



Standard Guide for Detection of Nucleic Acid Sequences by the Polymerase Chain Reaction Technique¹

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INTRODUCTION

This guide applies to the detection of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequences by the polymerase chain reaction (PCR) technique. The PCR is used as a tool in many molecular biology laboratory settings and for diverse reasons, for example, for amplification and detection of nucleic acid sequences. There is an abundance of publications addressing laboratory procedures and specific protocols for various applications. The field of PCR is advancing so rapidly that it is necessary to frequently modify and update these procedures and specific protocols. This guide consists of guidelines, recommendations, basic considerations, criteria, and principles that should be employed when developing, utilizing, or assessing PCR procedures and specific protocols for the amplification and detection of nucleic acid sequences.

This guide was developed by Subcommittee E48.02 on Characterization and Identification of Biological Systems. The 1997 edition of this guide was developed in collaboration with DIN (German Institute for Standardization) Committee E9 on Serodiagnosis of Infectious Diseases and Diseases of the Immune System, Department for Medical Standards (NAMED).

This guide assumes a basic knowledge of molecular biology. It assumes the availability of basic references in PCR for general procedures (see Refs 1-7)² and the ability to search the literature for target-specific protocols.

1. Scope

1.1 This guide covers guidelines, recommendations, basic considerations, criteria, and principles to be employed when developing, utilizing, or assessing PCR procedures and specific protocols for the amplification and detection of nucleic acid sequences. This guide is not intended to be a standard procedure with a list of requirements for PCR detection of nucleic acids. This guide is intended to provide information that will assist the user in obtaining quality and reliable data.

1.2 Nucleic acid targets for PCR include DNA, as well as RNA; RNA sequences are suitable targets for PCR following reverse transcription of the RNA to complementary DNA (cDNA). This type of amplification technique is known as reverse transcription-PCR (RT-PCR).

1.3 This guide has been developed for use in any molecular biology/biotechnology laboratory. This includes, but is not limited to, laboratories that specialize in the diagnosis of human, animal, plant, or bacterial diseases.

1.4 This guide conveys the general procedural terminology of PCR technology used for the detection of nucleic acids.

1.5 This guide is general; it does not cover the additional guidance that would be needed for specific applications, for example, for the PCR detection of nucleic acid sequences of specific microorganisms.

1.6 This guide does not cover details of the various methods that can be utilized to identify PCR-amplified DNA sequences.

1.7 This guide does not cover specific variations of the basic PCR or RT-PCR technology (for example, quantitative PCR, real-time PCR, multiplex PCR, and in situ PCR), and it does not cover details of instrument calibration.

1.8 **Warning**—Laboratory work involving certain clinical specimens and microorganisms can be hazardous to personnel. **Warning**—Biosafety level 2 (or higher) facilities are recommended for biohazard work (8). Safety guidelines should be adhered to in accordance with CLSI M29-A2 and other recommendations (8).

¹ This guide is under the jurisdiction of ASTM Committee E55 on Manufacture of Pharmaceutical Products and is the direct responsibility of Subcommittee E55.04 on General Biopharmaceutical Standards.

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² The boldface numbers in parentheses refer to the list of references at the end of this standard.