BACKGROUND

Internationally, there were 219 million cases of malaria in 2018 and 435,000 deaths. The most important first step in the management of patients with malaria is recognition of the diagnosis. In a western emergency department (ED), the returning traveler with a fever of unknown origin generates a long list of potential infectious diseases. To diagnose malaria, for many years,

A diagnostic evaluation of single screen testing for malaria in the returning traveler: A large retrospective cohort study

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Abstract

Background: Screening for malaria in the returning traveler has often required repeat testing; however, audit data suggest that patients have not been reattending. We sought to ascertain if this was safe by examining the diagnostic efficacy of a single screen consisting of a rapid diagnostic test (RDT) and a thin film.

Methods: We conducted a retrospective cohort study of patients with suspected malaria who attended in the past 5 years from two large teaching hospitals. We assessed the diagnostic accuracy of a single screen, reporting measures of sensitivity and specificity. To establish a reference standard, we cross-linked data with the national malaria registry held at Public Health England and regional centers.

Results: The cohort consisted of 1365 patients, of whom 33 opted out of the research and one did not have a complete initial screen. Of those 1331 screens there were 74 cases of Plasmodium falciparum (prevalence of 5.6%) and 104 of any malaria species (prevalence of 7.8%). Sensitivity for the detection of P. falciparum was 100.00% (95% confidence interval [CI] = 95.1 to 100), with a specificity of 99.4% (95% CI = 98.9 to 99.8). For the detection of any species of malaria the sensitivity was slightly lower due to the presence of one false negative; sensitivity was 99.0% (95% CI = 94.8 to 100) and specificity was 99.5% (95% CI = 98.9 to 99.8).

Conclusions: A single thin film and RDT is likely to be sufficient as a first screen for falciparum malaria in the returning traveler with important caveats. For those sent home from emergency departments, appropriate safety netting must be provided. Further prospective study is required to investigate this approach.

KEYWORDS
diagnostic accuracy, malaria, returning traveler
the use of serial thick or thin films has been the reference standard for diagnosis.2

The most recent Hematology Task Force guidelines recommend that when there is a strong clinical suspicion of malaria but the initial films are negative, repeat films should be made and examined after 12 to 24 h and again after an additional 24 h.3 For patients presenting to the ED who are discharged home, obtaining repeat films would require return visits to ambulatory care or outpatient clinics. This results in a cost and time implication for both the patient and the hospital.

In a Cochrane review, Abba et al.4 described rapid diagnostic tests (RDTs) by categories according to antigen targets. BinaxNOW is a type 2 RDT, meaning that it detects the presence of histidine-rich protein (HRP-2) and aldolase, allowing the detection of Plasmodium falciparum, or nonfalciparum species or a mixed infection with P. falciparum plus a nonfalciparum species. In their meta-analysis of the use of type 2 RDTs in endemic countries for the detection of P. falciparum they demonstrated a sensitivity of 96.0% (95% confidence interval [CI] = 94.0% to 97.3%). In lower-prevalence settings the diagnostic accuracy appears to persist with stand-alone RDT strategies, with sensitivities ranging from 93.3% to 97.7%. However, the parasitemias of the samples to which these sensitivities apply were not stated.4,7 These parasitemias are useful to contextualize the diagnostic accuracy of the RDT. Hypothetically, malaria may be more difficult to detect in low-parasitemia populations.

Since their introduction, RDTs have frequently been combined with microscopy in current clinical practice. This potentially allows an improved diagnostic sensitivity that may obviate the need for repeat testing on every patient, reducing inconvenience for them, improving efficiency for health services, and reducing the associated cost of repeat testing. The safety netting that is a core part of medical practice could be utilized here to direct patients to return if they feel unwell, potentially mitigating the harm from false negatives.

A local audit in a major teaching hospital ED examined the adherence to the malaria diagnostic standard of three serial screens over 72 hours over 10 years. During that time only 5.8% had the recommended three blood tests. These numbers were concerning; patients appeared to be opting not to return. This led us to question whether mandating repeat tests was truly clinically necessary. In this study we aimed to determine the diagnostic efficacy of a combined blood film and simultaneous RDT strategy, for the detection of Plasmodium falciparum and nonfalciparum malaria parasites in the setting of real-world practice of an ED.

METHODS

Study design and setting

We undertook a retrospective diagnostic accuracy study at Manchester Royal Infirmary and Royal Manchester Children’s Hospital, both of which are large university-affiliated major trauma centers situated in the northwest of England. Approval was obtained from the research ethics committee (19/NW/0236), and the confidentiality advisory group (19/CAG/0027).

Participants

We included all patients with suspected malaria who attended the adult or pediatric EDs at Manchester University NHS Foundation Trust over a 5-year period (2014–2019). Participants were identified from the laboratory database if they underwent testing for suspected malaria.

Index test

The patients are selected by emergency medicine clinicians who are suspicious of a diagnosis of infection by Plasmodium spp. This is defined by departmental guidelines as a returning traveler from an endemic area, with a fever or who is generally unwell. The clinical diagnostic pathway at the hospital mandated that each malaria test should consist of a thin film and RDT (BinaxNOW). The Manchester hospitals used BinaxNOW during the period of this study, regularly reviewing the test performance data. The blood film was examined by two trained laboratory biomedical scientists, only the final consensus result was recorded. Either a positive RDT or a positive film required samples to be sent to the local reference center, where thin and thick blood films were examined.

Reference standard

We retrieved all results of serial testing for malaria from local databases. Normal clinical practice in our unit dictates that all positive results are sent to the local reference center for confirmation of diagnosis and malarial species. The study database was then cross-referenced with the national malaria registry held at Public Health England (PHE). Diagnostic laboratories in England, Northern Ireland, and Wales are required to report positive malaria tests to the PHE Malaria Reference Laboratory (MRL).8 For cases in Scotland, reporting is to Health Protection Scotland. In the event of a negative screen, we examined any serial testing that had been conducted locally to identify in discrepancies.

Due to the high mortality and morbidity associated with P. falciparum an assumption was made that patients who originally tested negative would likely re-present and that the national registry could then be used to identify false negatives. A further assumption was made that patients infected with other species of malaria, but tested negative initially, would represent due to ongoing illness and universal free health care from the United Kingdom’s National Health Service.

Data collection

The local clinical database was used to extract the malaria screen results, regional center verification, demographics, physiologic
parameters, and full blood counts. The data were then collated in a central database for analysis.

**Data analysis**

The sample size was determined by the availability of relevant data. We planned to conduct measures of diagnostic accuracy including sensitivity, specificity, and positive and negative predictive values.

**RESULTS**

Over a 5-year period between March 2014 and 2019, a total of 2199 screens were conducted for 1365 unique patients. Thirty-three patients had requested that their records be excluded from research via a national opt-out program. Of those patients, only one was excluded for an incomplete initial screen, this left 1331 with a complete first screen (Figure 1). A total of 51.3% were male, and the average age was 30.6 years (range = 4 months to 89 years old; Table 1). There were 104 positive results from complete initial screens. Of those, 103 were single organisms, 74 were positive for *P. falciparum*, 23 *P. vivax*, six *P. ovale*, and one a mixed infection of *P. falciparum* with *P. ovale*. Of the completed and verified first screens, the background prevalence of *P. falciparum* was 5.6% (74/1331) and 7.8% (104/1331) for any species.

A total of 1331 patients had a complete first screen of both RDT and blood film. The blood film and RDT strategy identified all *P. falciparum* infections (see Table 2). This gave sensitivity and specificity of 100% (95% CI = 95.0% to 100%) and 99.4% (95% CI = 98.9% to 99.8%), respectively.

The blood film and RDT strategy did not identify all *Plasmodium* spp. infections; there was one false negative. The false-negative case was admitted and subsequently found to be positive on a serial screen conducted 14 hours later. The second screen also had a negative RDT but the film detected *P. ovale*. This gave sensitivity and specificity of 99.0% (95% CI = 94.8% to 100%) and 99.5% (95% CI = 98.9% to 99.8%), respectively.

**DISCUSSION**

Our findings suggest that negative results for the RDT and blood film, following a single blood test in the ED, excludes malaria sufficiently well to enable a safe diagnostic pathway when combined with safety netting. With the use of appropriate safety netting, advising patients strongly to return for follow-up testing if their symptoms persist, routine serial sampling for 3 days on every patient would not be required. In our study population this does not appear to increase the risk of a missed diagnosis and has the advantage of reducing inconvenience for patients and improving efficiency of health services. This study showed that there was already a reluctance of patients to reattend, and within the study period we did not find an increased risk for patients after a single screen with RDT and thin smear. We demonstrated an excellent sensitivity for *P. falciparum* of 1.00 (95% CI = 0.95 to 1.00) in our cohort of patients, with a mean parasitemia of 33,500 parasites/µL. Crucially, it was not sufficient to exclude all species of malaria, but the sensitivity was still high 99.0% (95% CI = 94.8 to 100). Given the far greater severity of *P. falciparum* versus *P. vivax*, *P. ovale*, and *P. malariae* (though not *P. knowlesi*), this is

| TABLE 1 Baseline characteristics |
|---------------------------------|
| Population | Malaria absent | *P. falciparum* present |
| Age (y) | 29 (12–44) | 35 (24–45) |
| Male | 604 (49.2) | 60 (81.1) |
| Non-UK residents | 1014 (82.6) | 41 (55.4) |
| Prevalence | — | 74 (5.6) |
| Parasitemia (parasites/µL), mean (95% CI) | — | 33,600 (24,500–42,700) |
| Parasitemia | — | 37,500 (5,000–45,000) |

Note: Data are reported as median (IQR) or n (%), unless otherwise reported.

**FIGURE 1** STARD diagram of study participants for *P. falciparum*. The national opt-out registry is cross-referenced when retrospective research is conducted NHS databases; any participants who have preemptively indicated that they do not wish to participate in research are excluded.
an advantageous diagnostic profile. Furthermore, the false-negative case was already admitted on clinical grounds and therefore came to no harm.

For those sent home from EDs, appropriate safety netting must be provided. Preferably this would be in a written form (see Data Supplement S1, available as supporting information in the online version of this paper, which is available at http://onlinelibrary.wiley.com/doi/10.1111/acem.14216/full), to ensure that those patients with a negative initial malaria screen whose illness continues in the absence of a viable alternative diagnosis present expeditiously for re-evaluation, including another malaria test.

The benefit of early exclusion of malaria is not only one of patient safety and satisfaction. There is a strong economic argument for where it is clinically safe to do so, in reducing the number of follow-up appointments. An outpatient visit has an economic cost to the hospital, £120,9 and to the patient’s economic activity, £59.10 If each patient were to have three screens, the estimated total excess cost in this cohort would have been £441,107.

Rossi et al.11 similarly examined the use of RDTs and blood films in combination to detect malaria in returning travelers to Switzerland, but they did not calculate any diagnostic accuracy statistics, possibly due to the lack of a reference standard. A multistage diagnostic process has been examined by Murungi et al.12 in an endemic population. They reported a mean parasitemia of 4410 parasites/µL (95% confidence interval 3,120 to 6,449 parasites per microliter).12 This included thin and thick films and then RDT adjudicated by polymerase chain reaction (PCR). A sequential testing regimen was used where only indeterminate RDT tests receive microscopy, with positive and negative RDT results being upheld. In a population with a mean parasitemia of 5000 parasites/µL (0.1%), they demonstrated a sensitivity of 95.5% (95% CI = 90.5% to 98.0%). In isolation, Gatti et al.13 found that in the detection of *P. falciparum* the RDT BinaxNOW, the technology of interest in this study, was 100% sensitive when compared to microscopy in a sample of 306 with a prevalence of 47.2% and a mean parasitemia of 1.12% (range = 0.001% to 16.0%; 56,000 parasites/µL [range = 50 to 800,000 parasites/µL]).

There is concern that HRP-2–based RDTs, such as the one used in this study, may fail to detect clinical cases of *P. falciparum* due to deletion of the HRP-2 and/or HRP-3 genes. Musa et al.14 examined a Sudanese population and found that nine of 26 samples were negative for the gene, this appears to vary by region as Bharti et al.15 found a rate of one in 1392 in Indian samples, and there were four of 93 in the analysis by Li et al.16 of samples from the China–Myanmar border. In the Amazon region of Peru and contiguous areas and in Eritrea, *P. falciparum* with HRP-2 and/or HRP-3 gene deletions are sufficiently common that RDTs based on alternative malaria antigens (albeit with lower sensitivity) have had to be introduced.17 Despite this evidence, this evaluation does not appear to support the notion of inadequate sensitivity of HRP-2–based assays in the returning traveler.

Looking forward for malaria diagnostics, loop-mediated isothermal amplification (LAMP) provides a powerful alternative for point of care detection. Mohon et al.18 used a biobank (50:50, positive:negative) to assess the accuracy of LAMP and found 100% sensitivity. In a study of LAMP for the diagnosis of imported malaria at the Hospital for Tropical Diseases in London, it demonstrated diagnostic sensitivity significantly superior to that of expert microscopy and close to that of nested PCR.19 Unlike malaria microscopy, its performance does not require lengthy training and LAMP has the potential to replace microscopy as the primary malaria screen in settings like the one described in this study, contributing greater confidence to the efficacy of a single visit strategy.

**LIMITATIONS**

The verification of negative results and subsequent detection of false negatives is a limitation of this study. To conduct this retrospective review we assumed that patients with untreated malaria would likely re-present to health care, particularly if they had *P. falciparum*. We could detect re-presentations by the laboratory route as diagnostic laboratories are required to report positive malaria tests to PHE. A capture–recapture study showed that the PHE MRL) surveillance system captures 56% (95% CI = 54%–58%) of malaria cases,20 similar to that in other industrialized countries.21 Ascertainment for *P. falciparum* was higher at 66% in the MRL study, so we would expect to detect a significant proportion of any cases missed in the malaria tests conducted in our unit.

This study was retrospective and as such is prone to selection and information bias. It is also the case that the single visit efficacy defined in this study depends on the availability of accurate laboratory diagnostic performance in morphologic diagnosis. The wider application of these findings requires a laboratory staff with sufficient skill and experience. This may not be the case in smaller hospitals so requires risk assessment before implementation in any single centre.

**CONCLUSION**

A single thin film and rapid diagnostic test is likely to be sufficient as a first screen for falciparum malaria in the returning traveler with

### TABLE 2 Diagnostic accuracy statistics of a blood film and RDT strategy for the diagnosis of *P. falciparum* and any species

|                | Sensitivity | Specificity | Negative predictive value | Positive predictive value |
|----------------|-------------|-------------|---------------------------|--------------------------|
| *P. falciparum*| 100 (95.1–100) | 99.4 (98.9–99.8) | 100 (99.7–100) | 91.4 (83.0–96.5) |
| Any malaria    | 99.0 (94.8–100) | 99.5 (98.9–99.8) | 99.9 (99.5–100) | 94.5 (88.4–98.0) |

Note: Data are reported as % (95% CI).
important caveats. The sensitivity of this screen and the detection of false negatives relies on the reattendance of patients. As such a prospective study should be conducted to further assess the safety of such an approach.

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CONFLICT OF INTEREST
The authors have no potential conflicts to disclose.

AUTHOR CONTRIBUTIONS
Charles Reynard, Richard Body, Michelle Brereton, and John Burthem—study design. Charles Reynard, Richard Body, Michelle Brereton, John Burthem, Katie Geary, Peter Chiodini, John McDermott, and Patricia van den Berg—manuscript writing.

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REFERENCES
1. World Malaria Report. World Health Organization. 2018. Accessed November 2, 2020. https://apps.who.int/iris/bitstream/handle/10665/275867/9789241565653-eng.pdf?ua=1.
2. Laloo DG, Shingadia D, Bell DJ, et al. UK malaria treatment guidelines 2016. J Infect. 2016;72(6):635-649.
3. Bailey JW, Williams J, Bain BJ, Parker-Williams J, Chiodini PL. The General Haematology Task Force of the British Committee for Standards in Haematology. Guideline: the laboratory diagnosis of malaria. Br J Haematol. 2013;163(5):573-580.
4. Abba K, Deeks JJ, Olliaro PL, et al. Rapid diagnostic tests for diagnosing uncomplicated P. falciparum malaria in endemic countries. Cochrane Database Syst Rev. 2011;2011(7):CD008102.
5. Bronner U, Karlsson L, Evengård B. Evaluation of rapid diagnostic tests for malaria in Swedish travellers. APIMS. 2011;119(2):88-92.
6. Durand F, Crassous B, Fricker-Hidalgo H, et al. Performance of the Now malaria rapid diagnostic test with returned travellers: a 2-year retrospective study in a French teaching hospital. Clin Microbiol Infect. 2005;11(11):903-907.
7. Fedele PL, Wheeler M, Lemoh C, Chunilal S. Immunochromatographic antigen testing alone is sufficient to identify asymptomatic refugees at risk of severe malaria presenting to a single health service in Victoria. Pathology (Phila). 2014;46(6):551-554.
8. O’Connell AM. Laboratory Reporting to Public Health England. Public Health England. 2016. Accessed November 2, 2020. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/739854/PHE_Laboratory_Reporting_Guidelines.pdf.
9. NHS to Trial Tech to Cut Missed Appointments and Save Up to £20 million. NHS England. 2018. Accessed May 20, 2020.
10. de Lacy J. The Potential Economic Impact of Virtual Outpatient Appointments in the West Midlands: A Scoping Study. NHS Midlands and Lancashire Comissioning Support Unit. 2018. Accessed May 20, 2020. https://www.strategyunitwm.nhs.uk/sites/default/files/2018-11/180813_Economic%20Impact%20of%20OP%20Appointments%20for%20WM%20CGGs_FINAL.pdf.
11. Rossi IA, D’Acremont V, Prod’Hom G, Genton B. Safety of falciparum malaria diagnostic strategy based on rapid diagnostic tests in returning travellers and migrants: a retrospective study. Malar J. 2012;11(1):377.
12. Murungi M, Fulton T, Reyes R, et al. Improving the specificity of Plasmodium falciparum malaria diagnosis in high-transmission settings with a two-step rapid diagnostic test and microscopy algorithm. J Clin Microbiol. 2017;55:1540.
13. Gatti S, Gramaglia M, Bisoffi Z, et al. A comparison of three diagnostic techniques for malaria: a rapid diagnostic test (NOW® malaria), PCR and microscopy. Ann Trop Med Parasitol. 2007;101(3):195-204.
14. Mussa A, Talib M, Mohamed Z, Hajijsa K. Genetic diversity of Plasmodium falciparum histidine-rich protein 2 (PfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests. BMC Res Notes. 2019;12(1):334.
15. Bharti PK, Chandel HS, Krishna S, et al. Sequence variation in Plasmodium falciparum histidine rich proteins 2 and 3 in Indian isolates: implications for malaria rapid diagnostic test performance. Sci Rep. 2017;7(1):1-8.
16. Li P, Xing H, Zhao Z, et al. Genetic diversity of Plasmodium falciparum histidine-rich protein 2 in the China-Myanmar border area. Acta Trop. 2015;152:26-31.
17. Poti KE, Sullivan DJ, Dondorp AM, Woodrow CJ. HRP2: transforming malaria diagnosis, but with caveats. Trends Parasitol. 2020;36(2):112-126.
18. Mohamed AN, Lee LD, Bayih AG, et al. NINA-LAMP compared to microscopy, RDT, and nested PCR for the detection of imported malaria. Diagn Microbiol Infect Dis. 2016;85(2):149-153.
19. Polley SD, González IJ, Mohamed D, et al. Clinical evaluation of a loop-mediated amplification kit for diagnosis of imported malaria. J Infect Dis. 2013;208(4):637-644.
20. Catchcart SJ, Lawrence J, Grant A, et al. Estimating unreported malaria cases in England: a capture–recapture study. Epidemiol Infect. 2010;138(7):1052-1058.
21. van Hest NA, Smit F, Verhave J. Underreporting of malaria incidence in the Netherlands: results from a capture-recapture study. Epidemiol Infect. 2002;129(2):371-377.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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