Detection limit in diagnostic electron microscopy of model suspensions

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Diagnostic electron microscopy (EM) is a frontline method for the detection of possible pathogens in clinical and environmental samples by using typical morphological features [1]. It allows a more or less unbiased view on the pathogen without the need for specific probes like antibodies, nucleic acids or information about the type-specific composition of the pathogen. To assess the detection limit of the two main techniques in diagnostic EM, i.e. negative staining and ultrathin sectioning, we have conducted several experiments using defined suspensions of bacterial endospores of *Bacillus subtilis* and Vaccinia virus as a model.

For negative staining we have used alcian blue treated grids and an adsorption time of 10 minutes. Application of the suspension directly onto the grid gave higher particle numbers than placing the grid on a droplet of the suspension. Detection of spores and Vaccinia virus using a systematic screen of 22 grid meshes was possible down to 10^6 particles per ml, i.e. 10^4 particles in the applied volume of 10 µl (Figure 1). Airfuge concentration of particles directly onto the grid, by using particular grid adaptors, decreased the detection limit to 10^5 particles per ml for spores while the limit for Vaccinia virus suspension dropped between 10^5 and 10^4 particles per ml.

Rapid diagnostic thin section electron microscopy of suspensions needs a particular preparation to prevent particles from dilution or loss during the different preparation steps. We have applied airfuge centrifugation for concentration and three different approaches for immobilization of particles in conjunction with a particular rapid embedding protocol using LR White as a resin [2]: (A) re-suspension of the pellet with 10 µl of low-melting point agarose; (B) centrifugation into a gel cushion of low melting point agarose; (C) *in situ* embedding of the pellet in LR White polymerized during centrifugation (Figure 2). To facilitate re-localization of pellets we have mixed 100 µl of the suspension with 10 µl of cationic gold particles. The detection limit of the different methods was determined by sectioning of the blocs and evaluation, whether the first plane of section contains relevant particles or not. With method (A) and (B) particles could be found in first sections of every bloc at a concentration of 10^7 particles per ml with generally more particles in a section with method (B) than with method (A). However, with method (C), *in situ* embedding of the pellet by polymerization of the LR White during airfuge centrifugation, the detection limit could be reduced to 10^6 particles per ml, for both model suspensions.

1. Hazelton & Gelderblom, Emerg. Infec. Dis. **9** (2003) p294.
2. Laue et al., J. Microbiol. Meth. **70/1** (2007) p.45.
3. We would like to acknowledge the valuable technical assistance of Silvia Muschter.

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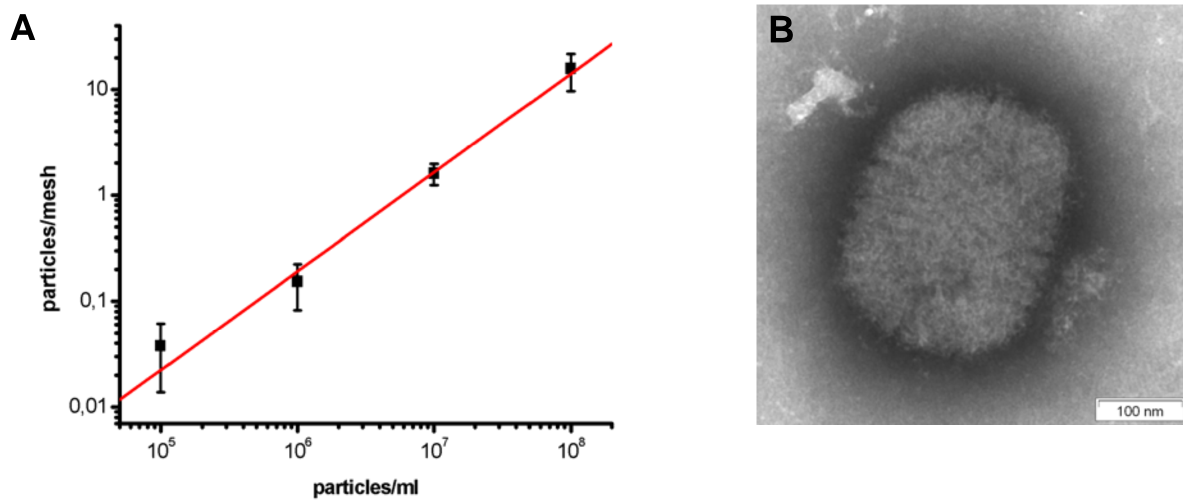

Figure 1. A Particle number per grid mesh plotted against concentration of virus particles in experiments where 10 µl of suspension were applied directly onto the grid. Detection likelihood of virus particles is 100% down to a concentration of 10^6 particles per ml, i.e. about 3-4 particles in 22 meshes observed. **B** Vaccinia virus particle stained with 1% phosphotungstic acid (PTA).

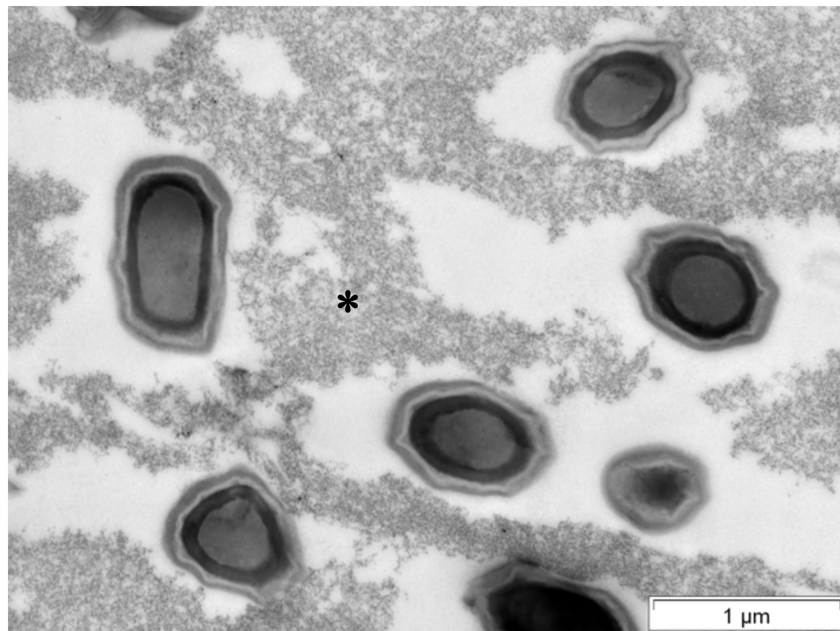

Figure 2. Section through spores of *Bacillus subtilis* after airfuge centrifugation and *in situ* embedding of the pellet in LR White. The spores are embedded into gold colloids (*) which were added to the suspensions to stain the pellet for a better visibility.

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