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Short communication

# Comparison of phenotypic and genotypic diagnosis of acute human bocavirus 1 infection in children

Nicola Isabelle Kols[a,b,*], Heli Aatola[c], Ville Peltola[d], Man Xu[e], Zaiga Nora-Krukle[e,f], Klaus Hedman[e,g], Aurelija Zvirbliene[h], Hanna Toivola[c], Tytti Vuorinen[i], Juha M. Koskinen[c], Andrea H.L. Bruning[j], Andreas Christensen[a,b], Maria Söderlund-Venermo[e], Janne O. Koskinen[c]

[a] Department of Medical Microbiology, Clinic of Laboratory Medicine, St. Olavs Hospital HF, Trondheim University Hospital, 7006, Trondheim, Norway
[b] Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, 7491, Trondheim, Norway
[c] ArcDia International Oy Ltd, 20521, Turku, Finland
[d] Department of Paediatrics and Adolescent Medicine, Turku University Hospital and University of Turku, 20521, Turku, Finland
[e] Department of Virology, University of Helsinki, and Helsinki University Hospital, 00290, Helsinki, Finland
[f] Institute of Microbiology and Virology, Riga Stradins University, LV-1067, Riga, Latvia
[g] Helsinki University Hospital, 00029, Helsinki, Finland
[h] Department of Immunology and Cell Biology, Institute of Biotechnology, Life Sciences Center, Vilnius University, LT-10223, Vilnius, Lithuania
[i] Department of Clinical Microbiology, Turku University Hospital and Department of Virology, University of Turku, 20521, Turku, Finland
[j] Department of Pediatric Infectious Diseases, Emma Children's Hospital, Academic Medical Center, 1105, Amsterdam, the Netherlands

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ABSTRACT

*Background:* Diagnosis of human bocavirus 1 (HBoV1) has been based on qualitative PCRs detecting HBoV1 DNA or detection of HBoV1 mRNA.
*Objective:* This study aims to assess whether a rapid and automated HBoV1 antigen test is suitable for diagnosis of acute HBoV1 infection.
*Study design:* HBoV1 antigen detection has been compared with quantitative HBoV1 DNA PCR and HBoV1 mRNA RT-PCR.
*Results and conclusion:* We conclude that HBoV1 antigen detection has higher clinical specificity and positive predictive value than HBoV1 DNA qualitative PCRs, yet a lower sensitivity than HBoV1 mRNA detection. Additionally, HBoV1 antigen detection is beneficial in its rapidity and availability as a point-of-care test.

## 1. Background

Human bocavirus 1 (HBoV1) is a common cause of respiratory tract infections (RTI) in young children and causes serious lower RTI in children younger than six years of age [1–5]. The diagnosis of HBoV1 infections has traditionally been based on qualitative PCRs detecting viral DNA. Recent studies have however shown that the clinical value of this approach is low due to persistence of HBoV1 DNA in the airways, leading to frequent detection of low concentrations in healthy children [6–8]. Clinical specificity of HBoV1 mRNA detection has been shown to be superior to that of HBoV1 DNA detection [6,9,10]. Detection of HBoV1 antigen is another diagnostic approach. An automated HBoV1 antigen test based on immunometric detection has recently been developed and has shown promise in preliminary studies [11]. Antigen detection is well suited for automation and point-of-care diagnostics, and may be beneficial to making an HBoV1 diagnosis by providing rapid and specific results.

## 2. Objectives

The aim of this study was to compare HBoV1 antigen detection with HBoV1 mRNA RT-PCR and quantitative HBoV1 DNA detection (qPCR) in nasopharyngeal swabs (NPS) from children with RTI.

## 3. Study design

NPS (N = 632) were collected from children admitted for RTI to the Pediatric Emergency Department of Turku University Hospital from November 2015 to October 2016. All samples were stored in mariPOC RTI sample buffer at −20 °C throughout the study period.

* Corresponding author at: Department of Medical Microbiology, Clinic of Laboratory Medicine, St. Olavs Hospital HF, Trondheim University Hospital, Postboks 3250 Torgarden, 7006, Trondheim, Norway.
*E-mail address:* nicola.isabelle.kols@stolav.no (N.I. Kols).

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The HBoV1 antigen test is part of a completely automated system for multianalyte identification of respiratory viruses and bacteria (influenzavirus A, influenzavirus B, respiratory syncytial virus, human coronavirus OC43, human metapneumovirus, human bocavirus 1, parainfluenzavirus type 1, parainfluenzavirus type 2, parainfluenzavirus type 3, adenovirus, *Streptococcus pneumonia*) and has IVD-CE approval for diagnostic testing (mariPOC® respi test, ArcDia International Ltd, Turku, Finland). It is an immunoassay based on separation-free two-photon excitation fluorometry and targets the HBoV1 capsid protein VP3 [12]. The mariPOC test reports strong positive samples in 20 min while low-positive and negative results are confirmed in 2 h. For mariPOC, NPSs were diluted in 1.3 mL of mariPOC RTI sample buffer for rapid prospective on-site testing on a random-access immunoassay analyzer. The test was in routine use at the Pediatric Emergency Department of Turku University Hospital for rapid point-of-care diagnostics of RTI throughout the study period. According to local guidelines, the mariPOC antigen test is used for all children hospitalized with acute RTI. This Pediatric Emergency Department serves a population of 70 000 children or adolescents (< 16 years of age) and receives patients mostly by referral from primary care clinics. The majority of children hospitalized with RTIs are younger than 5 years of age and have lower RTIs.

The Turku University Hospital approved the study with a waiver of informed consent by the Ethics Committee, as the study involved re-analysis of de-identified samples collected as part of routine care.

All samples were analyzed in duplicate with HBoV1 DNA qPCR and HBoV1 mRNA reverse-transcription (RT)-PCR, as described previously [10,13]. The cutoff for a high viral load in DNA qPCR was $> 10^6$ copies/mL.

Correlations between mRNA, high DNA load and antigen were measured by the phi coefficient of correlation. Sensitivity, specificity, and positive predictive value (PPV) for the antigen test were calculated by using mRNA RT-PCR and DNA qPCR as references.

## 4. Results

HBoV1 DNA, a high DNA load, mRNA, or antigen were detected in 96 (15.2%), 19 (3%), 17 (2.7%), and 13 (2.1%) of the 632 NPA samples, respectively (Table 1). Out of the 96 samples positive for DNA, only 19.8% had a high DNA load, 17.7% were positive for mRNA, and 13.5% for antigen. Of the samples with a high DNA load, 89.5% were positive for mRNA and 68.4% for antigen, whereas none of the low DNA-load samples harbored mRNA or antigen.

Positive HBoV1 antigen test results were available in 20 min for nine of the 13 samples, and the four remaining results were available in two hours.

Presence of HBoV1 antigen correlated well with both mRNA and high DNA load. The phi coefficient for the correlation between mRNA and antigen was 0.87, whereas it was 0.94 for mRNA and high DNA load.

Specificity and PPV of the HBoV1 antigen test were excellent, while the sensitivity was lower. With mRNA RT-PCR as reference, specificity, sensitivity, and PPV were 100%, 76.5%, and 100%, respectively. With high DNA load by qPCR as reference, the figures were 100%, 68.4%, and 100% (Table 2).

**Table 1**
Results of the HBoV1 diagnostic tests in relation to detection of HBoV1 mRNA and HBoV1 DNA quantity (N = 632).

| | | No. | HBoV1 mRNA + No. (%) | HBoV1 mRNA – No. (%) |
|---|---|---|---|---|
| HBoV1 antigen | Pos | 13 | 13 (100) | 0 |
| | Neg | 619 | 4 (0.6) | 615 (99.4) |
| HBoV1 DNA quantity | High[a] | 19 | 17 (89.5) | 2 (10.5) |
| | Low[a]/neg | 613 | 0 | 613 (100) |
| HBoV1 DNA presence | Pos | 96 | 17 (17.7) | 79 (82.3) |
| | Neg | 536 | 0 | 536 (100) |
| | | | HBoV1 DNA high load | HBoV1 DNA low load or negative |
| | | No. | No. (%) | No. (%) |
| HBoV1 antigen | Pos | 13 | 13 (100) | 0 |
| | Neg | 619 | 6 (1.0) | 613 (99.0) |
| HBoV1 mRNA | Pos | 17 | 17 (100) | 0 |
| | Neg | 615 | 2 (0.3) | 613 (99.7) |

[a] High HBoV1 DNA load: $\geq 10^6$ copies/mL NPA; low load: $< 10^6$ copies/mL NPA.

## 5. Discussion

The widely used qualitative HBoV1 DNA PCRs yield results of low clinical specificity and very low PPV [1,2,6,7,14]. We confirmed this. With mRNA RT-PCR as a reference, the qualitative DNA PCR specificity and PPV were 87.2%, and 17.7%, respectively (Table 2).

Serological tests based on detection of HBoV1-specific IgM and increases of IgG in paired serum samples are well-established reference methods in clinical studies [1,2,6,14]. However, sera were not obtained from the children included in this study. Instead, we used mRNA detection and high DNA load as the basis for calculating specificities, sensitivities and PPVs. mRNA is a marker of actively transcribing virus during an ongoing infection, and a rapidly declining DNA load is the normal outcome after an acute infection. The HBoV1 mRNA test has performed well in studies, providing support for its use as reference [6,7,9,10]. Furthermore, a high DNA load ($> 10^4$ or $> 10^6$ copies/mL) in nasopharyngeal secretions has been shown to be a better diagnostic criterion than mere DNA detection by endpoint PCR [8,14–16]. The two tests together should therefore form a good basis for evaluating the antigen test.

Detection rates of 15% for HBoV1 DNA, 2.7% for mRNA and 3.0% for high DNA load ($> 10^6$ copies/mL) in children with RTI are in agreement with previous reports in the literature [7,9,10,14,15]. Taken together, the results suggest that only about a fifth of the children with HBoV1 DNA in the nasopharynx has an acute HBoV1 infection.

In our study, the samples had been stored at −20 °C for up to two years, therefore some degradation of RNA cannot be excluded. The storage temperature recommended for RNA is usually −70 °C. The sensitivity of the HBoV1 antigen test could have been overestimated if RNA degradation had occurred. However, the mRNA positivity rate reflected that of the high DNA load, indicating that this effect was small. Furthermore, with an amplicon of 242 bp, the probability of degradation within the target sequence is low, explaining the limited effect of RNA degradation on the results.

Among adults with RTI, HBoV1 DNA is rarely detected. Sample sizes in studies on adults have been too small to conclude the clinical significance of HBoV1-DNA positivity [1,2].

We conclude that HBoV1 antigen detection is excellent for diagnosing acute HBoV1 infections in children. Compared with two independent reference assays, this antigen test shows substantially higher clinical specificity and PPV than the commonly used HBoV1 qualitative DNA PCRs. The sensitivity of the HBoV1 antigen test is lower than those of the PCRs, but it has the advantage of being a rapid test available for point-of-care use, with positive results usually available in 20 min, resulting in a faster diagnosis than traditional methods. This makes the test attractive for emergency room and outpatient use. A supplementary test, measuring HBoV1 DNA load, mRNA or specific antibodies, will be of value for higher sensitivity in an inpatient setting.

## Ethical approval

Not required.

**Table 2**
Performances of the HBoV1 diagnostic tests, with mRNA RT-PCR or DNA qPCR as reference markers.

| Diagnostic marker vs. reference | Specificity % (95 % CI) | Sensitivity % (95 % CI) | PPV % (95 % CI) |
|---|---|---|---|
| Antigen vs. mRNA | 100 (99.4 – 100) | 76.5 (52.7 – 90.4) | 100 (77.2 – 100) |
| Antigen vs. DNA quantity[a] | 100 (99.4 – 100) | 68.4 (46.0 – 84.6) | 100 (77.2 – 100) |
| DNA quantity[a] vs. mRNA | 99.7 (98.8 – 99.9) | 100 (81.6 – 100) | 89.5 (68.6 – 97.1) |
| mRNA vs. DNA quantity[a] | 100 (99.4 – 100) | 89.5 (68.6 – 97.1) | 100 (81.6 – 100) |
| DNA presence vs. mRNA | 87.2 (84.3 – 89.6) | 100 (81.6 – 100) | 17.7 (11.4 – 26.5) |

[a] High HBoV1 DNA load: $\geq 10^6$ copies/mL NPA. 95% CI, Confidence Interval (Wilson Score Method); PPV, positive predictive value.

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*Competing interests:* H.T. was previously employed by ArcDia International Ltd. (Turku, Finland).

## CRediT authorship contribution statement

**Nicola Isabelle Kols:** Investigation, Writing - review & editing. **Heli Aatola:** Methodology, Validation. **Ville Peltola:** Investigation, Resources, Formal analysis. **Man Xu:** Investigation, Funding acquisition. **Zaiga Nora-Krukle:** Investigation, Resources, Formal analysis. **Klaus Hedman:** Investigation, Funding acquisition. **Aurelija Zvirbliene:** Investigation, Funding acquisition. **Hanna Toivola:** Methodology, Validation. **Tytti Vuorinen:** Investigation, Resources, Formal analysis. **Juha M. Koskinen:** Conceptualization, Methodology, Validation. **Andrea H.L. Bruning:** Methodology, Validation. **Andreas Christensen:** Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Maria Söderlund-Venermo:** Investigation, Formal analysis, Writing - review & editing, Funding acquisition. **Janne O. Koskinen:** Project administration, Conceptualization, Methodology, Validation, Writing - review & editing, Funding acquisition.

## Declaration of Competing Interest

*Competing interests:* H.A, J.M.K., and J.O.K. are employed by ArcDia International Ltd. (Turku, Finland).

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