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Quality assurance and training programs for diagnostic electron microscopy of viruses

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Introduction

Electron microscopy of infectious diseases

Electron microscopy is a front-line method in the detection of emerging infectious diseases, like, for instance, SARS (Fig.1A) and in other cases where a rapid risk assessment is necessary [1; 2]. Moreover, it serves as an independent control for other more specific but usually more selective diagnostic methods. Negative staining electron microscopy is the routine method for a fast diagnosis of viruses within a couple of minutes [3]. Compact samples, that cannot be easily transferred into a suspension, can be processed for rapid thin section electron microscopy within a few hours [3]. By this approach even difficult to release intracellular viruses or masked viruses become accessible for a diagnosis. To prepare the health system for biological threats, like an outbreak or a bioterrorist attack, capabilities to perform diagnostic electron microscopy of viruses must be conserved and developed.

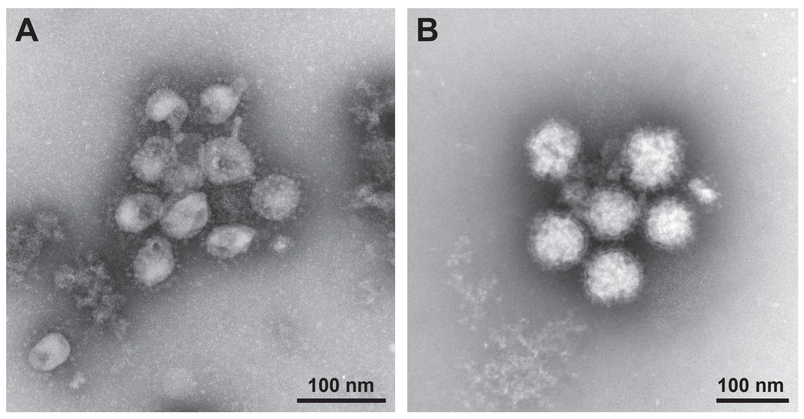

Fig.1 **A**: SARS-*coronavirus*. Isolate from a patient hospitalized in Frankfurt (2003). Negative staining (Andrea Männel, Hans Gelderblom). **B**: *Schmallenberg virus* (SBV). A recently described *orthobunyavirus* which infects cattle, sheep and goat, from an outbreak isolate (kindly provided by the Friedrich-Löffler-Institute). Negative staining with 1% phosphotungstic acid (Lars Möller).

Quality needs practice

Diagnostic electron microscopy of viruses relies on a diagnostic reference which is based on experience of the investigator. This fact implies that training is a major aspect of learning and preserving diagnostic capabilities. The importance of training is especially highlighted because most investigators are only rarely challenged by diagnostic cases(e.g. only in an emergency situation). As a consequence the Robert Koch Institute provides different programs for supporting basic and advanced training in diagnostic electron microscopy of viruses. The programs support beginners in diagnostic electron microscopy of viruses (Basic lab course) and allow experts to exchange knowledge and ideas (Workshop on electron microscopy in infectious diseases). The External Quality Assurance Program (EQA) delivers coded inactivated virus samples once a year for practicing.

External Quality Assurance Program (EQA)

Aim and scope of the EQA

The EQA provides six coded samples of inactivated virus suspensions once a year. Participation requires a registration, but is free of charge. Results should be returned within some weeks and participants receive a decode letter stating their individual result. The EQA is not a classical proficiency testing and no competition. However, it can be used to prove the quality of the diagnostic performance, e.g. to support accreditation. The idea is to provide training material which can be used by the participants to practice and to check their diagnostic capabilities. Samples cover viruses from emerging infectious diseases, possible zoonosis (Fig.1) and rather common viruses. A typical EQA panel is shown in Figure 2. EQA 24 was shipped to 103 participants in 29 countries and accomplished by 82 participants (Fig.3). Continuous participation improves diagnostic capability, which is supported by our data: 56 of 71 examined participants showed improvement of diagnostic performance in course of their first 5 participations at the EQA. Besides improving quality of diagnosis the EQA helps us to establish a worldwide network among colleagues which are interested in diagnostic electron microscopy of viruses.

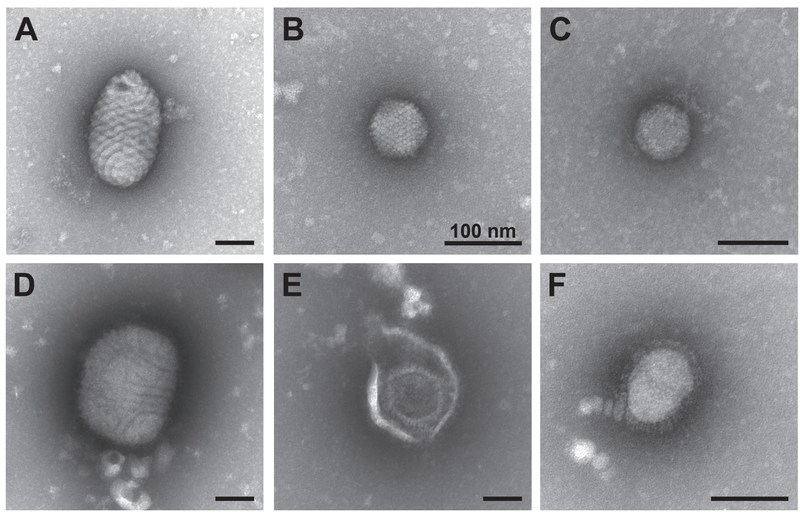

Fig.2 Representative electron micrographs from the six samples of the EQA 23. Some may be negative at all or contain double infections. **A:** *Pseudocowpox virus* (sufficient diagnosis *parapoxvirus*). **B:** *Human adenovirus* A (*adenovirus*). **C:** *Avian orthoreovirus* (*Reovirus*, other than *rotavirus*). **D:** *Vaccinia virus* (orthopox-like virus, not *parapoxvirus*). **E:** *Felid herpesvirus* 1 (*herpesvirus*). **F:** *Influenza* A *virus* (*orthomyxovirus*). all bars = 100 nm

Local distribution of participants at EQA 24

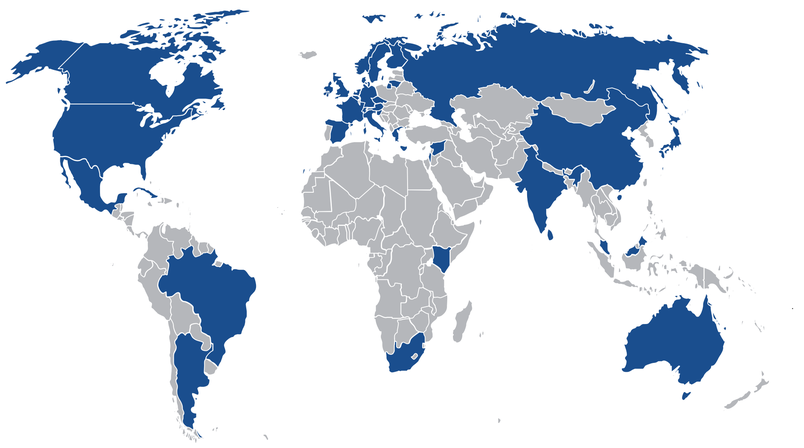

Fig.3 Participants of the EQA are localized in different countries (blue) all over the world.

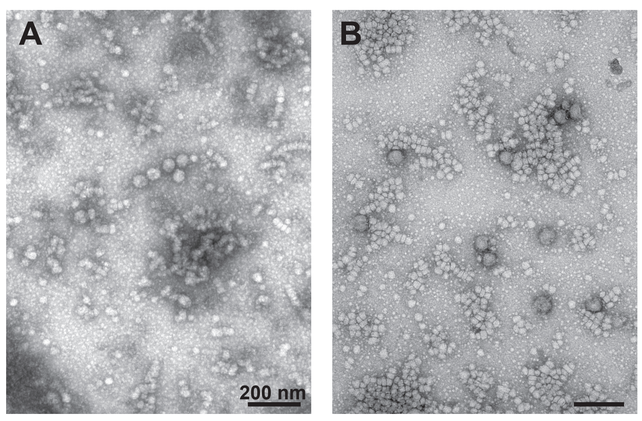

Fig.4 A very »dirty« sample prepared by using two different negative stainings. The challenge was to detect *Tick-borne encephalitis virus*, a *flavivirus*. **A**: Uranyl acetate (UAc) also positively stains the content which leads to a masking of the wanted structures and highlighting of others (e.g. lipoprotein). The picture is much more confusing than with the phosphotungstic acid staining, shown in **B**, where negative staining dominates. Nevertheless UAc is the best choice for high resolution work.

Preparation has an impact on detection

The quality of sample preparation may have influence on the detection of viruses. This is one particular aspect which the EQA addresses. Virus titer of a sample can be close to the detection limit [4]. In such a case, adsorption procedures and staining quality, as well as the proper use of enrichment techniques, are of importance. Staining parameters are also important, especially if small (Fig.4) or unusually big viruses (Fig.5) are a matter of a diagnosis. The EQA urges to cover all relevant aspects of the preparation and problems which could impair diagnosis, e.g. cell debris (Fig.4), multiple infections or even miscalibration of the microscope.

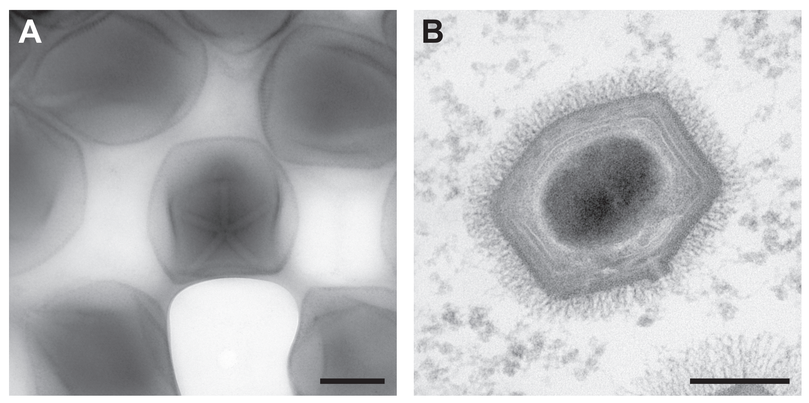

Fig.5 *Acanthamoeba polyphaga mimivirus*. **A:** Negative staining with 0.2% phosphotungstic acid. The standard staining solutions (concentration between 1% and 2%) cover typical strucutures of large viruses, e.g. the star structure of the *mimiviridae*. To reveal morphological details of large viruses, it is necessary to reduce the concentration of the staining solution. **B:** Thin sectioning EM reveals full detail of large viruses, but needs more preparation time than negativ staining EM. bars = 200 nm.

Training Program

Courses and workshops

The training program addresses different aspects of diagnostic electron microscopy of viruses. The basic lab course provides negative-staining electron microscopy of viruses from the scratch, starting with the preparation of grids, proper staining to immuno-negative staining. Advanced courses should extend the capabilities to more laborious methods, like, for instance, to the processing of compact samples by (rapid) ultrathin sectioning. Apart from practical courses, the Glienicke workshop gathers diagnostic investigators and researchers to exchange knowledge and experiences as well as aspects of basic and applied research in electron microscopy of infectious diseases. The meeting allows networking which is of particular importance in a field where experience is fading because of the availability of other techniques for routine diagnostics.

Overview about courses and workshops

- Basic lab course on diagnostic EM in infectious diseases (next course will be in spring 2013)
- Basic lab course on biomedical TEM (September 24–26th 2012)
- Advanced lab courses for members of the Global Health Security Action Group laboratory network (GHSAG-LN)
- Glienicke workshop on EM in infectious diseases-diagnostics and research (October 11–12th 2012, Berlin)

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Summary and Outlook

Diagnostic electron microscopy of viruses is still a timely and valuable approach in special cases. It is thus necessary to keep the standards by continuous practicing and networking. The different programs conducted by the Robert Koch Institute (supported by the German Society for Electron Microscopy, DGE) helps the community to remain prepared.

Literature

[1] Hazelton & Gelderblom, Emerg. Inf. Dis. 9 (2003): 294
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[3] Laue, Meth. Cell. Biol. (2010) 96:1.
[4] Laue & Bannert, J. Appl. Microbiol. 109 (2010): 1159

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