Evaluation of a new point-of-care test for influenza A and B virus in travellers with influenza-like symptoms
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ABSTRACT

Point-of-care (POC) tests for influenza facilitate clinical case management, and might also be helpful in the care of travellers who are at special risk for influenza infection. To evaluate influenza POC testing in travellers, a new assay, the ImmunoCard STAT! Flu A and B, was used to investigate travellers presenting with influenza-like symptoms. Influenza virus infection was diagnosed in 27 (13%) of 203 patients by influenza virus-specific PCR and viral culture. The POC test had sensitivity and specificity values of 64% and 99% for influenza A, and 67% and 100% for influenza B, respectively. Combined sensitivity and specificity were 67% and 99%, respectively, yielding positive and negative predictive values of 95%, and positive and negative likelihood ratios of 117 and 0.34, respectively. The convenient application, excellent specificity and high positive likelihood ratio of the POC test allowed rapid identification of influenza cases. However, negative test results might require confirmation by other methods because of limitations in sensitivity. Overall, influenza POC testing appeared to be a useful tool for the management of travellers with influenza-like symptoms.

Keywords Diagnosis, ImmunoCard STAT! test, influenza virus, point-of-care testing, rapid detection, travellers

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INTRODUCTION

Respiratory infections occur in c. 6–58% of all travellers [1], with ≥5% of these infections being caused by influenza viruses [2]. Consequently, influenza might be the most frequent vaccine-preventable infection among travellers [3]. Reasons for the high incidence of infection might be overcrowding during transportation or in accommodation [2], climatic conditions favourable to viruses during air travel [4], and the year-round presence of influenza viruses in tropical areas (http://www.who.int/mediacentre/factsheets/fs211/en). In addition to the risk of infection for individuals, the dissemination of influenza and other respiratory infections via travellers is an emerging concern [5]. Appropriate management of respiratory infections, e.g., influenza or SARS, depends on rapid and reliable diagnostic tests that can assist patient care by enabling early antiviral treatment, prophylaxis of contacts, and epidemiological investigations [6] (http://www.who.int/csr/disease/avian_influenza/guidelines/rapid_testing/en).

Several point-of-care (POC) influenza tests allow a more rapid and economical diagnosis than conventional laboratory methods, e.g., viral culture, PCR or direct immunofluorescence tests [7]. However, data concerning the performance of these POC tests are limited, and often derive from non-peer-reviewed manufacturers’ studies [8]. The WHO encourages POC testing in travellers and lists 19 available POC assays (http://www.who.int/csr/disease/avian_influenza/guidelines/rapid_testing/en). However, studies concerning the performance characteristics of POC tests for influenza in travellers have not been performed previously. Therefore, the aim of the present study was to evaluate a new POC assay in a population of travellers presenting with influenza-like symptoms.
PATIENTS AND METHODS

Between February 2005 and November 2006, individuals who attended the outpatient department of the Institute of Tropical Medicine and International Health, Berlin, Germany with influenza-like symptoms were tested for influenza virus. Depending on the travel history and clinical presentation, diagnostic tests for other infections, e.g., malaria and dengue fever, were also conducted. For the diagnosis of influenza, two nasal swabs were taken on dry rayon swabs (Copan Diagnostics Inc., Corona, CA, USA). One specimen from each patient was tested immediately with the ImmunoCard STAT! Flu A and B (Meridian Bioscience, Cincinnati, OH, USA) POC test according to the manufacturer’s instructions. This test, which works as a lateral flow immunochromatographic assay, based on monoclonal antibodies specific for influenza nucleoprotein, is able to differentiate between influenza A and B infections. After the POC test had been performed, the first swab was kept at −20°C as a back-up sample. The second specimen from each patient was sent to the German National Reference Center for Influenza, Berlin. To avoid desiccation during transport, 0.5 mL of sterile saline 0.9% w/v (Braun AG, Melsungen, Germany) was added. The samples were transported on the same day they were taken, or if transport was delayed, kept frozen at −20°C.

At the German Influenza Reference Center, all samples were immediately tested for influenza virus by PCR and viral culture as described elsewhere [9]. Changes in the standard protocol included the substitution of two primers (HA3-115, HA3-375) for detection of the haemagglutinin gene of influenza A/H3 by primers H3P-162 (5'-TCCTCATCAGATCCTTAGT) and H3P-291 (5'-ACAGTTGCTGTAGGCTTAGC), and of the primers for detection of influenza B viruses by primers BMP-1 (5'-GAGACACACATCCATCTCC-ACCTGC), BMP-2 (5'-CCACCCGAAACACAGTGTGAAAT) and probe BMP-72 FAM (5'-AGATGGAGAAGGCAAAGCAGGAACATAGC). In brief, following initial extraction of RNA with a QIAamp Viral RNA Kit (Qiagen, Hilden, Germany), material from the swabs was analysed using TaqMan PCR with the above primers and probe. Virus culture was performed on Madin–Darby canine kidney cells, and positive cultures were analysed further using the classic haemagglutination inhibition procedures [10].

For patients positive by the POC test and negative by PCR and virus culture, the respective back-up samples were retested to confirm the results. Samples were considered to be positive if influenza A or B virus was detected by influenza-specific PCR and/or virus culture. Travel-associated influenza was assumed if patients reported the onset of symptoms within 4 days of return from travel, and if the period between symptom onset and sampling was <7 days.

Data were analysed using SAS v.8.01 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

During the study period, samples from 203 individuals with a recent history of international travel were examined. All subjects reported influenza-like symptoms, including headache/myalgia (91%), fever (90%) and cough (69%). The subjects had an almost even gender distribution (51% male, 49% female) and had a median age of 37 years (range 4–80 years). Two subjects were aged <8 years. Influenza infection was diagnosed in a total of 27 (13.3%) individuals by influenza-specific PCR or virus culture. Influenza A and B virus were identified in 22 and six cases, respectively, with one double infection. All influenza A virus isolates, except two H1N1 strains, were subtyped as H3N2. Fourteen influenza-positive subjects did not fulfil the criteria for travel-associated influenza; the other 13 subjects acquired influenza infections during travel to Asia (84%), Africa (8%) and Latin America (8%).

The ImmunoCard STAT! Flu A and B POC test results were available in 20–25 min and are summarised in Table 1. Among the 22 subjects with influenza A virus, specimens from 14 individuals displayed a positive influenza A virus band, while eight subjects were not detected by the POC test. For ten subjects, only a weak or very weak influenza band was visible. Nine of these individuals were confirmed as positive by PCR or culture. One sample with a weak influenza A virus band was repeatedly negative by PCR and culture. Of the six individuals with influenza B virus, four were identified correctly and two were missed by the POC test. The subject with an influenza A and B double infection yielded only an influenza B virus band. Overall, the combined sensitivity and specificity of the POC test were 67% and 99%, respectively, resulting in positive and negative predictive values of 95%, and positive and negative likelihood ratios of 117 and 0.34, respectively. The ten cases of influenza not detected by POC testing did not differ from the other cases in terms of their clinical presentation or the period between symptom onset and diagnosis.

DISCUSSION

Rapid detection of influenza virus by POC assays facilitates clinical management and treatment decisions for individuals with possible influenza. However, the performance and impact of this approach depends on the epidemiological situation for a particular population [11] (http://www.who.int/csr/disease/avian_influenza/guidelines/rapid_testing/en). In terms of influenza, travellers
represent a complex population, since: (a) they might be exposed continuously in the tropics, or during a reversed season in the southern hemisphere; and (b) travelling involves close contact with individuals from different geographical areas under conditions that are favourable for the transmission of respiratory infections [12]. Although travellers are at increased risk, epidemiological data concerning influenza virus in travellers are scarce. A retrospective study reported a prevalence of 5.6% in travellers with respiratory symptoms [2], while a serological evaluation detected influenza seroconversion in 2.8% of all travellers, and in 12.8% of febrile travellers [13]. In the present study, influenza infection was found in 13.3% of the population of outpatients with influenza-like symptoms and a history of travel. If the analysis was limited to individuals with possible travel-acquired influenza, influenza virus was detected in 13 (15.5%) of 84 subjects. The different rates of influenza probably reflect different study populations and denominators, and therefore require further verification in prospective studies.

The WHO encourages the use of POC testing in travellers, since it allows rapid decisions to be made concerning the management of patients and contacts (http://www.who.int/csr/disease/avian_influenza/guidelines/rapid_testing/en). However, this recommendation has not yet been evaluated in clinical practice. The present study provides the first data concerning systematic POC testing in travellers using a newly developed POC test, the ImmunoCard STAT! Flu A and B assay.

A limitation of the study was the bias towards mild or moderate infections, since only outpatients were tested. In addition, the requirement for two nasal swabs might have caused discordant results because of an uneven virus distribution. However, it was decided to use this easy and relatively non-invasive sampling method because other techniques, e.g., nasopharyngeal aspirates, which might have resulted in specimens of higher quality, were impractical and almost impossible to use among adult outpatients.

The ImmunoCard STAT! Flu A and B test was easy and convenient to perform. Rapid results allowed prompt management and notification of cases. Faint or very faint test results, observed for ten subjects, were confirmed in all except one case, which highlighted the fact that even very faint bands must be regarded as positive. The interpretation of very weak results could be problematic in inexperienced hands and should be addressed by the manufacturer to avoid false-negative results. The assay reached sensitivity and specificity values comparable to those in previous evaluations of the same [14] or similar POC tests [15–18].

In general, influenza POC tests display high specificity rates, but moderate-to-low sensitivities, ranging from 29% to >90%, with a median sensitivity of c. 70% (http://www.who.int/csr/disease/avian_influenza/guidelines/rapid_testing/en), although lower sensitivities have been reported for influenza B tests [19]. Varying sensitivities might be test-specific, but could also reflect different study populations, sampling techniques and comparative assays. For example, POC test sensitivities in studies of children are often higher, which might be explained by increased shedding of virus in young patients [20,21]. In addition, test performance is better in high-prevalence months and in patients with severe infections [22]. Nasopharyngeal aspirates or washings have been shown to be superior to nasal or throat swabs [17,18]. Considering that 97% of the present study population consisted of adult outpatients, and that nasal swabs were used, it is likely that the sensitivity of the ImmunoCard STAT! Flu A and B assay might be higher with children and hospitalised patients, or if more invasive sampling was performed.
A high positive and negative predictive value of 95% was achieved with the present patient group. The excellent specificity and high positive likelihood ratio of the test allowed prompt and reliable diagnosis of positive cases. This not only facilitates the clinical management of influenza infection, but also helps to minimise unnecessary tests for other febrile tropical diseases, e.g., malaria or dengue fever, which often require repeated examinations. The cost-effectiveness of diagnostic strategies that include influenza POC tests has been demonstrated previously in other patient groups, e.g., children in emergency departments, where a reduction in both diagnostic tests and the use of antibiotics was observed [23–26].

The test sensitivity of 67% and negative likelihood ratio of 0.34 among the present population suggest that, in cases of high clinical suspicion, negative results need further confirmation by additional methods such as PCR or culture. In addition, since POC assays miss a substantial proportion of influenza cases, their general use as a surveillance or screening tool in patients with respiratory symptoms may pose a risk [6], but influenza POC tests might be able to rapidly identify human cases of avian influenza, e.g., in travellers returning from Asia [27]. However, to exclude or monitor human cases with new and more virulent influenza strains, more sensitive techniques such as real-time PCR are required. To generate more valid test results, POC assays with improved sensitivity should be developed. In addition, test markers indicating the quality of the tested respiratory specimen would be helpful in supporting negative test results. Finally, inclusion of other important respiratory pathogens would further improve the value of a POC assay in the management of patients with imported respiratory infections.

In summary, the acceptable performance characteristics of the influenza POC assay evaluated in this study indicate that it is a useful tool for the diagnosis of travellers with influenza-like or respiratory symptoms. At present, POC tests are the only inexpensive option available for timely diagnosis of influenza, which is essential for further decisions concerning the use of antiviral drugs and isolation procedures for patients and their contacts. Rapid confirmation of influenza infections in travellers also augments the exclusion of other imported infections that require immediate treatment or isolation measures, e.g., malaria or SARS.

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