Global **Malaria** Programme

False-negative RDT results and implications of new reports of *P. falciparum histidine-rich protein 2/3* gene deletions

MAY 2016 (REV. SEPTEMBER 2017) INFORMATION NOTE

TARGET READERSHIP

National malaria control programme managers and their implementing partners, procurement agencies, national regulatory authorities for in-vitro diagnostics and manufacturers of malaria rapid diagnostic tests (RDTs).

PURPOSE

To provide updated information on the implications of reports of *histidine-rich protein 2/3* (*pfhrp2/pfhrp3*) gene deletions in *Plasmodium falciparum* parasites for case management and to advise on procedures for investigating suspected false-negative RDT results.

BACKGROUND

Most of the currently available commercial RDT kits work by detecting a specific protein expressed only by *P. falciparum*, called HRP2, in the blood of people infected with falciparum malaria. The antibodies on the test strip recognize the PfHRP2 antigen but may cross-react with protein expressed by another member of the HRP gene family, *pfhrp3*, because of the strong similarity of the amino acid sequence. The general preference for PfHRP2-based RDTs in procurement is due largely to the finding in some studies that they are more sensitive and heat-stable than RDTs that detect other malaria antigens, such as plasmodium lactate dehydrogenase (pLDH) – pan (all species) or *P. falciparum*-specific – or aldolase.

World Health Organization

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In certain situations, HRP2-detecting tests are less sensitive, particularly for parasites that express little or no target antigen, resulting in a false-negative result. In 2010, Gamboa et al.[1] reported the first confirmed identification of *P. falciparum* parasites with *pfhrp2/pfhrp3* gene deletions, which expressed neither PfHRP2 or PfHRP3, in the Amazon River basin in Peru. Subsequent retrospective analyses[2] at different sites in the Loreto region of the Peruvian Amazon showed a statistically significant increase in the number (and percentage) of parasites with gene deletions between specimens collected in 1998–2001 (20.7%) and in 2003–2005 (40.6%). The prevalence of parasites with *pfhrp2/pfhrp3* gene deletions varies, however, from locality to locality. Publications followed from other countries, such as India, Mali and Senegal, but with much lower prevalence estimates, and some studies were based on a flawed design and/or had incomplete analyses.[3] There have been no reports of parasites failing to express pLDH or aldolase, the other antigens targeted by malaria RDTs, as these targets are essential enzymes for parasite metabolism and survival.

In light of recent reports of HRP2 deletions in parasites in several African countries, including the Democratic Republic of the Congo,[4] Eritrea,[5] Ghana,[6] Kenya,[7] Rwanda[8] and India,[9] WHO is providing guidance to RDT manufacturers, procurers, implementers and users on confirming (or excluding) new geographical foci of parasites with deleted *pfhrp2/pfhrp3* and on investigating other causes of suspected false-negative RDT results.

The guidance is updated to include the conclusions and recommendations of a WHO technical consultation on *pfhrp2/3* gene deletions in July 2016 and the results of round 7 of WHO malaria RDT product testing (http://www.who.int/malaria/publications/atoz/978924151268/en/).

POTENTIAL CAUSES AND INVESTIGATIONS INTO SUSPECTED FALSE-NEGATIVE RDT RESULTS

In most settings, genetic mutations like deletion of *pfhrp2/pfhrp3* in parasites are not likely to be the main cause of false-negative results in RDTs, and more studies are required to determine the true prevalence of these mutations. False-negative RDT results are more likely to be due to the procurement and use of poor-quality RDTs or use of the wrong comparator for the diagnostic test, such as poor-quality microscopy for cross-checking negative RDT results.[10] Poor transport and storage conditions for RDTs, with sustained exposure to high temperature, can affect their diagnostic performance. More rarely, operator errors during performance and/or interpretation of RDT results can result in false-negative results. Table 1 lists the product, operator, supply chain, host and parasite factors that can lead to false-negative RDT results and suggested means to investigate such cases. Many of the potential causes of false-negative results can be prevented or minimized by procuring good-quality RDTs, by improving the quality control of procured RDTs (lot verification) and by good training of users.

TABLE 1.
Causes of false-negative RDT results and investigative actions

CLASSIFICATION	CAUSE OF FALSE-NEGATIVE RDT RESULT	SUGGESTED ACTIONS
Operator factors	Operator error in preparing the RDT, performing the test or interpreting the result	Verify whether RDTs are used by untrained staff; assess RDT competence on site.
Use of an imperfect "gold standard" as a comparator	Thick or thin films from a patient with a negative RDT result are incorrectly interpreted as "positive" by microscopy.	Verify microscopy procedures and interpretation by a qualified microscopist.
Product design or quality	Poor sensitivity of an RDT due to poor specificity, affinity or insufficient quantity of antibodies. Poor packaging can result in exposure to humidity, which will rapidly degrade RDTs.	Inspect the instructions for errors; inspect the integrity of the packaging, including the colour indicator desiccant for evidence of moisture. Cross-check suspected false-negative RDT results against microscopy performed by two qualified microscopists or, if microscopy is not available, against a high-quality non-HRP2-detecting RDT; retrieve RDTs from affected areas, and send for lot testing to WHO- or FIND-recognized laboratories.*
	Poor visibility of test bands due to strong background colour on the test	Assess RDT performance and training on site; if the strong background colour persists, notify the manufacturer.
	Incorrect instructions for use	Review the instructions for use for accuracy.
Transport or storage conditions	Antibody degradation due to poor resistance to heat or incorrect transport or storage, e.g. exposure to high temperatures, freeze-thawing	Inspect temperature monitoring of RDT transport and storage chain to determine whether temperatures exceed maximum storage temperature, typically 30 °C or 40 °C or < 2 °C. If temperatures are not within those in the manufacturers instructions, send the RDTs to the WHO-FIND lot testing laboratory.* Train health workers to respect storage conditions, and improve storage places (e.g. add fans).
Parasite factors	Parasites lack or express low levels of the target antigen, i.e. HRP2	Patient samples are negative on an HRP2 test line of at least two quality-assured malaria RDTs **and** positive on the pan- or pf-pLDH test line of a combination RDT **and** the sample is confirmed to be positive microscopically for *P. falciparum* by two qualified microscopists. If these conditions are met, place fresh blood samples or dried blood spots (50-60 µL) on Whatman® 3MM filter paper or other collection cards, in frozen storage (-20 °C) until shipment for PCR and *pfhrp2/pfhrp3* gene analysis.
	Variation in the amino acid sequence of the epitope targeted by the monoclonal antibody	Repeat test with an RDT of a different brand or different manufacturer that targets the same antigen or an RDT that targets a different antigen, e.g. pan-pLDH or Pf-pLDH. Manufacturers may use monoclonal antibodies that target different epitopes of the same antigen.
Host parasite density	Very low parasite density or target antigen concentration	Perform high-quality microscopy, and record the parasite count; if high-quality microscopy is not available, repeat the RDT if symptoms persist.
	Very high parasite load (severe malaria) causing prozone effect (hyperparasitaemia and antigen overload)	Repeat testing with a 10 × and if needed a subsequent 50 × dilution of the sample, with dilutions in 0.9% NaCl **

Note:

* Information about lot testing can be found here: http://www.who.int/malaria/areas/diagnosis/rapid-diagnostic-tests/evaluation-lot-testing/en/

** Gillet et al. Prozone in malaria rapid diagnostics tests: how many cases are missed? Malar J 2011;10:166. https://doi.org/10.1186/1475-2875-10-166

Thousands of febrile children with negative RDT results have been followed up in several studies,[11,12] which showed no malaria-related deaths or hospitalizations. In many endemic areas, malaria prevalence rates have fallen to low levels, and the majority of accurately performed RDTs give negative results. Treatment of individuals with negative RDT results promotes drug resistance, wastes resources and can delay diagnosis of non-malaria causes of fever. In some circumstances, however, false-negative RDT results should be suspected, and an investigation should be carried out to determine the quality of the RDTs, the competence of the operator and/or the presence of *hrp2/hrp3* deletions.

When should false-negative RDT results be suspected for individual patients?

- A symptomatic patient with an initially negative RDT who presents with persistent signs or symptoms of malaria and repeated negative RDT results but a positive blood film interpreted by a qualified microscopist or a positive result with a different quality-assured RDT that targets a different *falciparum-specific malaria antigen (e.g. pf-pLDH) or is of the same brand but from a different lot.*
- A patient with signs or symptoms of malaria with a negative HRP2-based RDT result, who recently visited an area that is known to have a high prevalence of *pfhrp2/hrp3*-deleted parasites, such as Eritrea and Peru.

When should false-negative RDT results be suspected for a population living in a certain geographical area?

- Discordance between RDT and microscopy results, with ≥ 10–15% higher positivity rates by microscopy and routine quality control by cross-checking or when both tests are performed on the same individuals (e.g. during surveys).
- The national malaria control programme and/or the RDT manufacturer receives multiple formal complaints or anecdotal evidence of RDTs returning inaccurate results.

WHEN AND HOW SHOULD FALSE-NEGATIVE HRP2-DETECTING RDT RESULTS DUE TO SUSPECTED PFHRP2 DELETION BE INVESTIGATED?[13]

A *pfhrp2* deletion should be strongly suspected if a patient sample gives negative results on an HRP2 test line of at least two quality-assured malaria RDTs[14] **and** positive on the pan- or pf-pLDH test line when a combination test is used, **and** the sample is confirmed microscopically to be positive for *P. falciparum* by two qualified microscopists.

If a *pfhrp2* gene deletion is suspected and the conditions described above are met:

- Immediately inform the National Malaria Control Programme and WHO;
- Archive the labelled RDTs and slides in a dry, clean area;

- Collect at least 50 µL of blood (about one drop) onto filter paper (e.g. Whatman® 3MM) or appropriate collection cards optimized for DNA analysis;[15] air-dry filter paper or cards overnight in a clean environment, sealed in air-tight plastic bags with desiccant.[16]

- Confirm the presence of *P. falciparum* infection by PCR analysis according to established protocols and with appropriate standards and quality control measures.

- If PCR is positive, confirm *pfhrp2/hrp3* gene deletion by PCR and antigen analysis at laboratories experienced in this kind of assay. WHO/GMP can facilitate linkages with such laboratories and provide further guidance. Contact: cunninghamj@who.int, with the subject line: "Laboratory support for investigations into suspected pfhrp2/3 gene deletions".

IMPLICATIONS OF *PFHRP2/HRP3* MUTATIONS OR DELETIONS FOR PROGRAMMES

Attributing false-negative results to *pfhrp2/pfhrp3* deletion has significant implications for public health. Alternative RDTs will have to be procured, and case management decisions will have to be revised, with re-training in algorithms and RDTs. Therefore, all investigations must be carried out systematically and accurately.

Following confirmation of *pfhrp2* deletions in initial case investigations, blood collection surveys should be made of confirmed *P. falciparum* cases in the specific geographical region to determine the prevalence of parasites carrying gene deletions. Representative samples are required to establish reliable estimates of the prevalence of these parasites. In September 2017, WHO will publish a standard survey protocol for determining whether the number of *pfhrp2* deletions that cause negative HRP2 RDT results among symptomatic patients with confirmed *P. falciparum* malaria has reached a threshold for a change in diagnostic strategy. This protocol will include a sampling tool, report form and data entry templates.

ALTERNATIVES TO HRP2-BASED RDTS

If *pfhrp2* deletions are found to be prevalent among symptomatic individuals (lower 95% confidence interval is > 5%), as, e.g. in Eritrea and several countries in South America (Brazil, Colombia, Peru), country programmes will have to switch to RDTs that do not rely exclusively on HRP2 for detecting *P. falciparum*. A threshold of 5% was selected because it somewhere around this point that the proportion of cases missed by HRP2 RDTs due to non-hrp2 expression may be greater than the proportion of cases that would be missed by less-sensitive pLDH-based RDTs. A recommendation to switch is further informed by mathematical models that show whether parasites lacking *pfhrp2* genes will spread under HRP2-only RDT pressure; a switch may also be decided because of the complexity of procuring and training in use of multiple RDTs. Any change should be applied nationwide, although roll-out might be prioritized on the basis of the prevalence of *pfhrp2* deletions.

Table 2 illustrates the performance of RDTs evaluated in the WHO malaria RDT product testing programme[18] for diagnosis of *P. falciparum* malaria by detection of non-HRP2 antigens, namely *Plasmodium* lactate dehydrogenase (pLDH), pan (pan-pLDH; all species) and *P. falciparum*-specific (pf-pLDH). The products are coded by colour on the basis of whether they meet the recommended procurement critieria for detection of *P. falciparum* in test lines targeting HRP2, pf or pan-pLDH or both. In areas with both *pfhrp2*-deleted parasites and non-*pfhrp2*-deleted parasites, a combination RDT should meet minimum performance criteria for *P. falciparum* detection based on HRP2 and separately based on pf-pLDH. However, for surveys, owing to the scarcity of RDTs that meet performance criteria for Pf detection based on pf-pLDH, RDTs that detect pf-pLDH can be used if their panel detection score is > 90 at 2000 parasites/μL and their false-positive and invalid rates are < 2%. Further details of e.g. heat stability, false-positive results for non-*P. falciparum* infections and test band intensity should be consulted in product testing reports.

The current RDT product testing programme is based on *P. falciparum* culture and clinical samples that express HRP2. This is problematic for assessing the performance of products in which HRP2 and pf-pLDH are on the same test line (products 1, 4 and 6 in Table 2). These tests cannot be evaluated for both antigens; this can be done only when the two antigens are shown on separate test lines. To address this problem, WHO and collaborators are establishing a panel of wild-type and cultured *pfhrp2*-deleted parasites that include both *pfhrp3*-positive and *pfhrp3*-negative combinations, which will be tested in round 8 of WHO malaria RDT product testing. Thus, new data will become available on the performance of these dual antigen test lines as well as on other combination tests that have separate HRP2 and non-HRP2 Pf target antigens.

Where microscopy is available, services should be strengthened to ensure that parasitological confirmation of malaria continues during the transition to new RDTs and for investigations of new foci of suspected *pfhrp2*/*pfhrp3*-deleted parasites.

TABLE 2

Non-HRP2-based RDTs for detecting *P. falciparum* malaria evaluated in WHO malaria RDT product testing rounds 1–7

	PRODUCT	PRODUCT CODE	MANU-FACTURER	PANEL DETECTION SCORE [a]										ROUND	MEETS WHO PROCUREMENT CRITERIA
				200 parasites/µl					2000 parasites/µl						
				Pf samples [c]				Pv samples[d]	Pf samples[c]				Pv samples[d]		
				HRP2/pf-pLDH (dual antigen single test line)	HRP2 test line	pf-pLDH test line	pan-pLDH only test		HRP2/pf-pLDH (dual antigen single test line)	HRP2 test line	pf-pLDH test line	pan-pLDH only test			
	Pf only														
1	CareStart™ Malaria Pf (HRP2/pLDH) Ag RDTf	RMPM(U)-XXX7X	Access Bio, Inc.	91.0				NA	99.0	NA	NA	NA	NA	6	Yes , but only for HRP2 based Pf diagnosis; unknown performance of pf-pLDH alone for detection of *pfhrp2* deleted parasites
2	CareStart™ Malaria Pf (HRP2/pLDH) Ag Combo 3-Line [e]	RMSM-05071	Access Bio, Inc.	NA	94.0	38	NA	NA	NA	99.0	92.0	NA	NA	7	Yes, but only for HRP2 based detection of Pf or as screening test in surveys for *pfhrp2* deleted parasites
3	SD Bioline Malaria Ag P.f (HRP2/pLDH) [e, f]	05FK90	Standard Diagnostics, Inc.	NA	87.0	52.0	NA	NA	NA	100.0	97.0	NA	NA	6	Yes, but only for HRP2 based detection of Pf or as screening test in surveys for *pfhrp2* deleted parasites
4	SD BIOLINE Malaria Ag P.f. (HRP2/pLDH) 2 Lines	05FK130-40-0	Standard Diagnostics, Inc.	90.0	NA	NA	NA	NA	100.0	NA	NA	NA	NA	7	Yes, but only for HRP2 based Pf diagnosis; unknown performance of pf-pLDH alone for *pfhrp2* deleted parasites
	Pf and Pan														
5	CareStart™ MALARIA Pf/PAN (pLDH) Ag RDT	RMLM-05071	Access Bio, Inc.	NA	NA	73.0	NA	0.0	NA	NA	100.0	NA	71.4	7	No for Pf or Pv detection
6	CareStart™ Malaria Screen RDT	RMAM-05071	Access Bio, Inc.	93.0	NA	NA	NA	94.3	99.0	NA	NA	NA	97.1	7	Yes, but only for HRP2 based Pf diagnosis; unknown performance of pf-pLDH alone for detection *pfhrp2* deleted parasites

	PRODUCT	PRODUCT CODE	MANU-FACTURER	PANEL DETECTION SCORE [a]										ROUND	MEETS WHO PROCUREMENT CRITERIA
				200 parasites/µl					2000 parasites/µl						
				Pf samples [c]				Pv samples[d]	Pf samples[c]				Pv samples[d]		
				HRP2/pf-pLDH (dual antigen single test line)	HRP2 test line	pf-pLDH test line	pan-pLDH only test		HRP2/pf-pLDH (dual antigen single test line)	HRP2 test line	pf-pLDH test line	pan-pLDH only test			
	Pf and Pv														
7	BIOCREDIT Malaria Ag Pf/Pv (pLDH/pLDH)	C60RHA25	RapiGEN Inc.	NA	NA	75.0	NA	100.0	NA	NA	98.0	NA	100.0	7	Yes
	Pf, Pf and Pv														
8	SD Bioline Malaria Ag P.f/P.f/P.v [e]	05FK120	Standard Diagnostics, Inc.	NA	84.0	36.0	NA	91.4		100.0	98.0		100.0	6	Yes, but only for HRP2 based detection of Pf or as screening test in surveys for *pfhrp2* deleted parasites
	Pan only														
9	CareStart™ Malaria PAN (pLDH) Ag RDT[f]	RMNM(U)-XXX7X	Access Bio, Inc.	NA	NA	NA	84.0	88.6	NA	NA	NA	99.0	97.1	5	Yes
10	Advantage Pan Malaria Card	IR013025	J. Mitra & Co. Pvt. Ltd.	NA	NA	NA	77.0	100.0	NA	NA	NA	98.0	100.0	5	Yes

UK, unknown
NA, not applicable
Pf, *Plasmodium falciparum*
Pv, *Plasmodium vivax*
pan, *Plasmodium* species
Pvom, *Plasmodium vivax, ovale* and *malariae*

Meets procurement criteria for case management

Does not meet procurement criteria for case management

Meets criteria only for use in surveys for pfhrp2 deletions

PERFORMANCE MEASURE	RECOMMENDED WHO PROCUREMENT CRITERIA
Panel detection score for Pf and Pv 200p/µL samples	≥ 75%
False-positive rate against clean-negatives	<10%
Invalid rate	<5% of tests conducted

a According to methods of WHO malaria RDT product testing a sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive

b The total number of times a positive result for malaria was generated when it should not have been

c Round 1, n=79; Round 2, n=100; Round 3, n=99; Round 4, n=98; Round 5, n=100; Round 6, n=100; Round 7, n=100

d Round 1, n=20; Round 2, n=40; Round 3, n=35; Round 4, n=34; Round 5, n=35; Round 6, n=35; Round 7, n=35

e PDS presented in the table is based on a HRP2 test line and Pf-pLDH test line. The overall result at 200 p/µl based on positive HRP2 or pf-pLDH test line is 88 for 05FK90; 85 for 05FK120 and at 2000p/µl it is 99 for RMSM-05071; 100 for 05FK90; 100 for 05FK120

f Indicates a WHO prequalified product

INTERIM WHO RECOMMENDATIONS

1. Suspected false-negative RDT results should be investigated.

2. *Pfhrp2/3* gene deletions should be suspected and the national malaria control programme and WHO informed when:

   a. a sample from an individual tests negative on the HRP2 line of at least two quality-assured malaria RDTs and positive on the pan- or pf-pLDH test line of a combination RDT and the sample is confirmed by microscopy to be positive for *P. falciparum* by two qualified microscopists;

   b. in a programme, the rates of discordance between the results of RDTs and microscopy are systematically ≥ 10–15%, with higher positivity rates in microscopy, where quality is controlled routinely by cross-checking or both are performed on the same individuals (e.g. during surveys) and/or when the national malaria control programme receives multiple formal complaints or anecdotal evidence of RDTs that give false-negative results for *P. falciparum*.

3. When *hrp2/hrp3* gene deletions have been reported, the baseline prevalence should be determined in the affected country and neighbouring countries. This may require specific surveys or adaptation of planned surveys, such as therapeutic efficacy studies. In September 2017, WHO will publish a standard survey tool for determining the prevalence of false-negative HRP2-based RDT results secondary to *pfhrp2* gene deletions.

4. Confirmatory evidence of deletions should include PCR for *pfhrp3*, in addition to PCR for *pfhrp2*, as HRP3 proteins can show cross-reactivity in HRP2-based RDTs; however, analysis of flanking genes for *pfhrp2* (and *pfhrp3*) and serological confirmation of the absent HRP2 antigen (by ELISA or a second brand of RDT) are optional.

5. A nationwide change to an RDT that includes non-HRP2 target antigens for *P. falciparum* is recommended when the lower 95% confidence interval of the prevalence of symptomatic patients carrying *pfhrp2*-deleted parasites (causing false-negative HRP2 RDT results) is ≥ 5%. If *pfhrp2* deletions are confirmed but the prevalence is < 5%, it is recommended that a change be planned over a longer period, as it is anticipated that *pfhrp2/3*-deleted parasites will persist and spread. A repeated survey after 1–2 years will inform a prioritized roll-out of RDTs that include non-HRP2-based antigens.

   In all other cases, if *pfhrp2* deletions are confirmed in samples from any source, the suggested action is to establish the prevalence of false-negative HRP-based RDT results secondary to *pfhrp2* deletion through representative surveys.

6. Well-preserved archived specimens may be analysed to identify the existence and geographical location of *pfhrp2/pfhrp3*-deleted parasite populations.

7. In the absence of confirmed reports of *pfhrp2/pfhrp3* gene deletions, it is not recommended that new initiatives be taken to find these gene deletions, unless they are prompted by findings described under 2 above.

WHO/GMP RESPONSE

Given the complexity of investigating suspected false-negative RDT results and the risk that parasites that do not express HRP2/HRP3 emerge but are not detected, WHO is conducting the following activities:

- preparing a plan of action for surveillance and response to the emergence and spread of *pfhrp2/3* gene deletions (October 2017);
- preparing standard protocols (and tools) for conducting baseline surveys to determine whether the prevalence of *pfhrp2* deletions that cause negative HRP2 RDT results among symptomatic patients with confirmed *P. falciparum* malaria has reached a threshold for a change in diagnostic strategy (September 2017);
- establishing a panel of *pfhrp2/3*-deleted parasites (cultured and wild-type) for evaluating the performance of non-HRP2 Pf-detecting RDTs;
- establishing a network of laboratories to review and build consensus on laboratory methods for characterizing *pfhrp2/3* gene deletions and linking reference laboratories with field investigators to ensure reliable, accurate reporting of *pfhrp2/*3 gene deletions;
- working with relevant groups to adapt planned surveys to include collection of blood samples for molecular testing for malaria, including analysis of *pfhrp2/pfhrp3*, based on WHO-recommended protocols. Areas affected by these mutations, including neighbouring countries, will be a priority;
- working with research groups that hold collections of recently archived samples to screen for the presence of *pfhrp2/pfhrp3*-deleted parasites;
- rigorously reviewing manuscripts submitted for publications and published reports of *pfhrp2/pfhrp3* deletions to determine the accuracy of claims; and
- encouraging test developers and RDT manufacturers to improve the performance of pLDH-based tests and identify new target antigens.

Endnotes

1. Gamboa D, Ho MF, Bendezu J, Torres K, Chiodoni PL, Barnwell J et al. A large proportion of *P. falciparum* isolates in the Amazon region of Peru lack *pfhrp2* and *pfhrp3*: implications for malaria rapid diagnostic tests. PLoS One. 2010:5:e8091.
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3. Cheng Q, Gatton M, Barnwell J, Chiodini P, McCarthy J, Bell D et al. *Plasmodium falciparum* parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting. Malar J. 2014;13:283.
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6. Amoah LE, Abankwa J, Oppong A. Plasmodium falciparum histidine rich protein-2 diversity and the implications for PfHRP 2-based malaria rapid diagnostic tests in Ghana. Malar J. 2016;15:101.

7. Beshir KB, Sepúlveda N, Bharmal J, Robinson A, Mwanguzi J, Obukosia Busula A et al. *Plasmodium falciparum* parasites with 1 histidine-rich protein 2 (*pfhrp2*) and pfhrp3 gene deletions in two endemic regions of Kenya (submitted for publication).

8. Kozycki CT, Umulisa N, Rulisa S, Mwikarago EI, Musabyimana JP, Habima JP et al. False-negative malaria rapid diagnostic tests in Rwanda: impact of *Plasmodium falciparum* isolates lacking *hrp2* and declining malaria transmission. Malar J. 2017;16:123.

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10. Kahama-Maro J, D'Acremont V, Mtasiwa D, Genton B, Lengeler C. Low quality of routine microscopy for malaria at different levels of the health system in Dar es Salaam. Malar J 2011;10:332.

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12. Baiden F, Webster J, Tivura M, Delimini R, Berko Y, et al. Accuracy of rapid tests for malaria and treatment outcomes for malaria and non-malaria cases among under-five children in rural Ghana. PLoS ONE 2012;7(4): e34073.

13. Cheng Q, Gatton M, Barnwell J, Chiodini P, McCarthy J, Bell D et al. *Plasmodium falciparum* parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting. Malar J. 2014;13:283.

14. Quality-assured RDTs are selected on the basis of WHO-recommended procurement criteria, lot-tested before field deployment by a WHO- or FIND-recognized laboratory and transported and stored in accordance with the manufacturer's recommendations.

15. Stored blood slides and used RDTs could be used as sources of DNA, but they are not ideal.

16. The desiccant in the RDT cassette packaging can be used.

17. Gatton ML, Dunn J, Chaudhry A, Ciketic S, Cunningham J, Cheng Q. Use of PfHRP2-only RDTs rapidly selects for PfHRP2-negative parasites, with serious implications for malaria case management and control. J Infect Dis. 2017. doi: 10.1093/infdis/jix094.

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19. To allow for sampling variation.