Catching the common cold

*Rapid detection and epidemiology of respiratory viruses*

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Chapter 5

Rapid detection and monitoring of human coronavirus infections


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ABSTRACT

Human coronaviruses (HCoVs) are increasingly recognized as important respiratory pathogens associated with a broad range of clinical outcomes. This report describes a newly developed assay to rapidly detect HCoV infections. With the new rapid test we correctly identified and monitored four HCoV infections in patients with respiratory tract infection symptoms.
INTRODUCTION

Coronaviruses (CoVs) are large, enveloped, single-stranded, positive-sense RNA viruses which belong to the family of the *Coronaviridae*. Although the first two human CoVs, CoV-229E and CoV-OC43, were already discovered in the 1960s, no special attention was given to them as infections were primarily self-limiting and only associated with mild common cold symptoms (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, Severe Acute Respiratory Syndrome (SARS), which turned out to be caused by a CoV (2). Late 2004, a novel CoV -NL63- was isolated from two children suffering from respiratory symptoms in the Netherlands, followed by the discovery of CoV-HKU1 in a patient with pneumonia. In 2012, the Middle East Respiratory Syndrome coronavirus (MERS-CoV) was identified and acknowledged as 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 outcomes. Molecular techniques, specifically polymerase chain reaction (PCR), have been the method of choice for diagnosing CoV infections but encounter several disadvantages. (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 co-detected with other respiratory viruses and the contribution of positive CoV PCR-results to disease severity is not always clear (5, 6).

Despite the high morbidity and mortality associated with infections caused by some specific CoVs and the frequent detection of CoV in patients with respiratory infections, there is currently 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 concentrations in confirmed CoV patients, using a newly developed assay for the rapid detection of CoV infections.

METHODS

During the influenza season 2015-2016, all employees from a work community of ten persons in Turku, Finland were asked to directly contact one of the research team members when respiratory tract infection symptoms developed. After verification of symptoms and informed consent nasopharyngeal swabs and information on clinical symptoms were collected daily from onset until disappearance of symptoms. After collection, swabs were immediately tested.
with a mariPOC\textsuperscript{®} respi test. mariPOC\textsuperscript{®} (ArcDia Int. Ltd, Turku, Finland) is an automated and multianalyte antigen detection test system that enables rapid detection of acute infections. mariPOC\textsuperscript{®} respi test is able to detect nine respiratory viruses (influenza A and B viruses, respiratory syncytial virus, adenovirus, human metapneumovirus, parainfluenzavirus type 1-3, human bocavirus) and \textit{Streptococcus pneumoniae} from one nasopharyngeal sample at the point-of-care (7). An assay to detect CoV nucleoprotein antigens was recently added to the mariPOC\textsuperscript{®} respi test for research use. This new CoV antigen test has an analytical sensitivity of 2 ng/ml for OC43 recombinant antigen. It cross-reacts with NL63 and 229E but not with other common respiratory pathogens or normal flora.

mariPOC\textsuperscript{®} test results are typically reported as qualitative. For this study we used the semi-quantitative property of the mariPOC\textsuperscript{®} analysis to report CoV-antigen-levels. For verification of results, samples were sent to two laboratories (Laboratory of Clinical Virology, Academic Medical Center, The Netherlands, and the National Institute for Health and Welfare (THL), Finland) for PCR-testing with respectively a multiplex RT-PCR (8) and a coronavirus-species-specific RT-PCR (5).

**RESULTS**

From December 2015 till March 2016 four out of ten otherwise healthy employees developed respiratory illness symptoms and tested positive for CoV in the mariPOC\textsuperscript{®} assay. PCR confirmed these results. The four CoV were identified as CoV-OC43. Antigen measurement results from (almost) daily collected samples are shown in Figure 1. Antigen secretion correlated relatively well with symptom severity, especially with fever. Patient 1 secreted CoV-antigen at measurable levels for six days from onset of symptoms. Symptoms persisted for nine days and consisted of cough, fever, and fatigue. Fever decreased after day 5. Patient 2 suffered mainly from fever and cough, which diminished after day 6. In patient 3 only rhinitis persisted for six days, but cough was absent after day 4. Symptoms in patient 4 were only mildly present for four days with cough and fatigue, but no fever. All samples with measurable CoV-antigen levels in mariPOC\textsuperscript{®} were also positive by PCR.
**DISCUSSION**

In conclusion, during influenza season 2015-2016, four patients with confirmed coronavirus infection were monitored with the newly developed CoV antigen assay. The assay reacts with CoV-OC43, -229E and -NL63 but does not differentiate them. For the purpose of this study we further typed the CoVs and all four infections were CoV-OC43. This is in line with previous studies reporting CoV-OC43 as the most prevalent CoV in certain countries (9, 10).

Because of its frequent detection and the sometimes severe complications associated with CoV infection (11, 12) new diagnostic methods to rapidly identify these infections are needed. Here we showed that the new CoV test of the mariPOC® platform correctly identified and enabled monitoring of CoV infections. Compared to nucleic acid amplification based tests, the new antigen test could potentially identify patients in whom CoV is the real cause of the infection since it measures the virus itself and the antigen level needed for detection is achieved only during the acute phase of the infection. However, larger studies are needed to confirm these findings and further determine the diagnostic accuracy of the new assay, not only for CoV-OC43, -229E and -NL63, but also for the other CoVs, i.e. –HKU1, MERS- and SARS-CoV.
Monitoring of the antigen concentrations suggested that viral load peaked around the third and fourth day after symptom onset which confirms the findings in the experimental study by Adney et al (2014) in camels (13). Sampling in adults should therefore be done within the first four days of symptom onset in order to ensure maximum sensitivity of antigen detection testing. Prompt testing and diagnosis also maximizes the potential to affect treatment decisions such as prescribing virus specific drugs, and withholding prescription of antibiotics. The new rapid test might therefore be a valuable contribution to patient care.

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**Conflict of interest**

HA, HT and JOK are R&D employees at ArcDia International Oy Ltd. The other authors declare no conflict of interest.
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