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Numerous variables are associated with PCR, and modifications in technique exist from laboratory to laboratory for any one type of PCR format. Consequently, variation in results among different laboratories can easily occur when PCR is used in experiments or diagnostic assays. In addition, the possibility of false-positive results attributable to contamination and false-negative reactions caused by enzyme inhibition are potential problems for PCR use in clinical diagnostic laboratories.[1] Therefore, standardized PCR procedures are needed to ensure reliable results. Standardized procedures can help to avoid confusion, mistakes, and complications, and they encourage efficiency, reproducibility, and quality control. The objective of this overview is to describe a number of efforts presently under way by national and international organizations to standardize PCR procedures. The overview does not tabulate or compare specific requirements or recommendations from different standards organizations, because most of the PCR standards are presently still in development and, consequently, are unavailable to the public.

What is a laboratory standard? There are different types of standards and different definitions of standards, depending on the organization that established the definition. The two general types of standards that are described in this overview are written laboratory standards (standardized procedures) and laboratory standard solutions or reagents. The latter type of standard is well known and does not require clarification. A written laboratory standard is essentially a written description of a particular laboratory procedure on which a group of individuals agreed. If a standard for a particular laboratory procedure has been developed properly and has been well written, then individuals working in different laboratories should be able to use the standardized procedure and obtain comparable results.

There are different types of written laboratory standards, depending on the organization that developed them and the purpose of the standards. They range from voluntary consensus standards to mandatory government regulatory standards. Consensus standards are generally prepared by standards organization committees of representatives, for example, from industry, universities, and government. Some consensus standards are only a set of recommendations that offer guidance but do not establish a fixed procedure, that is, "guidelines" or "guides." Others are definitive procedures that produce test results such as "test methods." Government regulatory standards are usually developed by government employees with government and nongovernment input obtained through a formal notification and response process. In some cases, government agencies adopt voluntary consensus standards and convert them to mandatory regulatory standards. Most of the PCR written standards discussed in this overview are voluntary consensus guidelines.

Readers should be made aware of the date of completion of this overview (December 1993), because the pace of PCR technology advancement and standards development is so rapid. This overview represents my own viewpoints and not the views, opinions, or endorsements of the U.S. Food and Drug Administration, the U.S. Public Health Service, or the U.S. Department of Health and Human Services.

MULTICENTER COLLABORATIVE TRIALS

Multicenter collaborative trials for establishing quality control of PCR provide useful standards-related data and are consequently an important component in the development of PCR standards. Several such trials have been reported and are briefly described below. Authors of the reports of these trials have emphasized the value of quality control, such as using positive and negative controls, and the importance of using standardized PCR procedures that have been developed through collaborative efforts.

Sheppard et al.[2] determined the sensitivity and specificity of PCR for the detection of human immunodeficiency virus type 1 (HIV-1) proviral DNA in five different laboratories. This was the first reported multicenter, blinded, proficiency trial of PCR for amplification of HIV-1 DNA. Each participating laboratory examined ~200 coded specimens with known HIV-1 infection status. Although each laboratory used the same basic PCR method, the procedures, primers, and reagents were not standardized, and each laboratory used its own protocol. HIV-1-specific hybrids between amplified DNA and radioactively labeled oligonucleotide probes were identified by autoradiography. Some false-positive (overall rate of 1.8%) and false-negative (overall rate of 0.8%) results and sample classification errors were reported. The authors recommended that any PCR test for clinical use should be standardized with respect to reagents and laboratory procedures and that laboratories should participate in ongoing blinded proficiency monitoring.

Defer et al.[3] performed a multicenter

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(seven French laboratories) collaborative trial to assess the sensitivity and specificity of the laboratories' own PCR protocols and to determine optimal PCR conditions for detecting HIV-1 DNA. Each laboratory tested blind two coded panels (standardized reagent solutions, which usually consist of dilutions of the test substance) of peripheral blood mononuclear cell samples collected from HIV-1 seropositive individuals and seronegative individuals at high or low risk of HIV infection. Each laboratory was allowed to use its own PCR protocol. For one of the two panels tested, participating laboratories were required to use a specific primer pair. ^{32}P-Labeled HIV-1-specific DNA probes were used to detect amplified DNA products. False-positive and/or false-negative results were reported in all participating laboratories. Variations in results reported between laboratories were believed to be attributable to such factors as differences in PCR protocol, laboratory errors, and DNA contamination. The authors emphasized the importance of positive and negative controls and the need for standardization of PCR procedures among different laboratories.

Zaaijer et al.[4] performed an international, multicenter (31 laboratories) collaborative trial to test the reliability of PCR detection of hepatitis C virus (HCV) RNA. Only 5 of 31 laboratories generated perfect results with the entire panel of coded test samples. The other laboratories experienced some false-negative results due to lack of sensitivity and some false-positive results due to contamination. The authors recommended the use of negative control samples to monitor contamination and weak-positive samples to guarantee sensitivity. The goal of the authors is to develop a standardized procedure for PCR detection of HCV RNA. They consider comparison of PCR results between laboratories participating in the trial a first step toward this goal. The authors stated that reports of the presence of HCV should be interpreted with care until reliable HCV RNA detection becomes widely available.

Another study that was performed to assess reference reagents for HIV-1 PCR[5] is described in a different section of this overview (United Kingdom Medical Research Council).

GUIDELINES FOR FORENSIC LABORATORIES

Guidelines for the use of PCR in forensic laboratories have been in development by the Technical Working Group on DNA Analysis Methods (TWGDAM) (representatives from various national crime investigation laboratories and other organizations) and the California Association of Criminalists Ad Hoc Committee on DNA Quality Assurance (representatives of California crime investigation organizations and other laboratories). These two groups published the first guidelines for PCR in 1991 with special emphasis on quality assurance for DNA typing in crime laboratories.[6] The guidelines include sample preparation requirements, validation of PCR-based DNA procedures, recommendations concerning equipment, materials, facilities, controls, reports and proficiency testing to demonstrate quality performance, and other information. For further information, contact James J. Kearney, TWGDAM Chairman, FBI Laboratory, Washington, D.C. 20535.

WORLD HEALTH ORGANIZATION

A World Health Organization (WHO, Geneva, Switzerland) international committee of PCR experts was organized in 1990 by WHO's Global Program on AIDS to make recommendations to WHO on research needs for standardization of PCR, particularly with respect to detection of HIV. The WHO Technical Working Group on Standardization of PCR prepared a report in 1990 (unpubl.; S. Osmanov, WHO, pers. comm.) that described recent developments and applications of PCR for diagnostic and research purposes, a consensus statement on the use and limitations of PCR as a diagnostic and research tool, and recommendations to WHO on research needs for further evaluation and standardization of PCR, training, and proficiency level testing programs.

The Technical Working Group recommended that PCR should not be used as a routine diagnostic test for HIV infection until further international collaboration on assessment and standardization of PCR has been carried out. The committee encouraged laboratories interested in using PCR for diagnosis of HIV to periodically test coded and noncoded reference panels to demonstrate reproducibility and satisfactory sensitivity and specificity. The committee recommended that WHO should make available coded and noncoded panels of DNA containing different amounts of HIV provirus and other PCR reference reagents through WHO collaborating centers on AIDS, including the National Institute for Biological Standards and Control (London, UK), Institute Pasteur (Paris, France), and National Institute of Allergy and Infectious Diseases (National Institutes of Health, Bethesda, MD). The WHO Steering Committee on Diagnostics is continuing activities in the area of PCR standardization (S. Osmanov, WHO, pers. comm.).

UNITED KINGDOM MEDICAL RESEARCH COUNCIL

The Medical Research Council (MRC) PCR Reference Centre, AIDS Collaborating Centre, National Institute for Biological Standards and Control (NIBSC; South Mimms, Potters Bar, Hertfordshire, EN6 3QG, UK) is involved with research on development and standardization of PCR, particularly with regard to HIV diagnosis. Functions of the Centre include being one of the WHO AIDS collaborating centers and providing advice and guidance on the performance of PCR. In 1992 the Centre published results of an international collaborative study, which they sponsored, to evaluate their complete set of PCR reference reagents for detection of HIV-1 by PCR and a proficiency panel of 10 coded DNA template standard solutions.[5]

In the above study, 26 laboratories analyzed the 10 coded DNA template standards using the PCR reference reagents and protocols provided. Template standards included a titration series prepared from a plasmid containing an incomplete HIV-1 genome, which was diluted in human chromosomal DNA as carrier. Although half the participating laboratories correctly assessed the coded samples, the other half apparently lacked quality control procedures; the authors reported that the overall false-positive result rate for the study was 9.3%, and the overall false-negative result rate was 7.4%. Twelve of the participating laboratories obtained no false-positive results. All of these 12 laboratories detected a sample containing 10 molecules of template DNA. The set of characterized reference reagents and template standard solutions is available for quality control assessment of HIV-1 PCR on request from the MRC AIDS Directed Program at NIBSC (Hertsfordshire, UK).

Recently, the PCR Reference Centre completed an international collaborative study to assess their set of HIV-1 DNA reference standards using a commercial diagnostic test kit for the detection of HIV-1. The Centre has also published a comprehensive review on quality control of PCR that includes internal quality control procedures, special considerations for diagnostic PCR assays, and external quality assurance schemes for PCR assays.[7] According to these investigators, quality control of PCR assays begins with good "in-house" procedures, well-trained personnel, and well-designed laboratories. The investigators also indicated that the introduction and use of well-characterized commercial kits will probably improve the situation with regard to diagnostic PCR quality control and quality assurance.

DEUTSCHES INSTITUT FÜR NORMUNG

The Deutsches Institut für Normung [DIN (German Institute for Standardization, Berlin, Germany)] is a voluntary consensus standards organization that establishes written test standards for materials, products, systems, and services. It is a member of the International Organization for Standardization (ISO) and the European Committee for Standardization (CEN). DIN's committee E9 (Immunology; Serodiagnostics of Infectious and Immunological Diseases) has nearly completed development of a general consensus standard that applies to the diagnostic detection of human infectious and immunological diseases by PCR.

The purpose of the general PCR standard ("Serodiagnosis of Infectious and Immunological Diseases; Polymerase Chain Reaction; Terminology, General Methods-Specific Requirements"), presently in draft form, is to establish terminology and minimum requirements to assure comparable and reproducible PCR test results among various laboratories. It is meant primarily for use in a clinical diagnostic laboratory, but it has information of value for any PCR laboratory. This standard alone will not be sufficient for use by anyone in a clinical diagnostic laboratory; the combination of the general standard plus a specific standard, for example, for detection of a specific microorganism or disease, is needed for practical utility. The development of a specific PCR standard for the detection of HIV genomic DNA (or RNA, after reverse transcription to cDNA) is also in progress by this committee.

The DIN general PCR standard defines the terminology used in the standard, describes the principle of PCR, discusses requirements for patient sample collection and preparation, and gives performance requirements concerning amplification of nucleic acid sequences (genomic DNA or DNA from reverse-transcribed RNA) and detection of amplified DNA. Also covered are recommendations for avoiding carryover and requirements concerning supplies, equipment, chemicals, reagents (including primers and probes), controls, and evaluation plus interpretation of assay results.

One question that arose during development of this diagnostic standard and that caused considerable debate was, How many different target sequences for amplification should be required to establish diagnostic reliability of a test result? A consensus was reached by the committee members that the required value depends on the particular type of test specimen being assayed for and that this requirement should not be specified in the general PCR standard. Another concern was standardizing the type and number of controls that should be included in a test assay. Present (December 1993) consensus agreement by committee members is that at least three controls should be included: (1) a positive control (definite number of target sequence copies to be determined individually for each target selected); (2) a negative control (nucleic acid, but no target sequence included), and (3) a reagent control (all reagents, but no nucleic acid).

The general PCR standard is not yet available as an official DIN standard but should be available in 1994. Further information about the draft standard can be obtained by contacting DIN Deutsches Institut für Normung e.V. (Burggrafenstr. 6, Postfach 1107, D-10772 Berlin 30, Germany).

AMERICAN SOCIETY FOR TESTING AND MATERIALS

The American Society for Testing and Materials (ASTM, Philadelphia, PA) is a voluntary consensus standards organization that establishes written national standards in all areas of life, including materials, products, systems, and services. ASTM's Committee E-48 on Biotechnology is developing a standard guide for detection of nucleic acid sequences by PCR. This guide ("Standard Guide for Detection of Nucleic Acid Sequences by the Polymerase Chain Reaction Procedure"), presently in draft form, is meant for use in any biotechnology laboratory involved with PCR detection of DNA sequences (or RNA following reverse transcription to cDNA). It could be used, for example, in a laboratory that detects plant or animal viruses or bacteria, or in a laboratory that uses PCR for any of a variety of research or medical applications. The objective of the guide is to describe the laboratory procedures that would be used to detect nucleic acid sequences by PCR, to identify the amplified segments of the sequences, and to establish the criteria necessary to assure comparable and reproducible results among different laboratories.

This guide is a general PCR standard guide and does not cover specific applications, for example, the PCR detection of a specific microorganism. It is meant to be used together with specific PCR guides to be developed by ASTM in the future. The draft guide includes sections on PCR terminology, principle of the method, detection material, laboratory precautions against contamination or carryover, recommendations concerning supplies, equipment, chemicals, and reagents, assay procedure, controls, and quality assurance. This general PCR guide is not yet available as an official ASTM standard. Further information about this draft standard can be obtained by contacting ASTM (1916 Race Street, Philadelphia, PA 19103).

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS

The National Committee for Clinical Laboratory Standards (NCCLS, Villanova, PA) is a voluntary consensus standards organization that promotes the development of written national standards and guidelines in the area of clinical laboratory testing. The organization's Subcommittee on Molecular Microbiology is developing guidelines on "Specifications for Molecular Microbiology Methods for Infectious Diseases." The guidelines, presently in draft form, but nearly in final form, will encompass several nu-

cleic acid-based tests, including nucleic acid amplification technologies, and will cover specimen handling, inhibitors and interfering substances, quality assurance, proficiency testing, specimen collection, contamination, test optimization, interpretation of results, different types of amplification formats, recommendations for manufacturers and clinical laboratories, and other aspects. Further information about these draft guidelines can be obtained by contacting NCCLS (771 E. Lancaster Ave., Villanova, PA 19085).

NATIONAL INSTITUTES OF HEALTH

The AIDS Research and Reference Reagent Program was established by the National Institute of Health's (NIH's) National Institute of Allergy and Infectious Diseases (NIAID) to provide reagents for AIDS research to investigators throughout the world and to be one of the WHO AIDS collaborating centers. This resource is acquiring standard reagents and panels of reagents useful in establishing quality control/quality assurance performance specifications. PCR standard reagents are periodically available to investigators free of charge, when registration is approved. A PCR panel consisting of an HIV-1 genomic DNA titration series is available for PCR quality control/quality assurance. A reagent program catalog can be obtained by contacting the Division of AIDS, NIAID (6003 Executive Boulevard, Bethesda, MD 20892).

The Division of AIDS of the NIAID supports the ongoing development of a quality control program for HIV-related virological measurements.[8] This program complements the NIAID-associated multicenter AIDS Clinical Trials Group (ACTG) involved in national AIDS drug treatment clinical trials. Standardization of HIV-1 DNA and RNA PCR assays is under development as part of the program according to H.J. Lin and F.B. Hollinger [Baylor College of Medicine, Houston, TX (pers. comm.)]. More information about the program can be obtained by contacting the NIAID Division of AIDS (see address above).

U.S. FOOD AND DRUG ADMINISTRATION

Participation of U.S. Food and Drug Administration (FDA) employees in PCR standardization activities has thus far been primarily indirect, for example, through participation in voluntary standards organizations such as ASTM, NCCLS, and DIN. The FDA regulates commercial DNA amplification-based in vitro diagnostic kits used to detect human diseases in clinical laboratories. Although FDA regulatory standards have not been developed for PCR diagnostic kits, a "Guidance Document" has been prepared by FDA's Center for Devices and Radiological Health. This document provides general guidance to manufacturers concerning the information needed by the FDA for evaluating applications for in vitro diagnostic devices intended to detect microorganisms in human specimens using nucleic acid amplification methods. The document entitled "Review Criteria for Nucleic Acid Amplification-Based In Vitro Diagnostic Devices for Direct Detection of Infectious Microorganisms" is available on request from the Division of Small Manufacturers Assistance, Center for Devices and Radiological Health, FDA (HFZ-220) (Rockville, MD 20857).

DISCUSSION

As readers of this journal are aware, PCR is rapidly evolving and continuously undergoing procedural modifications. Different types of PCR-based formats are being developed, for example, quantitative competitive PCR,[9] and different applications of the procedure continue to be identified. Therefore, continued PCR standards development can be expected, and frequent updating of completed standards will be necessary. Because there are several different PCR standards being developed, requests for international harmonization of these standards can be expected.

If you are interested in incorporating quality control/quality assurance into your PCR protocols, and you are not required by your laboratory to follow specific requirements or guidelines, then you may wish to (1) contact the standards organizations mentioned in this overview that are appropriate with regard to your interests and the goals of your laboratory, whether biotechnology, diagnostics, forensics, or other, and request available draft or completed (published) standards or guidelines; (2) follow recommended precautions for avoiding carryover contamination, such as those discussed by Kwok and Higuchi;[10] (3) use an established protocol for eliminating contaminating amplicons carried over from previous reactions, such as the "UNG" procedure,[11] if possible and appropriate for your laboratory; (4) study results of the multicenter collaborative trials mentioned in this overview; (5) obtain and utilize an available coded panel of test substances with controls periodically to demonstrate quality performance ("external quality assurance"), if available, or if not available, design your own coded panel; and (6) read relevant articles, for example, on good laboratory practice for PCR instrumentation[12] and on quality control of PCR.[7]

If you have PCR expertise and wish to participate in the development of present or future PCR standards, contact the standards organization that is most suited to your interests. I predict that such organizations will welcome your participation. Furthermore, you might find it a worthwhile, educational, and enjoyable experience.

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