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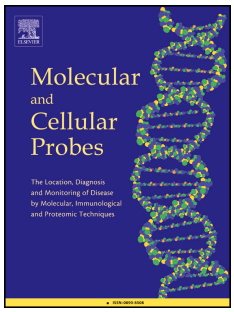

Assessment of NS1 gene-specific real time quantitative TaqMan PCR for the detection of Human Bocavirus in respiratory samples

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Assessment of NS1 gene-specific real time quantitative TaqMan PCR for the detection of Human Bocavirus in respiratory samples

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Abstract

Human Bocaviruses (HBoV) were associated with respiratory diseases. Here, we assessed a TaqMan®-based PCR for the detection of all four HBoV subtype infections with a sensitivity up to 15 copies/reaction. To evaluate this assay on clinical samples, 178 nasopharyngeal aspirate specimens from pediatric cases were analyzed and HBoV genome was detected in 13 out of 178 patients with a viral load range between $1.6x10^3$ and $9.4x10^7$ copies/ml. These results indicated that this method could be used as an alternative technique for the diagnosis of HBoV infection.

Methods, Results and Discussion

Human Bocavirus (HBoV) is a single strand DNA virus classified into the family *Parvoviridae*, subfamily *Parvovirinae*, and genus *Bocavirus* [**1**]. HBoV is a major pathogen in viral respiratory and gastrointestinal infections, detected worldwide without any significant geographic prevalence. Genetic analyses provided the recognition of four HBoV subtypes [**1-2**]. The first subtype (HBoV-1) is generally detected in the respiratory tract infection whereas HBoV-2, 3 and 4 subtypes are more frequently associated to gastrointestinal infections [**2-4**]. The pathogenesis of HBoV is still unclear but the first consistent model was shown in respiratory infection. In this case, HBoV is transmitted by respiratory route and replicates in the respiratory epithelia of patients invading their blood stream. Subsequently, HBoV may localize in the gastrointestinal district from blood or ingestion. Viral spreading is then possible through coughing and/or stool. Interestingly, several studies demonstrated that HBoV can be co-detected in respiratory secretions or stool with other viruses in symptomatic cases suggesting a secondary role as bystander agent for HBoV [**2-5**]. Conversely, recent observations showed that HBoV is directly involved in pathogenetic effects and can determine severe clinical consequences, when it productively infects newborns or very young children [**1,6-8**]. The increasing importance of HBoV in the human pathology requires adequate molecular techniques for the diagnosis of direct HBoV infection. In this paper, we assessed a new real time TaqMan®-based quantitative PCR (qPCR) method based on the use of oligonucleotide pairs, able to detect all four HBoV subtypes. Furthermore, this technique was evaluated on nasopharyngeal secretion samples obtained from pediatric patients with respiratory symptoms.

To develop this specific TaqMan®-based qPCR for the detection of HBoV genome, we analyzed HBoV genome sequences belonging to all four HBoV subtypes available from National Center for Biotechnology Information (NCBI) sequence database using ClustalW to select specific region conserved in all HBoV strain genomes (**Figure 1A**).