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Perspective

A Brief Description of How Reverse Transcription Polymerase Chain Reaction (RT-PCR) Used in Molecular Biology

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DESCRIPTION

Reverse transcription polymerase chain reaction (RT-PCR) is a laboratory technique that combines reverse transcription of RNA into DNA (also known as complementary DNA or cDNA) with polymerase chain reaction amplification of specified DNA targets (PCR). For in vitro nucleic acid amplification, RT-PCR uses RNA as a starting material. Reverse transcriptase is an RNA-dependent DNA polymerase that uses RNA as a template to catalyze DNA synthesis. Complementary DNA is the final product (cDNA). Because cDNA is not degraded by RNAase, it is more stable than RNA.

In RT-PCR, the starting RNA is destroyed, dsDNA is generated, and PCR amplification is carried out as usual. RNA extraction kits for manual and automated RNA purification are available, and when used in conjunction with RT-PCR, RNA analysis in the clinical laboratory is nearly as fast and sensitive as PCR-based DNA amplification. RT-PCR is extensively utilized in the diagnosis and quantification of RNA viral infections (e.g., human immunodeficiency virus and hepatitis C virus) as well as the study of mRNA transcripts produced by translocations found in non-lymphomas, Hodgkin's leukemia, and sarcomas. In the coming years, gene expression profiling is expected to have a significant impact on molecular diagnostics, and it will rely on RNA analysis utilizing RT-PCR and possibly high-density arrays.

Molecular diagnostic assays for Plasmodium falciparum parasites are increasingly being employed in clinical trials and field surveillance investigations to enable ultrasensitive identification of infection. The detection limits of ribonucleic acid (RNA)-based tests targeting 18S rRNA have been reported to be as low as a single infected red blood cell (RBC) in a significant volume of blood. However, validation testing at such limiting doses is impeded by the so-called Poisson distribution of such uncommon events, which can lead lab technicians to set the limit of detection incorrectly higher (i.e., less sensitive) than the assay can detect. We wanted to show that the Plasmodium 18S rRNA quantitative reverse transcription PCR has analytical sensitivity (qRT-PCR).

A laboratory method used to make many copies of a specific genetic sequence for analysis. It converts a specific piece of RNA into a matching piece of DNA using an enzyme called reverse transcriptase. Another enzyme called DNA polymerase then amplifies (makes a lot of copies of this strand of DNA). The amplified DNA copies can be used to determine whether a gene is producing a certain mRNA molecule. The reverse transcription-polymerase chain reaction can be used to examine for specific modifications in a gene or chromosome, as well as the activation of specific genes, which can aid in the diagnosis of diseases like cancer. It can also be used to investigate the RNA of specific viruses, such as the Human Immunodeficiency Virus (HIV) and the hepatitis C virus, to aid in the diagnosis and monitoring of infections. RT-PCR is another name for reverse transcription polymerase chain reaction.

Fluorescence Activated Cell Sorting (FACS) was used on synchronous P. falciparum cultures doubly stained for DNA and RNA and was followed by qRT-PCR on the individual sorted cells spiked with negative whole blood. Over 95% of individual single-ring infected RBCs were detected by qRT-PCR. The formally measured median 18S rRNA content per individual ring-stage P. falciparum parasite was 9,550 copies (interquartile range 8,130-12,300). Thus, one can confidently rely on Plasmodium 18S rRNA qRT-PCR to detect one parasite per 50-µL blood sample.

Expand TM Reverse Transcriptase is an RNA-directed DNA polymerase that can amplify cDNA fragments up to 13.5 kb in a two-step RT-PCR test. The enzyme is a genetically modified form of the reverse transcriptase from the Maloney Murine Leukemia Virus (M-MuLV-RT). RNase H activity is reduced to undetectable levels by a point mutation in the RNase H sequence. When compared to employing the native M-MuLV-RT, there are noticeable gains in terms of higher numbers of full-length cDNA transcripts and longer transcripts.

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