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Multi-author Reviews

Methodological developments in nucleic acid diagnosis

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Introduction

U. B. Göbel and E. Stackebrandt

Institut für Mikrobiologie und Hygiene, Universitätsklinikum Charité, Medizinische Fakultät, Humboldt-Universität, Clara-Zetkin-Str. 96, D-10098 Berlin (Germany) and DSM (German Collection of Microorganisms and Cell Cultures), Mascheroder Weg 1b, D-38124 Braunschweig (Germany)

At the turn of the century in vivo 'amplification', i.e. isolation, growth and continuous cultivation of pure cultures of bacteria on artificial media, was a prerequisite for the emergence and success of the new field of microbiology. Microscopic examination and 'classical' culture techniques, later supplemented by physiological, serological and chemical tests to identify the organism of interest were used for research and diagnostic purposes. With the advent of molecular biology during the last thirty years and the development of recombinant DNA techniques we are seeing the beginning of a new era of microbiology. Hybridisation[2], cloning[1] and sequencing techniques[4] are being used increasingly in pathogenicity research, vaccine development, phylogeny, systematics and taxonomy, and diagnostic microbiology. Most recently, the introduction of in vitro nucleic acid amplification, i.e. the polymerase chain reaction (PCR), described in Science[3], and its application in almost all areas of molecular biology including diagnostic procedures has initiated a revolution in microbiology, particularly in clinical microbiology and microbial ecology.

Dozens of research papers initially described the application of nucleic acid-based detection methods for the diagnosis of infectious diseases. An ever-increasing number of commercial companies are developing and producing reagents and instruments for molecular diagnosis in the clinical microbiology laboratory. Due to active commercial promotion and overenthusiastic initial reports, a plethora of publications describing the benefits and unlimited applicability of this technology have since appeared. To provide a critical forum for discussion of the benefits and problems of these techniques the 'Deutsche Gesellschaft für Hygiene und Mikrobiologie' (DGHM), the 'Gesellschaft für Virologie' (GfV) and the 'Vereinigung für Allgemeine und Angewandte Mikrobiologie' (VAAM) organised a poster workshop on applications of nucleic-acid based diagnosis, held in Berlin on November 6, 1993. The extended abstracts collected here provide a broad coverage of the practical implementation, pitfalls, and dangers of nucleic-acid amplification, sample preparation, and analysis of the amplification products for a variety of specific applications, ranging from detection of specific cultivable and uncultivable organisms to molecular typing and phylogenetic analysis.

1 Cohen, S. N., Chang, A. C. Y., Boyer, H. W., and Helling, R. B., Construction of biologically functional bacterial plasmids in vitro. Proc. natl Acad. Sci. USA *70* (1973) 3240–3244.

2 Hall, B. D., and Spiegelman, S., Sequence complementary of T2-DNA and T2-specific RNA. Proc. natl Acad. Sci. USA *47* (1961) 137–146.

3 Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, P., Horn, G. T., Mullis, U. B., and Erlich, H. A., Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science *239* (1988) 487–491.

4 Sanger, F., Nicklen, S., and Coulson, A. R., DNA sequencing with chain termination inhibitors. Proc. natl Acad. Sci. USA 74 (1977) 5463–5467.