Protocol for virus isolation

Key point:

1. Sample should be fresh, less freeze and thaw is better.

2. Cell lines used for virus isolation should be maintained at good state.

Materials:

1. Cell line: VeroE6, Huh7

2. DMEM medium, Fetal bovine serum (FBS), Anti-anti (Gbico,REF15240-062)

3. Filter(0.45 μm), cell culture plate (24 wells)

Seeding of plates

1. The day prior to isolations seed 24 wells plate as follows

VeroE6 $1X10^5$ cells per well

Huh7 $1.5X10^5$ cells per well

Isolation Procedure

(1) Sample: Oral swab or bronchoalveolar lavage fluid,

(2) Liquid samples were filtered with filter (0.45 μm),

(3) Remove the medium of the cell plates,

(4) Add 200 μL filtered samples each well, each sample inoculate two wells,

(5) Incubate at 37 ˚C for 1 hour,

(6) Add DMEM medium and add FBS with 2% final concentration,

(7) Also add anti-anti to a final concentration of 2 times,

(8) Incubate at 37 ˚C, check the plate everyday.

Result

1. With cytopathic effect, identify the virus.

2. Without cytopathic effect, infect next generation as first round.