be used at 50 µg/ml (maximum is 200 µg/ml). Trade name is Garamycin™. 40 µg/ml [0.1 ml in 100 ml media (long-term cultures: TR + ST)] from Shering or Elkins-sinn at UWMC Pharmacy. All cultures from affected incubators are evacuated and quarantined; affected cultures are treated and unaffected cultures from the incubator are monitored. Sterilization of the incubator is then done. Important: All contamination and corrective actions are recorded in QC summary sheet. Culture contamination is also recorded in the comments section of the individual culture in the GCS database. (Table: CULTURE, Field: COMMENTS)
3. **Contamination avoidance:** 1 Spantab tablet is added to H2O to all wet incubators to discourage fungal growth. Incubators are sterilized semiannually. For antibiotics, 2 µl Normocin, 5µl Fungin, or 10-50 units of penicillin plus 10-50 µg of streptomycin (or 100 units of penicillin) and 0.025 µg fungizone are added to each ml of media (see below). Each specimen is divided into at least 2 independent cultures. All cases must have different incubators, different lots of media and different feeding schedules to safeguard loss of culture due to poor media or contamination.
4. **Media contamination control: used in all lab prepared media** (Invivogen):

Normocin™ 100µg/ml final concentration

Fungin™ 10-50µg/ml final

Alternative antibiotic reagents (Gibco):

Penicillin 1 U/ml final

Streptomycin 1 µg/m1 final

Fungizone 0.25 µg/ml final

1. **Other methods and guidelines:** 1.Consult J. Paul's book "Tissue Culture," for various antibiotic concentrations for tissue contamination (see References). 2. Yeast contamination has a specific smell and, usually a poor recovery rate. See Mold contamination below for treatment. Discard if possible. 3. Mold contamination that has resisted anti-fungal can be treated with Mycostatin™ 30 U/ml. Mycostatin does not go into solution completely (for lab use from Squibb from UWMC Pharmacy). 4. Clinical Microbiology (NW-120) can determine what the organism is and to what it is sensitive: call (206) 598-6147. (Provide patient's name and use budget # for Pathology-Cytogenetics 08-7092.) For mycoplasma see below.
   1. **Mycoplasma Treatment**
2. **Treatment**

Use Plasmocin™ (Invivogen) at 25µg/ml for two weeks in the infected culture to eliminate infection. A 1000X solution specific for treatment (ant-mpt) is provided by Invivogen. After a positive infection finding prevent Mycoplasma infection by using Plasmocin™ 2.5µg/ml on a regular basis in cell culture. A 100X solution specific for prophylactic use (ant-mpp) is provided.

***Note:*** All vessels that come in contact with specimens or cultures from specimens are biohazardous and should be discarded in appropriate containers following the guidelines for disposal.

REFERENCES

1. Russell, WC et al., A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses. *Nature* 253:461-462, 1975.
2. Barch, MT, Knutsen, T, Spurbeck, JL. The AGT Cytogenetics Laboratory Manual. Raven Press, 3rd edition, 1997
3. Freshney, RI. Culture of Animal Cells, Ch 18, Contamination. Wiley-Liss, 4th edition, 2000.
4. Paul, J. Cell and Tissue Culture, 5th Edition. Edinburgh, Churchill Livingstone, 1975.

Written By: Director Approval:

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Cytogenetics Supervisor