SARS-CoV-2 – Remarks for Diagnostic EM

Authors: Michael Laue, Lars Möller (Robert Koch Institute, Berlin, Germany)

Inactivation

We use our validated inactivation protocol (2% PFA in Hepes; Möller et al. 2015 https://doi.org/10.3390/v7020666) for inactivation of SARS-CoV-2 cell culture supernatant. Tests of the inactivation efficiency using cell cultures showed full efficiency, even at high titres. However, number of experimental replications still is low.

Negative Staining

Our standard procedure (doi 10.5281/zenodo.1468676) resulted in sufficient visualization of the virus morphology using cell culture supernatant. PTA staining gave a better structural representation than UA staining (Fig. 1). Airfuge enrichment was possible, but usually not necessary with cell culture supernatants. Inspection of patient samples (suspensions recovered from swabs with and without transport medium) were not successful so far, even from samples which showed low CT-values (high RNA copy number) in real-time PCR. The reason is unclear so far and under investigation. Direct application of transport medium or PBS extracts of swabs on grids gave insufficient staining quality/visibility due to precipitation or aggregate formation (even in the PBS extracts). Dilution of the sample suspension by a factor of 5 with HEPES buffer followed by airfuge concentration directly onto the grid, resulted in a good staining quality which allowed detection of bacteria but no doubtless detection of SARS-CoV-2 particles. In swab samples, which were spiked with fixed virus from cell culture, detection of virus particles was immediately possible.

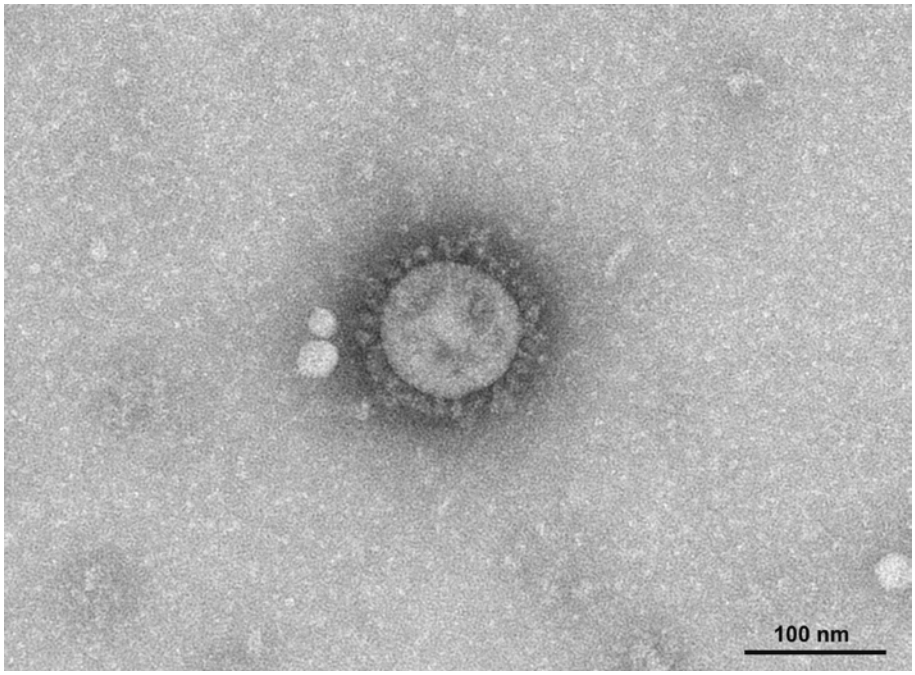

Figure 1 SARS-CoV-2 (isolate SARS-CoV-2/Italy-INMI1) from Vero E6 cell culture. Negative staining, 1% PTA. Image: Tobias Hoffman, Robert Koch Institute.

The distribution of particle size in PTA stained samples is shown in Figure 2. Median maximal particle size (including peplomers) is 121 nm (Min = 77.3; Max = 153; N = 100). Size without peplomers is 90.2 nm (Min = 52.8; Max = 112; N = 100)

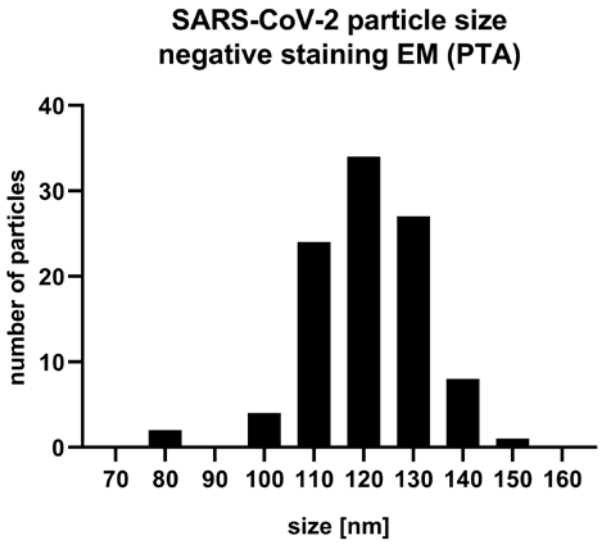

Figure 2 SARS-CoV-2 Particles size distribution

Ultrathin sections

Detection of SARS-CoV-2 viruses in Vero E6 cell culture was possible by using a standard protocol for fixation (2.5% glutaraldehyde in Hepes buffer for at least 2 h, RT) and resin embedding (post-fixation in osmium tetroxide, tannic acid, uranyl acetate; epon embedding) (Laue 2010 Methods Cell Biology 96:1). Median of maximal particle size including peplomers is 128 nm (Min = 111; Max = 167; N = 52), and 95 nm (Min = 83; Max = 135; N = 54) without peplomers. Investigation of structural details of entry, replication, assembly or release events are under way using different cells and tissue.

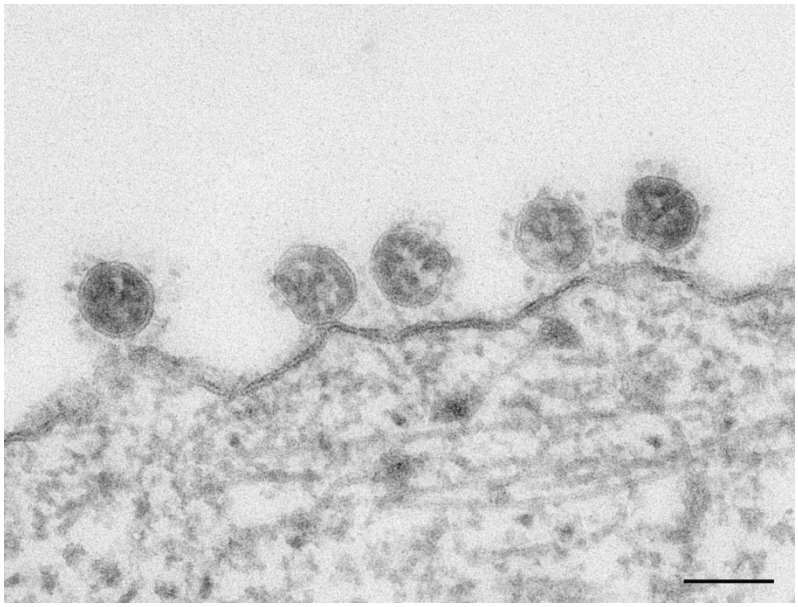

Figure 4 SARS-CoV-2 particles at the surface of a Vero E6 cell. Bar = 100 nm. Image: Tobias Hoffman, Robert Koch Institute.