

Real-Time NASBA

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Abstract

NASBA is an isothermal nucleic acid amplification method that is particularly suited to detection and quantification of genomic, ribosomal or messenger RNA. The product of NASBA is single-stranded RNA of opposite sense to the original target. The first developed NASBA methods relied on liquid or gel-based probe-hybridisation for post-amplification detection of products. More recently, real-time procedures incorporating amplification and detection in a single step have been reported and applied to a wide range of targets. Thus real-time NASBA has proved to be the basis of sensitive and specific assays for detection, quantification and analysis of RNA (and in one case DNA) targets. Molecular beacons have been utilised for detection of NASBA products in all published real-time procedures whether for commercially-available kits or for in-house diagnostic assays. As experience in design of such fluorescent-labelled probes increases and fluorimeters suitable for their detection become widely available, real-time NASBA methodology will be confirmed as a suitable alternative to other real-time amplification methods such as reverse transcriptase PCR (RT-PCR).

Introduction and Background to the Methodology

NASBA technology has provided an alternative method to standard procedures with a broad application for the amplification and detection of a range of nucleic acid targets (Compton, 1991). The majority of applications have been developed for detection and analysis of RNA targets including viral genomes, viroids, ribosomal RNA (rRNA) and messenger RNA (mRNA). Advantages of NASBA above methods such as RT-PCR include fast amplification kinetics and selective amplification of RNA in a background of DNA. The amplification is isothermal and the single-stranded RNA amplicons produced can be used directly in subsequent rounds of amplification or probed for detection without the need for denaturation or strand separation. Thus thermocycling is not required for NASBA.

Sample Preparation for NASBA

Amplification inhibitors and RNA integrity are the main cause of concern when preparing clinical specimens for NASBA. There is no need to degrade associated DNA if non-spliced mRNAs are to be analysed by a transcription-mediated amplification system such as NASBA since the standard method does not utilise temperatures which would denature and allow amplification of DNA. The method first detailed by Boom and colleagues (Boom *et al.*, 1990) is widely used for extraction of RNA for use in NASBA and reagents for this are commercially available. One advantage of this procedure is that dilute clinical samples can be concentrated during the extraction and the approach has been validated for a wide range of specimen types.

NASBA Primers and Probes

As for other amplification-based procedures, NASBA requires two target-specific oligonucleotides suitable for use as primers in the amplification phase. A region for probe-specific detection of amplified products also needs to be identified. Design of primers and probes for NASBA, whether for use in end-point or real-time detection methods,

