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Letter to the Editor

Wide spectrum of referral routes for acute hepatitis E infections

Dear Editor,

Horn et al.[1] recently highlighted the difficulties of obtaining accurate epidemiological data on hepatitis E virus (HEV) infections in Europe. This may be partly due to the wide spectrum of referral routes by which such patients are diagnosed with HEV infection, making the capture of such data across different specialties problematic.

Hepatitis E virus is a non-enveloped, single-stranded RNA virus that causes acute hepatitis, and presents in similar ways to hepatitis A (fever, lethargy, fatigue, abdominal pain, jaundice). It has four common genotypes (G), with G1 and G2 mainly infecting man, and G3 and G4 infecting a variety of animals.[2] However, now human HEV infections have been reported with all 4 genotypes.[3] Like hepatitis A it is transmitted via the faecal-oral route and has traditionally been more associated with travel in HEV-endemic countries. However, in recent years, HEV has been found to be widespread in the UK swine population and related pork products on sale to the public.[4,5]

Recent 2016 recommendations for the routine screening of HEV in blood and organ donors,[6] have increased awareness that HEV infection may also be a cause of acute hepatitis. This differential diagnosis is of particular importance in pregnant women, in whom HEV infection can cause high mortality.[7]

We present recently diagnosed HEV cases from different specialties, together with HEV sequence analysis, demonstrating the variety of referral routes for this infection.

Case 1 was a 78-year-old male with rheumatoid arthritis, who was on treatment with methotrexate. He was admitted under the elderly care physicians with an 8-week history of lethargy, weight loss and painless jaundice. On admission, his liver function tests (LFTs) were: alanine transferase (ALT) 1008 IU/L, alkaline phosphatase (ALP) 226 IU/L, and total bilirubin (BIL) 46 µmol/L. He was discharged with a working diagnosis of methotrexate-induced hepatitis. However, subsequent testing showed that he was HEV IgM and IgG positive, with an HEV RNA level of 22,000 IU/mL, of genotype 3e, with closest sequence similarity to a human HEV infection from France in 2006 (Fig. 1). There was no history of recent overseas travel.

Case 2 was a 63-year-old male with hypertension and diabetes mellitus (DM), who presented to his general practitioner (GP) with abdominal pain and jaundice. On presentation, his LFTs were: ALT 1374 IU/L, ALP 522 IU/L, BIL 98 µmol/L. He was then referred to the hospital on-call surgical team and admitted. Subsequent testing showed HEV IgM and IgG positive, with an HEV RNA level of 32,000 IU/mL, of genotype 3c, with closest sequence similarity to an HEV infection in a wild boar from Germany in 2006 (Fig. 1). There was no history of recent foreign travel.

Case 3 was a 70-year-old male with chronic lymphocytic lymphoma admitted under the adult haematology team, with a history of a mildly elevated ALT for 8 months, which had been presumed to be either due to his chemotherapy or lymphoma. He developed an acute LFT deterioration: ALT 468 IU/L, ALP 96 IU/L, BIL 21 µmol/L, and subsequently tested HEV IgM and IgG positive, with an HEV RNA level of 330,000 IU/mL, of genotype 3c, with closest sequence similarity to a human HEV infection from France in 2011 (Fig. 1). He had only visited Scotland in the preceding 3 months.

Cases 4 was a 76-year-ol d female with type 2 DM, who presented to her GP with a 2-3 week history of fatigue, epigastric pain and loose bowel motions. Her LFTs were acutely deranged: ALT 1582 IU/L, ALP 148 IU/L, BIL 14 µmol/L, and on further testing was found to be HEV IgM equivocal and IgG negative, with an HEV RNA level of 1,700,000 IU/mL, of genotype 3c, with closest sequence similarity to a human HEV infection from Germany in 2011 (Fig. 1). Her only recent travel had been within the UK.

Case 5 was a 38-year old female who presented to her GP with a 4-week history of fatigue, abdominal pain and jaundice, following a diarrhoeal illness. She was referred to the hospital medical team and then managed as an outpatient. On admission her LFTs were: ALT 1846 IU/L, ALP 124 IU/L, BIL 75 µmol/L. Following her second outpatient review, her HEV serology was requested, which showed: HEV IgM and IgG positive, with an HEV RNA level of 15,000 IU/mL, of genotype 1a, with closest sequence similarity to a human HEV infection from Japan in 2014 (Fig. 1). She had only just arrived in the UK from India 2 weeks earlier.

Case 6 was a 46-year-old male with DM and depression, who was found to have deranged LFTs during a routine GP visit: ALT 1862 IU/L, ALP 399 IU/L, BIL 28 µmol/L. The patient had been purchasing testosterone supplements online and an initial diagnosis of drug-induced hepatitis was made. At follow-up, his HEV serology was tested which showed: HEV IgM and IgG positive, with an HEV RNA level of 84,000 IU/mL, of genotype 3c, with closest sequence similarity to a human HEV infection from the UK in 2014 (Fig. 1**).** He had no history of recent travel.

For HEV genotyping, patient serum or plasma HEV RNA was amplified, sequenced, and subjected to phylogenetic analysis across a 1.3 kb region of the open reading frame 2 (corresponding to ORF2 amino acids 179–660) as previously described.[4] Briefly, total nucleic acid was extracted from 200 µL serum or plasma using the MagNA Pure 96 automated extraction platform (Roche Diagnostics Ltd., Burgess Hill, England). Following cDNA creation a hemi-nested PCR was performed. Sanger sequencing of PCR products was undertaken using a Beckman CEQ8000 sequencer (Beckman Coulter UK Ltd., Wycombe, England), with generated sequences assembled

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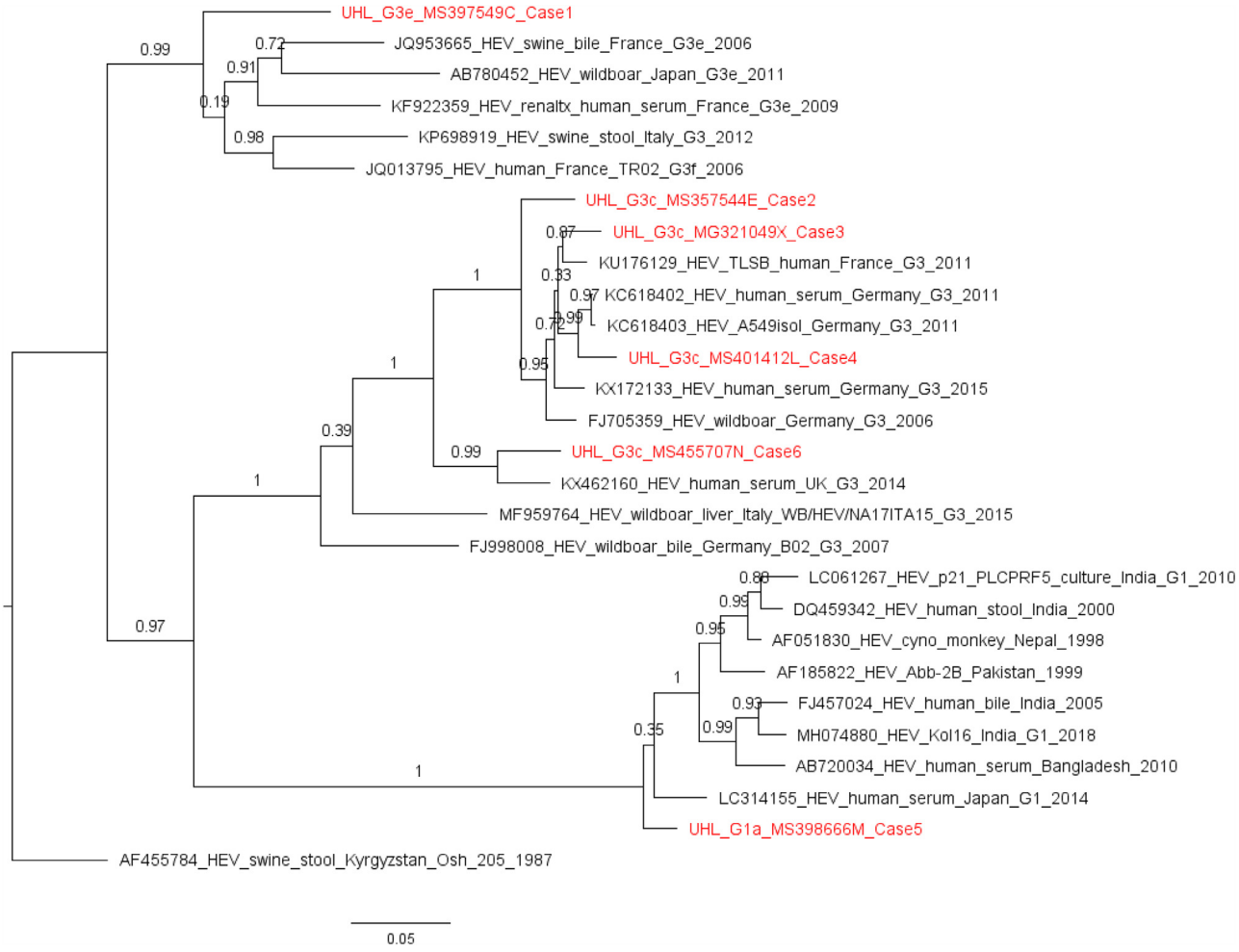

Fig. 1. Patient HEV sequences were aligned against the top 100 most closely related sequences from GenBank (identified using BLAST: https://blast.ncbi.nlm.nih.gov/Blast.cgi), using BioEdit v7.0.4 (http://www.mbio.ncsu.edu/bioedit/bioedit.html) (final length 1304 bp). The phylogenetic tree was drawn using a general time reversible (GTR) model of evolution within FastTree v2.1.10 (http://www.microbesonline.org/fasttree/), and displayed with Figtree v v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). Sequences in red were from the patients described in the main text (Cases 1–6). The numbers by the branches are Shimodaira-Hasegawa (SH) statistical support values (as implemented in FastTree), indicating the robustness of the branch shown – the higher the value the more certain that branch is to exist.

using DNAstar (version 11.2.1; Lasergene). The resulting HEV sequence analysis is described in Fig. 1.

The recent introduction of routine HEV screening for blood and organ donors in the UK,[6] together with the recognition of chronic HEV infection,[8] and the presence of HEV in food products,[5] is gradually increasing the awareness of HEV as a differential diagnosis for acute hepatitis – as demonstrated by the patients described here. These patients were eventually diagnosed with HEV infection after being referred by a variety of clinical specialties: elderly care, surgical, haematology and GPs (i.e., primary care), often with an incorrect, initial or delayed diagnosis.

All the non-foreign travel-related HEV infections (Cases 1–4, 6) were of genotype 3, with only one genotype 1 (Case 5) infection in a patient with recent travel to an HEV-endemic country. The genotype 3 sequences were all accompanied by the same reference laboratory comment, that the sequence was “highly homologous to HEV sequences from sporadic indigenous hepatitis E cases in the UK”, demonstrating that an HEV genotype that was previously mostly associated with pigs and European wild boars is now the predominant cause of human non-travel-related HEV infections in the UK.

Fortunately, this case series also shows that there is an increasing awareness of HEV, across multiple specialties, as a cause of acute hepatitis, leading to increased screening, which improves our surveillance and epidemiological data for a better understanding how this zoonotic infection is spreading in the human population.

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Fluoroquinolone use and associated adverse drug events in England

Dear Editor,

We read with great interest the article published in this journal that addressed the complex interactions of antimicrobial stewardship, bacterial drug resistance, sepsis and the resultant lay press coverage of these topics in the United Kingdom (UK) currently.[1] Oral fluoroquinolones have been extensively used to treat a wide range of bacterial infections for many years, some of which have been complicated by sepsis, and have been predominately used in the primary care setting. Concerns for overuse of these drugs with resultant worsening bacterial resistance, more adverse drug reactions (ADR), and the increased risk of *Clostridium difficile* infection have been a focus of educational interventions for clinicians, the public, and policymakers.[1,2]

Earlier trend studies[3–7] involving segments of the population in the UK have been reported and provide a profile of fluoroquinolone use, but have inconsistent results with no detailed information on types of fluoroquinolone-related ADR. Therefore, the aim of the current investigation was to better define contemporary trends in fluoroquinolone use and provide a characterization of reported ADR in a large, population-based investigation from England.

Data for all oral antibiotics prescribed in the community setting (outside hospitals) in England between 2010 and 2017 were extracted from the Prescription Cost Analysis data held by NHS Digital (https://digital.nhs.uk/data-and-information/publications/statistical/prescription-cost-analysis). Clinicians who issued the prescriptions included physicians, nurse practitioners, and other health care providers, including dentists. For this investigation, a focus on fluoroquinolones was conducted with data for each oral fluoroquinolone included.

National Yellow Card Interactive Drug Analysis Profile data from the Medicines and Healthcare Products Regulatory Agency (MHRA) (http://yellowcard.mhra.gov.uk/iDAP/) were interrogated and data extracted for all reported ADR to fluoroquinolones that were administered orally, or where the route of administration was not stated (reactions to drugs administered parenterally, topically or by other routes were excluded), between 2010 and 2017, to define the rate of ADR with the ADR rate/million prescriptions calculated. In addition, an investigation of the different organ systems involved in an ADR was done for comparative purposes among the different oral fluoroquinolones that had been prescribed during the study period.

Overall, there were over six million prescriptions for oral fluoroquinolones issued in the community setting for the eight years of the study period and ciprofloxacin accounted for 91.6% of prescriptions. Remarkably, there was a 29.8% decline (880,970 for 2010 to 618,229 for 2017) in ciprofloxacin prescriptions over this period with a decline noted in each of the study years (Fig. 1). Prescriptions issued for the other oral fluoroquinolones also declined over the study period, except for levofloxacin and ofloxacin where prescriptions increased the last two years of the study.

For every million prescriptions issued over the study period, 250 resulted in an ADR of which 39, 205, and 6 were designated as non-serious, serious, or fatal, respectively. For ciprofloxacin, musculoskeletal reactions were the most frequently reported serious reactions ($n = 889$) (Table 1), with tendon disorders being the most common of these ($n = 248$). Nervous system disorders were the next most frequently reported serious reactions ($n = 602$) of which paresthesia/dysesthesia/peripheral neuropathy was most common ($n = 196$) followed by headaches ($n = 55$) and seizures ($n = 43$). Of the 25 fatal ciprofloxacin-related reactions reported, 5 were reported as “sudden death”, 6 as infections, of which 3 were attributed to *C. difficile* infection, and 4 as psychiatric disorders resulting in suicide.

Previous surveys from England have yielded varied results and are likely due, in part, to the variability in healthcare settings of patient cohorts included in databases previously used for investigation.[3–7] For example, in one large investigation of 98% ($n = 158$) of acute hospitals in the National Health Service that included data from IMS Health, a data warehouse, between 2009 and 2013, fluoroquinolone use increased 1.6%.[3] Notably, there were large variations in data comparisons between individual hospitals and use included all routes of fluoroquinolone administration. In contrast, data retrieved from the UK THIN database for 2000–2015, which included six percent of the UK’s General Practice patient population, demonstrated a reduction in fluoroquinolone use in recent years.[7] By inclusion of all community-prescribed oral fluoroquinolone use in England in the current work, the trend analysis is likely to be more accurate than that of prior reports.

In response to largely post-approval ADR,[2] serial warnings have been released to inform clinicians and patients of potential risks of fluoroquinolone use, which could have impacted fluoroquinolone use in the current investigation, along with educational and institutional interventions. Although uncommon, some of these ADR can be chronic and result in disabling effects on patients who suffer a diminished quality of life.

Fluoroquinolone use has been associated with an increased risk of development of *C. difficile* infection, which can be recurrent and severe complications can occur, including sepsis and death, the latter of which was seen in the current investigation. This has been

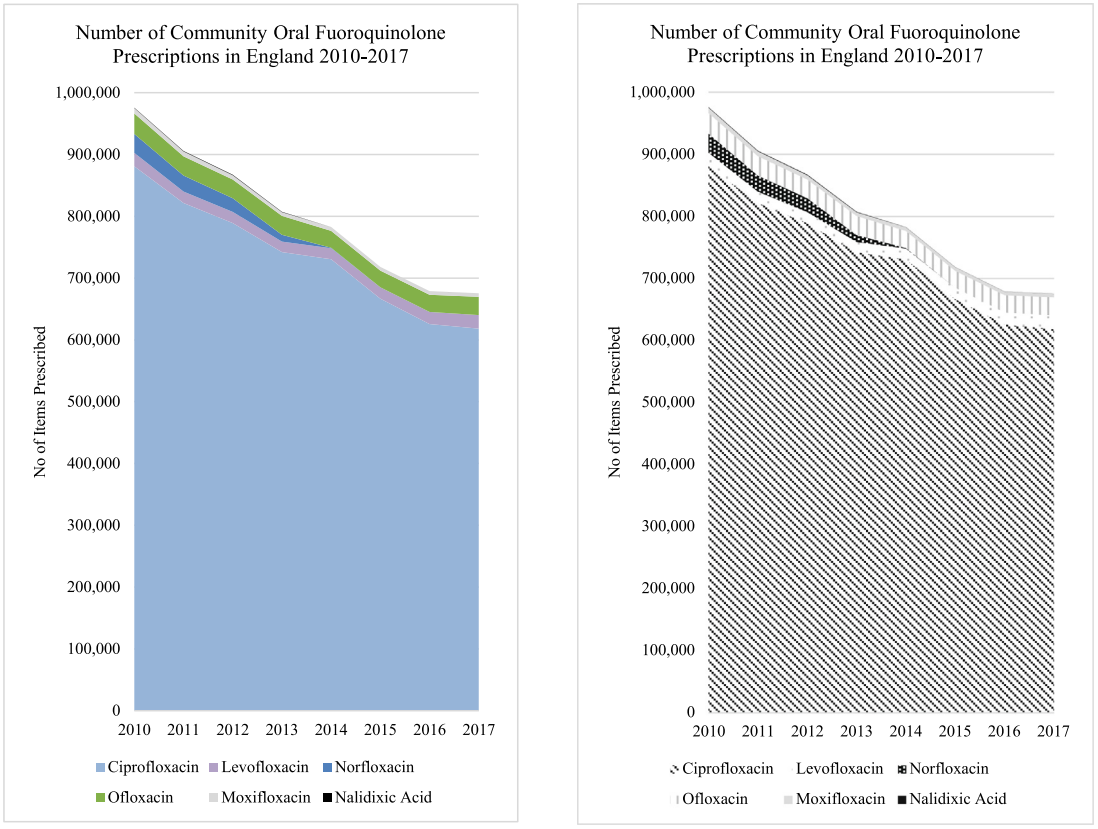

Fig. 1. Number of community oral fluoroquinolone prescriptions in England, 2010–2017 (both color and B&W versions).

the subject of national campaigns in the UK to reduce the use of fluoroquinolones and have likely impacted the trend data of fluoroquinolone use described in our study.[4,8,9]

The development of fluoroquinolone resistance among aerobic gram-negative bacilli has reduced use of this drug class and could have contributed to the trend data displayed in the current investigation. Interestingly, with the reduction in fluoroquinolone "burden" on bacteria seen in clinical practice, improvement in *in vitro* susceptibility of some organisms has been described.[10]

A major limitation of this work is that information is lacking so that we are unable to identify causal factors responsible for the de-

Table 1
Different types of serious and fatal adverse reactions reported for oral fluoroquinolones between 2010 and 2017.

Type of Adverse Reaction	Ciprofloxacin		Other Fluoroquinolones		All Fluoroquinolones		No of Events/million Fluoroquinolone Rx	
	Serious ADR	Fatal ADR	Serious ADR	Fatal ADR	Serious ADR	Fatal ADR	Serious ADR	Fatal ADR
Blood and lymphatic system disorders	42	1	8	0	**50**	**1**	**7.8**	**0.2**
Cardiac disorders	85	2	25	1	**110**	**3**	**17.2**	**0.5**
Congenital, familial and genetic disorders	4	0	0	0	**4**	**0**	**0.6**	**0.0**
Ear and labyrinth disorders	83	0	22	0	**105**	**0**	**16.4**	**0.0**
Endocrine disorders	1	0	3	0	**4**	**0**	**0.6**	**0.0**
Eye disorders	122	0	29	0	**151**	**0**	**23.6**	**0.0**
Gastrointestinal disorders	347	1	96	0	**443**	**1**	**69.1**	**0.2**
General disorders and administration site conditions	570	7	140	2	**710**	**9**	**110.7**	**1.4**
Hepatobiliary disorders	36	2	9	0	**45**	**2**	**7.0**	**0.3**
Immune system disorders	43	0	11	1	**54**	**1**	**8.4**	**0.2**
Infections and infestations	148	6	42	4	**190**	**10**	**29.6**	**1.6**
Injury, poisoning and procedural complications	177	0	68	0	**245**	**0**	**38.2**	**0.0**
Investigations	153	0	46	0	**199**	**0**	**31.0**	**0.0**
Metabolism and nutrition disorders	63	0	16	0	**79**	**0**	**12.3**	**0.0**
Musculoskeletal and connective tissue disorders	889	0	263	0	**1152**	**0**	**179.7**	**0.0**
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	2	0	0	0	**2**	**0**	**0.3**	**0.0**
Nervous system disorders	602	0	180	3	**782**	**3**	**122.0**	**0.5**

(*continued on next page*)

Table 1 (*continued*)

Type of Adverse Reaction	Ciprofloxacin		Other Fluoroquinolones		All Fluoroquinolones		No of Events/million Fluoroquinolone Rx	
	Serious ADR	Fatal ADR	Serious ADR	Fatal ADR	Serious ADR	Fatal ADR	Serious ADR	Fatal ADR
Pregnancy, puerperium and perinatal conditions	0	0	0	0	**0**	**0**	**0.0**	**0.0**
Product issues	3	0	0	0	**3**	**0**	**0.5**	**0.0**
Psychiatric disorders	409	4	149	2	**558**	**6**	**87.0**	**0.9**
Renal and urinary disorders	81	0	13	1	**94**	**1**	**14.7**	**0.2**
Reproductive system and breast disorders	26	0	1	0	**27**	**0**	**4.2**	**0.0**
Respiratory, thoracic and mediastinal disorders	98	0	40	1	**138**	**1**	**21.5**	**0.2**
Skin and subcutaneous tissue disorders	353	2	68	0	**421**	**2**	**65.7**	**0.3**
Social circumstances	10	0	3	0	**13**	**0**	**2.0**	**0.0**
Surgical and medical procedures	4	0	0	0	**4**	**0**	**0.6**	**0.0**
Vascular disorders	48	0	10	0	**58**	**0**	**9.0**	**0.0**
All Reactions	**4399**	**25**	**1242**	**15**	**5641**	**40**	**879.9**	**6.2**

Notes: These data represent the number of different types of adverse event recorded between 2010 and 2017 for each fluoroquinolone type. Each individual/prescription may be associated with more than one type of adverse event. ADR = Adverse Drug Reaction, Rx = Prescriptions.

clining use of oral fluoroquinolones in the community-based practice in England. As cited previously, use of the Yellow Card system to identify ADR is dependent on passive reporting and is biased by the likelihood that non-severe reactions are less often reported, as evidenced by the data presented in the current survey.

Based on findings presented herein, there has been a marked reduction in oral fluoroquinolone use in the ambulatory care setting in England. Ciprofloxacin has accounted for the bulk of this use; reports of serious ADR coupled with antimicrobial stewardship programs that have advocated for diminished use of oral fluoroquinolones have likely been responsible for the decline, although cause-and-effect was not evaluated.

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Transparency declarations

All authors have no declarations of conflicts of interest.

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Impact of a poorly performing point-of-care test during the 2017-2018 influenza season

A recent study by Vilca et al.[1] demonstrated the burden of influenza infection in pregnant women in Spain. Bedside or point-of-care testing (POCTs) allows earlier influenza detection and treatment, though the performance of such tests versus their cost is often difficult to balance for optimal, cost-effective clinical utility.

Table 1
Performance of BD Veritor A + B point-of-care test during 2017-2018 influenza season. RSV – respiratory syncytial virus; Sens – sensitivity; Spec – specificity; ND – not done.

Ward/Centre	Mid-season (Feb 2018)						End-of-season (May 2018)					
	Influenza A		Influenza B		RSV		Influenza A		Influenza B		RSV	
	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec
Acute respiratory ($n = 575$)	21.1	90.1	1.7	100	ND	ND	22.78	90.52	2.46	100	ND	ND
Cystic fibrosis/ long-stay respiratory ($n = 49$)	0	100	0	100	ND	ND	0	95.8	0	100	ND	ND
Adult haematology ($n = 45$)	100	96.9	0	98.45	ND	ND	66.7	95.2	0	97.6	ND	ND
Paediatrics ($n = 177$ – influenza; 176 - RSV)	60	96.5	62.5	100	79.5	98.45	57.14	96.47	70	100	81.81	97.6

Respiratory virus POCTs are becoming more common in hospitals, with most targeting influenza and/or respiratory syncytial virus (RSV). A reliable positive influenza result allows infection control and clinical teams to quickly isolate and treat patients, or discharge patients home on antivirals to avoid the risk of infecting other patients or staff.

However, an unreliable test (i.e. giving false influenza positive–FP, or false negative–FN results) can cause a lot of disruption, with patients being unnecessarily isolated and treated on the basis of a FP result, or being nursed in a shared bay, potentially causing an outbreak, based on a FN result. Hence the importance of the initial choice, and subsequent performance monitoring of any selected POCT throughout the season.

For the 2017-2018, we used the influenza nucleoprotein (NP) antigen-based BD Veritor Flu A + B test (Becton Dickinson UK Ltd., Berkshire, England) on adult haematology (bone marrow transplant), acute and chronic (including cystic fibrosis) respiratory, and general paediatric (which included RSV testing) wards. Each clinical team was left to use the test on patients of their choice, depending on perceived clinical and infection control needs.

After a respiratory swab (adults) into virus transport medium (VTM, Sigma-Virocult, Medical Wire, Wiltshire, UK), or a nasopharyngeal aspirate (younger children) were collected for the POCT, the residual was tested, as routine, on the Virology laboratory PCR-based assay. This respiratory virus panel (AusDiagnostics UK Ltd., Chesham, UK) detected: influenza A/B, RSV A/B, entero/rhinoviruses, parainfluenza viruses types 1-4, adenoviruses, rhinoviruses, coronaviruses (229E, OC43, NL63, HKU1), human metapneumoviruses).

The POCT and the 'gold standard' laboratory results were compared at mid-season (Feb 2018) and end-of-season (May 2018) (Table 1). Whilst the specificity of the influenza A, B and RSV remained mostly within the POCT kit-specified limits across all patient populations, the sensitivities varied dramatically from 0-100%.

In the acute respiratory patients the influenza A sensitivity was only 21–23% and < 3% for influenza B throughout the season. This indicated that the POCT and laboratory tests did not agree on the positive results of most samples, particularly for influenza B. By end-of-season, on the POCT, there were 47/575 FP, and 61 FN test results for influenza A; 0/575 FP and 119/575 FN test results for influenza B.

In the chronic respiratory patients, there was no influenza A or B positive result agreed upon by both the POCT and the laboratory test, hence a sensitivity of 0% for both viruses. By end-of-season, there were 2/49 FP and 1/49 FN test results for influenza A; 0/49 FP and 5/49 FN test results for influenza B.

In the adult haematology patients, there were only 2 influenza A patients confirmed as positive by both POCT and laboratory testing (100% sensitivity, mid-season), with an additional FN on the POCT by end-of-season (6.7% sensitivity), but no influenza B positive patients confirmed by either test (0% sensitivity). Overall, by end-of-season, there were 2/45 FP and 1/45 FN for influenza A; 1/45 FP and 4/45 FN for influenza B.

The best overall performance by the POCT was in the paediatric population, with influenza A and B sensitivities of 57–60% and 62–70%, respectively, with 6/177 FP and 3/177 FN for influenza A; 0/177 FP and 3/177 FN for influenza B. The RSV sensitivities (79-82%) were within the range reported in the POCT kit insert, with 4/176 FP and 16/176 FN.

Previous studies published for this POCT reported higher sensitivities for influenza A (70-80% to > 90%) and B (67-77%, to > 90%),[2-5] though the performance of any test can vary seasonally, with changes in the circulating viruses.

Most of the FN results on the POCT were likely due to low viral loads, however, other causes may be possible. Some samples were sequenced as part of routine UK surveillance (Respiratory Virus Unit, Colindale, London, UK), and a limited sequence analysis showed the presence of the influenza A/H3N2 V197I NP mutation in some patient samples (**Figure S1**), though none of these had also been tested on the POCT. This mutation contributes to escape from the NP-specific cytotoxic T lymphocytes.[6-8] In fact, among the Oct 2017-Apr 2018 UK influenza A/H3N2 sequences available in influenza sequence databases (Global Initiative on Sharing All Influenza Data-GISAID: https://www.gisaid.org/), 462/500 had already acquired the V197I NP mutation compared to just 46/455 in 2016/2017. This coincides with the markedly reduced sensitivity of BD Veritor test seen this season (most of the POC-tested samples were not routinely sequenced).

For influenza B, one of our patient samples contained a novel V62A NP mutation (**Figure S2**). Sequences downloaded from the same database showed that this mutation has increased in proportion recently, from 32.4% (11/34) during Oct 2016-Apr 2017, to 49.6% (204/411) during Oct 2017-Apr 2018. The potential impact of these influenza A/H3N2 or B NP mutations on diagnostic assays targeting this region warrants further investigation. However, the impact on individual commercial assays will be difficult to assess as this requires proprietary knowledge of the assay design.

Reasons for the FP results are more difficult to determine, but some may have been due to occasional transcription errors of the POCT results by ward staff. This was difficult to confirm as the older version BD Veritor test kit that was used had no facility to record the result or the operator details electronically. All results were hand-written by the ward staff in results books kept beside the POCT.

Whatever the reasons, the poor performance of this POCT during this season led to some patients (with FP results) being unnecessarily treated and isolated, and others (with FN results) being left untreated and nursed in open bays, with the potential to cause outbreaks.

As a result of this experience, for this season, we are looking at alternative, more sensitive and specific, PCR-based (or similar) molecular detection POCTs.[9,10]

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2018.10.013.

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Rapid evolving H7N9 avian influenza A viruses pose new challenge

Dear editor,

Recently, the hemagglutinin characteristics, pathogenicity, and antigenic variation of highly pathogenic (HP) H7N9 avian influenza viruses (AIVs) were reported in this journal.[1] H7N9 AIVs have been endemic in chicken since their emergence in China in February 2013,[2] and have triggered five epidemics of human infections.[3,4] At first, the 2013 H7N9 viruses were nonpathogenic in chickens. However, some H7N9 viruses transitioned from low to high pathogenicity for chicken during the 5th wave.[5, 6] These H7N9 HPAIVs were not only destructive to poultry, but also lethal to humans. In addition, H7N9 HPAIVs isolated in ducks in the past year can be systemically replicated and shed viruses in ducks.[1, 7] Thus, in this study, we briefly assess the evolutionary patterns seen for H7 AIVs in ducks.

We collected 2768 non-redundant H7 sequences isolated in China from the Global Initiative on Sharing Avian Influenza Data (GISAID) database (www.gisaid.org), National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov/genomes/FLU), and the Influenza Research Database (FluDB) (www.FluDB.org). The median-joint network showed that the H7Nx viruses (H7N1, H7N2, H7N3, H7N6, H7N7, H7N8 and H7N9) have circulated in ducks in China during the past decade (duck H7Nx lineage, Fig. 1). Duck H7Nx contributed HA to chicken H7N9 (2013 chicken H7N9 lineage, Fig. 1).[2] Since then (2013), the H7N9 LPAIVs have been circulating in domestic chicken and have caused human infections. These chicken H7N9 viruses, though, cannot replicate efficiently in ducks,[8] a conclusion that was supported by surveillance data, which showed that the H7N9 viruses were mainly isolated from chickens and humans (Fig. 2A). However, a few viruses were iso-

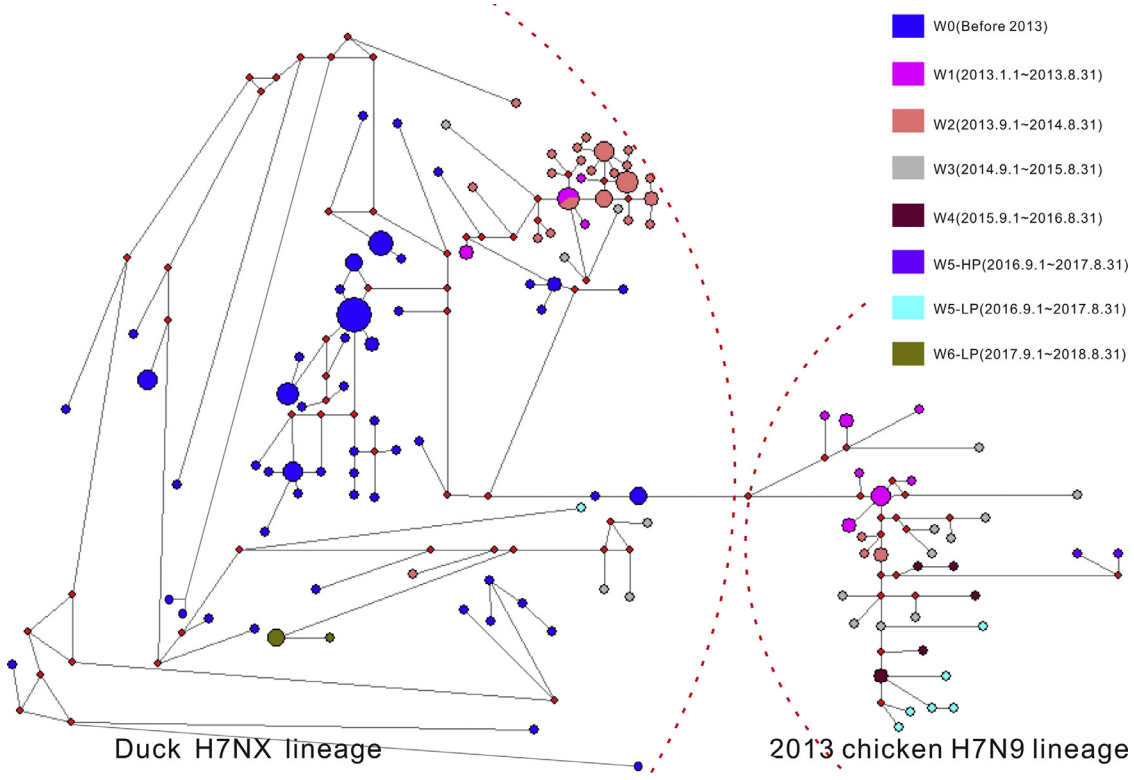

Fig. 1. Phylogenetic network of HA gene for duck H7 AIVs. The median-joint network of HA sequences was constructed with Network 5.0 (http://www.fluxus-engineering.com/sharenet.htm).

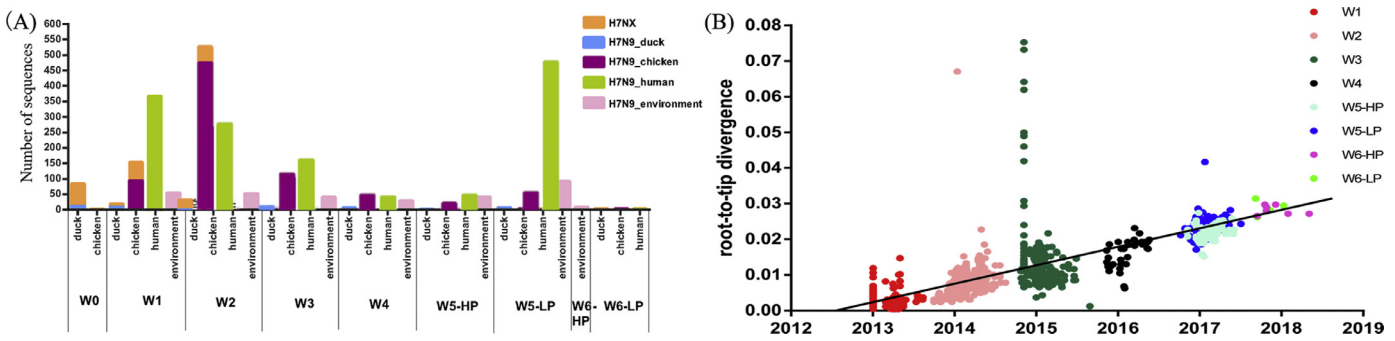

Fig. 2. Hosts of H7Nx AIVs and their mutation ratios. (A) Hosts of all H7Nx sequences isolated in China.[2] Regression of the root-to-tip divergence estimated from the HA gene of H7N9 AIVs.

lated in ducks with each wave of chicken H7N9 (wave 1: 12 isolates; wave 2: 4 isolates; wave 3: 10 isolates; wave 4: 6 isolates; wave 5: 8 isolates). In addition to the chicken H7N9 lineage, ducks also harbor other H7Nx viruses (duck lineage) that have been isolated since 2013 (wave 1: 7 isolates; wave 2: 33 isolates; wave 3: 6 isolates; wave 5: 1 isolate; wave 6: 4 isolates). These duck lineage H7Nx viruses are very diversity (HA sequences sharing 59.0%–100% identity) compared to the chicken H7N9 lineage (HA sequences sharing 94.2%–100% identity). This suggests that although ducks are not the major host of 2013 H7N9, a duck H7 lineage which is distant from the 2013 H7N9 viruses is circulating in ducks in China. Whether these H7 viruses can contribute their genes to chicken H7N9 should raise our attention.

The 2013 H7N9 viruses cannot replicate efficiently in ducks.[8] However, H7N9 HPAIVs isolated in ducks in the past year (wave 6) can systemically replicate and shed virus, but showed no or moderate pathogenicity in ducks.[1,7] This new finding suggests that ducks therefore can act as a silent carrier for the H7N9 AIVs. This would make the control of H7N9 influenza viruses extremely difficult. Ducks are raised in great number in open fields in China, thus have many opportunities to contact domestic chickens and wild birds. Aquatic birds are the natural hosts for all AIV subtypes, thus, if H7N9 viruses extended their host range to ducks, this should raise concerns that these viruses might circulate among ducks, chickens and wild birds, and lead to greatly increased diversity in these viruses. Indeed, H7N9 have reassorted with duck AIVs and a novel H7N2 virus was detected.[7] Human infection by a novel H7N4 virus was found in December 2017.[9] Further dissemination by wild birds along flyways to new areas, and new reassortments are possible and need our concern. Thus, control of this subtype in ducks is critically needed.

The evolution of the HA gene of H7N9 viruses has shown a clock-like pattern since 2013 (Fig. 2B), suggesting that the HA

gene sequences of H7N9 evolve at a relatively constant rate over time. A bivalent H5/H7 inactivated vaccine was used in chickens in September 2017. After vaccination, the mutation ratio did not have a significant change. The seed virus for the H7 Re1 vaccine is based on a 2013 H7N9 LPAIV (A/pigeon/Shanghai/S1069/2013). Whether the accumulated genetic changes over time will lead to antigenic change needs further monitoring.

Vaccination of chickens has successfully decreased the prevalence of H7N9 viruses in chicken,[7] however, we face new challenges with these rapidly evolving H7N9 viruses, due to their circulation in ducks, and genetic changes. Measures to prevent these viruses from continuing to circulate in ducks should be on the way. Further monitoring is critical to detect genetic changes and antigenic variants of these viruses and to assess the effectiveness of the current vaccine.

Conflict of interest

The authors declare not conflict of interest.

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Bronchoalveolar lavage *Aspergillus* Galactomannan lateral flow assay versus *Aspergillus*-specific lateral flow device test for diagnosis of invasive pulmonary Aspergillosis in patients with hematological malignancies

Dear Editor,

We read with interest the paper by Heldt and colleagues[1] who report on the benefit of combining multiple biomarkers and tests including galactomannan (GM) ELISA assay from bronchoalveolar lavage fluid (BALF) as well as the BALF *Aspergillus* specific Lateral Flow Device Test (LFD) for the diagnosis of invasive pulmonary aspergillosis (IPA) in patients with hematological malignancies.

Importantly, early diagnosis and treatment of IPA remains the most important factor to reduce mortality and improve outcome.[2–4] As an important limitation, GM testing is limited in particular by varying turnaround times (up to 3 days and more in some centers), dependent on the number of specimens to be tested and the distance/duration of transport between the clinical setting and the laboratory where the test is performed.[5] This limitation has in part been overcome by the newly formatted and European conformity (CE)-marked *Aspergillus* specific LFD, which detects an extracellular mannoprotein antigen secreted exclusively during active growth of *Aspergillus* species via the JF 5 monoclonal antibody.[6–9] This test has been shown to increase performance for the diagnosis of IPA when used in combination with the galactomannan assay.[6–9] Very recently a second point-of-care test for IPA, which detects galactomannan, was developed and CE-marked (*Aspergillus* Galactomannan LFA). This test may overcome the limitation of long turnaround time by conventional galactomannan ELISA testing and may further facilitate point-of-care diagnosis of IPA. The objective of this study was to evaluate the new *Aspergillus* Galactomannan Lateral Flow Assay (LFA) and compare its performance against the *Aspergillus* specific LFD and other biomarkers for the diagnosis of IPA in patients with hematological malignancies.

A total of 24 BALF samples obtained from 24 patients with underlying hematological malignancies (1 patient with proven IPA, 8 patients with probable IPA, 5 patients with possible IPA, two patients meeting mycological and host criteria for IPA but without typical radiological signs [as defined by the revised European Organization for Research and Treatment of Cancer / Mycoses Study Group (EORTC/MSG) definitions], and eight patients not fulfilling IPA criteria) were included in this analysis. IPA was classified according to the revised EORTC/MSG criteria with one modification: exclusion of beta-D-Glucan as mycological criterion.[10] BALF samples were obtained between September 2016 and September 2018 at the University of California San Diego, United States. GM (Platelia Aspergillus Ag ELISA; Bio-Rad Laboratories, Munich, Germany) and culture were performed prospectively in all BALF

Table 1
Demographic data and underlying diseases of the study population.

	Probable or proven IPA ($n=9$)	No evidence for IPA ($n=8$)	Possible IPA ($n=5$)	Mycological and host factors for IPA but no typical radiological signs ($n=2$)
Female (n, %)	3 (33%)	5 (63%)	2 (40%)	0 (0%)
Age, years (median, range)	70 (24–78)	56 (32–75)	49 (20–62)	34 (21–46)
Underlying diseases (n, %)				
Acute myeloid leukemia	2 (22%)	3 (38%)	–	–
Multiple myeloma	2 (22%)	1 (13%)	1 (20%)	–
Acute lymphocytic leukemia	1 (11%)	2 (25%)	2 (40%)	1 (50%)
Myelofibrosis	1 (11%)	–	–	–
Non-hodgkin lymphoma	1 (11%)	2 (25%)	1 (20%)	1 (50%)
Melodysplastic syndrome	2 (22%)	–	1 (20%)	–
Allogeneic Stem Cell Transplantation	4 (44%)	2 (25%)	1 (20%)	1 (50%)
Autologous Stem Cell Transplantation	2 (22%)	1 (13%)	1 (20%)	1 (50%)

Table 2
Performance of the *Aspergillus*-specific Lateral Flow Device Test (LFD), the *Aspergillus* Galactomannan Lateral Flow Assay (LFA), Galactomannan (GM), and fungal culture in bronchoalveolar lavage (BALF) for diagnosis of invasive pulmonary aspergillosis (IPA) in patients with hematological malignancies. Sensitivity and specificity for probable/proven IPA versus no IPA, as well as test positivity in cases of possible IPA and those who fulfilled mycological and host criteria of IPA but not clinical criteria are displayed.

Biomarkers/tests and combinations	Probable/proven IPA ($n=9$) versus no IPA ($n=8$)		Test positivity in cases with possible IPA ($n=5$)	Test positivity in cases with mycological and host factors for IPA but no typical radiological signs ($n=2$)
	Sensitivity	Specificity		
Aspergillus-specific LFD 15 Min	78% (7/9)	100% (8/8)	40%	100%
Aspergillus-specific LFD 25 Min	89% (8/9)	88% (7/8)	40%	100%
Aspergillus Galactomannan LFA 30 Min	89% (8/9)	88% (7/8)	20%	50%
BAL GM 0.5 ODI cut-off	89% (8/9)	100% (8/8)	0%	100%
BAL GM 1.0 ODI cut-off	78% (7/9)	100% (8/8)	0%	100%
BAL culture	11% (1/9)	100% (8/8)	0%	0%
Aspergillus-specific LFD 15 MinAND/OR *Aspergillus* Galactomannan LFA 30 Min	89% (8/9)	88% (7/8)	40%	100%
Aspergillus-specific LFD 25 Min AND/OR *Aspergillus* Galactomannan LFA 30 Min	100% (9/9)	75% (6/8)	40%	100%

samples. Randomly selected GM positive and negative samples were thereafter stored at −20 °C and tested between August and September 2018 for the *Aspergillus*-specific LFD (OLM Diagnostics, Newcastle upon Tyne, UK), and the *Aspergillus* Galactomannan LFA (IMMY, Norman, Oklahoma, USA). Stored BALF samples where thawed, vortexed, and tested according to the manufacturer's instructions. For the *Aspergillus*-specific LFD, clear BALF was centrifuged only, while not clear BALF was pretreated according to the manufacturer's instructions, and 70 μL of supernatant was added to the test. Results were read 15 and 25 min later and scored as either -, +, ++, or +++. For the *Aspergillus* Galactomannan LFA, BALF samples were pretreated, heated, and centrifuged. Test strips were then inserted into 80 μL of sample following the manufacturer's instructions. Results were read after 30 min and scores given ranging from 0 (i.e. negative), to 4 (highly positive). Results of both the LFD and LFA were each read by two interpreters who were blinded to IPA status, GM ELISA, and culture results.

Statistical analyses were performed using SPSS 25 (SPSS Inc., Chicago, IL, USA). A two-sided P-value of less than 0.05 was considered statistically significant. The Human Research Protections Program at the University of California, San Diego approved the study protocol and all study-related procedures.

A total of 24 samples from 24 patients were included in the analysis. Demographic characteristics and underlying diseases of the study population are displayed in Table 1. A total of 10/24 (42%) of patients were receiving mold-active antifungal prophylaxis/therapy at the time of the BALF procedure. Performance of the *Aspergillus*-specific LFD, *Aspergillus* Galactomannan LFA, BALF culture, Galactomannan ELISA, as well as combinations of the assays are depicted in Table 2.

Both the *Aspergillus*-specific LFD and the *Aspergillus* Galactomannan LFA showed high sensitivities and specificities for probable/proven IPA versus no IPA, with sensitivities of close to 90% for the *Aspergillus*-specific LFD read after 25 min (LFD 25 min) and the *Aspergillus* Galactomannan LFA, and a specificity of 100% of the *Aspergillus*-specific LFD read after 15 min (LFD 15 min) (Table 1). Sensitivity reached 100% when either the *Aspergillus*-specific LFD (25 min) or the *Aspergillus* Galactomannan LFA resulted positive, with a specificity of 75% when the assays were used in combination. The *Aspergillus*-specific LFD and the *Aspergillus* Galactomannan LFA also gave a positive result in a proportion of the possible cases (e.g. in one of the possible cases both tests gave strong positive results, ++ and 2, respectively) and the cases who fulfilled host criteria, had a positive BALF GM (4.77 and 1.51 ODI respectively), but did not have typical radiological criteria on chest CT (tests results were +++ and 2 in the case with the higher BALF GM level). This indicates that these POC tests may be useful in differentiating between those possible IPA cases.

The strength of the result also provided some information for both the LFD and LFA. The single false positive test results for the LFD and the LFA were only low level positive (i.e., + and 1, respectively). BALF GM levels were significantly higher in those with at least a ++ positive test result ($n=7$) versus + positive test results ($n=4$) with the *Aspergillus*-specific LFD 15 min (median 4.77 ODI versus median 1.05 ODI; $p=0.042$). The same was true for the *Aspergillus*-specific LFD 25 min (median 3.62 ODI in the 8 cases with a ++ or stronger positive test result versus median < 0.5 ODI in the 5 cases with a + test result; $p=0.019$). In contrast there was no significant difference BALF GM levels when comparing scores of 1 versus 2 or higher for the *Aspergillus* Galactomannan LFA (median 2.46 ODI versus median 1.37 ODI; $p=0.8$).

Given the importance of early and reliable diagnosis of IPA for targeted and successful treatment, rapid tests allowing for point-of-care diagnosis of IPA are an extremely attractive tool.

Here we evaluated for the first time a newly CE-marked *Aspergillus* Galactomannan LFA and compared performance to the recently CE-marked *Aspergillus*-specific LFD. We found that both tests were highly sensitive and specific for diagnosing IPA in patients with hematological malignancies. Future studies should investigate the diagnostic performance of both point-of-care tests in larger prospective patient cohorts.

Conflicts of interest

M. Hoenigl received research grants from Gilead and honoraria from the Research Practitioner Network and the Mycoses Study Group. R. Taplitz has served on a Merck Advisory Board. All other authors have nothing to declare.

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