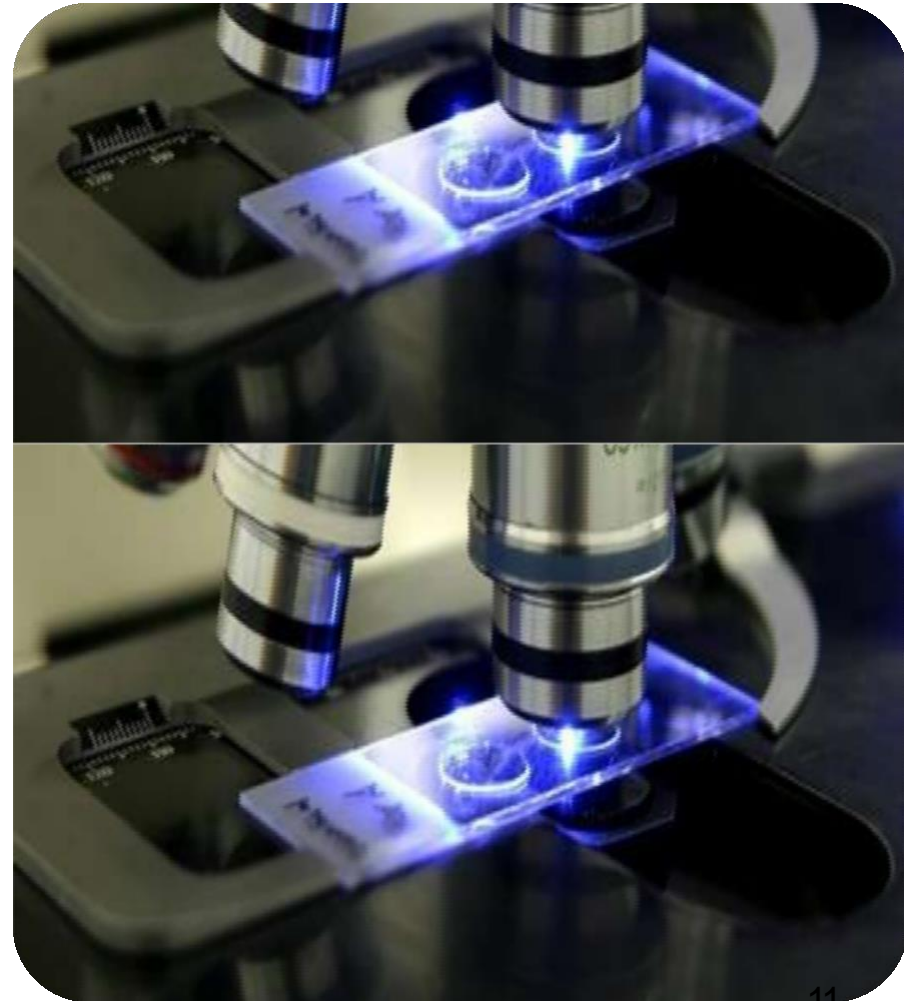


SARS-CoV 2 Virus Culture

Compiled by Dwi Wahyu Indriati

Diagnostic Methods in Virology

1. Direct Examination
2. Indirect Examination (Virus Isolation)
3. Serology



Why we need to culture this virus??

- Antiviral Research
- Vaccine development research
- Pathogenesis research (include virus transmission, severity of illness , virus titer, what organ the virus can spread)
- Virus stability research

CDC, 2020 (<https://www.cdc.gov/coronavirus/2019-ncov/lab/grows-virus-cell-culture.html>)

Virus Isolation

- SARS-CoV-2 was first isolated from bronchoalveolar lavage from a patient with pneumonia in Vero E6 and Huh7 cells
- The viral identity was confirmed by immunofluorescence using the now-known cross-reactive anti-SARS-CoV-2 N antibody, RT-qPCR and metagenomics sequencing
- The infectivity of the isolated virus was confirmed by virus neutralization assay using sera from convalescing patients
- Virus isolation is not recommended as a routine diagnostic procedure because it has low sensitivity, time consuming and require BSL-3 containment

Virus Isolation

- SARS-CoV-2 is also culturable in several cell lines such as human airway epithelial, vero E6, Vero CCL-81 and Huh-7 cells
- Vero E6 cells express high levels of angiotensin-converting enzyme 2 (ACE2) which has been identified as a key cell receptor for SARS-CoV-2 infection
- Vero E6 also expresses TMPRSS2 (transmembrane serine protease 2) levels that are 10-fold higher than in human normal lung tissue. It showed that TMPRSS2 protease is important in SARS-CoV-2 infection

Virus Isolation

- Harcourt and co-workers showed that SARS-CoV-2 produced distinct plaques in Vero E6 cells, but plaques in Vero CCL-81 were not as clear thus suggesting that the Vero E6 cells might be the best choice for the propagation, quantification and study of plaque phenotypes of different SARS-CoV-2
- 90 SARS-CoV2 patient samples were cultured with Vero cells (CCL-81) during 4 days and then the cytopathic effect was evaluated. Of these 26 samples demonstrated viral growth but there was no growth in samples with a Ct value >24 . It suggests that samples with low Ct values will be successful for viral isolation
- Huh-7 and HEK-293T cells showed only modest viral replication whereas no virus replication was detected in either A549 or EFK3B cells

Virus Isolation

- Other cell line which permissive to SARS-CoV-2
 1. BGM cell: kidney cell
 2. Vero/hSLAM: kidney cell
 3. MA104: kidney cell
 4. Vero 81: kidney cell
 5. Vero E6: kidney cell
 6. LLC-MK2: kidney cell
 7. Caco-2 : cells from colorectal adenocarcinoma

Wurtz et al 2021. Culture of SARS-CoV-2 in a panel of laboratory cell lines, permissivity and differences in growth profile. *Eur J. of Clin Microbiol and Infectious Disease*.

Virus Isolation

- Other cell line which permissive to SARS-CoV-2
 1. BGM cell : MEM + 10% FBS in 37 °C 5% CO₂
 2. VERO/hSLAM : MEM + 5% FBS in 37 °C 5% CO₂
 3. MA104: MEM + 10% FBS in 37 °C 5% CO₂
 4. VERO 81: MEM + 4% FBS in 37 °C 5% CO₂
 5. VERO E6: MEM + 10% FBS in 37 °C 5% CO₂
 6. LLC-MK2 : M199 +1% FBS in 37 °C 5% CO₂
 7. Caco-2 : DMEM + 10% FBS +1% AA in 37 °C 5% CO₂

Virus Isolation

- Other cell line which permissive to SARS-CoV-2
 1. BGM cell : Morphology: epithelial-like, cell culture properties: adherent
 2. Vero/hSLAM :Morphology: fibroblastic, cell culture properties: adherent
 3. MA104 : Morphology: epithelial, cell culture properties: adherent
 4. Vero 81: Morphology: epithelial, cell culture properties: adherent
 5. Vero E6 : Morphology: epithelial, cell culture properties: adherent
 6. LLC-MK2 : Morphology: epithelial, cell culture properties: adherent
 7. Caco-2 : Morphology: epithelial, cell culture properties: adherent

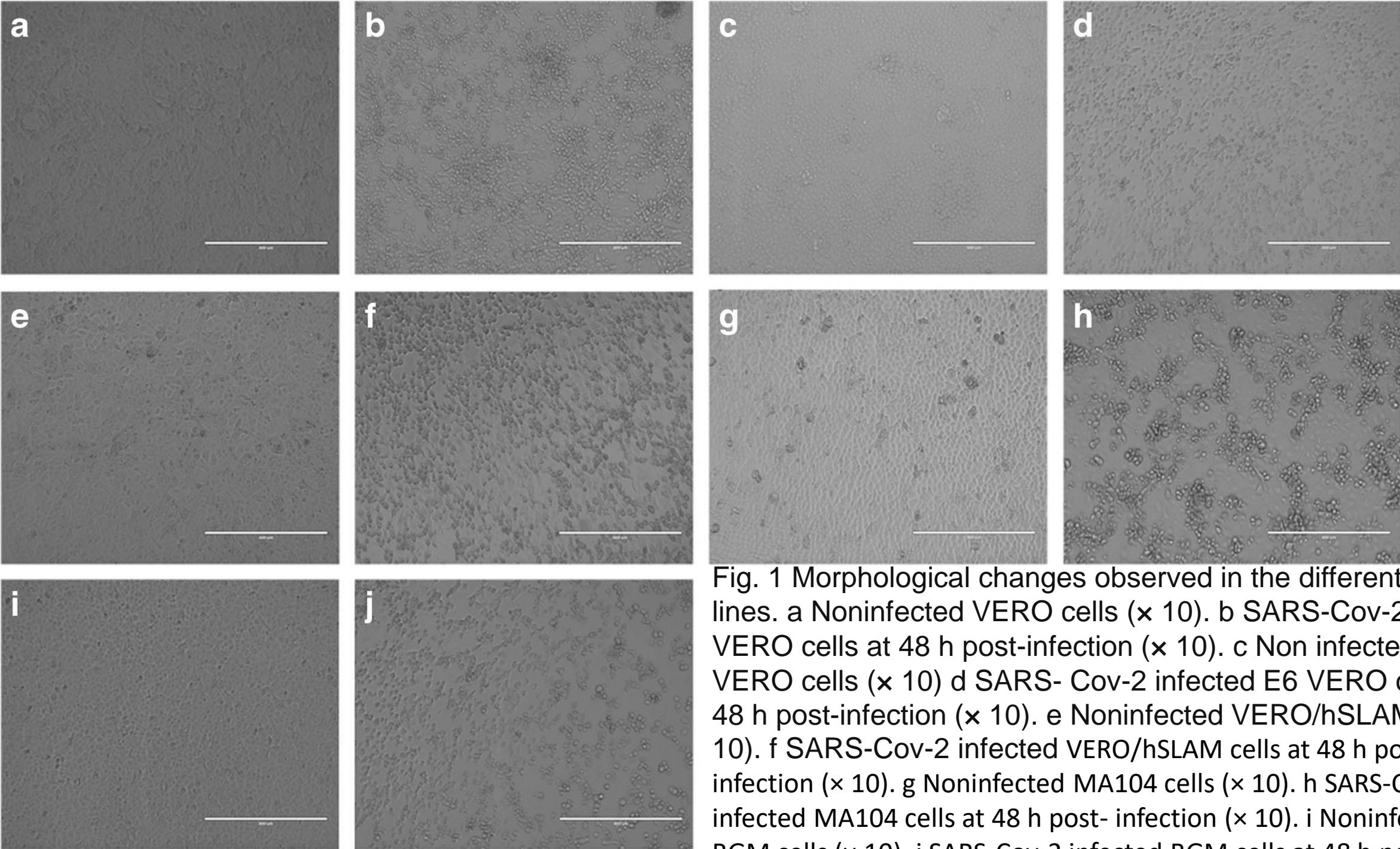


Fig. 1 Morphological changes observed in the different cell lines. a Noninfected VERO cells ($\times 10$). b SARS-Cov-2 infected VERO cells at 48 h post-infection ($\times 10$). c Non infected E6 VERO cells ($\times 10$) d SARS- Cov-2 infected E6 VERO cells at 48 h post-infection ($\times 10$). e Noninfected VERO/hSLAM cells ($\times 10$). f SARS-Cov-2 infected VERO/hSLAM cells at 48 h post-infection ($\times 10$). g Noninfected MA104 cells ($\times 10$). h SARS-Cov-2 infected MA104 cells at 48 h post- infection ($\times 10$). i Noninfected BGM cells ($\times 10$). j SARS-Cov-2 infected BGM cells at 48 h post-

