Malaria diagnosis: blood films, Rapid Diagnostic Tests & the emergence of *P. falciparum* HRP2 deletion strains

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UK Health Security Agency

Learning objectives:

Laboratory diagnosis of malaria - BSH guidelines 2022 - summary

- Microscopy
- RDTs: antigen

Antigen-detecting RDTs

Case studies

1. RDT - false positive Pf finding – complex travel history
2. RDT - false negative

Pf HRP2 story

BSH Laboratory Diagnosis of Malaria Guidelines (revised 2022)

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BJHaem

GUIDELINE

British Society for Haematology guidelines for the laboratory diagnosis of malaria

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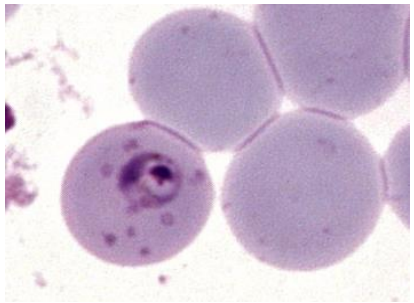
Abstract

The laboratory diagnosis of malaria depends on skilled examination of well-stained thick and thin blood films. Rapid diagnostic tests are a useful supplement and the use of nucleic acid-based testing in diagnostic laboratories should also be considered. These British Society for Haematology guidelines update the 2003 guidelines for malaria diagnosis. Training, quality control, incidental diagnosis, differential diagnosis and reference laboratory referral are considered.



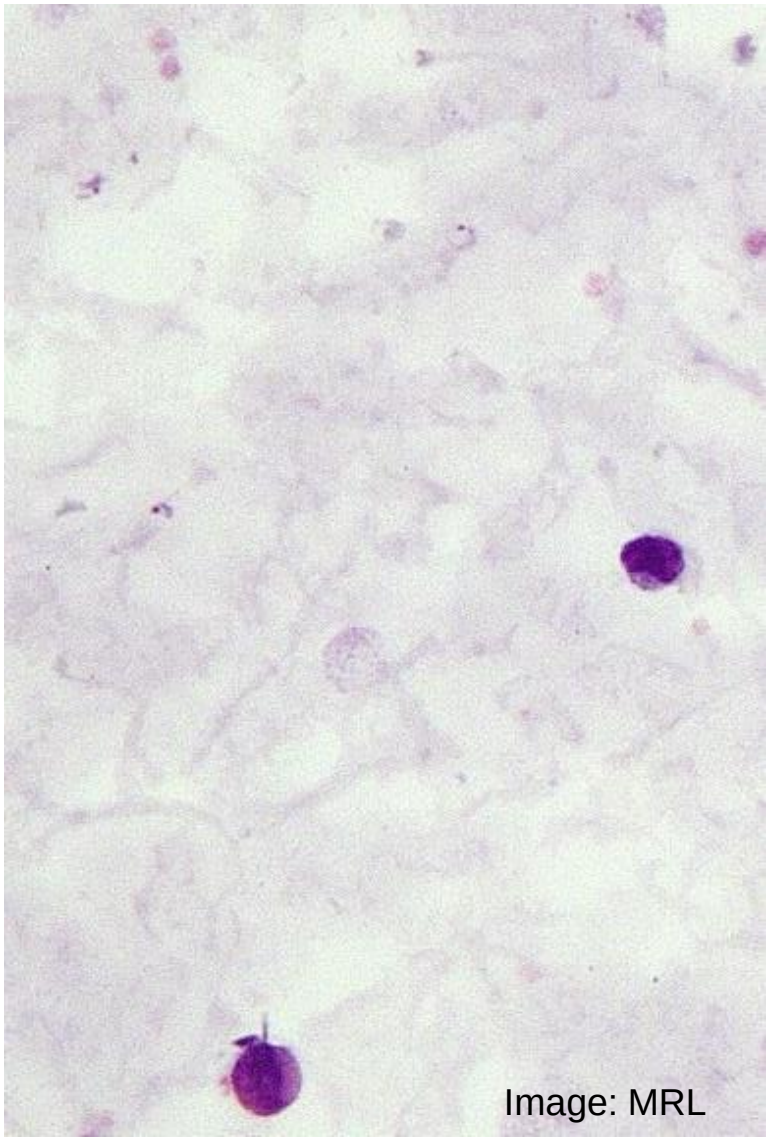


Image: MRL

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BSH Laboratory Diagnosis of Malaria Guidelines (revised 2022)

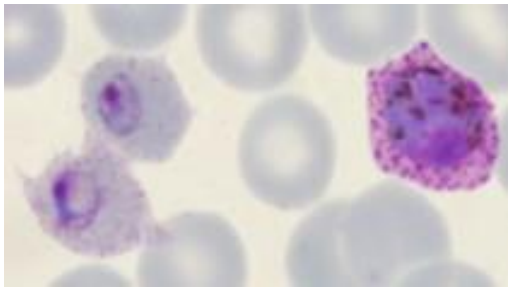
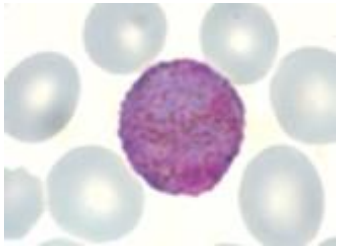
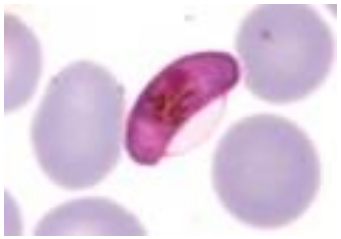
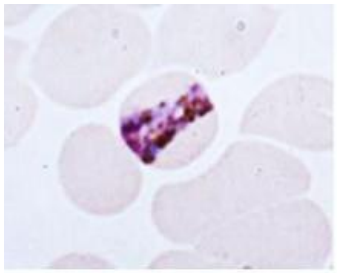
SUMMARY OF RECOMMENDATIONS - Microscopy:

- **Gold standard**

- **Thick films** stained with either Giemsa or Field's stain.
  > two trained staff, each viewing a minimum of 200 high-power fields
  > if positive, the species should be determined by examination of a thin film

- **Thin films** should be stained with a Giemsa stain at pH 7.2
  > If Pf or Pk seen, the %P should be estimated and reported

- Typically takes 40-60mins from receipt in lab
- Requires trained & experienced staff

Images: MRL

BSH Laboratory Diagnosis of Malaria Guidelines (revised 2022)

SUMMARY OF RECOMMENDATIONS – RDTs for malarial antigen

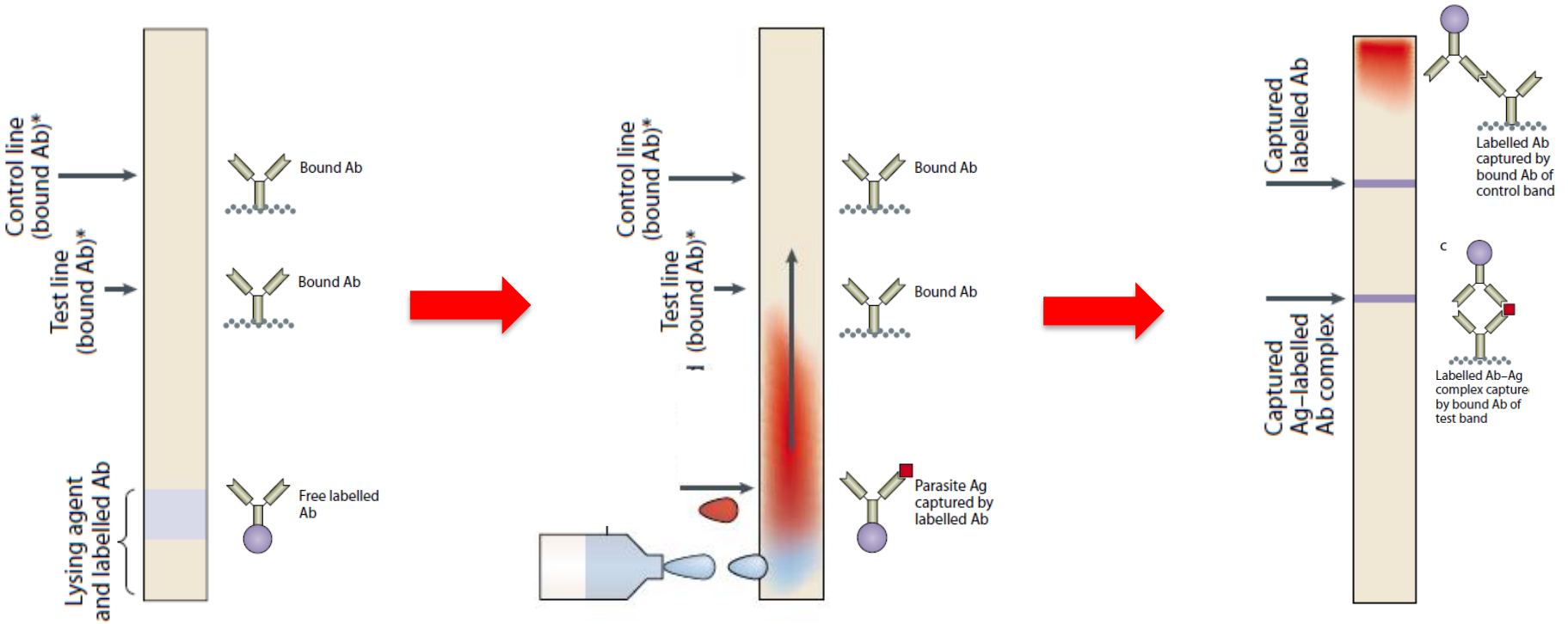
Rapid diagnostic tests (RDTs)

- Widely used by UK labs as a preliminary, supplementary test
- Can be performed by relatively inexperienced staff
- <30 mins to perform, POCT or lab setting
- should always be followed up by microscopy, including out of hours
- Various tests, detect combinations of antigens
  eg Pf **HRP2** (Pf histidine-rich protein) and
  Pan-**LDH** (lactate dehydrogenase, all species)
- Issues……

Images: MRL

RDTs

Ag detecting malaria RDTs



General layout

Positive sample & chase buffer added

Ag from +ve sample coupled to dye-labelled Ab captured by immobilised Ab on test line

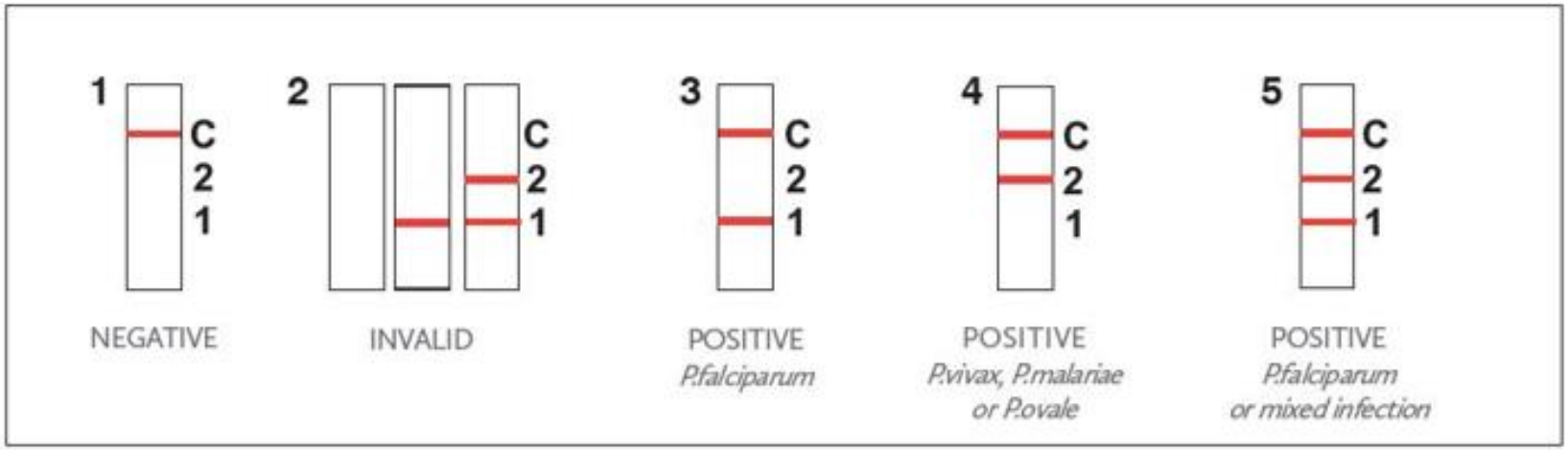
Figure adapted from WHO (2008) *Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 1*

Ag detecting malaria RDTs

Most malaria RDTs used in UK are “three-band” tests ie detect control plus 2 Ags, e.g.:

- Carestart Rapydtest Pf/Pan Pf-HRP2 (1) + Pan-LDH (2)

- Binax NOW Malaria Pf-HRP2 + Pan-aldolase
- OptiMAL-IT Pf-LDH + Pan-LDH



Schematic from Carestart kit insert

Ag detecting malaria RDTs - issues

Malaria Ag RDTs > similar sensitivity as thick blood film ie < 100p/uL (Pf, Pv)

- interpretation not always correct

- **false positives** >> *Pf*HRP2 **antigen persists** following treatment

- **false negatives** >> Low parasitaemia / genetic variation / gene expression
P. ovale P. malariae

Emerging strains of *P. falciparum* with HRP mutations

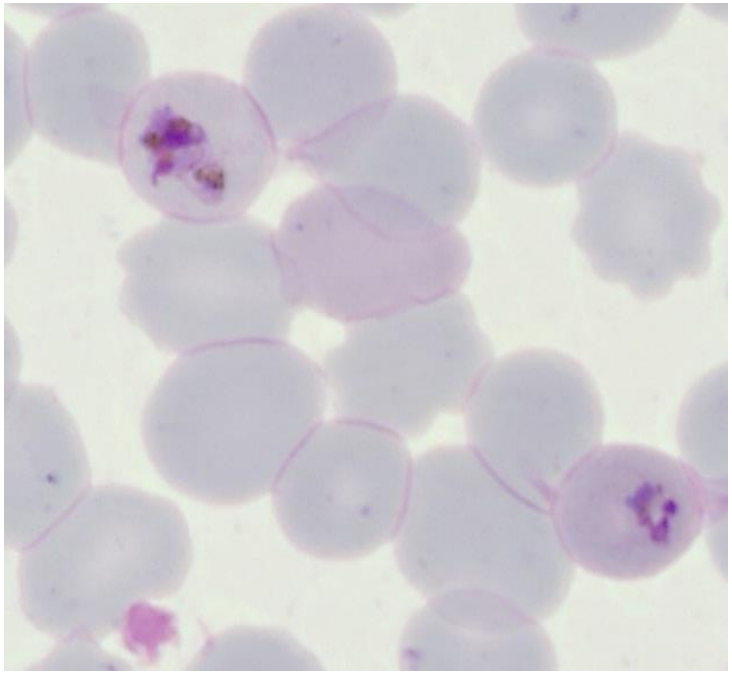
CASE 1

Case I

- 30y old man presented to A&E in mid September with 5 day h/o rigors & fever
- Patient had returned 8 days previously from a 2 week trip to **Indonesia**
- Patient reported that he had also travelled to **Ghana** (late August)
- No prophylaxis had been taken for either trip

- The sending lab had diagnosed *P. malariae*

- Blood films (made 01/09/23, EDTA sample 30/08/23) & EDTA blood were sent to the MRL for confirmation. This is an example field of what we saw:

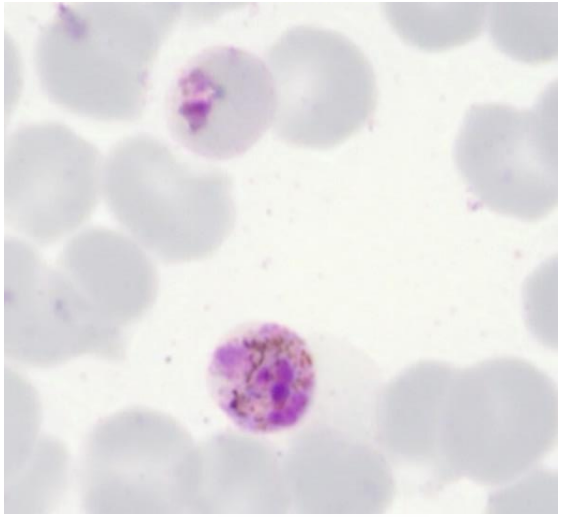


Images: MRL

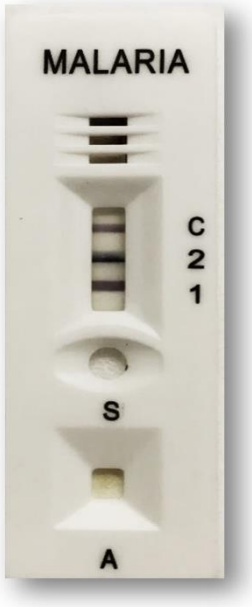
Case I

Immediate concerns:

- odd parasite morphology > *? P. knowlesi* (travel to Indonesia,)
- very young rings present & some with sparse dots > unable to exclude *P. falciparum* (travel to Ghana)



- The sending lab also reported a positive Carestart malaria RDT result (but didn't say positive for what….)
- MRL Carestart RDT:
- **>> PCR…..**



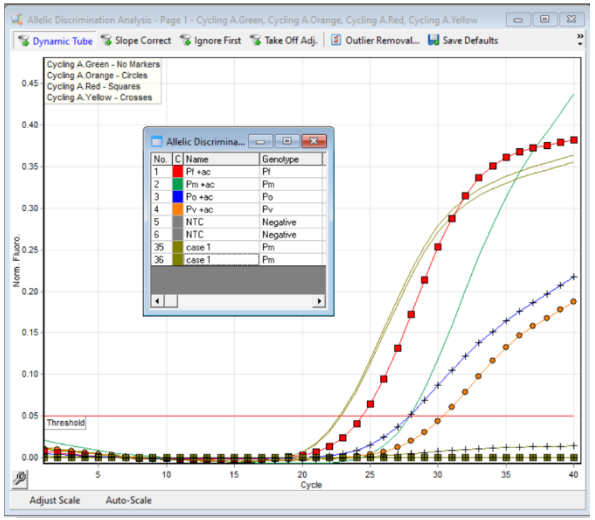
Images MRL

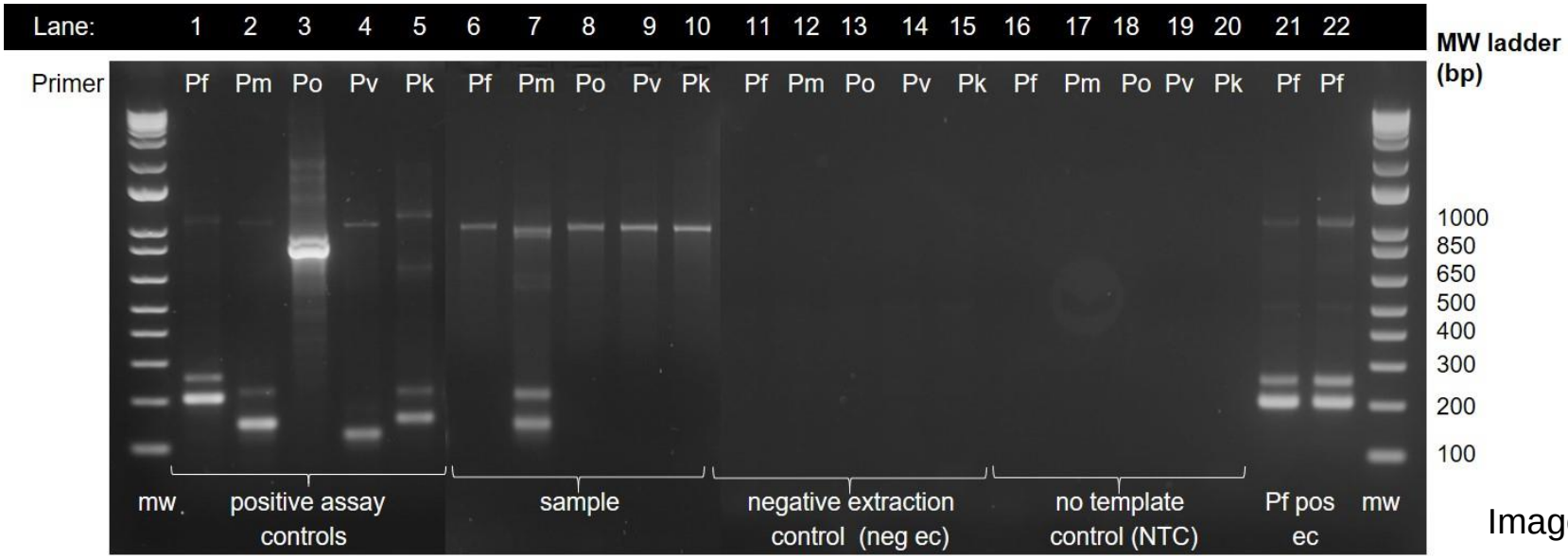
Case I

PCR:

Plasmodium malariae (only) DNA detected

No DNA persisting from treated Pf infection
>> Pm ? new infection





Images: MRL

Case I

- On further enquiry: the patient reported having received unspecified treatment for fevers (?malaria) whilst in Africa.

>> take a comprehensive history: clinicians, let the lab know this

- BF: positive for malaria parasites but morphology not clear as films made from old blood.

Make blood films from freshly taken blood <6h old
Make extra slides to send to the Ref Lab in case needed.
Send original slides if blood too old to make fresh films

- RDT: Pf antigen persisting from previously treated infection (diagnosed & treated in Africa).

>> repeat films to monitor for recrudescence

CASE 2

Case 2

- 55y old Sudanese man presented to A&E OOH with symptoms suggestive of malaria.
- The patient had returned from a 3 week stay in Sudan
- The patient had started feeling unwell 6 days before leaving Sudan and had a malaria diagnostic test* there which was negative.
- In the lab, the on-call BMS carried out an RDT – **pan *Plasmodium* antigen** - and a provisional diagnosis of ***P. vivax*** was reported

* for Pf only.

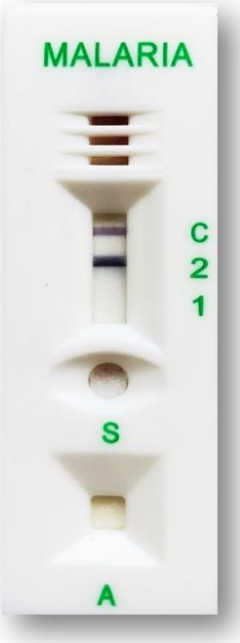


Image: MRL

Case 2

- Patient was quite poorly >> urgent examination of the blood films

  > revised diagnosis, *P. falciparum*

- patient was moved to ITU

- Samples sent to the MRL

  > *Pf* confirmed by microscopy

  & PCR

False negative RDT

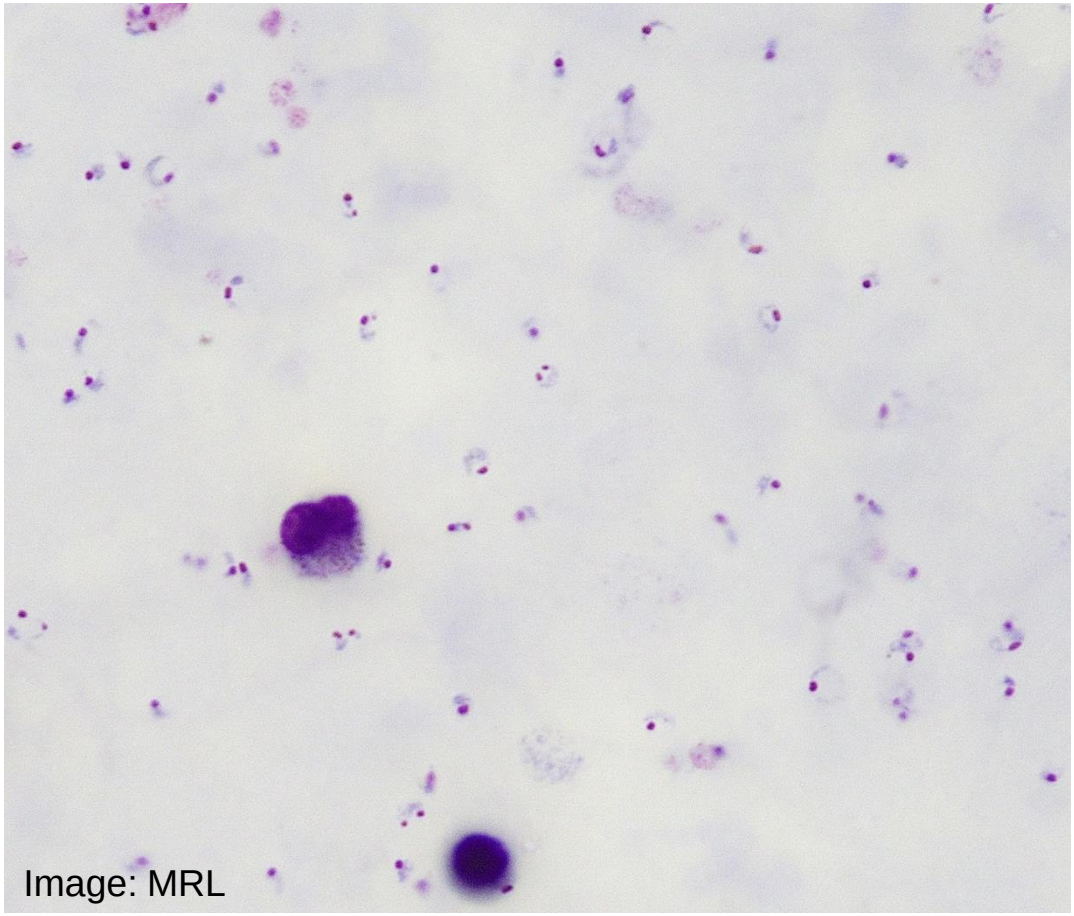
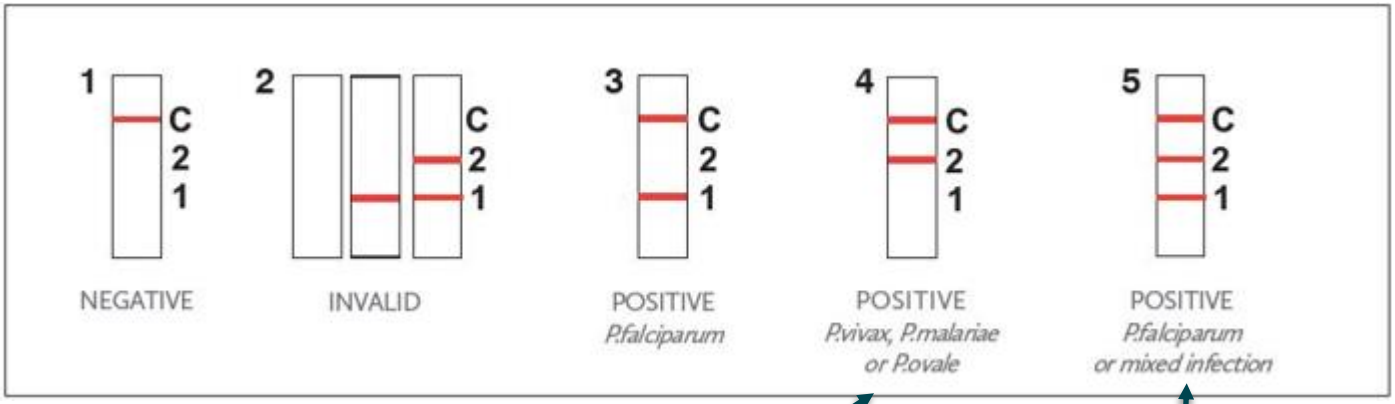


Image: MRL

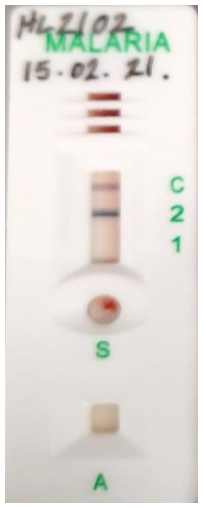
Case 2 - MRL investigation of discrepant RDT results



C = control

1 & 2 = Ag captured (differs between tests)

1 = Pf HRP2
+
2 = pan LDH



Pf = Pf LDH
+
P = pan LDH

Images: D. Nolder, MRL, LSHTM

Plasmodium falciparum HRP2 and RDTs

UKHSA MRL, Sutherland, Beshir & Drakeley labs, LSHTM

Why HRP2?

- Abundantly expressed >> most sensitive detection in WHO testing rounds
- Heat stable

➢ Majority of RDTs use this Ag

First reports of deletions from Peru 2010

Now reported from many countries in South America, Africa and Asia

Evolving ?

Plasmodium falciparum HRP2 and RDTs

UKHSA MRL, Sutherland, Beshir & Drakeley labs, LSHTM

Sequencing & genomic analysis >>

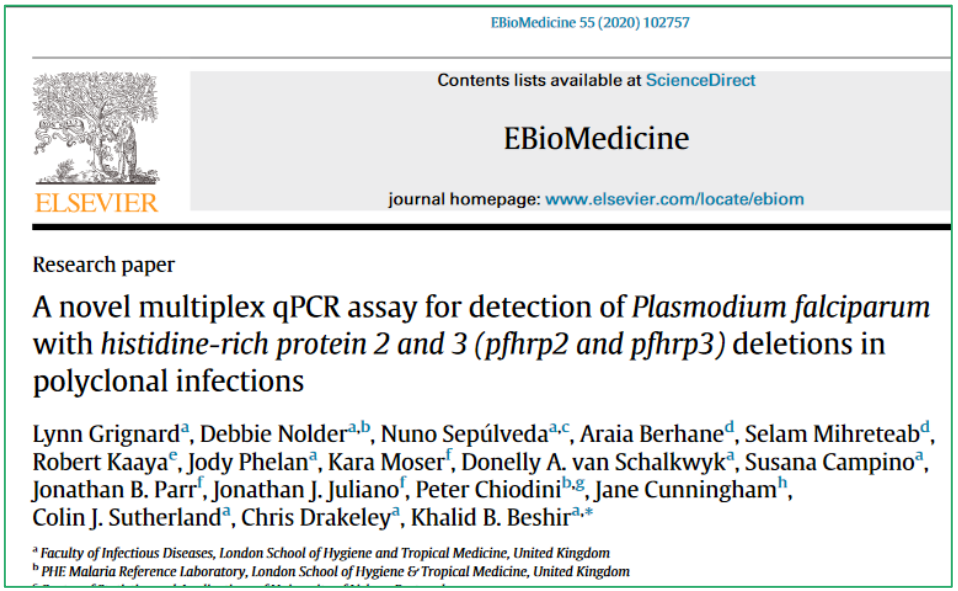
- Partial and full deletions of HRP2 can occur
- HRP2 (c/s 8) has a paralogue, HRP3 (c/s 13), with shared epitopes >> cross reactivity by RDT antibodies
- if %P is high enough, HRP3 is detected even in the absence of HRP2
- If both HRP2 & HRP3 are absent >> no reactivity on HRP2-based tests

- <u>Screening world-wide</u>:

  co-ordinated surveillance required

  WHO

  >> new assays eg



EBioMedicine 55 (2020) 102757

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.elsevier.com/locate/ebiom

Research paper

A novel multiplex qPCR assay for detection of *Plasmodium falciparum* with *histidine-rich protein 2 and 3 (pfhrp2 and pfhrp3)* deletions in polyclonal infections

Lynn Grignard[a], Debbie Nolder[a,b], Nuno Sepúlveda[a,c], Araia Berhane[d], Selam Mihreteab[d], Robert Kaaya[e], Jody Phelan[a], Kara Moser[f], Donelly A. van Schalkwyk[a], Susana Campino[a], Jonathan B. Parr[f], Jonathan J. Juliano[f], Peter Chiodini[b,g], Jane Cunningham[h], Colin J. Sutherland[a], Chris Drakeley[a], Khalid B. Beshir[a,*]

[a] Faculty of Infectious Diseases, London School of Hygiene and Tropical Medicine, United Kingdom
[b] PHE Malaria Reference Laboratory, London School of Hygiene & Tropical Medicine, United Kingdom

False negative RDTs

Increased cases of Pf RDT fails?

10+ cases in MRL since 2020

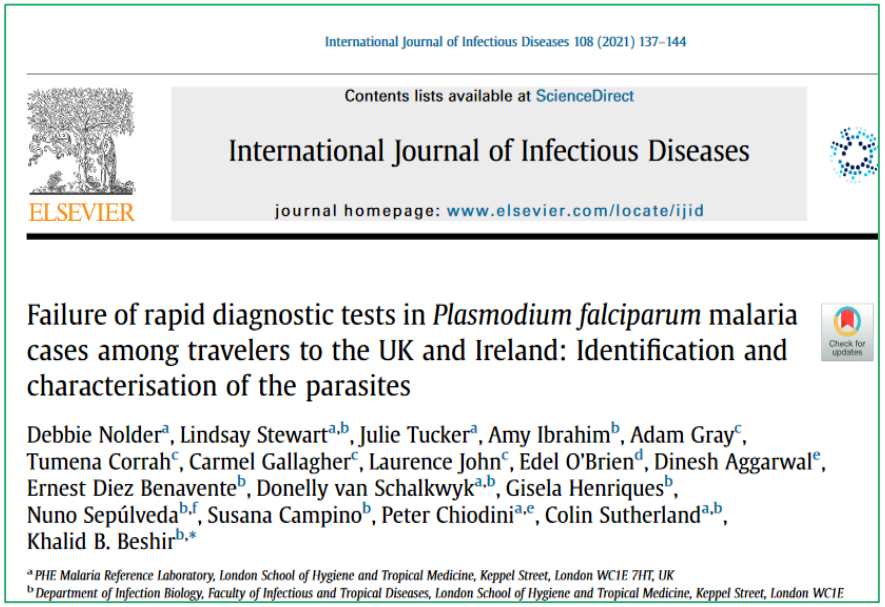
5 reported in 2021

- **Low %P > ? sensitivity of test (1)**
  ….some assays better than others
  …. All should > +ve at ~100p/uL (0,002%)

- **High %P > ? prozone effect (1)**
  ….rare
  ….Pf HRP2 assays only
  See Gillet *et al* 2011 Mal J

- **Pf HRP 2 +/- HRP3 gene deletion (3)**
  ….ongoing analysis of archived samples

>>> All East Africa



International Journal of Infectious Diseases 108 (2021) 137–144

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Failure of rapid diagnostic tests in *Plasmodium falciparum* malaria cases among travelers to the UK and Ireland: Identification and characterisation of the parasites

Debbie Nolder[a], Lindsay Stewart[a,b], Julie Tucker[a], Amy Ibrahim[b], Adam Gray[c], Tumena Corrah[c], Carmel Gallagher[c], Laurence John[c], Edel O'Brien[d], Dinesh Aggarwal[e], Ernest Diez Benavente[b], Donelly van Schalkwyk[a,b], Gisela Henriques[b], Nuno Sepúlveda[b,f], Susana Campino[b], Peter Chiodini[a,e], Colin Sutherland[a,b], Khalid B. Beshir[b,*]

[a] PHE Malaria Reference Laboratory, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK
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BSH Laboratory Diagnosis of Malaria Guidelines (revised 2022)

SUMMARY OF RECOMMENDATIONS – RDTs for malaria **DNA**

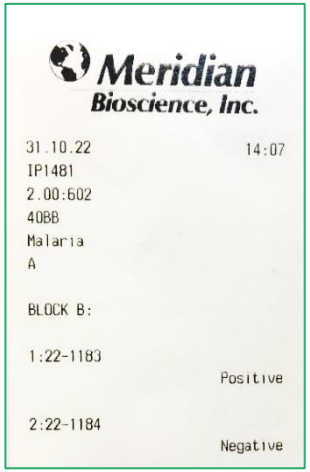
- Consideration should be given to the use of **nucleic-acid-based RDTs** eg LAMP

>> detects **DNA** of *Plasmodium* spp.:

>> highly sensitive (sim to PCR) but not quantitative

>> does not differentiate spp.

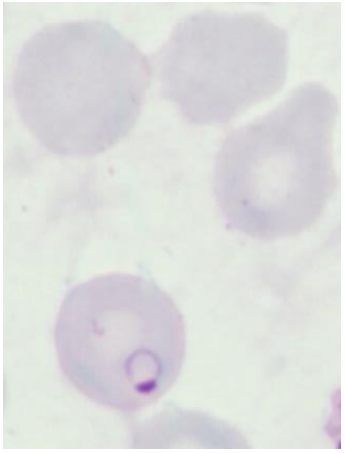
>> DNA can persist following treatment.



Images: MRL

BSH Laboratory Diagnosis of Malaria Guidelines (revised 2022)

- Be alert to possibility of malaria when not specifically requested: **incidental finding**



- Be aware of **geographical ranges of species** and **diagnostic issues eg with RDTs**

- Be aware of **other blood parasites**, especially *Babesia*, particularly when intra-erythrocytic parasites are seen but travel history and / or RDT results do not indicate a diagnosis of malaria.

- Participate in **External Quality Assessment** ie one of the UK NEQAS schemes ☺

Thanks !

UK Health Security Agency

Malaria Reference Laboratory (Director: Prof. Peter Chiodini)
Prof. Colin Sutherland (MRL Clinical Scientist)
Claire Rogers (Head of MRL / DPL),
MRL / DPL molecular team: Helen Liddy, Lucy Smith & Lindsay Stewart
Colleagues in the MRL / DPL (Emma, Sarah, Rita, Dawn, Helena, Saba, Keir, Karen)

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

HRP2 / 3:
Dr. Khalid B. Beshir
Dr. Lynn Grignard
Prof. Chris Drakeley
Dr. Nuno Sepúlveda

Malaria culture:
Lindsay Stewart
Dr Don van Schalkwyk
Gisela Henriques

Genomic analysis:
Prof. Susana Campino
Dr. Ernest Diez Benavente
Dr. Amy Ibrahim

And not forgetting….

Clinical teams & sending laboratories

Thank you !!

UK NEQAS
International Quality Expertise

Keep sending the samples in !

- 3mLs EDTA blood please
- Pre-treatment if possible
- Doesn’t have to be fresh