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Conclusions

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At present we are witnessing a transition from traditional culture based methods requiring the recovery of microorganisms on solid or liquid media towards modern molecular genetic techniques based upon the identification of taxon-specific nucleic acid sequences. At first, most microbiologists not actively working in the field and overwhelmed by dozens of enthusiastic reports on various research applications believed in the power of nucleic acid hybridization and amplification techniques to completely replace standard techniques within a short time. Undoubtedly molecular genetic methods broaden the scope of microbiological research as well as determinative and applied microbiology, including clinical and ecological microbiology. This is not only due to the great specificity and sensitivity of probing, the PCR technology and the speed and ease of automation, but also and primarily to the fact that molecular genetic analysis can be applied to the detection of both cultivable and uncultivable microorganisms. However, initial enthusiasm has been replaced by a more realistic view, which is mirrored by the selection and content of the papers presented at this workshop.

First of all, there was a general consensus about special indications for the application of molecular genetic tests, which can be summarized as follows. Molecular analysis cannot and should not replace standard techniques, but complement those where culture techniques are either not available or not practical. They are a great help if biochemical and/or serological identification is lengthy or unreliable. There will be a niche for these techniques in the clinical laboratory where rapid diagnosis is required, to avoid invasive diagnosis, unnecessary or even dangerous therapy and the spread of new plagues or nosocomial infections.

Secondly, all participants agreed that much effort has to be put into standardization of these tests before they can reliably replace standard procedures. Of major concern was the preparation of samples. Points to consider include the specimen, the organism, nucleic acid target sequences and the appropriate hybridization or amplification protocol. For all subsequent steps it is necessary to take into account the type of specimen, e.g. blood, serum, urine, cerebrospinal fluid or bio[illegible]ies, the presence of inhibitors, and the volume t[illegible]cessed. Often, pretreatment of the sample is r[illegible]der to free or enrich nucleic acid target s[illegible]v magnetic separation techniques. Some require very complex and labour-intensive processing. This consequently limits the application of these procedures in a clinical environment, where often hundreds of samples have to be processed at once. Of major concern is the inability of the present methods to quantify members of a mixed culture in any environmental sample. This critically influences the sample size. Some samples require sterilization prior to further processing of the specimen to avoid any health hazard for the laboratory personnel. Ideally, this pretreatment weakens rigid cell walls for the penetration of probes and for the release of nucleic acids, as happens with mycobacteria. Some specimens may even require combined chemical and mechanical lysis, e.g. ultrasonication or treatment with glass-beads.

The selection of appropriate target sequences has been grossly neglected by many people, particularly those who are applying amplification protocols. It is not sufficient to uncritically use probes and primers described in literature. Very often microheterogeneity of target sequences or their distribution within a given taxon has not been evaluated sufficiently. This is particularly true when virulence genes, plasmid-encoded genes or insertion elements are used as targets. The choice of phylogenetic markers, such as *r*-RNA or rRNA genes may be beneficial. The latter have proven to be of great value for the characterization of unknown and as yet uncultivable organisms or the analysis of microbial populations. However, it should be noted that rRNA does not distinguish between all species, as has been found among pathogenic bacilli, mycobacteria and enterobacteria. Probes developed to detect individual subgroups of *Clostridium botulinum* and non-pathogenic relatives are useless if exclusively pathogenic strains need to be detected. There are rare exceptions where other genes or the intragenic spacer region located between the 16S and 23S rRNA genes should be used. It is worth noting that great care should also be taken, if comparative rRNA sequence analysis is used to describe complex microbiota. Sample processing, the efficiency of restriction enzyme digestion, ligation and transformation, the choice of cloning systems and the number of clones to be analyzed to obtain a statistically sound result, have still not been investigated in detail. Generally, interpretation of the results is not a simple matter and does require stringent quality controls.

The same is true in the clinical situation. The high level of sensitivity, allowing for the detection of a single organism requires careful clinical interpretation of the laboratory result. There is always the possibility of accidental contamination, transient colonization, latent infection or mere persistence of microbial nucleic acids from dead or moribund microorganisms. Interpretation of these 'DNA/RNA footprints' remains difficult. The identity of an organism must be verified by specific probes in situ in the environment. The clinical relevance of such observations has to be determined.

In conclusion, molecular genetic analysis allows for sensitive detection of cultivable and uncultivable organisms including the description of unexpected biodiversity in polymicrobial infections and environmental microbiota. It provides means for reliable taxonomic i.e. phylogenetic classification and rapid, unambiguous molecular typing for tracking the outbreak and spread of new diseases and nosocomial infections by using fluorescent-labelled probes. Special care should be taken before this new technology is implemented into clinical microbiology. The clinical usefulness of the information has to be determined. Accuracy must be evaluated continuously. Guidelines for the interpretation have to be formulated. To allow for cost-efficiency, niches should be defined where these new techniques are useful and economical. This means in consequence that care should be taken in the application of the tests and in the interpretation of their results.