Rapid detection and monitoring of human coronavirus infections

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Abstract

Human coronaviruses (CoVs) are increasingly recognized as
important respiratory pathogens associated with a broad range of
clinical diseases. We sought to increase the insight into clinically
relevant CoV infections by monitoring antigen concentrations in
six confirmed CoV-positive patients using a newly developed
assay for rapid detection of CoV OC43 infections. Antigen
positivity lasted 3 to 6 days in secondary infections and 13 days
in primary infection. CoV infections are clinically diverse, are
common, and cannot be diagnosed from clinical symptoms alone.
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Introduction

Coronaviruses (CoVs) are large, enveloped, single-stranded,
positive-sense RNA viruses that belong to the *Coronaviridae*
family. Although the first two human CoVs—CoV-229E and
CoV-OC43—had already been discovered in the 1960s, no
special attention was given to them because infections were
primarily self-limiting and were only associated with symptoms
of the mild common cold [1]. Since 2000, several new CoV
types have emerged. In 2003, the World Health Organization
issued a global alert about a deadly new infectious disease, se-
vere acute respiratory syndrome, which turned out to be
caused by a CoV [2]. In late 2004 a novel CoV, NL63, was
isolated from two children with respiratory symptoms in the
Netherlands, followed by the discovery of CoV-HKU1 in a
patient with pneumonia. In 2012, the Middle East respiratory
syndrome CoV was identified and was acknowledged to be one
of the most dangerous respiratory viruses for humans [3,4].

As a result, CoVs are increasingly recognized as important
pathogens associated with a broad range of clinical diseases.
Previous studies have reported CoV-OC43 to be the most
prevalent CoV in many countries [5,6]. Virus isolation in cell
culture and more recently molecular techniques, specifically
PCR, have been the method of choice for diagnosing CoV in-
fections, but they have several disadvantages [4,7]. Commercial
PCR-based methods are often relatively expensive, they require
technical expertise and the presence of viral RNA or DNA
does not always reflect acute disease. Moreover, using PCR,
CoVs are frequently codetected with other respiratory viruses,
and the contribution of positive CoV PCR results to disease
severity is not always clear [8,9].

Despite the high morbidity and mortality associated with in-
fections caused by some specific CoVs and the frequent detec-
tion of CoV in patients with respiratory infections, there is no
rapid method available that can detect clinically relevant CoVs in
humans. The aim of this study was to increase our insight in
clinically relevant CoV infections by monitoring antigen con-
centrations in confirmed CoV patients using a newly developed
assay for the rapid detection of CoV-OC43 infections.

An assay to detect species-specific CoV-OC43 nucleopro-
tein antigens was introduced to the mariPOC respi test in 2017.
mariPOC (ArcDia Int. Ltd., Turku, Finland) is an automated and
multianalyte antigen detection test system that enables rapid
detection of acute infections [10−12]. Besides the recently
added CoV-OC43, the mariPOC respi test is able to detect
nine respiratory viruses (influenza A and B viruses, respiratory
syncytial virus, adenovirus, human metapneumovirus, para-
influenzavirus type 1−3, human bocavirus) and *Streptococcus
pneumoniae* from one nasopharyngeal sample at the point of
care. The new CoV antigen test has an analytical sensitivity of 2
ng/mL for OC43 recombinant antigen. The test cross-reacts
with neither HKU1, NL63, and 229E nor with other common
respiratory pathogens or microbiota. It is therefore unlikely to
cross-react either with Middle East respiratory syndrome CoV
or severe acute respiratory syndrome CoV. According to the

manufacturers' specification, the clinical specificity of the test is 99.4% (n = 160) compared to PCR.

For this study, we used the semiquantitative property of the mariPOC analysis to obtain CoV antigen levels by extrapolation from a standard concentration curve. For verification of the results, samples were sent to two laboratories (Laboratory of Clinical Virology, Academic Medical Center (AMC), The Netherlands; and the National Institute for Health and Welfare (THL), Finland) for PCR testing with a multiplex RT-PCR [13] and a CoV-species−specific RT-PCR [8], respectively.

Case description

We prospectively followed six otherwise healthy immuno-competent Finnish volunteers who developed respiratory

illness symptoms and tested positive for CoV-OC43 in the mariPOC assay between December 2015 and December 2016. Informed consent was obtained from patients or their parents before enrolment. After verification of symptoms, nasopharyngeal swabs were collected daily from onset of disease until disappearance of the symptoms. The patients were negative for all other ten pathogens covered by the mariPOC respi test. After mariPOC analysis, all samples were frozen at −20°C, and aliquots were sent to the AMC and THL for confirmation of the results. Antigen measurement results from (almost) daily collected samples are shown in Fig. 1. Antigen secretion correlated relatively well with symptom severity. The clinical characteristics of the CoV-positive patients are provided in Table 1. All samples with measurable CoV antigen levels in mariPOC were also positive by both PCRs.
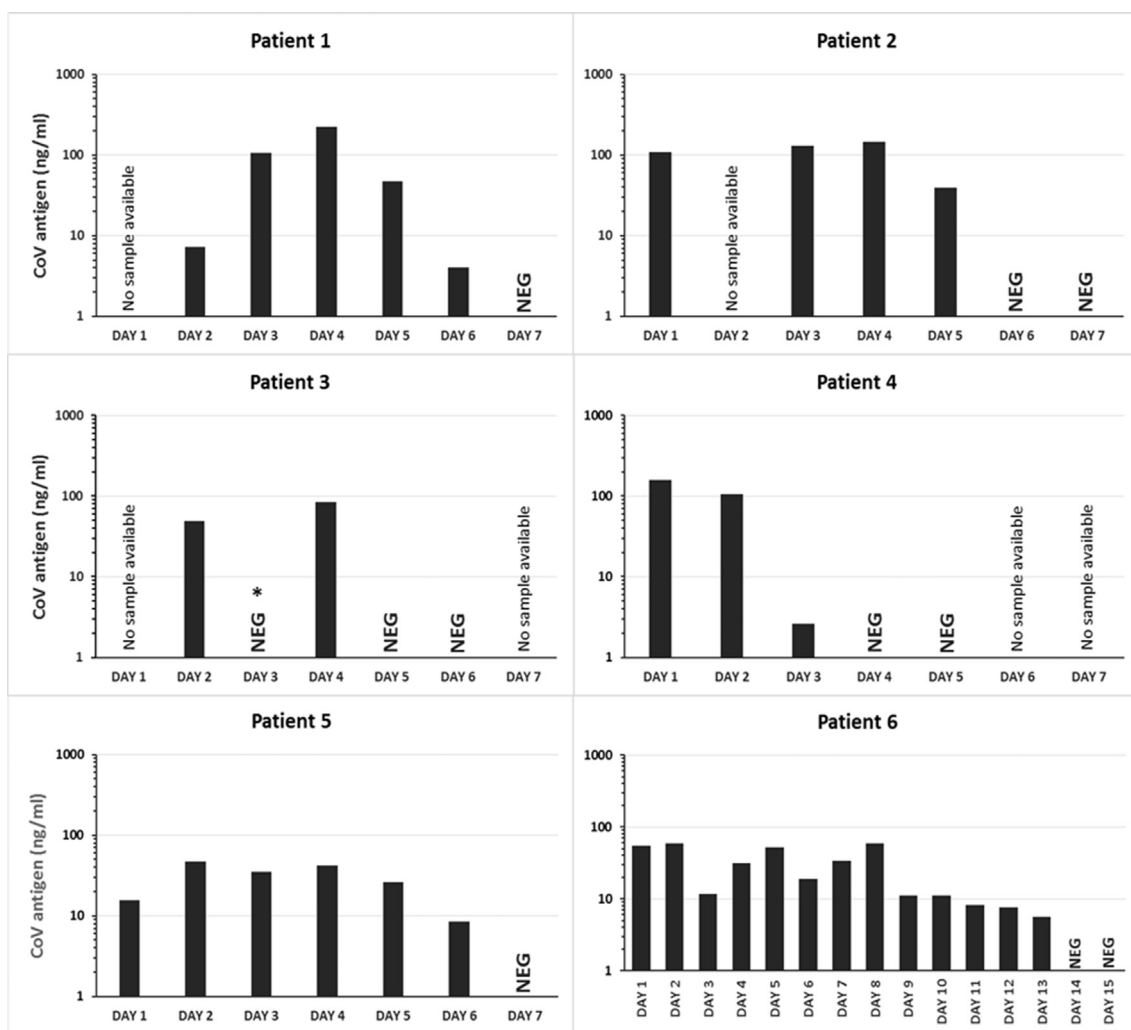
FIG. 1. Detection of coronavirus antigen by mariPOC in six patients with respiratory tract infection symptoms. Results are shown from date of symptom onset. Bars marked with 'NEG' display samples with a mariPOC signal below cutoff for positive finding. One sample obtained in middle of positivity period (marked with asterisk) was also negative by PCR, suggesting that sample collection was unsuccessful.