Tissues, Pathology, and Diagnostic Microscopy

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Diagnostic electron microscopy of pathogens in emergency situations

M. Laue[1]

[1]Robert Koch Institute, ZBS 4, Berlin, Germany

lauem@rki.de
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Electron microscopy serves as an important method for the diagnosis of pathogens in emergency situations that pose a considerable risk to the public health, like outbreaks of dangerous infectious diseases or cases of bioterrorism [1]. The role of electron microscopy in those situations is to provide rapid information about the pathogens involved, especially in cases where no indication about the pathogen exists. Diagnostic electron microscopy is a generic „catch all“ method which visualizes all objects of a diagnostic sample and guides a further, more specific diagnosis by other methods. In addition, it serves as a valuable independent control to minimize the risk of false diagnoses.

Negative staining electron microscopy is the method of choice for a rapid diagnosis in emergency situations, because preparation is simple and quick and allows the assessment of a few samples within a short time, usually well below one hour [2]. If compact or dense samples must be analysed, rapid thin section techniques can be employed. Efficient protocols provide a diagnosis within a few hours (e.g. 2 h [3]).

Diagnostic features of many pathogens are well documented and serve as a reference which allows to assign pathogens to a certain systematic group [4]. This usually works even if the pathogen has been modified by mutation because structural features are usually rather conservative. However, specificity of the diagnosis is comparatively low and depends on the group investigated. While viruses can be identified down to the family or rarely genus level, discrimination of bacteria is only possible at more general levels. In some groups of parasites (e.g. Microsporidia) the number of possible infectious species in humans is rather low and structural features allow a diagnosis down to the species level. At any case, electron microscopy provides valuable information about the presence of microbiological objects and their morphological features which supports obtaining a final diagnosis.

The contribution will give an overview about methods (including data on detection limit and inactivation procedures), applications (bioterrorism, outbreaks, zoonosis; compare Figure 1) and new developments (combination with spectroscopy; compare Fig. 2) in diagnostic electron microscopy of pathogens.

1. P. R. Hazelton and H.R. Gelderblom, Emerging Infectious Diseases 9 (2003), p.294.
2. M. Laue, Methods in Cell Biology 96 (2010), p.1.
3. M. Laue, B. Niederwöhrmeier, N. Bannert, Journal of Microbiological Methods 70 (2007), p.45.
4. A. Curry, in „Diagnostic electron microscopy: A practical guide to interpretation and technique“, eds. J.W. Stirling, A. Curry and B. Eyden, (Wiley, Chichester) (2013), p.181.
5. M. Laue and G. Fulda, Journal of Microbiological Methods *(in press)*

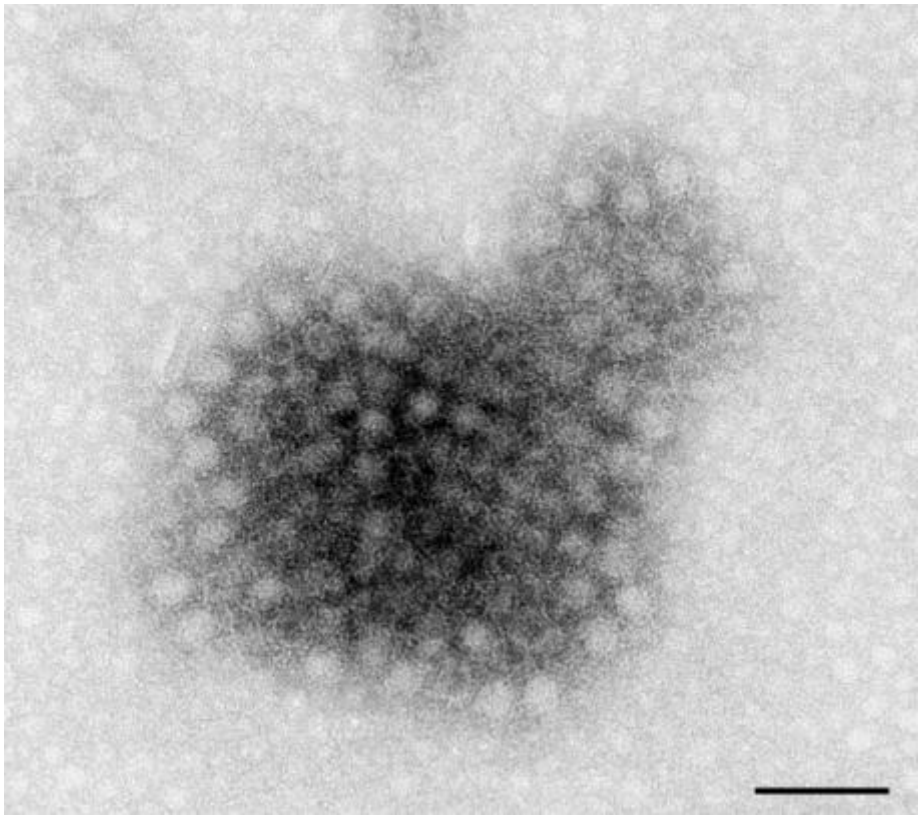

Figure 1. A cluster of Norovirus particles from a stool sample that was analysed during the large outbreak (over 11.000 cases) of gastroenteritis in Germany, 2012. Diagnostic electron microscopy was used to check samples for other viruses, because only a fraction of samples were PCR-positive for Norovirus. Bar = 100 nm.

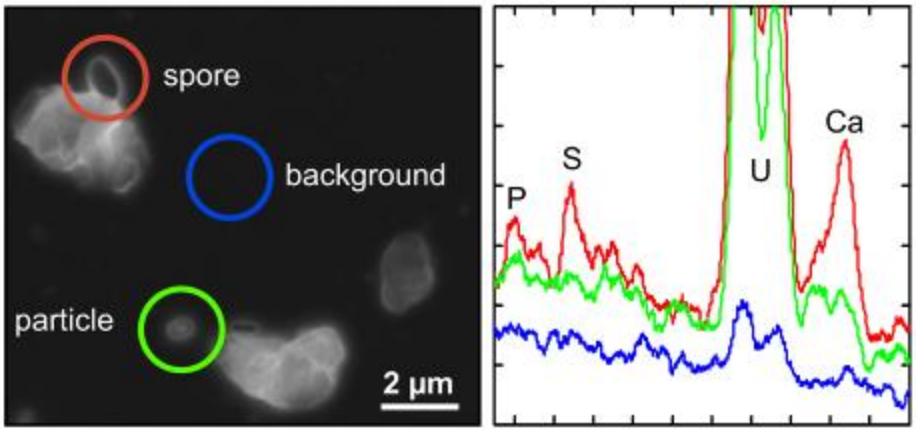

Figure 2. Diagnosis of putative bioterrorist samples for bacterial spores by scanning electron microscopy combined with X-ray microanalysis. Analysis of a control sample, which has been spiked with spores of *Bacillus subtilis* as a positive control, is shown. Left Suspicious particles are identified based on morphological criteria. Right Spectra from X-ray microanalysis (counts versus energy are plotted) allow to decide clearly between spores (red circle/graph) and other constituents, like the particle highlighted with the green circle, because spores reveal a typical element signature comprising of phosphorus (P), sulphur (S) and a prominent amount of calcium (Ca) [5]. The uranium (U) peak derives from the contrasting of the sample with uranyl acetate. Measurement of the background (blue circle/graph) gives information on dissolved material that has spread over the entire sample.