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Modern microbiological techniques and their use in dialysis fluid systems: What are the benefits?

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Modern microbiological techniques using characterization of DNA are increasingly used in ecological microbiology to describe the diversity of microfloras. The benefit is that systems can be described with a much higher degree of reality. In dialysis, one application is the evaluation of fluid-system disinfection strategies.

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Introduction

Since 1990, concern about the microbiological quality of dialysis fluids has been increasing, and the requirements in standards and recommendations set by authorities are becoming more stringent. An important issue in these discussions has been the method used to detect microorganisms. Classically, microorganisms have been detected with the use of different types of culture techniques. Indeed, at present, most microbiological analyses are performed with the use of only insensitive culture methods.[1,2]

In order to form a visible colony, noted as a colony-forming unit, a microorganism must be able to grow under the culture conditions offered. The problem with the use of culture methods to determine bacterial colonization is, in short, that there are many microorganisms but only a very limited number of culture conditions used — usually just one. Just consider that there are some 1 100 acknowledged genera of bacteria, with about 7 000 separate species described in the literature.[3] Using a single standardized culture technique,[1,2] it can be estimated that fewer than 30% of these species will be able grow. For dialysis water, some countries have established specific criteria for the numbers of certain bacterial species permitted. But the 'identification' (or rather characterization) and quantification of a certain species is often laborious and often very imprecise when dealing with an environmental microflora. Other microorganisms, such as yeast, molds, algae, and protozoa, are notoriously difficult to grow in culture. There is a real need of methods more sensitive than the culture methods developed by Robert Koch in 1881.[4]

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What are 'modern techniques'?

Modern techniques for the identification and quantitation of microorganisms from their DNA have been developed since the polymerase chain reaction (PCR) technique became available. The PCR technique makes it possible in principle to replicate a single DNA molecule sufficiently to have enough DNA for further analytical work such as gel electrophoresis.

In principle, work could be done with either DNA or RNA, but for practical reasons DNA is preferred, as mRNA is very readily and rapidly degraded by RNAses. Conversely, the DNA molecule is a rather stable structure and can stand all the required chemical treatments. It is degradable by DNAses. In practice its 'survival time' is judged to be about 3 days. As all cells do have DNAs, it is essential to destroy the DNAses immediately after the sampling.

All techniques start with a sampling procedure to obtain bacterial (or other microorganism) DNA followed by PCR amplification to obtain enough 16S ribosomal DNA (rDNA) for further analysis.[5–7] Several methods can be used then to characterize the rDNA (the techniques discussed here are only for prokaryotic organisms):

(1) temperature gradient gel electrophoresis,[8] in which different DNAs can be separated on the basis of the melting point of the DNA (which depends on guanine–cytosine content);
(2) density gradient gel electrophoresis,[8,9] in which different DNAs can be separated on the basis of their density;
(3) restriction fragment length polymorphisms, which can be obtained and compared with libraries.

Characterization of microflora. The modern use of 16S rDNA, temperature gradient gel electrophoresis, density gradient gel electrophoresis, and restriction fragment length polymorphisms allows, in principle, the determination of the presence of all species present in the dialysis sample. The description of a species is very precise, as it is accurate down to the strain level without any problem. Determination of the composition of the microflora present will be much more complete as compared with conventional techniques currently used, as shown by Gomila *et al.*[5].

Limitations of the DNA-based methods. Unfortunately, accurate quantification of the microflora is in principle not possible, as it is generally not known how many cells contributed to the original DNA. Further, no distinction can be made among living culturable organisms, living non-culturable organisms, inactive organisms, and dead organisms so long as the DNA is not damaged.

Discussion

The use of modern DNA-based techniques for evaluation of environments for

their microbiological content is becoming more common in research work. The benefit is that all the different microfloras present can be detected. In principle, only one undamaged DNA molecule from each species is required. These modern DNA techniques are clearly superior to the conventional cultivation techniques for identification of the different species present. Especially, the search for specific pathogens in different environments is facilitated. A limiting factor at present for DNA techniques (as well as for conventional characterization) in analysis of environmental microflora is the limited number of organisms characterized in the reference libraries. This will, however, be overcome with time. Reference libraries can be built up.

The modern DNA techniques do not compete with the conventional cultivation technique in quantifying a microflora. One still cannot obtain results such as the number of bacteria present per milliliter. But it is also difficult to quantify microflora in a fluid system with the use of conventional culture techniques, as microorganisms tend to form colonies on inner surfaces of the fluid systems because of biofilm formation.[10] These microorganisms will of course not show up in any analysis of a fluid phase.

The DNA techniques are still very laborious in comparison with conventional culture techniques and require a specially equipped laboratory. They are presently used mostly for research or special tasks rather than for routine surveillance of dialysis water supplies. A no-growth or a low-growth result with a non-optimal culture technique is usually accepted as satisfactory even if no preventive and effective disinfection program is in place.

The value of the DNA-based approach at present is that a fluid system in dialysis can be evaluated from a hygienic point of view by a more sensitive technique. This is very useful in deciding on or discussing a disinfection program or strategy for a fluid system. The conventional cultivation technique will not reveal the full range of the microflora present in the fluid. In addition, in those areas where additional requirements exist (for example, testing for the presence of *Pseudomonas aeruginosa*), the DNA techniques are useful.

Conclusion

Development of DNA-based methods for analyzing microorganisms present in dialysis solutions has been a major advance and offers important supplemental information above and beyond what can be obtained from standard culture methods. For the time being, the major advantages of the modern DNA techniques are for the detection of specific species that are to be kept under control and for the process of deciding on a disinfection strategy, as these techniques can show a more complex and complete image of the microbiological reality.

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