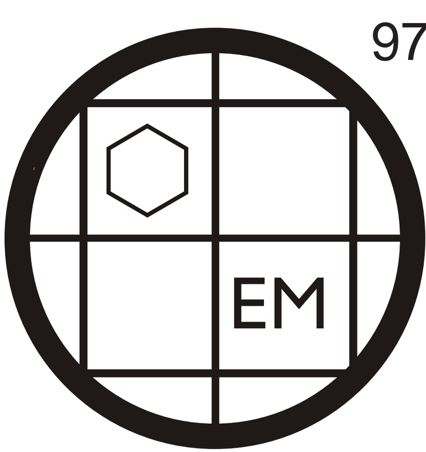
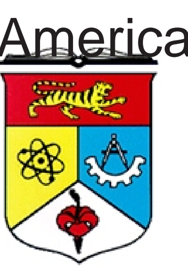
970 DOI: 10.1017/S143192760550494X Microsc Microanal 11(Suppl 2), 2005 Copyright 2005 Microscopy Society of America





Consultant Lab for Diagnostic EM in Infectious Diseases
Diagnostic EM in Infectious Diseases: Update 2004

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Role of EM in infectious diseases lab diagnostics

The emergence in 1997 of Coxsackie virus, 2001 of Nipah virus in Malaysia and the emergence in 2003 and 2004 of severe infections, e.g. human monkeypox, H5N1-avian influenza, Ebola and SARS, underlines the need for rapid and safe lab diagnostics. Likewise, preparedness for BT (6 -13) and the needs in medicine and vet medicine (1-5) require efficient lab diagnostic tools.

Compared to other methods, EM by its "open view" does not need special reagents or programs, is capable to reveal all agents contained in the sample, also smallest viruses, and visualizes even agents that were not considered beforehand. The morphological differential diagnosis (DD) between different agents (Fig. 1) is often sufficient. Diagnostic EM should not be restricted to viral agents alone, as was learned during the anthrax attacks in 2001.

Indications for diagnostic EM

As routine diagnostics is performed mainly by highly specific, sensitiv high through-put systems, EM can be restricted to solve specific and urgent diagnostic questions. EM should be used "front-line", in parallel with other methods. Samples are taken directly from the patient (Figs. 1, 3) as vesicle fluids, urine, stool etc., or after culturing (Fig. 5) or as "environmental sample", e.g. "dust" collected from a BT - suspected envelope (Fig. 2).

Indication	Example
"rapid diagnosis" of infectious agents	"direct EM", e.g. in emerging diseases, BT or conditions of immuno-compromised patients
shortening "classical lab routines" after culturing a suspect agent	analysis of cell culture supernatants, e.g. Nipahvirus, meta-pneumovirus, SARS; bacterial isolates
search for otherwise undetectable agents	lack of specific reagents, lack of diagnostic methods
need for "catch-all-method" and / or rapid differential diagnosis	lack of clinical hints or broad group of possibly involved agents, e.g. BT "environmental" samples, diarrhoea
QC in lab diagnostics, GMP-production of biomedicals	fulfilling GLP/ GMP rules: test for specificity of antigens, antibodies, methods, exclusion of contaminants

Advantages, limitations and quality control (QC)

Preparation for diagnostic EM by two-step negative staining and evaluation by TEM needs less than 15 min until a safe positive DD ("this is an orthopoxvirus", "this is a herpesvirus": Fig. 1) is achieved. Distinctive differences in morphology between the viruses of the relevant virus families help to a rapid and accurate DD. Likewise, morphological details of bacteria are used for a "front-line" diagnosis (Fig. 2) (5). This rapid group diagnosis can be sufficient for the clinican: in the lab it helps to direct the further characterization to the relevant family of agents.

There are limitations reported as typical for diagnostic EM:
(1) insensitivity: high particle concentrations, i.e. > 10^6/ ml are needed for diagnosis
(2) requires a great degree of skills, dedication and experiences
(3) is based on the use of expensive, technically demanding equipment
(4) low sample through-put, i.e. EM is not compatible with a mass-screening of samples.

Such arguments are "relative": e.g., there are efficient EM preparation and enrichment techniques (1, 3, 5, 8, 9, homepage: Consul Lab). Working within a diligent and intelligent crew is - most often - a pleasure. Instrumental and technical support is often found in the neighborhood (1, 2) and low sample through-put furthers the concentration on the really important problems, i.e. there are good reasons to keep EM within the lab diagnostic repertoire. Rapid EM is supported by tele-microscopy and rapid embedding techniques.

Continuous education and QC guarantee the performance and reliability of diagnostic EM. The Consultant Lab, established in 1997, together with the Arbeitskreis EM-Erregerdiagnostik of the DGE (AK-EMED), are running since 1995 annual Workshops on diagnostic EM and 1 to 3 Basic Lab Courses (homepage: Consul Lab, ref. 14). We help in organizing special Labor-Meetings. Since 1993, twice a year an External Quality Assurance Scheme is run (5,6). The number of participants and countries are still growing in parallel to the needs for diagnostic preparedness (Figs. 1, 2). By these means we expect to assure the performance of EM as a uniquely rapid and accurate lab diagnostic tool.

Fig. 3: Sample collection for smallpox diagnosis

(1) Formaldehyde (FA) inactivated vesicle smear on microscopic slide

(2) Infectious vesicle fluid aspirated in needle and syringe

(3) Vesicle fluid FA-inactivated in a vial after collection by syringe

(4) Grid touched to fluid or base of lesion for „direct" EM: FA- or GA-inactivation

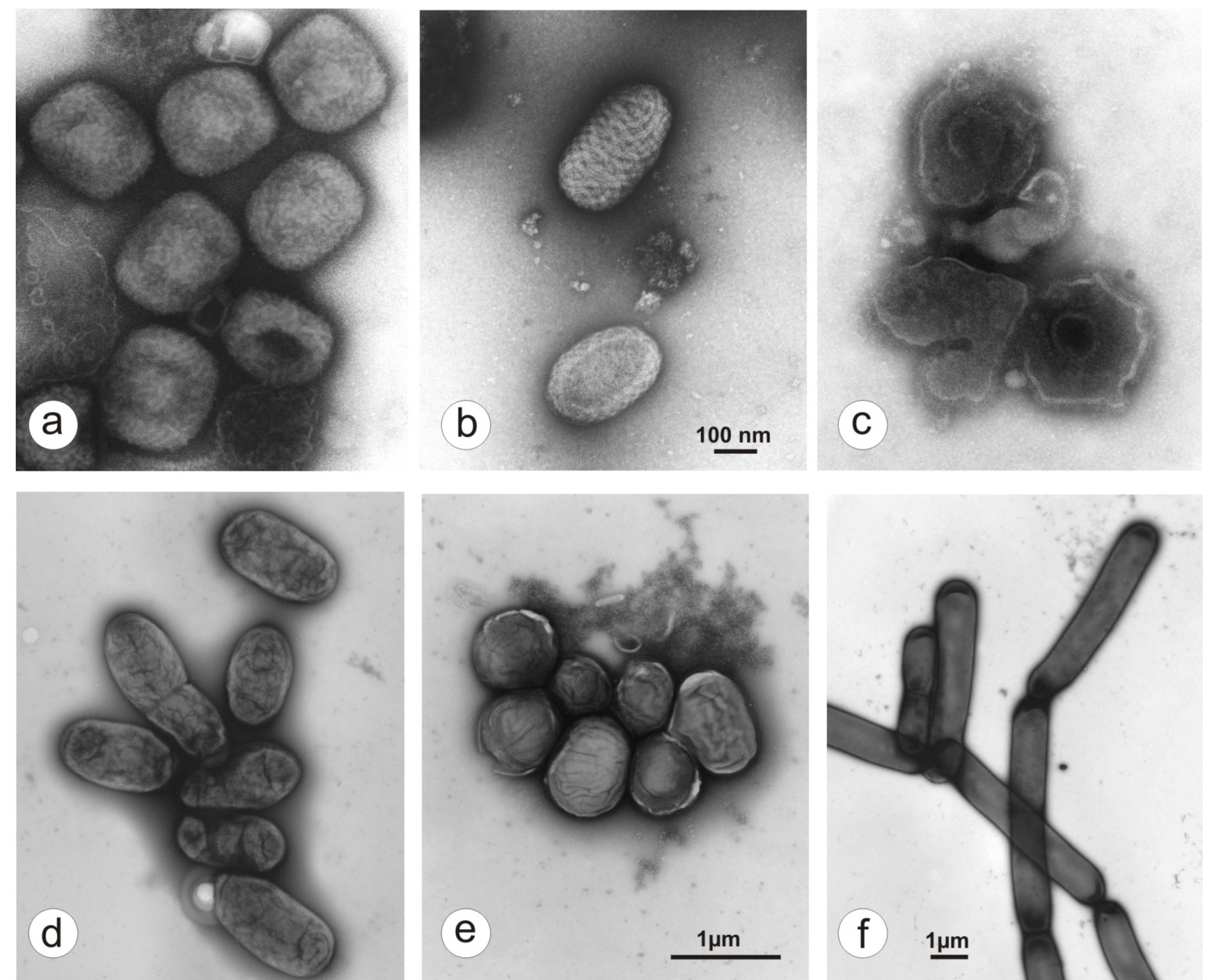


Fig. 1: Rapid DD electron microscopy with viruses and bacteria

a. Ectromelia mouse orthopoxvirus. The first virus ever shown by EM (von Borries, B., Ruska, E., and Ruska, H.: Klin. Wochenschr. 17, 921-925 (1938). Cell culture grown, formaldehyde- (FA-) inactivation, PTA staining

b. Parapoxvirus from cattle, propagated in a diagnostic cell culture FA-inactivation, UAc staining

c. Herpesvirus particles from a human febrile vesicular rash disease. Direct EM of blister fluid revealed aggregates of enveloped particles typical of chickenpox (german: Windpocken; most important DD to smallpox). FA-inactivation, UAc staining

Electron microscopy of candidate bioterror bacterial agents:

d. *Yersinia pestis,* FA-inactivation, UAc staining

e. *Francisella tularensis,* FA-inactivation, UAc staining

f. *Bacillus anthracis*: vegetative forms, FA-inactivation, UAc staining

Fig. 2: Negative staining of *B. subtilis* spores: a model for anthrax

Spores at a concentration of 10^9/ml of environmental *B. subtilis*: a model for the "forbidden" spores of *B.anthracis.* Staining with UAc using special hydrophilic grids (Alcian blue) results in an even and efficient distribution of spores.

Inactivation: 2 hrs, 10 % formaldehyde plus 0,05 % glutaraldehyde

Negative staining: 1% Uac

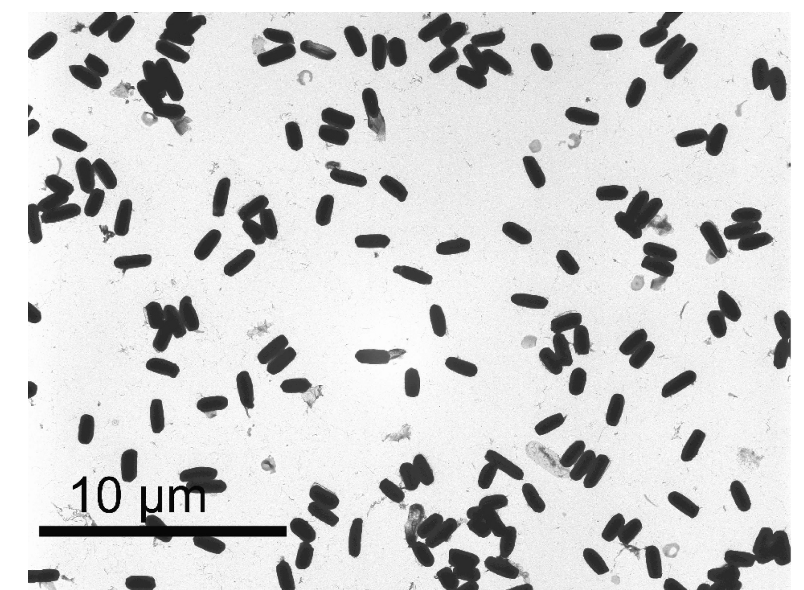


Fig. 4: External Quality Assurance Scheme for EM Viral Diagnosis (EQA-EMV)

- 16 EQA runs since 1994
- participants: physicians, veterinarians, universities, governmental institutions, industry, army
- EQA 1 – EQA 3: distributed in Germany
  since EQA 4: distributed in Europe
  since EQA 6: distributed Worldwide
- supported by: ESCV, ESVV (Europe) INSTAND, DVV, GfV, DGHM, DGE, AVID - DVG (Germany)
- registered with EPTIS

- Participants in EQA 16:
  112 laboratories from 32 countries
  81 laboratories from Europe
  37 laboratories from Germany

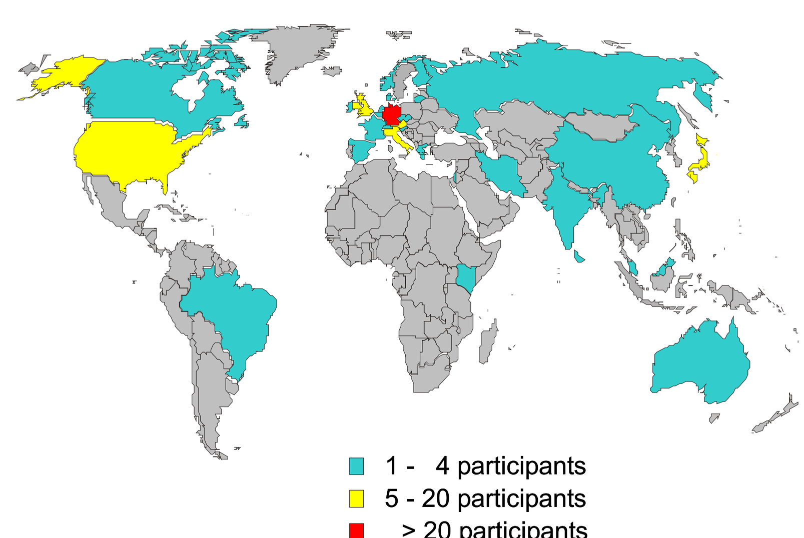


Fig. 5: Immuno-negative staining of SARS

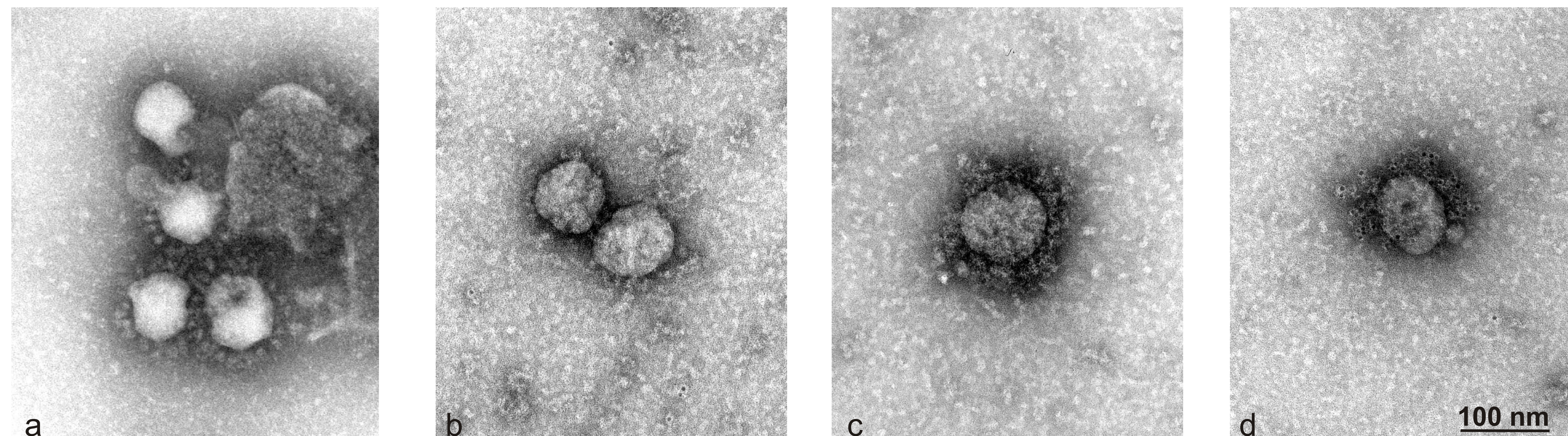
Negative staining and on grid-immuno-negative staining of SARS corona virus. SARS virus Frankfurt (a generous gift of Profs Doerr and Rabenau, Frankfurt and Dr. Niedrig, RKI) was used as un-purified cell culture supernatant - after fixation with 4% formaldehyde.

a. PTA staining reveals distinctive peplomers on the virion.

b. Incubation with "normal" human serum (1:20) does not lead to any Ab-decoration nor to gold-labelling. UAc

c. Direct un-labelled immuno-EM: the SARS patient serum (1:50, without using secondary gold-labelled Ab) reveals a dense fringe of primary Ab bound to the virion. UAc

d. Indirect immuno-EM: incubations with patients serum (1:20) followed by gold-labelled secondary Ab results in both decoration and gold-labelling. UAc



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(16) www.bt.gov/labissues/index.asp (click: Negative staining EM protocol for rash illness)
(17) www.bt.cdc.gov/agent/smallpox/lab-testing (click: Specimen collection guidelines)

https://doi.org/10.1017/S143192760550494X Published online by Cambridge University Press

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