

# Nucleic Acid Sequence-Based Amplification using molecular beacons for quantification of enterovirus RNA

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**Quantificazione dell'enterovirus RNA mediante Nucleic Acid Sequence-Based Amplification utilizzando sonde molecular beacons**

## INTRODUCTION

Enterovirus (EV) infections are very common in young children and lead to a wide spectrum of clinical presentations. Most cases are asymptomatic or mild, and usually recover without any special medication. However, especially in children, in the elderly, and in immunocompromised patients, infection can lead to death (3).

Rapid detection of enterovirus infections is essential in the management of different respiratory pathologies.

Molecular approaches have opened the way to such rapid, but also specific and sensitive, diagnostic tests. In particular, nucleic acid sequence-based amplification (NASBA) assays have been demonstrated to be more sensitive for detection of enteroviruses than RT-PCR (2, 1).

The aim of this study was to develop a molecular beacon-based real time NASBA assay for detection and quantification of enterovirus.

## METHODS

Primers and probe oligonucleotide sequences were obtained from literature. Standards production provided different stages.

In particular, enterovirus coxsackie B4 RNA was extracted by using the automatic extractor NucliSENS easyMAG platform (bioMérieux).

Amplification reactions were carried out using the reagents from the NucliSens EasyQ Basic Kit (bioMérieux) and the the EasyQ incubator (bioMérieux).

Primers and probes were modified by adding 60% dimethyl sulfoxide (DMSO) for oligonucleotides stabilization and different primers, probes and KCl concentrations were tested.

In particular we used two sets of primers (200 nM) and two probes (30 nM) and the amplifica-

tion reactions were performed at 80 mM KCl. Amplification product was digested with DNase (*Invitrogen*) and purified using RNagent kit (*Promega*).

Ten fold dilutions were performed in order to obtain  $10^{10}$  to  $10^0$  RNA copies/reaction.

Dynamic range, sensitivity and detection limit were evaluated.

A quantity of  $10^5$  of an internal control (UIA) was added to reaction mixture.

## RESULTS

EV-RNA standard curves showed a dynamic range from  $10^8$  to 100 copies ( $R^2 = 0.994$ ) and a sensitivity of 10 copies.

Limit of detection was 1 copy.

## CONCLUSIONS

In conclusion NASBA assay resulted sensitive, specific and more suitable for quantification of EV. It could represent an useful tool for rapid detection of enteroviruses.

## REFERENCES

1. Leone G, van Schijndel H, van Gemen B, Kramer FR, Schoen CD. Molecular beacon probes combined with amplification by NASBA enable homogeneous, real-time detection of RNA. *Nucl Acids Res* 1998; 26: 2150-5.
2. Patterson SS, Casper ET, Garcia-Rubio L, Smith MC, Paul JH. Increased precision of microbial RNA quantification using NASBA with an internal control. *J Microbiol Methods* 2005; 60: 343-52.
3. Pei-Chieh W, Li-Min H, Chuan-Liang K, Tsui-Yen F, Ai-Ling C, Luan-Yin C. An outbreak of coxsackievirus A16 infection: comparison with other enteroviruses in a preschool in Taipei. *J Microbiol Immunol Infect* 2010; 43: 271-7.

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