Medical Virus Molecular Diagnosis

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Abstract

The development of molecular techniques, particularly the use of the polymerase chain reaction, has transformed the detection of infectious diseases (PCR). Because of the method's great sensitivity and ease of use in detecting any known DNA sequence, it has found widespread use in the biological sciences. Real-time PCR assays have lately made significant contributions, with the addition of a fluorescent probe detection system resulting in increased sensitivity over conventional PCR, the ability to confirm the amplifcation product, and the capacity to quantify the target concentration. Furthermore, nucleotide sequence analysis of the amplifcation products has aided epidemiological studies of infectious disease outbreaks and the monitoring of infection treatment outcomes, particularly for viruses that mutate often. The applications of qualitative and quantitative real-time PCR, nested and multiplex PCR, nucleotide sequence analysis of amplifed products, and quality assurance with nucleic acid testing (NAT) in diagnostic laboratories are discussed in this study.

Keywords: Virology • Probe detection • Medicine • Allergy

Introduction

The discovery of molecular tools has transformed the diagnosis of human virological disease, as it has most bioscience disciplines. While the impact of this influence has been felt for well over a decade, the change is far from complete. Although different molecular approaches have been available for decades, the technologies have only recently advanced to the point that they may be used for diagnostic purposes. The development of real-time PCR, which combines product detection and confirmation using highly sensitive hybridisation probes, as well as target quantification if needed, in a very rapid experiment, has been the most important. In addition, gene sequencing has made a significant contribution. The use of molecular systematics to directly assist patient management has been facilitated by the combination of inexpensive and rapid sequencing chemistries, computer-based phylogenetic analysis software, and electronic interrogation of Internet-accessible gene sequence databases, both for individual patients as in drug resistance testing, and within the community as in epidemiological analysis of infectious outbreaks. The scope of this study precludes a thorough examination of all molecular approaches available or in use for the detection of all medically significant viruses. However, it is critical to first focus on the general issue of ensuring result correctness, as any test would be pointless without it. Following that, specific typical examples of the application of molecular methods for the diagnosis and management of human virological disorders will be shown.

Assurance of the quality of the outcome

The quality of the results is critical, and it can only be achieved if all parts of the test technique are thoroughly understood [1]. The design, development, and validation of the test for the range of specimen types, collection conditions, and sample quality for which the test is expected to function begins with the design, development, and validation of the test for the range of specimen types, collection conditions, and sample quality for which the test is expected to function. These performance standards are typically specified for commercial test kits, however before in-house tests can be used, each aspect must be thoroughly evaluated and validation analyses documented. Regardless of the source of the test, the ongoing performance must be closely checked and problems reported to ensure that the test's result quality is maintained. Laboratory design and personnel competency are other important considerations. Quality assurance issues are usually outlined in national standards and guidelines, which must be followed in order to achieve good laboratory practise and are frequently mandated in order to obtain statutory certification [2-3].

The causes of false results

To ensure test performance, each assay batch must include sufficient positive and negative controls. They must be chosen to assure the validity of each step: nucleic acid extraction, reagent master-mix preparation, nucleic acid aliquoting, amplifcation, and detection. The use of weakly positive control material, with nucleic acid concentrations just beyond the limit of detection, should be used to evaluate the performance of the entire test procedure, including extraction, to ensure test sensitivity is maintained for each batch. A negative patient sample is also necessary, and it should be processed last at each step, after any positive controls, to avoid sample contamination. Negative controls should be strewn throughout large batches of assays [4-5]. A negative water control in the mastermix, not opened during the aliquoting of nucleic acid, is also recommended to ensure that the mastermix is uncontaminated. Even if the controls are performing well, clusters of positive results, particularly after a substantially positive sample, should be treated with caution until the suspect samples can be re-extracted and retested to confirm the result.

Contamination

Understanding the sources of misleading results is necessary for their eradication. The possibility of contamination of new tests with amplifed product (amplicon) from prior amplifcations of the same target sequence offers by far the biggest risk, and is the most prevalent source of false positive results with PCR-based testing, because the same techniques are run repeatedly [6-7]. To avoid contamination, it is critical that the functions of (a) PCR reagent storage and master mix preparation, (b) nucleic acid extraction, (c) nucleic acid addition to PCR mixes, and (d) PCR amplifcation and post-PCR manipulation, such as running gels, sequencing, or cloning of products, are physically separated, with independent airfows. Each should, ideally, be carried out in different rooms. To avoid contamination of set-up regions with previously amplifed product, a unidirectional workfow from one function to the next is required. Each department needs its own pipettors, equipment, consumables, and personal protective equipment for the workers, such as lab coats and gloves. Staff must be well-trained and adhere to strict sterile procedures. If racks must be returned to the unidirectional workfow, they must first be disinfected for 4 hours in 2 percent sodium hypochlorite or the equivalent. At all stages, PCR-certified fltered tips should be used, and reagent, sample, and PCR tubes should be kept covered, only opening when absolutely necessary. Because the latter criteria cannot be met, the usage of ganged cap strips should be avoided. Wiping down work locations and pipettes with 2 percent sodium hypochlorite disinfectants after each use, as well as irradiating work areas with UV light, is also recommended [8-10]. For stages 1–3, a biohazard hood is recommended, both to shield the operator from potentially infectious agents and to limit the possibility of sample contamination. Smaller particles, such as viruses and nucleic acid molecules, are expected to be efficiently retained by the HEPA filters in such cabinets, which are normally rated to 0.3Mm. Viruses are frequently aggregated, linked to, or confined within larger particles such as cells, and molecular interactions such as electrostatic attraction and Brownian motion, rather than size exclusion, are more likely to maintain nucleic acid molecules efficiently.

However, cabinets used to manipulate post-amplifcation products, such as during two-tube nested amplifcation, should be externally vented to avoid recirculation of any amplicons that pass through the filters into the exhaust. The use of dUTP instead of dTTP in the PCR master mix, as well as the use of Uracil-DNA Glycosylase (UNG) to degrade previously amplifed uracil-containing contaminating amplicons, a method commonly included in commercial kits, should be considered to eliminate contamination, though it is only effective when the level of contamination is low. If quick examination of the result is not practicable, PCR samples should be kept at -20°C. Furthermore, using dUTP and UNG for one-step RT-PCR is troublesome, necessitating the use of a heatstable reverse transcriptase active above 60°C to prevent cDNA degradation before PCR. Assay sensitivity may be reduced as a result of such RT-PCR needs and circumstances. Multiple tests are routinely performed on samples sent to a diagnostic laboratory. Before the specimen is sent for virological testing, it is usually subjected to biochemical or haematological tests conducted by auto-analysers. During injection sampling, such auto-analysers can cross-contaminate specimens. Setting aside an aliquot specifically for molecular testing eliminates the problem, but it also adds the potential of transcribing and aliquoting errors, as well as the possibility of specimens being too small to split properly. The presence of amplifcation reaction inhibitors in the sample, such as haemoglobin, lactoferrin, and bile salts, is the most common cause of erroneous negative results. Because the nucleic acid contained in the silicagel membrane can be cleaned very well before elution, most commercial nucleic acid extraction techniques incorporating a spin or vacuum column allow for efficient removal of inhibitors. Similarly, approaches that use magnetic beads to trap and keep nucleic acid produce high rates of recovery and purity. Some samples, however, will remain inhibitory, and these samples must be identified using effective inhibition controls. To offer a meaningful assessment of the presence of inhibitors, such control nucleic acid should be present only at levels close to the assay's limit. We prefer to run a duplicate inhibition control for each sample spiked with the low positive control since integrating both a separate inhibition control target and the test target in a multiplex reaction can reduce amplifcation sensitivity. However, if maximal sensitivity is not required, such multiplex, simultaneous testing of test and inhibitory control targets may be appropriate. If the rate of inhibition has been determined to be very low, inhibition controls in specific specimen types can be skipped. Samples containing inhibitors can be further treated with chelating agents, such as Chelex 100 chelating resin (Bio-Rad Laboratories, Hercules, CA, USA), to remove divalent metal cations, or diluted until the inhibition is no longer detectable, but keep in mind that these steps will reduce the test sensitivity. If inhibition is a concern during the development phase due to the nature of the materials being tested, such as faeces, amplifcation facilitators such as bovine serum albumin and betaine could be investigated to reduce inhibition problems. Furthermore, some polymerases may be more resistant to inhibitors than others.

Target homology

Primer and probe target sites should ideally come from areas of the genome that are conserved throughout the viral strains to be detected. To ensure broad responsiveness of the test for all wild strains of the virus, this is frequently done to functionally limited areas within protein-encoding non-structural genes. This isn't always doable, though.

RNA viruses come in a wide variety of strains in the wild. Although known strain diversity at the target location is accommodated in the primer and probe design, the genetic diversity of some viruses, such as caliciviruses and HIV, exceeds the test's ability to detect all strains without sacrificing sensitivity.

Conclusion

As molecular approaches have progressed and made a significant contribution to the diagnosis of human viral disease, there has been a growing recognition of the importance of quality assurance in order to generate clinically useful results. Furthermore, the targeted nature of PCR has some inherent constraints that are difficult to regulate, particularly in virology, where the diverse composition of the viral genome can make test design and performance difficult. Nonetheless, being aware of these limitations will lead to a better knowledge of this new technology and enable the full potential of these current diagnostic tools to be realised.

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