I. PURPOSE:

The purpose of this procedure is to outline the usage instructions for vesphene, a strong antimicrobial solution.

II. SAFETY:

Personnel handling this need to have eye protection, gloves and disposable gown since the use of Vesphene is primarily carried out in BSL2+ laboratories.

III. PROCEDURE:

**Processing Lab**: have in the hood 2 buckets: one for pipettes (that one will have liquid) and one for tubes (that one will collect inactivated tubes)!

**Squirt Bottle**: 4 ml Vesphene IIse + fill up with water until the textured line (500 ml)
- Add 4 ml of concentrated Vesphene IIse to 500 ml water and initial/date the squirt bottle every 7 days or as needed.

**Aspirating Flasks**: 5 full pumps (15ml) of Vesphene IIse
- Add fresh concentrated Vesphene IIse and initial/date the flask every day, or as needed.
- Do not allow the flask volume to exceed 1800 ml (capacity = 2000 ml).

**Hood Buckets**: Two liners + 4 ml Vesphene IIse in 500 ml water (content of a Squirt Bottle)
- You can also use 1L bottle to have diluted Vesphene under the hood for frequent use (8ml + 1000ml)
- Decontaminate eppendorf and cryo-tubes/tips/serologicals with diluted Vesphene IIse and discard in hood bucket.
- At the end of the day, aspirate out the liquid, remove serological pipettes to coffins, tie up the liner, and transfer the waste from the hood bucket into the container outside the hood that collect the trash to be autoclaved.
- Change the liner and initial/date on the waste container every 7 days or as needed.

**10 ml Vacutainers**:
- After collecting the blood from the vacutainer, fill it with diluted Vesphene IIse from the Squirt bottle, let it stand for 20 minutes and aspirate off the liquid.
- Discharge them into the basket for the tubes in the hood.

**15mL or 50mL Falcon tubes with remaining RBCs following Ficoll**:
- Add diluted Vesphene IIse from squirt bottle to volume of 30ml.
- Plug with gauze and put in single liner hood bucket.
At end of day, or when it is full, close with tape and discard the bag in the large waste basket to be autoclaved.

IV. REFERENCES/ ADDITIONS/ NOTES:

VESPHENE IIse is a strong antibacterial, antimicrobial reagent

**Proper dilution 1:128**

Add 4 ml of concentrated Vesphene IIse reagent to 500 ml of water.

One pump=3 ml of Vesphene IIse – MAKE SURE THAT you use 3ml Pump or based dilution on volume