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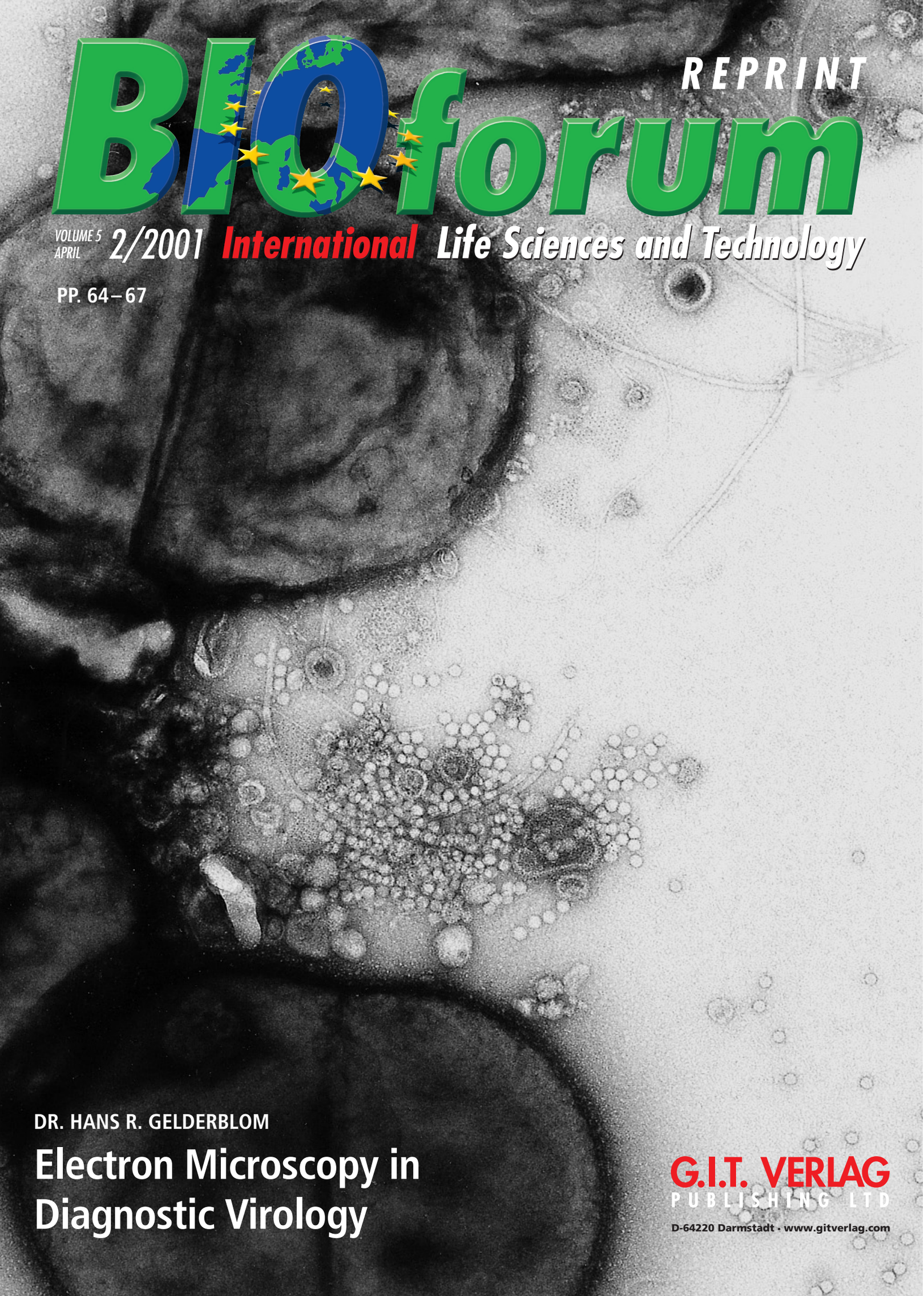

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Electron Microscopy in Diagnostic Virology

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Electron Microscopy in Diagnostic Virology

In the laboratory diagnosis of infectious diseases, diagnostic electron microscopy (EM) shows two principal advantages compared to alternative test systems: It excells by rapidity and an undirected "open view" allowing in clinically or epidemiologically critical situations a rapid diagnosis. EM can also shorten the routine cell culture diagnosis and may help in the quality control of the diagnostic routine. To exploit fully its potential, EM should be integrated into the diagnostic routine and executed in a quality-controlled way to guarantee its performance.

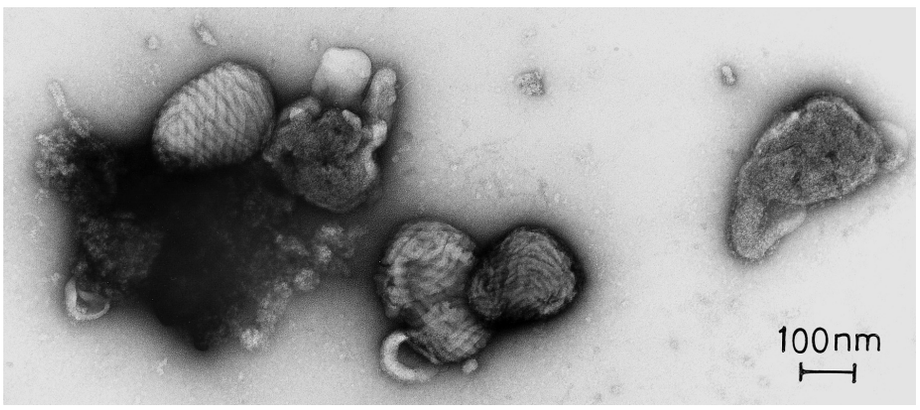


Fig. 1: Parapox- and paramyxovirus: diagnostic specimen derived from a cell culture after negative staining with 1 % uranyl acetate. Parapoxviruses, e.g. Orf, milker´s nodule virus, sealpox and others, appear slightly ovoid and reveal distinct surface tubules surrounding the complete virion. Paramyxoviruses (particle to the right) are enveloped by a flexible lipid membrane, studded with tiny surface projections and contain a helical ribonucleoprotein 18 nm in diameter (seen to the upper left). Magnification: x 67,000

On the role of electron microscopy in biosciences

Different from the situation in material sciences, electron microscopy (EM) in life sciences has lost much of its former role. The apparent loss in availability and performance is only partly due to short-sighted economic decisions – a main factor is the introduction of modern, highly effective molecular analytical techniques (antibody-, antigen-, autoradiographic-, nucleic acid-assays, chip technology and spectroscopy [for review: 8]). Also confocal laser scan microscopy, definitely a powerful tool, is often presented as a full substitute for EM, a judgement flatly wrong if spatial accuracy, i.e. higher resolution information is required.

Today, EM in life sciences is often regarded as a tedious, complicated and old fashioned discipline – introduced more than 60 years ago [1, 14; for review: 7].

A further retreat of EM out of life sciences, however, will have harsh consequences. Already today deficits in understanding cell biology and medically relevant processes are clearly stated and ways out of the apparent crisis are requested [5]. Indeed, a renaissance of EM in life sciences is to be expected. As an imaging technique EM allows a direct and open view into the objects under study. While alternative analytical techniques – with high efficiency – determine just selected, single properties, EM scrutinizes all constituents in the object in their mutual relation at a molecular resolution. The undirected visualization by EM provides a thorough insight and easy understanding of structure-function relations. The inherent "open view" allows controlled observations at the same specimen and helps to elucidate also relations that have not been under direct scrutiny.

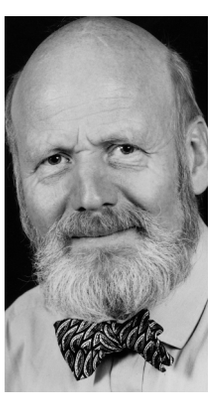
The renaissance of EM is favoured also by a number of modern EM preparation techniques which broaden the field of application considerably [8, 10]. When genomics and proteomics, todays trendy waves, are ebbing again, questions as to protein localization, function and interaction at the molecular level will be raised. To localize the functional components of the cell and their relationship at high resolution, immuno EM, cytochemistry, autoradiography, freeze preparations techniques, stereology, image processing and 3D-reconstruction will be required.

Keywords

electron microscopy, virus diagnosis, indications, quality control

On the role of morphology in the lab diagnosis of infectious diseases

The eye, the sense of sight, is most essential for recognizing the surrounding world and thus also for the understanding of causal relationships (greek: dia gnosis = look through). Only during the last half of the 19th century – applying advanced light microscopic techniques – the diagnosis of infectious diseases and the study of their natural history became possible on a rational, scientific base. Many bacteria were detected and described as the cause of a number of severe, transmissible, i.e. infectious diseases, e.g. anthrax, tuberculosis, cholera, diphteria, and typhoid fever [7]. The etiological role of the isolates was proven by fulfilling what was later called the Koch-Henle postulates: (1) the isolation of the "germ" from the diseased tissue as a single colony growing on artifi-



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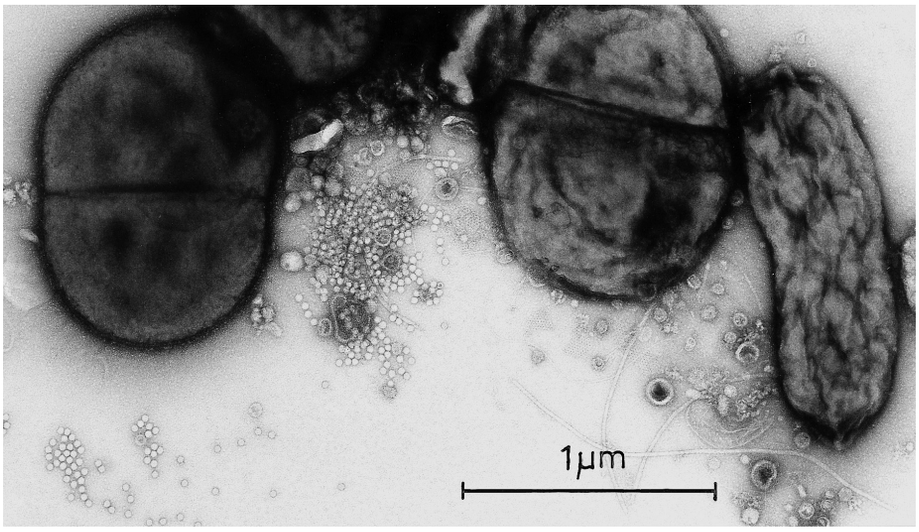

Fig. 2: Diagnostic stool specimen from an outbreak of epidemic gastro-enteritis caused by Norwalk-like caliciviruses. Besides the bacteria, the 33 nm measuring isometric virus particles are seen. Negative staining with 1 % uranyl acetate. Magnification: x 33,000

cial growth media, (2) the presentation of both uniform colony growth and germ morphology (as judged by light microscopy), and (3) the experimental infection by the isolate of laboratory animals verifying the primary symptoms of the disease.

Yet from a number of transmissible diseases, e.g. poliomyelitis, influenza, measles, chickenpox and others, neither an infectious agent could be isolated nor shown by light microscopy, hold back in bacteria-tight filters or sedimented in conventional centrifuges. Therefore, soon a class of much smaller agents was surmised. Starting only in 1938, with the introduction of the EM with its 500-fold higher resolution compared to the light microscope, these until then "negatively" defined agents and their morphology became accessible [7, 13, 14]. Three factors, EM, virus susceptable cell cultures and the ultracentrifuge, during the 60th and 70th allowed the description of a great number of human, animal, plant, and bacterial viruses. Today, more than 30,000 different viruses are known. They are classified according to genome properties and particle morphology – as the latter is a direct consequence of the structural viral genes – using morphological criteria into 56 virus families. From these, 26 families are specific for vertebrates, 21 for human beings [12].

In EM viral diagnosis, size and fine structure of a suspect structure are assessed. If a particle is recognized as virus specific, it is – at the same time – assigned to a specific virus family (Fig. 1). The assignment to a family is in most cases sufficient for the clinician – often as a differential diagnosis resulting e.g. in the isolation (barrier nursing) of the patient and/or the use of antiviral therapy.

The preparation for EM diagnosis requires conventional negative staining, a rapid and technically simple procedure. A droplet of the diagnostic suspension is adsorbed onto a support grid and dried down together with a solution of the contrast-stain (a heavy metal salt) as a thin film [for review – also on the use of ultrathin sections of infected cells in EM viral diagnosis: 2, 4, 6]. The undirected, "open view" of conventional transmission EM recognizes structures down to a size of 15 nm. This lower limit comprises also the smallest viruses [12]. Negative staining and the evaluation at the EM need less than 15 min until a diagnosis is met – provided the necessary high particle concentrations are present in the suspension. However, using enrichment procedures also specimens containing less then 10^7 particles/ml can be evaluated and, if required, also typing of the isolate can be achieved using immuno EM [2].

History of EM viral diagnosis

The morphological diagnosis is mainly used in virology, however, it can be applied also to the wider field of microbiology if conventional techniques fail or turn out too time consuming. In virology, diagnostic EM has shown a change of paradigms. Initially applied in poxvirus diagnosis, it helped later, starting in the 60th, to characterize a great number of isolates grown in diagnostic cell cultures as new viruses. When next – starting in the middle of the 70th – microscopists dared to apply the "clean instrument to dirty body excretions" (Fig. 2), a whole new virus world was detected. Astro-, calici-, corona-, and rotaviruses of men and animal were described [3, 9, 11]. Since EM is not suitable for mass screening, alternative diagnostic means, e.g. ELISA and PCR techniques, have been and are being developed for the routine detection of new agents. Owing to high efficiency, specificity and sensitivity the alternative tests will narrow further the role of EM in the lab diagnosis of infectious diseases. Nevertheless, because of the "open view" and the rapidity, EM will not become completely substituted e.g. by broadband multiplex PCR-techniques.

Table 1: Indications for EM in diagnostic virology

Indications and applications	Examples and explanations
Rapid Viral Diagnosis in Medicine and Veterinary Medicine	in clinically critical situations, e.g. chickenpox suspected in a newborn ward or immunodeficiency due to organ transplantation or HIV infection, that often is associated with the appearance of new clinical syndromes. Important in animal breeding: e.g. enteritis due to rota- or coronavirus
direct electron microscopy	most efficient preparation – directly from the lesion onto the grid
rapid diagnosis in case of "emerging infections"	in epidemiologically critical situations
in case of failure of the routine laboratory diagnosis	when conventional diagnostic means miss a fastidious agent, e.g. in case of non-cultivable viruses
"open view" "catch-all-method"	when a variety of different agents may be involved as in non-bacterial epidemic gastro-enteritis (differential diagnosis: rota-, adeno-, astro-, Norwalk-like-, and other caliciviruses, coronaviruses)
to shorten the conventional cell-culture based virus diagnosis	identification of the agent by EM in culture supernatants or from diagnostic cells broken up by freezing and thawing (when a cytopathic effect becomes apparent indicating the propagation of an agent)
quality control of routine lab diagnosis	internal quality assurance of other techniques used in the laboratory
quality control in the production of medically applicable biomaterials	required following the GLP rules, e.g. in the production of vaccines. EM covers up possible contaminations and thereby the production line gains in biological safety

Indications for diagnostic EM

EM should form an integral part of the routine laboratory diagnosis in order to operate in a most effective way. In a proper setting, EM by its "open view" will assure the quality of the diagnostic routine and shorten the procedures. To clarify "difficult" syndromes and as an instrument of rapid viral diagnosis, EM is hardly to be substituted. The gain in time is an important factor in the control of infectious diseases. Finally, EM is involved also in the controlled preparation of biomaterials for medical use. Here contaminations become detectable and the production gains considerably in biological safety. The present indications for the application of diagnostic EM are summarized in Table 1.

Quality control in EM virus diagnosis

As other techniques in a medical laboratory, also EM diagnostics should be performed in a quality controlled way. To this end, we have established since 1994 in cooperation with respective national and European societies in infectious diseases a program consisting of three constituents. Besides external quality control schemes (EQA-EMV) performed twice a year, we organize national and international workshops and basic laboratory courses in diagnostic EM [3, 11]. In the present 10th EQA run 92 participants from 27 countries are enlisted (Fig. 3) working mainly in medicine and veterinary medicine at universities and Public Health Institutions. The number of participants is still increasing. Serving as the German Konsiliarlaboratorium for rapid EM viral diagnosis we help other groups with advice or directly in difficult diagnostic situations. Further information on our activities may be gained directly from the author, at the homepage www.electron-microscopy.org of the Arbeitskreis EM-Erregerdiagnostik (AK-EMED) of the German Society for Electron Microscopy or at www.rki.de/INFEKT/ENIVD/EMQM/EMDIAG.HTM

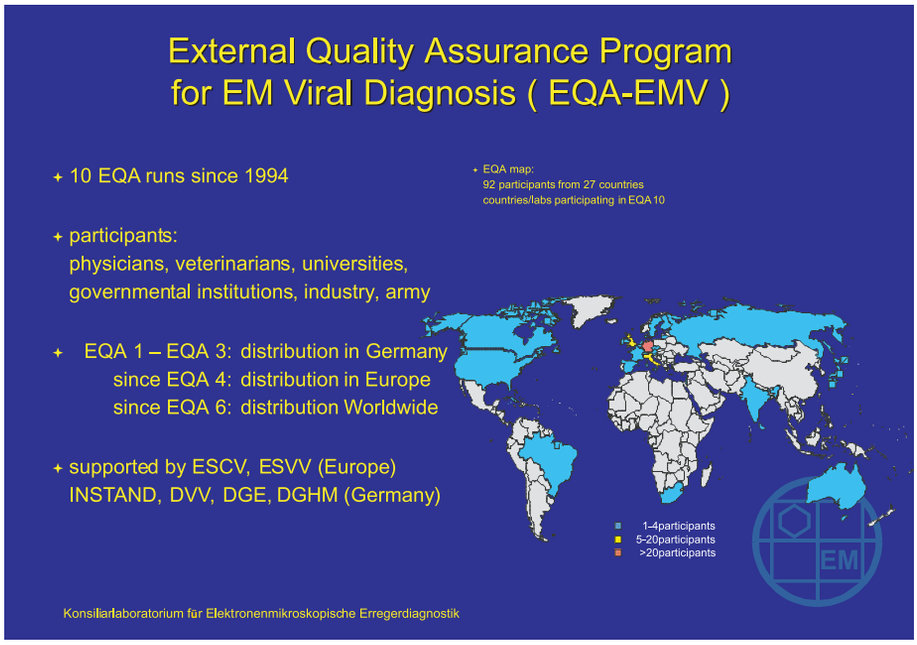

Fig. 3: World map showing the distribution of the participants in EQA-EMV 10

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