1.0 PURPOSE

The purpose of this procedure is to describe the use of basic aseptic technique.

2.0 RESPONSIBILITY

2.1 It is the responsibility of each employee working in a laboratory setting to follow this procedure when working inside a BSC and on the bench-top when executing applicable testing.

3.0 SAFETY

3.1 All applicable safety procedures within the laboratory will be followed per DES Guidelines

3.2 Human tissue and blood products are potentially infectious and must be handled according to OSHA regulations and universal precautions for preventing transmission of blood-borne pathogens. Liquid aerosol and physical contact with human tissue, fluids and blood products are potentially hazardous for infection from HIV and hepatitis.

4.0 TERMINOLOGY

4.1 **Aseptic Technique** – Preventive measures taken to prevent microbial contamination of the personnel, the environment they are working in or contamination of the specimen they are working on.

4.2 **BSC** – Biological Safety Cabinet – A piece of equipment that is specially designed to provide a microbe-free work area. A BSC draws air through the front of the working area into the vent system; it is then recirculated and expelled (See Figure 1).

4.3 **Disinfected item** – An item that is sterile inside its packaging but the outside has been disinfected with an approved disinfectant (e.g. 70% IPA). The outside of the item is not sterile.

4.4 **PPE** – Personal Protective Equipment – materials worn to protect individuals from exposure to chemicals or materials (e.g. safety glasses, laboratory coats).

4.5 **Sterile** - The state of being free of viable microorganisms.

4.6 **Sterile Operator** – An operator who only works within the sterile field.

4.7 **Working end or surface** – The area of an item that has direct material contact.

5.0 EQUIPMENT

5.1 BSC

6.0 MATERIALS AND REAGENTS

6.1 Sterile Alcohol, 70%

7.0 PRELIMINARY SETUP

7.1 Basic aseptic technique requires that analysts and technicians be constantly aware of their own actions and surroundings to ensure sterility during the execution of all practices under the BSC and on the laboratory bench-top. It requires that each individual be conscious of not only their own practices but also those of their fellow operators. Analysts should point out incorrect techniques and in this way improve the overall aseptic technique of the quality control department.

7.2 Analysts and all people who enter the laboratory must don PPE including:

7.2.1 Clean laboratory coats

7.2.2 Safety glasses or corrective eye glasses

8.0 PROCEDURE

8.1 Application of aseptic technique for the cleaning and operation of the BSC.

8.1.1 Clean BSC and while cleaning the BSC be sure to remove all materials in order to clean all surfaces of the BSC.

Note: The light should be cleaned when the BSC is disinfected.

8.2 Application of aseptic technique during all procedures performed under the BSC.

8.2.1 Before beginning a procedure under the BSC tie long hair back.

8.2.2 Before entering the BSC don sterile gloves and sterile sleeve covers. Open sleeve covers so that they are ready to put on. Spray gloves with 70% IPA and then don sterile sleeve covers. When removing sterile sleeve covers from the packaging, take care not to touch any part of the sleeve covers that may come into contact with the BSC. Spray hands with 70% IPA prior to entering the BSC.

8.2.3 Do not touch hair, face, safety glasses, laboratory coat or any other item outside the BSC with gloved hands. If any items touch gloves or sleeve covers, replace gloves and sleeve covers and spray with 70% IPA prior to reentry to the BSC. For example if holding a pen to document steps one must change gloves prior to entering BSC again.

8.2.4 When placing hands into the BSC for the first time, keep hands still with fingers spread wide for approximately one minute prior to manipulating items

within the hood. This practice allows airflow to return to normal before activity.

8.2.5 While working under the BSC ensure to make slow deliberate movements so that proper airflow is maintained. Do not make any quick movements.

8.2.6 Work 6 inches into the BSC from the front grate.

8.2.7 Do not rest any part of the arms that come in contact with the sterile field on the edge of the BSC while working and do not allow arms to come into contact with the bottom of the sash while working.

8.2.8 When passing items in to the BSC ensure that aseptic technique is followed as below.

8.2.8.1 Inspect all items for tears, holes, and seal integrity prior to being passed into the BSC. Any item found with a defect could be sterility compromised. Discard and replace compromised item immediately.

8.2.8.2 If item is packaged in a paper or cardboard box do not pass the box into the BSC.

8.2.8.3 When introducing double wrapped items to the BSC, pass the items in such a way as to ensure to remove the outer wrapping so that it does not contact the inner item. Also be sure that the gloved hand does not touch the inner item and that the inner item does not come in contact with any surface outside the BSC as it is introduced.

8.2.8.4 If a sterile operator is already working in the hood, he or she may take the item without touching the outer wrap, and then place the item into the BSC.

8.2.8.5 Individually spray all other materials and items passed into the BSC with 70% alcohol or wipe with an alcohol-saturated wipe and introduce so that the sprayed surface does not contact any surface outside the BSC.

8.2.9 Spray gloved hands with 70% IPA each time when entering the BSC.

8.2.10 In order to reduce the risk of possible contamination the operator once working under the BSC should minimize the number of times that one must go in and out of the BSC.

8.2.11 Organize and place all materials passed into the BSC in such a way that the airflow of the BSC is not interrupted. Keep materials away from the return air grills to ensure proper airflow.

8.2.12 Do not crowd the BSC with materials.

8.2.13 Arrange the items in the BSC in a logical pattern from clean to dirty to avoid passing contaminated materials through airflow above clean materials.

8.2.14 Do not place clean or unused materials in close proximity to used items.

8.2.15 Immediately dispose of any small contaminated items into a discard container. Promptly remove any larger contaminated items or other items meant of disposal from the work space and place in the biohazardous waste.

8.2.16 Do not touch open sterile ends of ports, caps, bottles, or tubes. Do not touch the working ends or surfaces of spoons, spatulas, bowls, jars, lids, bottles, pipets, tubes, etc. Always grasp items as far away from the working end as practicable to allow manipulation. If any contact with a working end or surface occurs the item must be discarded and replaced immediately.

8.2.17 Do not allow objects or hands to pass over any opened items (e.g. open bowls, bottles, jars, tubes, etc).

8.2.18 Keep bottles, jars, tubes capped until ready to use. Gently lift cap, without touching the bottle, jar, or tube insert pipet and carefully draw up or dispense solution. Remove pipet without touching the bottle, jar, or tube and then cap the containers.

8.2.19 Cap should remain in the operator's hand whenever possible with the opening facing downwards so that particulates cannot fall into the cap.

8.2.20 Do not allow pipet tips to come in contact with rims of bottles, jars, or tubes. If this occurs discard and replace pipet before continuing with process. Do not rest pipet in bottle between uses, discard and use a new pipet each time. If the pipet becomes loose during operation discard and replace with a new pipet.

8.2.21 When aspirating a flask using a vacuum stand the flask on end. Tilt flask so the medium pools on the topside of the flask. Aspirate the medium using caution to not touch the sides.

8.2.22 Once all work inside the BSC is complete and operator exits the BSC gloves should be discarded when possible.

8.3 Application of aseptic technique during all procedures performed on the bench-top.

8.3.1 Prior to beginning a procedure clean bench-top with 70% IPA

8.3.2 Remove any miscellaneous equipment and materials that are not needed for the procedure.

8.3.3 Do not touch open sterile ends of ports, caps, bottles, or tubes. Do not touch the working ends or surfaces of spoons, spatulas, bowls, jars, lids, bottles, pipets, tubes, etc. Always grasp items as far away from the working end as practicable to allow manipulation. If any contact with a working end or surface occurs the item must be discarded and replaced immediately.

8.3.4 Do not allow objects or hands to pass over any opened items (i.e. open bowls, bottles, jars, tubes, etc).

8.3.5 Keep bottles, jars, tubes capped until ready to use. Gently lift cap, without touching the bottle, jar, or tube insert pipet and carefully draw up or dispense solution. Remove pipet without touching the bottle, jar, or tube and then cap the containers.

8.3.6 Cap should remain in the operator's hand whenever possible with the opening facing downwards so that particulates cannot fall into the cap.

8.3.7 Do not allow pipet tips to come in contact with rims of bottles, jars, or tubes. If this occurs discard and replace pipet before continuing with process. Do not rest pipet in bottle between uses, discard and use a new pipet each time. If the pipet becomes loose during operation discard and replace with a new pipet.

8.3.8 When aspirating a flask using a vacuum stand the flask on end. Tilt flask so the medium pools on the topside of the flask. Aspirate the medium using caution to not touch the sides.

8.4 General notes to operators performing aseptic procedures.

8.4.1 Do not handle chairs, footstools, or other items with the hands. Push or pull the item with the foot.

8.4.2 Place items into open trashcans, do not touch trashcans or push trash into the trashcans.

8.4.3 Do not pick up items off of the floor. Once an item is on the floor it should remain there until the end of the process. Be sure to push it out of the way with the foot to avoid possible tripping hazards.

9.0 DOCUMENTATION

9.1 Figure 1, BSC vertical flow diagram

Figure 1, BSC Vertical Flow Diagram