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Sensitivity of Reverse Transcription: Polymerase Chain Reaction

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Abstract

RT-PCR uses RNA as starting material for in vitro macromolecule amplification. The discovery of retroviral reverse transcriptase in the early 1970's ultimately made RT-PCR possible. Reverse transcriptase is an RNA-dependent DNA polymerase, catalyzing DNA synthesis using RNA as the template. The end product is known as complementary DNA (cDNA).

Keywords: Hepatitis C virus • DNA amplification • Non-Hodgkin's lymphomas

Commentary

Complementary DNA (cDNA) isn't subject to RNase degradation, making it more stable than RNA. In RT-PCR, the starting RNA is subsequently degraded, dsDNA is produced, and PCR amplification proceeds in the usual manner. RNA extraction kits for both manual and automatic RNA purification exist and, when combined with RT-PCR, make RNA analysis within the clinical laboratory virtually as rapid and equally sensitive as PCR-based DNA amplification. RT-PCR is commonly used in the diagnosis and quantification of RNA virus infections (e.g., human immunodeficiency virus and hepatitis C virus) and the analysis of mRNA transcripts such as those produced by translocations commonly associated with non-Hodgkin's lymphomas, leukemias, and sarcomas. Gene expression profiling is likely to have a major impact on molecular diagnostics in the coming years and will depend on RNA analysis using RT-PCR and possibly high-density arrays.

RT-PCR is becoming an increasingly important tool for the diagnosis of various diseases. Evaluations of RT-PCR are often performed either by agarose gel electrophoresis, or by real-time techniques (RT-qPCR). As laboratory equipment is becoming reliable and also cheaper, coupling of liquid handling robotics for macromolecule isolation and RT-qPCR is becoming practicable. Another advantage of both the quality gel-based RT-PCR and therefore the RT-qPCR is that, thanks to their high sensitivities, pooled samples are often tested. Especially, the utilization of RT-qPCR allows rapid and reliable testing of herds at the perimeter of an epidemic so as to avoid preemptive slaughter. Despite the benefits that RT-PCR methodology may have over conventional diagnostic tests, it's extremely susceptible to false negative or false positive results. False negative results can arise when the macromolecule is degraded, or when the reaction mixture contains inhibitors. Thanks to its high sensitivity, false positive results may arise from contaminations, either from sample to sample or from other sources. This suggests that before diagnostic laboratories can replace any test, their RT-PCR protocols need to be validated, and regular participation in proficiency testing must prove that performance of the methods used is accurate. Additionally, specification concerning sensitivity of the detection must be defined. This is often important when samples are pooled. In summary, analytical performance must be adequate to or better than that of the quality method.

How the RT-PCR Works?

Reverse transcription PCR (RT-PCR) uses mRNA instead of DNA because the starting template. First, the enzyme polymerase uses the mRNA template to supply a complementary single-stranded DNA strand called cDNA during a process referred to as reverse transcription. Next, DNA polymerase is employed to convert the single-stranded cDNA into double-stranded DNA. These DNA molecules can now be used as templates for a PCR reaction as described above. The value of RT-PCR is that it is often won't to determine if an mRNA species is present during a sample or to clone a cDNA sequence for a subsequent experiment.

Elementary Components of a RT-PCR Technique

Reverse Transcription PCR (RT-PCR) was developed to amplify, isolate or identify RNA sequences. The principle is to convert RNA into its complementary DNA sequence by polymerase, to synthesise a second strand with DNA polymerase, and eventually to get a ds cDNA molecule which can be amplified by PCR in the normal way. RT-PCR is both specific and sensitive, and is particularly suitable as a means of detecting a small number of copies of the target RNA. Also during this case there's an increasing risk of contamination, due to the upper number of operations to be performed. Establishing amplicon identity is achieved by either sequencing or by hybridization with labeled probes. Recently, certain thermo stable reverse transcriptase have become available; these can allow reverse transcription to be performed at an elevated temperature (typically 60 °C), which improves reaction efficiency by minimizing the secondary structure of the template RNA. The polymerase (a recombinant enzyme derived from T. thermophilus) functions both as a reverse transcriptase and as a DNA

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polymerase. An application of RT-PCR in food toxicant detection has been documented within the case of some pathogenic RNA viruses, particularly the NLVs (Norwalk-like viruses). Reverse Transcription PCR (RT-PCR) was developed to amplify, isolate or identify RNA sequences. The principle is to convert RNA into its complementary DNA sequence by polymerase, to synthesise a second strand with DNA polymerase, and eventually to get a ds cDNA molecule which can be amplified by PCR in the normal way. RT-PCR is both specific and sensitive, and is particularly suitable as a means of detecting a small number of copies of the target RNA. Also during this case there's an increasing risk of contamination, due to the upper number of operations to be performed. Establishing amplicon identity is achieved by either sequencing or by hybridization with labeled probes. Recently, certain thermo stable reverse transcriptase have become available; these can allow reverse transcription to be performed at an elevated temperature (typically 60 °C), which improves reaction efficiency by minimizing the secondary structure of the template RNA. The polymerase (a recombinant enzyme derived from T. thermophilus) functions both as a reverse transcriptase and as a DNA polymerase. An application of RT-PCR in food toxicant detection has been documented within the case of some pathogenic RNA viruses, particularly the NLVs (Norwalk-like viruses).

Conclusion

Given the number of choices available for every aspect of RT- PCR, it may be difficult to determine what detection chemistry, quantitation method, normalization gene, etc., to use. Although every experimental situation is unique and requires specialized consideration, some general guidelines can be suggested. In terms of quantitation method (absolute versus relative), the majority of users will not require absolute data such as copy number of transcripts or nanograms of DNA, and therefore, relative quantitation will suffice.

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