

TRANSMISSION ELECTRON MICROSCOPY OF THE SARS CORONAVIRUS

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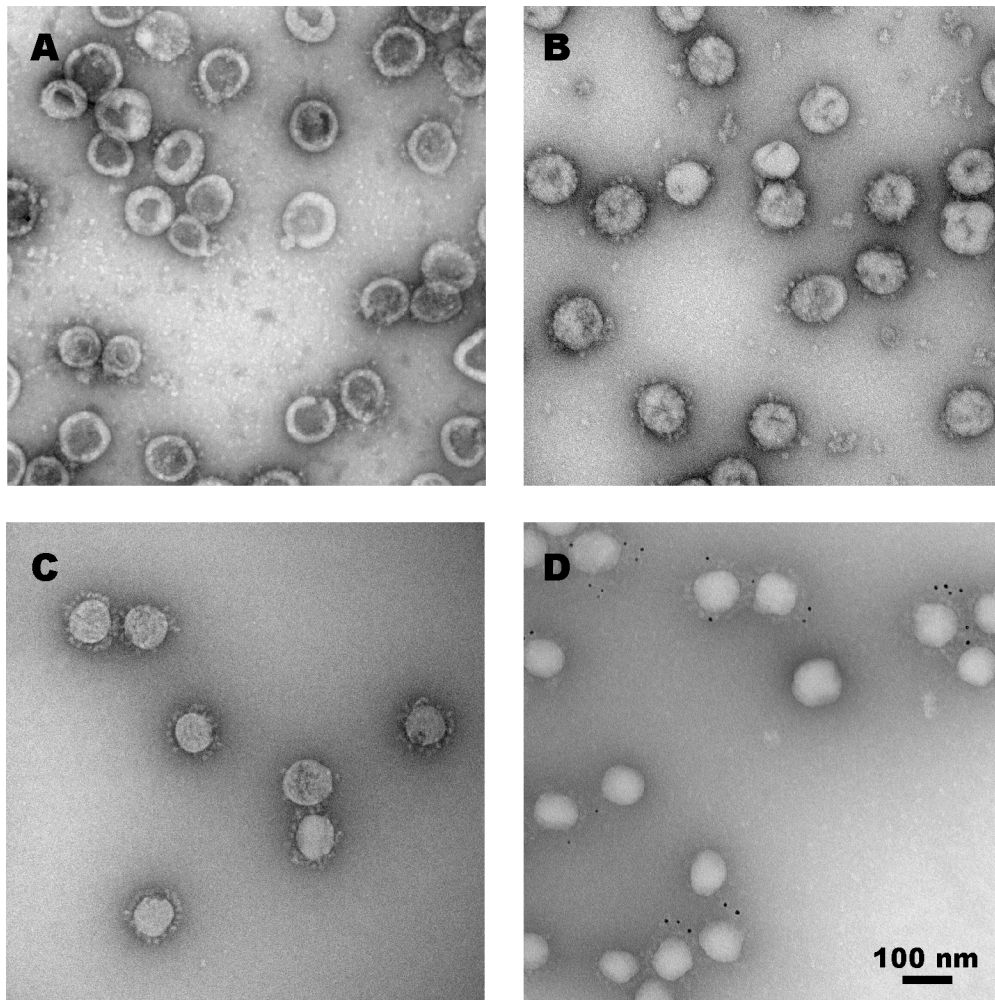
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The recent outbreak of atypical pneumonia referred to as severe acute respiratory syndrome (SARS) affected several countries during the 2002-2003 winter flu season. The causative agent was named publicly by the world health organization as the “SARS coronavirus” on 16 April 2003. The virus was sequenced by May 2003 by two independent groups [1,2], followed by others. Both studies identified that the “SARS agent” was a unique coronavirus. The coronavirus genus is broken up into three serologically distinct groups. The SARS coronavirus sequence indicated that this coronavirus was in a new genus phylogenetically distinct from the other three groups. The SARS coronavirus RNA genome encodes several structural proteins including; the spike glycoprotein (S), membrane glycoprotein (M), small envelope protein (E), and the nucleocapsid phosphoprotein (N). Recent investigations have indicated the virus has four structural proteins, sixteen non-structural proteins, and eight accessory proteins [3]. One of the challenges that face a diagnostic virology laboratory is a balance between rapid detection, structural preservation, and the appropriate fixation or inactivation for BSL2 and BSL3 pathogens. Particularly challenging are enveloped viruses that are sensitive to both mechanical and chemical conditions used in their purification. In this abstract we present our preliminary findings that we have obtained on the structure of SARS coronavirus.

We have grown the SARS coronavirus (Tor3 isolate) in Vero E6 cells in BSL3 containment. The virus was subsequently purified by iodoxanol gradient centrifugation. Western blotting positively identified the S and N proteins in the purified fractions. These fractions were then dialysed to remove the iodoxinol, and the virus was inactivated by gamma-irradiation. We then imaged the SARS coronavirus in a FEI Tecnai 20 TEM at 200 kV. In this investigation we stained the virus with uranyl acetate (A,B), methylamine tungstate (C), and methylamine vanadate (D). In all experiments the grids were treated with glow discharge prior to virus adsorption and negative staining. The SARS coronavirus is spherical, with an average envelope diameter of ~90nm. The spikes on the virus extend ~15 nm from periphery of envelope, and there is a densely staining core in the virus interior. We have found using a combination of stains and immuno-labelling gave us the best overview of the structure of the SARS coronavirus.

References

1. Marra M.A., *et al.*, Science. 300, 1399-1404, (2003).
2. Rota P.A., *et al.*, Science. 300, 1394-1399, (2003).
3. Bartlam, M. *et al.*, Curr. Opin. Struct. Biol. 15, 664-672, (2005).



Images of the SARS coronavirus: (A) Unfixed SARS coronavirus has been stained with uranyl acetate, in these images a prominent dense core was evident in the viruses. In the remainder of the images (B-D) the virus was fixed prior to staining with 1% paraformaldehyde and 0.025% glutaraldehyde. (B) When fixed and stained with uranyl acetate the dense core seen in (A) is not as visible. Spikes were visible on the surface for both uranyl acetate stained specimens (A,B). (C) Staining with methylamine tungstate resulted in the best contrast for the viral spikes. (D) The virus was immuno-labelled with a monoclonal antibody for the spike protein, with a 10nm gold label on the secondary antibody. Methylamine vanadate was the best counter stain for the virus, in this image the gold particles are clearly visible and can be interpreted in the context of the viral particle.