VERO-E6 CELLS (BSL-2/ML-II)

1. Transfer the Flask with cells from the incubator into the BSC.
2. In the BSC the cells are trypsinized with 2 ml trypsin.
3. Transport the cells to the incubator and incubate for 5 minutes at 37°C with 5% CO_2.
4. Transfer the Flask with trypsinized cells from the incubator into the BSC.
5. Resuspend the cells with 8 ml cell culture medium.
6. Transfer the suspended cells into a 15 ml tube.
7. Centrifuge the tube with cells for 4 minutes at 1200 rpm.
8. After centrifugation remove the medium.
9. Add 10 ml cell culture medium into the 15 ml tube with cells.
10. Resuspend the cells.
11. Count the cell to $1.5x10^5$ cells/ml

Dit kan je doen 1 voor dat je de cellen nodig hebt. je zou virus verdunning en cellen ook te gelijk kunnen toevoegen dan moet de cel concentratie 2.0x105 cells/ml zijn.

INOCULATION OF SARS-CoV-2 ON VERO-E6 CELLS

1. Transfer the prepared cells from the incubator into the BSC.
2. In the BSC the cells are infected with clinical specimen or virus stock by pipetting the specimen or virus stock into the flask or plate.
3. Transport the cells to the incubator and incubate for 48 hours at 37°C with 5% CO_2.
4. Every 48 hours transport the cells to the inverted microscope and apply check for Cytological Pathological Effect (CPE).
5. When the CPE is 4 stop the virus culture by aliquoting the virus culture into 2 ml microtubes.
6. Wipe down the microtubes with disinfectant.
7. Transport the aliquots to the -80°C freezer.

SAMPLES FOR NUCLEIC ACID EXTRACTION

1. Transfer from the freezer the samples that will be nucleic acid extracted into the BSC.
2. Transfer the filled out microtubes with lysisbuffer into the BSC.
3. Vortex the sample.
4. Pipet 200 ul sample into the filled out lysisbuffer.
5. Vortex the microtube with lysisbuffer and sample.
6. Incubate for 20 minutes at room temperature.
7. Vortex every 5 minutes the microtube with lysisbuffer and sample.
8. Vortex the microtube with lysisbuffer and sample.

The Virus is now inactivated and can be transported out of high containment.