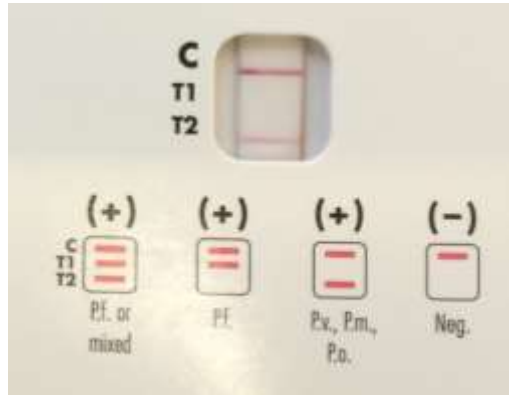


# Imported malaria from Africa with false negative rapid diagnostic test for *P. falciparum* due to deletion of the histidine-rich protein 2 gene



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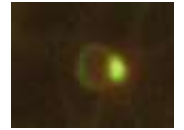
<sup>1</sup>Department of Medical Microbiology and  
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Academic Medical Center, Amsterdam, The  
Netherlands

# Malaria

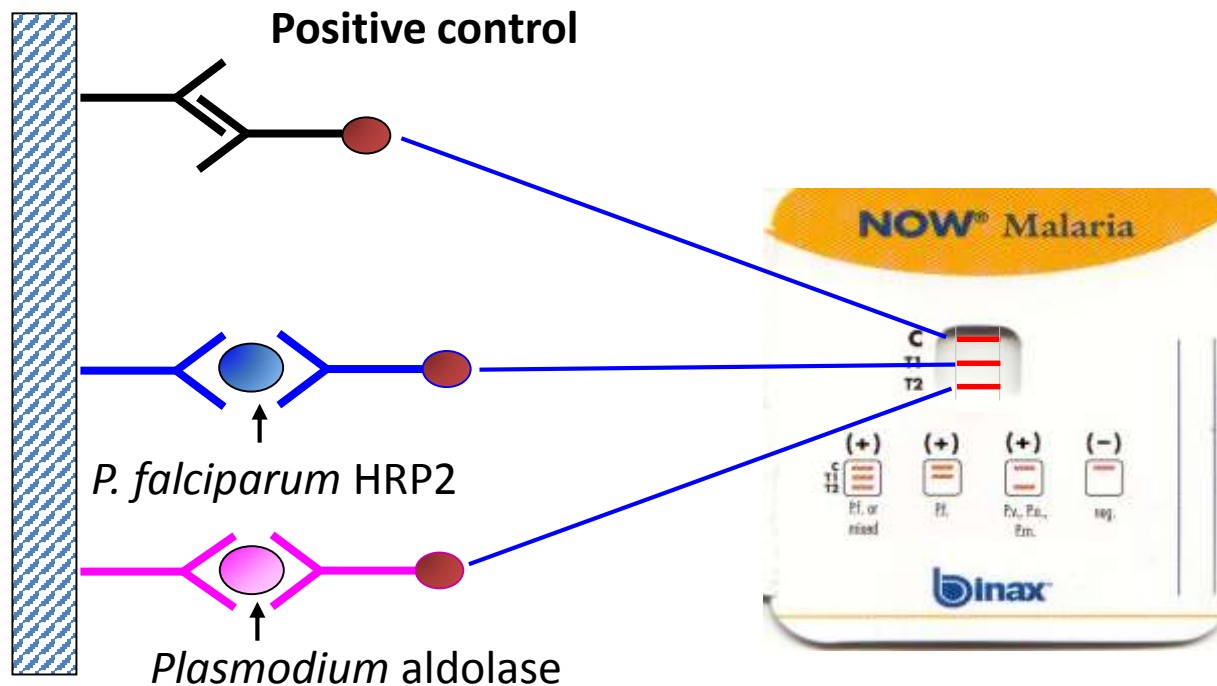
- 25 April 2017: World Malaria Day
- Devastating disease in tropical countries with 212 million cases and an estimated 429,000 deaths worldwide in 2015
- In Europe, malaria is an imported disease: returning travellers and immigrants, 6,000-10,000 per year
- Different species require different treatment/management
  - *Plasmodium falciparum*
  - *Plasmodium vivax*
  - *Plasmodium ovale*
  - *Plasmodium malariae*
  - *Plasmodium knowlesi*

# Malaria diagnosis

- Thick smear (sensitivity +++, speciation +)
- Thin smear (sensitivity ++, speciation +++)
- Quantitative buffy coat (QBC) (sensitivity +++++, but no speciation)
- Rapid diagnostic tests based on antigen detection (sensitivity *Pf* +++, non-*Pf* +)

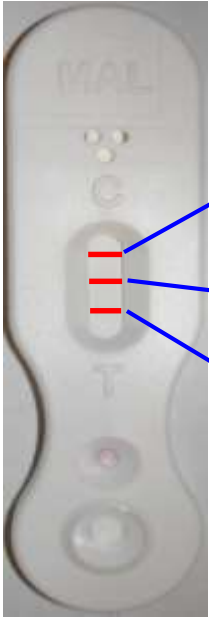


# Rapid Diagnostic Test (RDT): antigen detection e.g. BinaxNOW<sup>®</sup> Malaria



# Most RDTs use HRP2 antibodies for *P. falciparum* detection

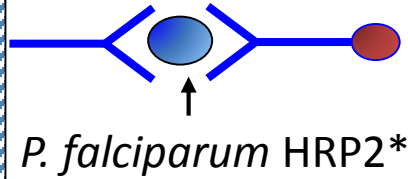
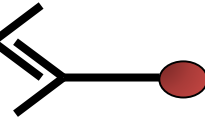
ICT Malaria Dual Test



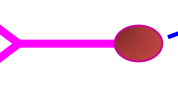
*Plasmodium* lactate dehydrogenase



Positive control

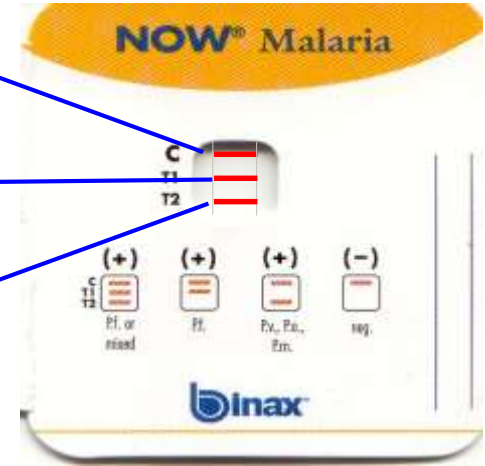


*P. falciparum* HRP2\*



*Plasmodium* aldolase

BinaxNOW® Malaria



\* some HRP2 antibodies cross-react with HRP3

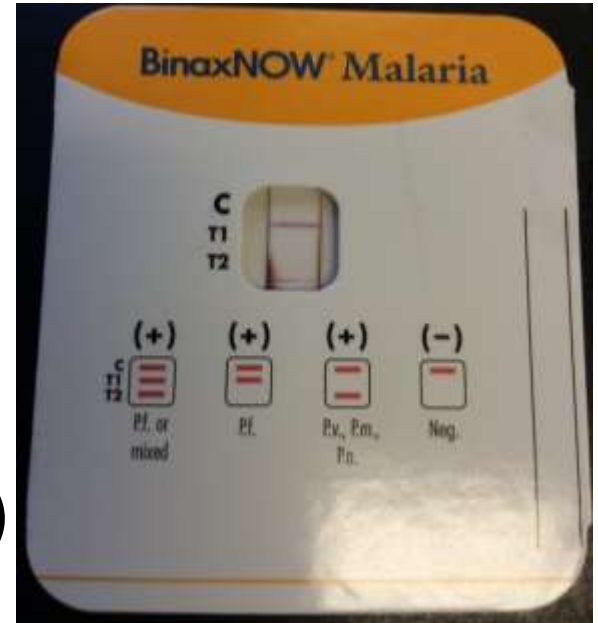
# Our patient

- September 2016
- 77 year old male, living in NL, born in Eritrea, visited Eritrea for >1 month ~14 days prior to presentation
- Presented with fever

# The initial tests in our patient

## Malaria tests:

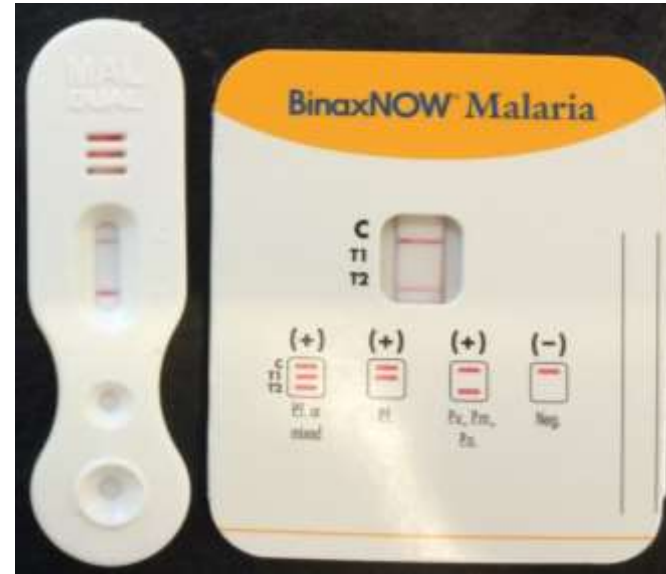
- Quantitative buffy coat: malaria parasites
- Thick smear: *P. falciparum* parasites
- Thin smear: *P. falciparum* parasites
- Parasitemia 1.6%
- RDT: BinaxNOW® Malaria: PfHRP2 (T1) negative, pan-aldolase (T2) positive



# Additional tests

## Malaria tests:

- Quantitative buffy coat: malaria parasites
- Thick smear: *P. falciparum* parasites
- Thin smear: *P. falciparum* parasites
- Parasitemia 1.6%
- RDT: BinaxNOW® Malaria: PfHRP2 (T1) negative, pan-aldolase (T2) positive
- Repeated BinaxNOW® (same results)
- Additional RDT: ICT Malaria Dual: PfHRP2 negative, pLDH positive
- Real-time PCR: *P. falciparum* DNA, Cp=30
- Illumigene® Malaria test positive





# Possible causes of false negative RDT results

| Classification                  | Cause of false negative RDT result  |
|---------------------------------|---|
| Product design or quality       | Poor detection sensitivity of a RDT product due to poor specificity, affinity, or insufficient quantity of antibodies used in the RDT |
|                                 | Poor visibility of test bands due to high background colour on the test   |
|                                 | Incorrect instructions for use  |
| Transport or storage conditions | Antibody degradation due to poor durability to heat or to incorrect transport or storage  |
| Operator factors                | Operator error in preparing the RDT, performing the test, or interpreting the result  |
| Host parasite density           | Very low parasite densities/target antigen concentrations   |
|                                 | Prozone effect (hyperparasitemia/antigen overload)  |
| Parasite factors                | Variation in the amino acid sequence of the epitope targeted by the monoclonal antibody   |
|                                 | Parasites lacking the target antigens or expressing reduced levels of the target antigens   |

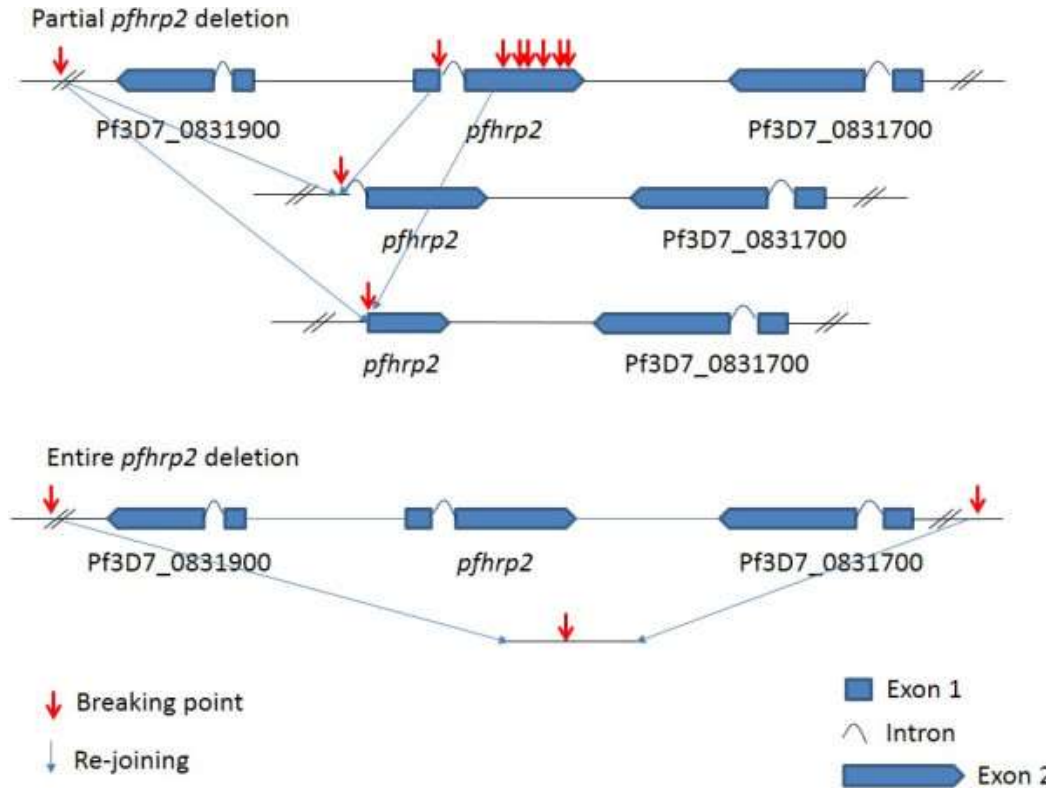
# Possible causes of false negative RDT results

| Classification                  | Cause of false negative RDT result   |
|---------------------------------|--|
| Product design or quality       | Excluded:<br>other tests from the same batch by the same operator worked well,<br>other operators obtained identical results,<br>other RDT showed similar results                    |
| Transport or storage conditions |  |
| Operator factors                |  |
| Host parasite density           | Very low parasite densities/target antigen concentrations<br>Prozone effect (hyperparasitemia/antigen overload)  |
| Parasite factors                | Variation in the amino acid sequence of the epitope targeted by the monoclonal antibody<br>Parasites lacking the target antigens or expressing reduced levels of the target antigens |

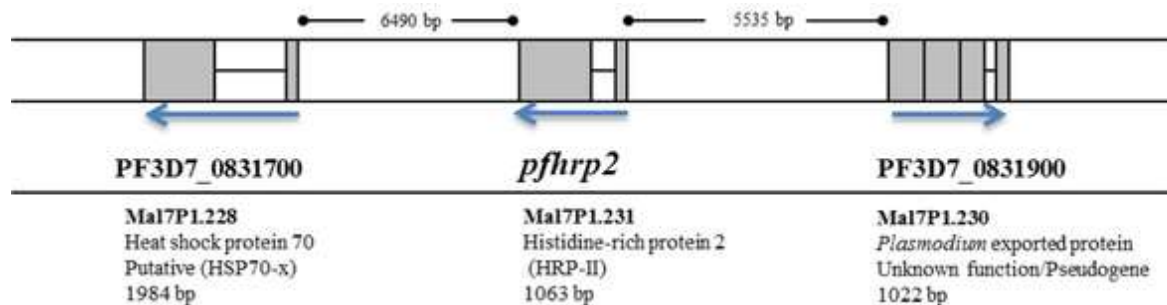
# Possible causes of false negative RDT results

| Classification                  | Cause of false negative RDT result   |
|---------------------------------|--|
| Product design or quality       | <p style="text-align: center;">Excluded:<br/> other tests from the same batch by the same operator worked well,<br/> other operators obtained identical results,<br/> other RDT showed similar results</p> |
| Transport or storage conditions |  |
| Operator factors                |  |
| Host parasite density           | <p style="text-align: center;">Parasitemia was 1.6%: sufficient for a positive test, too low for prozone effect</p>  |
| Parasite factors                | <p>Variation in the amino acid sequence of the epitope targeted by the monoclonal antibody</p>   |
|                                 | <p>Parasites lacking the target antigens or expressing reduced levels of the target antigens</p>   |

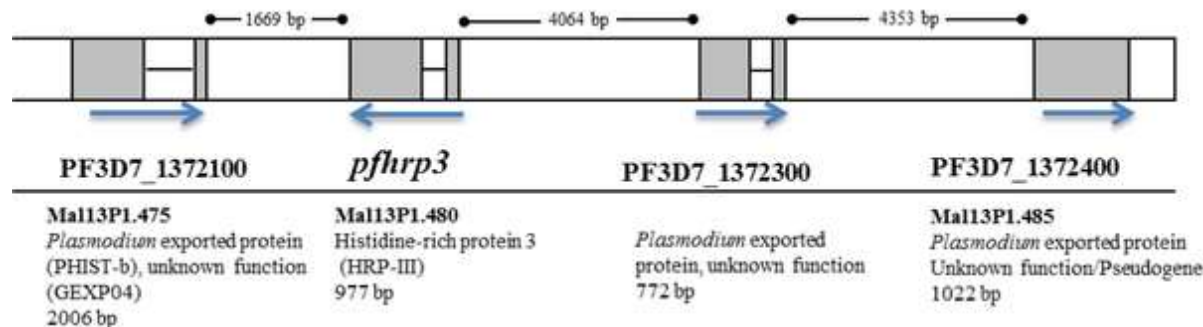
# Absence of PfHRP2 and PfHRP3 in South American *P. falciparum* parasites is caused by (partial) deletion of the encoding genes



## Schematic of the structure of *pfhrp2* and *pfhrp3* and their respective neighboring genes.



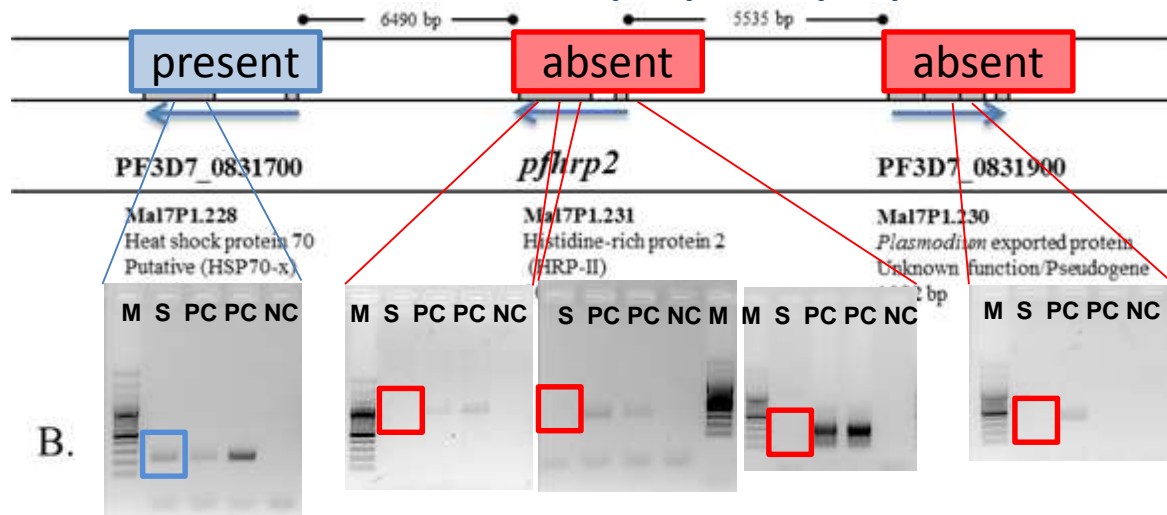
*pfhrp2*  
locus



*pfhrp3*  
locus

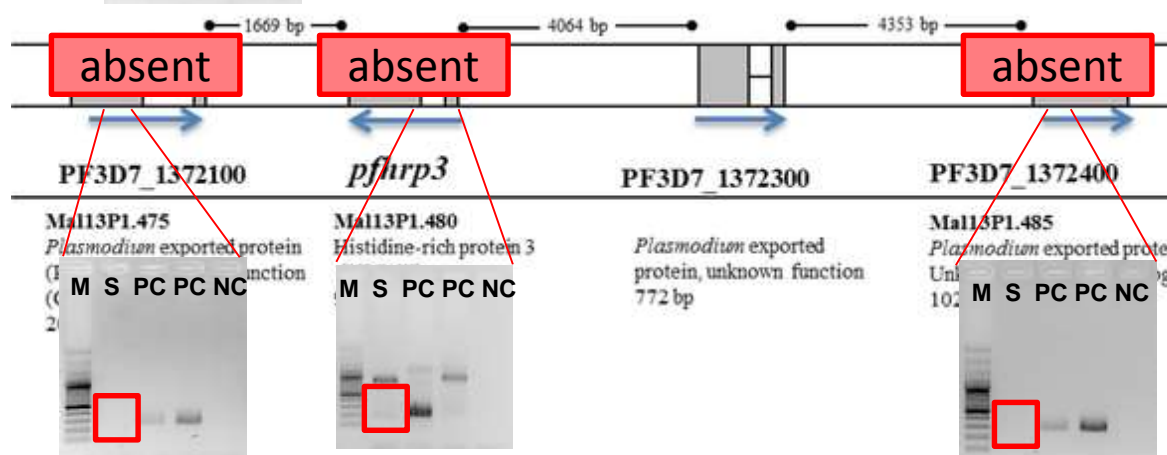
Test for presence of genes by performing (nested) PCRs for *pfhrp2* and *pfhrp3* genes and flanking regions, using similar amount of DNA from other patients as positive controls.

# Schematic of the structure of *pfhrp2* and *pfhrp3* and their respective neighboring genes.



*pfhrp2*  
locus

Control for DNA integrity and PCR amplification: 2 unrelated genomic loci



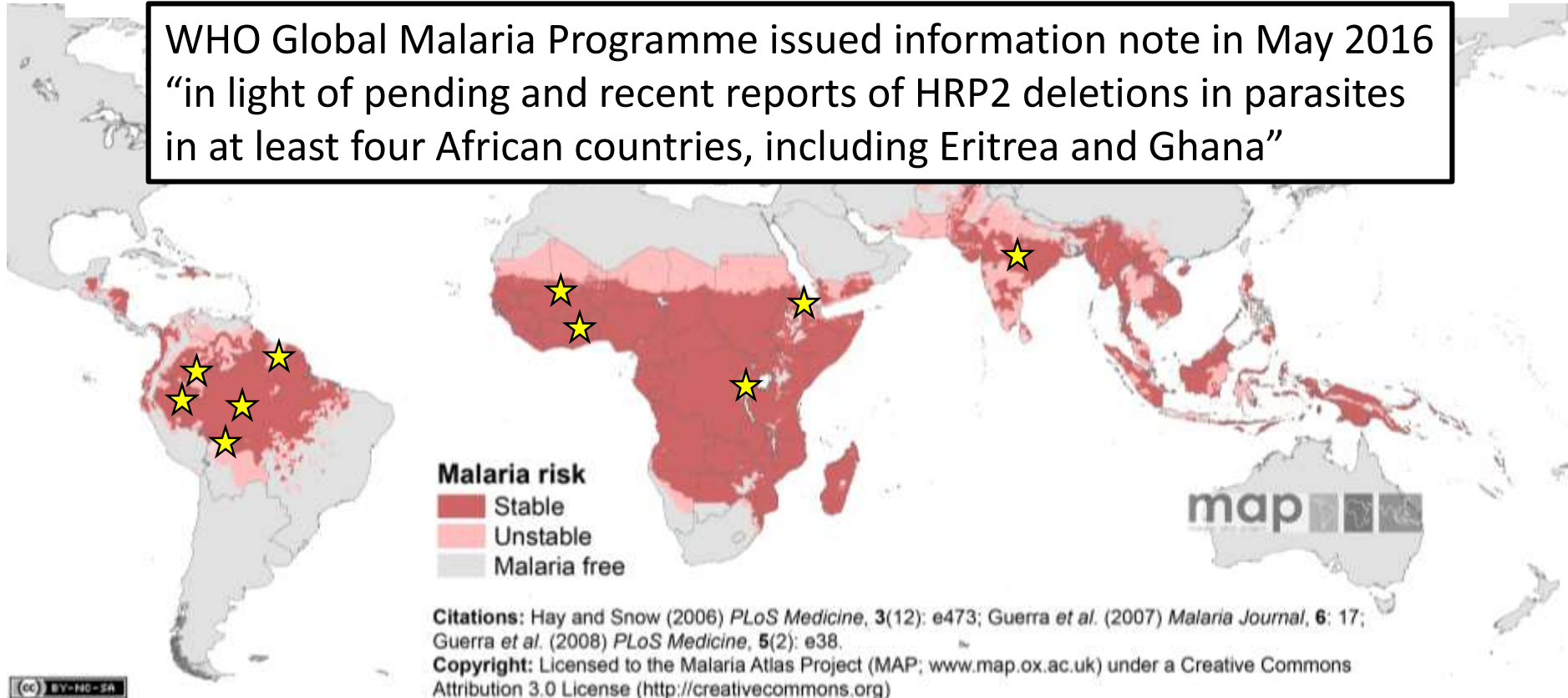
*pfhrp3*  
locus

# Conclusion and Discussion -1-

- The *P. falciparum* from our patient from Eritrea lacks reactivity to HRP2 monoclonal antibodies in RDT due to deletion of the HRP2 and HRP3 genes
- Non-reactivity results in **false negative results** or **incorrect species assignment** in tests detecting both Pf HRP2 and a pan-Plasmodium antigen, which may lead to suboptimal treatment

# Countries where HRP2 negative *P. falciparum* were reported

WHO Global Malaria Programme issued information note in May 2016  
“in light of pending and recent reports of HRP2 deletions in parasites  
in at least four African countries, including Eritrea and Ghana”



★ HRP2 negative *P. falciparum* reported, prevalence up to 40%



# Conclusion and Discussion -2-

- The *P. falciparum* from our patient from Eritrea lacks reactivity to HRP2 monoclonal antibodies in RDT due to deletion of the HRP2 and HRP3 genes
- Non-reactivity results in **false negative results** or **incorrect species assignment** in tests detecting both Pf HRP2 and a pan-Plasmodium antigen, which may lead to suboptimal treatment
- HRP2 negative *P. falciparum* are no longer confined to South America.
- Do not rely on HRP2 reactivity alone for *P. falciparum* detection in imported cases, keep confirming negative RDT results by microscopy