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# Molecular testing for detection of asymptomatic *Plasmodium* infections

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May 2024

## Disclosures

- Dr. Galelis an employee and shareholder of Roche Diagnostics
- cobas®Malaria was licensed by the US FDA for donor screening on March 19, 2024
- cobas®Malaria is not yet commercially available

# Malaria

## Transmission



Infection caused by *Plasmodium* parasites



Transmitted to humans by *Anopheles* mosquitoes



Parasites infect red blood cells

## Side effects



- Infection can cause severe anemia
- Other organs systems can be impacted causing other, sometimes fatal, symptoms
- Recurrent infections in endemic areas can result in asymptomatic chronic infection with low level parasitemia (“semi-immune”)



There are many *Plasmodium* species. Most human infections are due to 5 species:

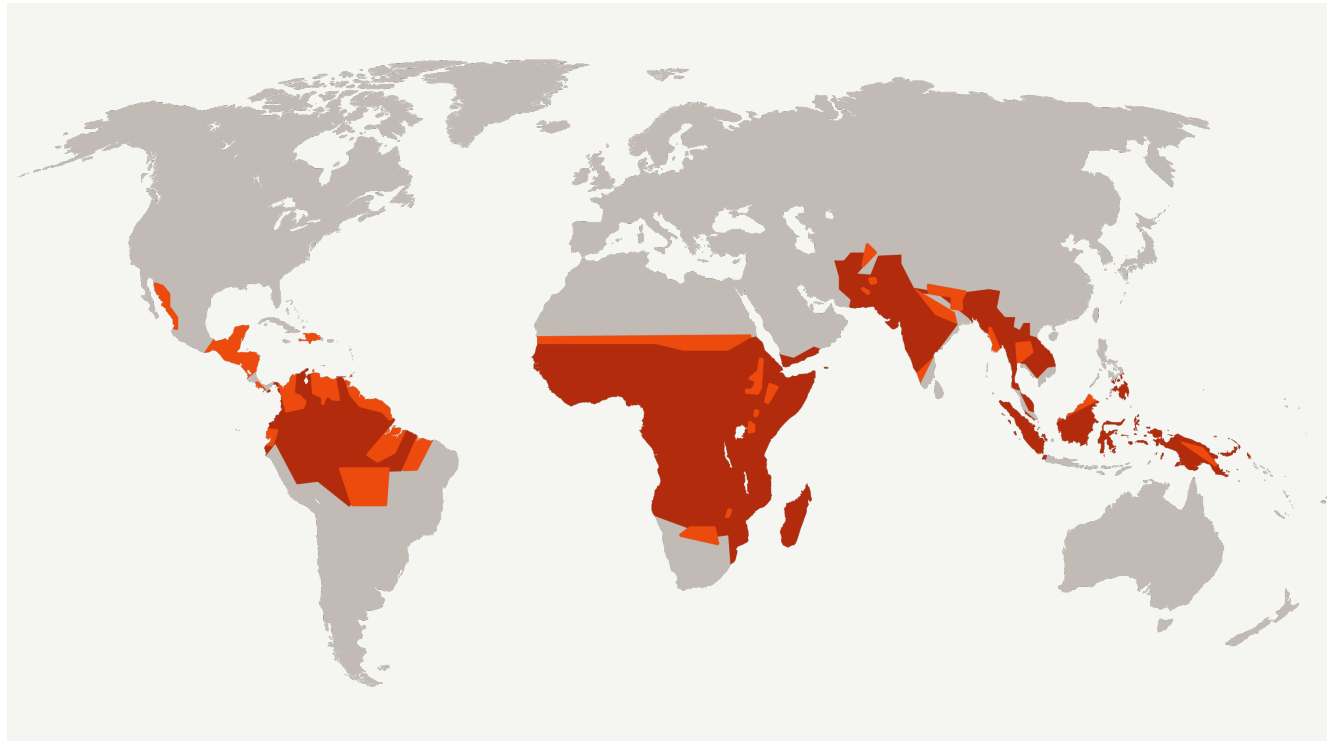
*P. falciparum*, *P. vivax*,  
*P. malariae*, *P. ovale* and  
*P. knowlesi*

# Transfusion-transmitted malaria (TTM)

Worldwide risk

Global rates of malaria<sup>1</sup>

■ High risk ■ Limited risk



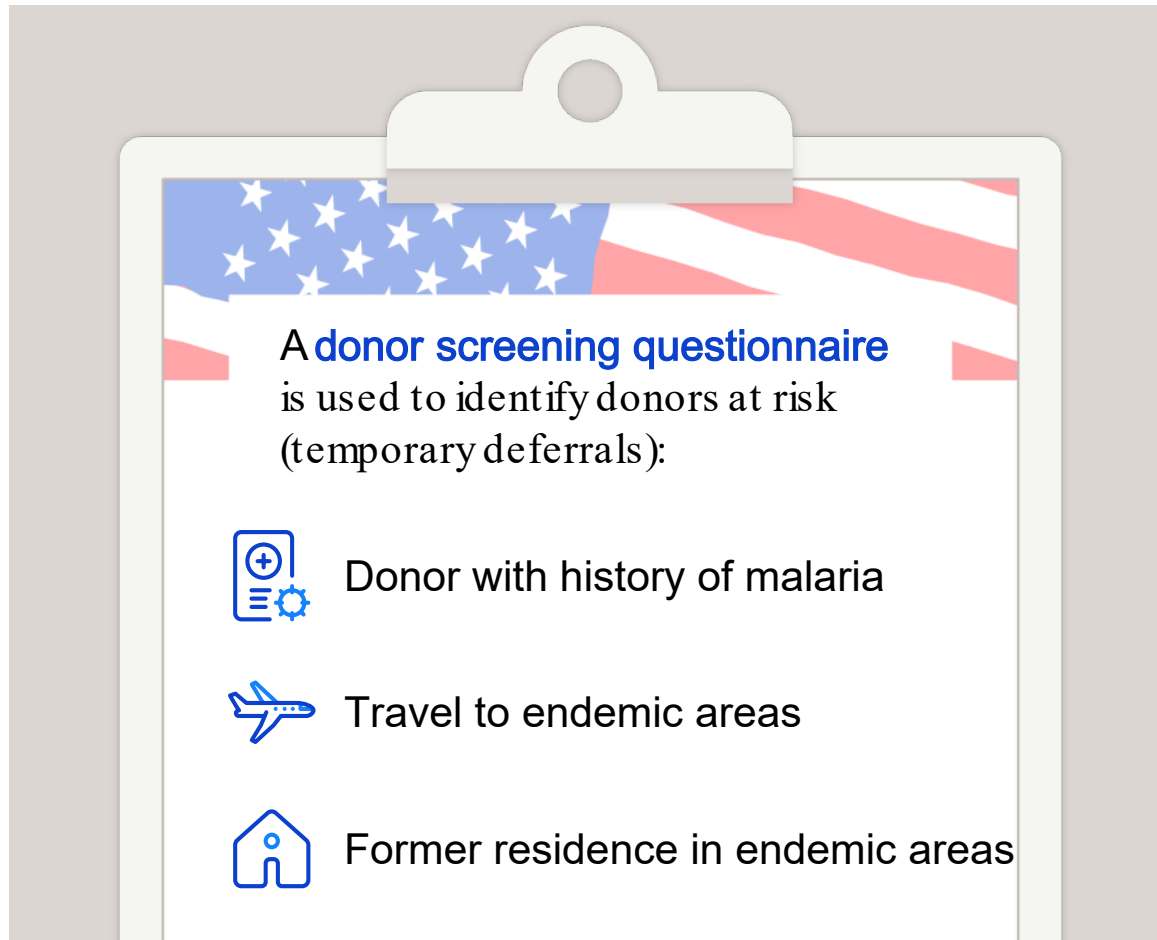
Transfusion-transmission of malaria can occur in both endemic and non-endemic areas. In non-endemic areas, transfusion-transmission is due to:

- Individuals who travelled to or resided in endemic areas
- Chronically infected immigrants from endemic areas
- Recent concern about the potential for local transmission




1. Malaria. International Association for Medical Assistance for Travellers. Available at: <https://www.iamat.org/risks/ malaria/> (Accessed: April 2024).

# Current mitigation strategy

## United States



A donor screening questionnaire is used to identify donors at risk (temporary deferrals):

-  Donor with history of malaria
-  Travel to endemic areas
-  Former residence in endemic areas



### Challenges:

- Imperfect reliability of donor information
- Large number of potential donations lost from individuals who are unlikely to be infected
- Incomplete protection from chronically infected former residents
- Deferral of former residents can impair access to donors whose red cell types may be needed to support patients from that region.
- No current strategy for blood safety in context of local transmission episodes

# Current diagnostic testing methods



## Microscopy and antigen tests

- Sensitivity approx 100,000 parasites /mL
- Intended for use in febrile patients to determine whether *Plasmodium* is the cause of the fever



## DNA based molecular tests

- Detect *Plasmodium* genes (1–5 copies/parasite)
- Laboratory-developed PCR tests.
- Sensitivity approx. 1,000–6,000 parasites /mL. Limited by number of gene copies and by sample volume
- Documented improved detection of asymptomatic infections compared to microscopy or antigen



## Ribosomal RNA (rRNA) based molecular tests

- Detect ribosomal RNA (estim. 7,400 copies/parasite<sup>1</sup>)
- Predicted sensitivity: If there is one parasite in the sample it would be detected

1. Seilie A, et al., Am J Trop Med. Hyg 2019;100(6):1476

# *Plasmodium* nucleic acid in plasma



There is evidence that *Plasmodium* nucleic acid can also be found in plasma/serum:

Examples:

- Use of stored serum samples for retrospective diagnosis
- Brazil: testing of donor plasma samples in pools of 6 with locally produced assay<sup>2</sup>



Nature of nucleic acid in plasma is unclear:

- Parasite fragments?
- Extracellular vesicles?



It is possible that nucleic acid could be detected in a donor whole blood sample **even if no parasite is captured!**

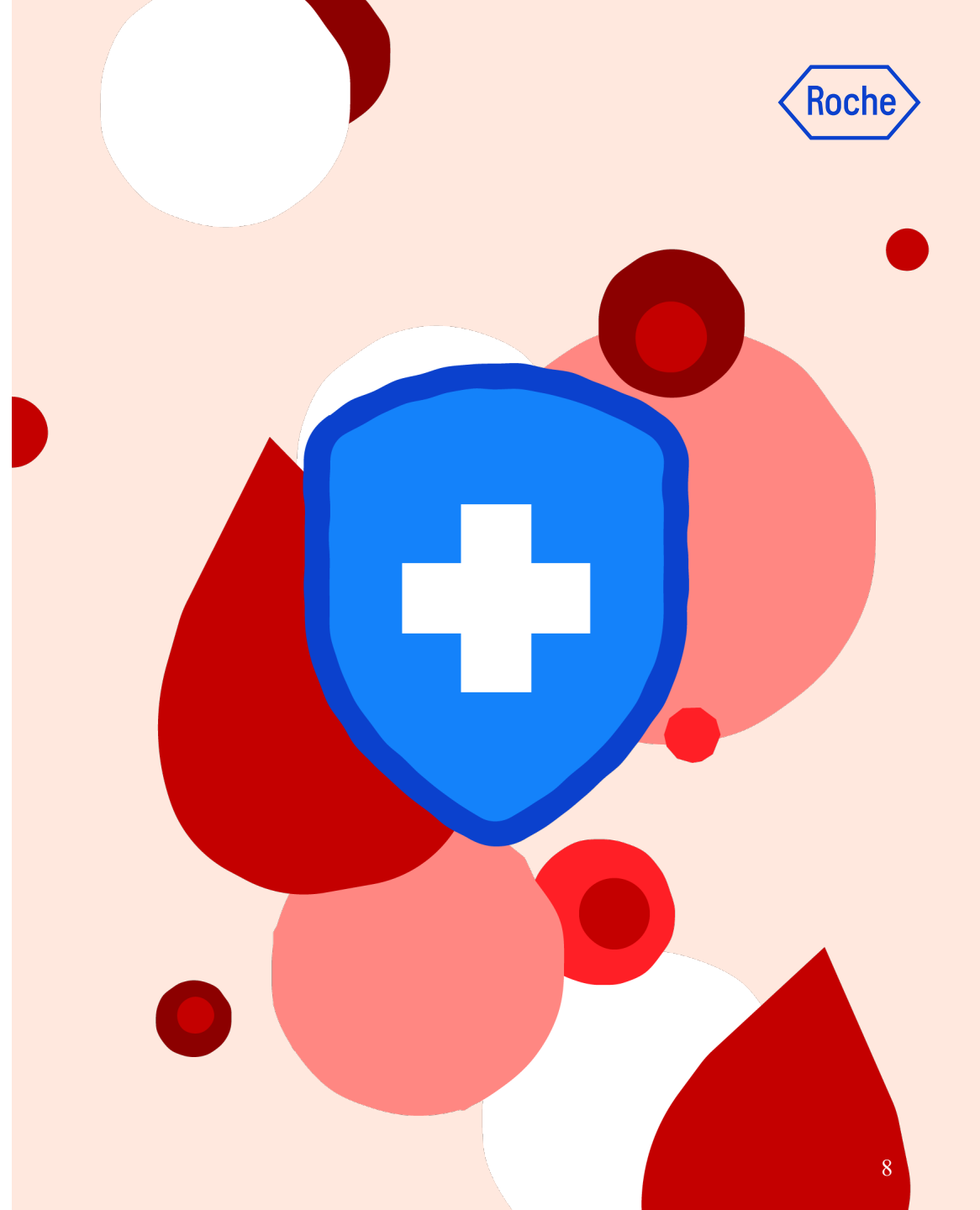
1. Bharti AR, et al., Am J Trop Med Hyg 2007;77:4446.  
 2. Costa E., et al., Transfusion 2024;64:56809



cobas® Malaria design goal  
High sensitivity, specific *Plasmodium*

### Intention:

Tool to enable preservation of  
blood safety while increasing  
donor availability and diversity



# Roche high sensitivity malaria PCR assay

## Design goals



Target

Ribosomal RNA (rRNA) and DNA  
rRNA is reported to be present in  
thousands of copies per parasite<sup>1</sup>  
Rationale: if a parasite is present in  
the sample, it should be detected!



Detect

Detection to include the 5 main  
species known to infect humans:  
*P. falciparum*, *P. vivax*, *P. malariae*,  
*P. ovale* and *P. knowlesi*



Identify

*Plasmodium* parasites are inside  
RBCs: sample type is whole blood,  
not plasma  
Use Roche Whole Blood Collection  
Tube developed for the cobas®  
Babesia test

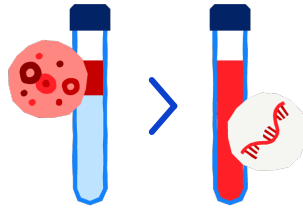
1. Seilie A, et al., Am J Trop Med. Hyg 2019;100(6):1476

# cobas® Malaria workflow



Whole blood collection

Approximately 1.1mL of whole blood is collected into tubes containing lysis buffer and preservatives



Lysis of red blood cells

The red blood cells and any parasites are lysed and the nucleic acid is stabilized



Fully automated sample preparation, NAT amplification/detection/analysis

The tube is placed on the cobas® 6800/8800 Systems and tested using ready to use malaria specific cobas® reagents

# Analytical sensitivity of cobas® Malaria

*P. falciparum* culture, intact infected red blood cells (iRBCs)




*P. falciparum* culture, iRBC concentration quantitated by microscopy, was serially diluted in whole blood



1.1 mL aliquots of specific concentration levels were transferred into Roche Whole Blood Collection tubes, and the sample was tested by cobas® Malaria on the cobas® 6800/8800 Systems

The observed concentration for 95% probability of detection (2.9 iRBC/mL) is essentially the concentration needed to have a 95% probability of capturing one iRBC in the test sample, based on Poisson distribution

↓

 95% probability of detection by PROBIT	2.9 iRBC/mL (95% CI 2.4-3.8 iRBC/mL)
50% probability of detection by PROBIT	0.6 iRBC/mL (95% CI 0.5-0.7 iRBC/mL)

# Analytical sensitivity of cobas® Malaria

## 5 species using recombinant RNA (aRNA)



Analytical sensitivity for the ribosomal RNA of each of the 5 species was assessed using recombinant particles encoding a single copy of *Plasmodium* target rRNA encapsulated by bacteriophage coat protein (“armored RNA,” aRNA)

aRNA particles were serially diluted in specimen diluent and tested in 71 or 72 replicates

**Similar sensitivity** was demonstrated across the 5 species

- Range of 95% limits of detection: 23.7–59.0 aRNA particles/mL
- Differences negligible compared to number of copies per parasite

# Detection of positive samples



cobas® Malaria utilizes a dual target PCR design that targets highly conserved regions of ribosomal RNA sequences



In silico analysis predicts robust detection of the species claimed



Detection of the 5 species was confirmed by wet lab testing using clinical samples, culture supernatants, and armored RNA constructs

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These studies are described in the package insert

# cobas® Malaria

## Clinical specificity

Whole blood samples from volunteer donors in the US were collected in the Roche Whole Blood Collection tube and tested by cobas® Malaria on the cobas® 6800/8800 Systems.

Results:



20,187 donations were tested by individual sample testing



No reactive donations



Specificity 100% (95% CI 99.982% to 100%)



# Samples from asymptomatic individuals in Nigeria



Study population asymptomatic study participants in Edo State of Southern Nigeria. Samples collected in August/September 2021 (rainy season)



Fresh blood tested by microscopy and antigen



1.1 mL of EDTA whole blood from each participant was inoculated into a Roche Whole Blood Collection tube. Material was frozen and shipped to US for testing



Samples tested in US by cobas® Malaria and in-house alternative NAT (AltNat)



199 samples evaluable

4 samples (2.0%) positive by microscopy and antigen

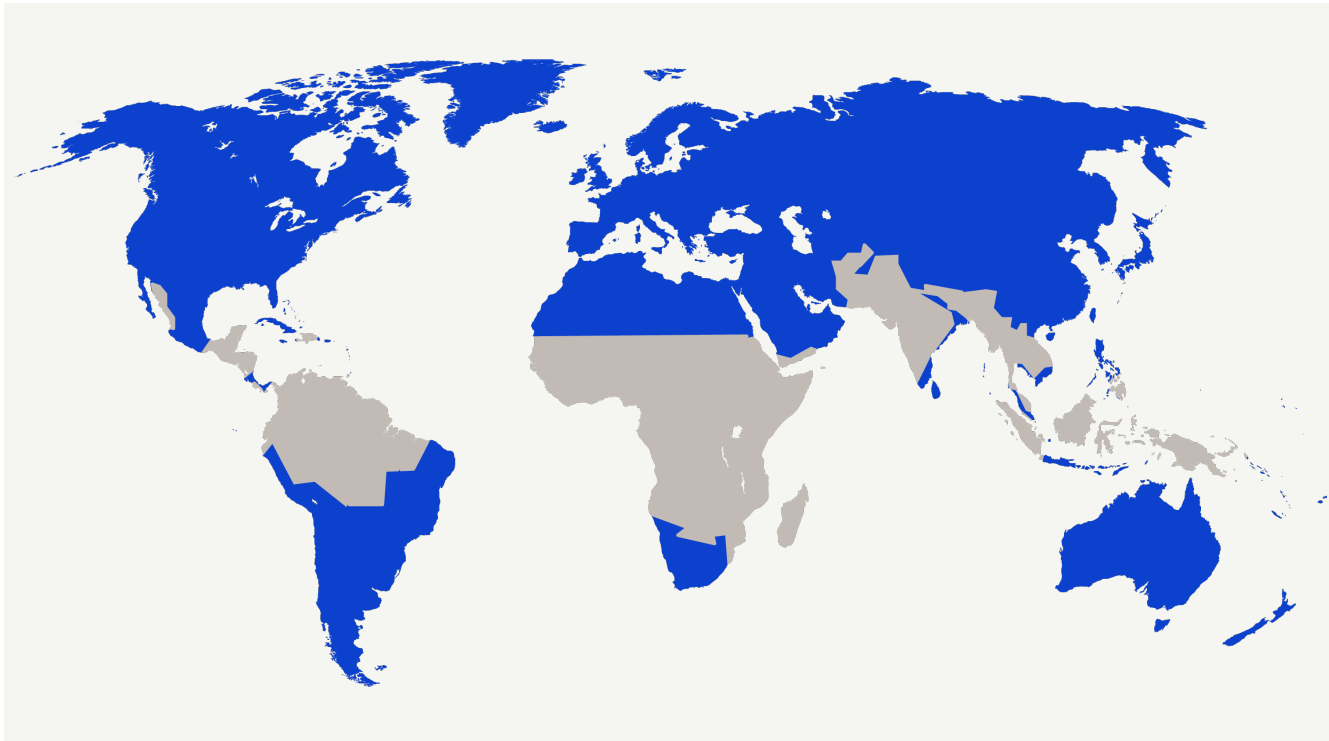
76 samples (38.2%) reactive on cobas® Malaria and confirmed by NAT

(These include the 4 samples that were positive by microscopy/antigen)



# Detection of asymptomatic *Plasmodium* infections in non-endemic areas

 Non-endemic



Asymptomatic *Plasmodium* infections are rarely identified in the US and other non-endemic areas



Much of what we know about the laboratory detectability of these infections is from donors identified as the cause of transfusion-transmitted malaria

# Review: Laboratory detectability of donors identified as the source of TTM in nonendemic areas

## US, Canada, and Europe

### Methods:

- Identified all published cases of TTM in US, Canada, and Europe since 2010
- Authors and labs were contacted to solicit missing details about sample types and lab methods.
- Summarized results of tests performed on samples retained from the donation causing the TTM and/or on fresh follow-up (f/u) samples

### Cases identified



Results of molecular testing (DNA-based PCR assays) were reported for 12 of the 13 implicated donors

# Donors implicated in TTM: PCR results

## Cases in US and Canada



- PCR for Cases 7 performed at US CDC; Case 8 at Natl Ref Center for Parasitology, McGill • Sensitivity 3,000–6,000 parasites/mL
- Laboratory-developed PCR assays

Case #	Country, year, species	Donor risk	Fresh f/u sample	Retained sample from index donation		
				Blood segment	Plasma	Undefined sample type
1	US, 2010, Pf	Former resident of Benin, 4 yr after departure				
2	US, 2011, Pm	Former resident of Liberia, 15 yr after departure				
3	US, 2016, Pf	Former resident of Democratic Rep of Congo, multiple tra back to Africa most recently 16 mo prior to donation				
4	US, 2017, Pf	Former resident of Togo, 2.8 yr after departure				
5	US, 2017, Po	Former resident of Cameroon, 2 yr after departure				
6	(BMT) US, 2018, Pf	BMT donor traveled to Ghana 1 yr prior to donation; malaria-like sx on return, microscopy neg, not treated				
7	US, 2020, Pf	Former resident of Nigeria, 4 yr after departure				
8	Canada, 2022, Pf	Former resident of W. Africa, 12 yr after departure				

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8	Canada, 2022, Pf	Former resident of W. Africa, 12 yr after departure	+			

+ Positive
 - Negative

\*Positive nested PCR, borderline PCR. \*\*Blood segments had been stored multiple weeks in the refrigerator

# Donors implicated in TTM: PCR results

## Cases in Europe



- 3 tested with laboratory developed PCR assays, one with commercial PCR, test results not reported for one case
- PCR sensitivity similar to assays used by US CDC

Case #	Country, year, species	Donor risk	Fresh f/u sample	Retained sample from index donation		
				Blood segment	Plasma	Undefined sample type
9	Netherlands, 2011, Pm	Travel (more than 4r prior to donation?)				
10	France, 2012, Pf	Former resident of Benin, 12 after departure				
11	France, 2015, Pm	Former resident of Comoro Islands, more than 3 after departure				
12	Italy, 2019, Pm	Missionary, more than 10 after departure from endemic areas				
13	Austria, 2019, Pf	Donor traveled to Uganda 2k prior to donation, became febrile 1 wk after donation and was diagnosed with malaria†				

†Case 13: test results not reported

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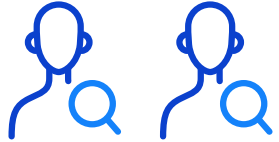
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†Case 13: test results not reported

+ Positive - Negative

# Donors implicated in TTM: PCR results

## Conclusions



The PCR assays used in these case investigations were able to detect *Plasmodium* infection in all donors tested except for two donors.

- These two donors were tested only on samples likely to have deteriorated from prolonged refrigerated storage.



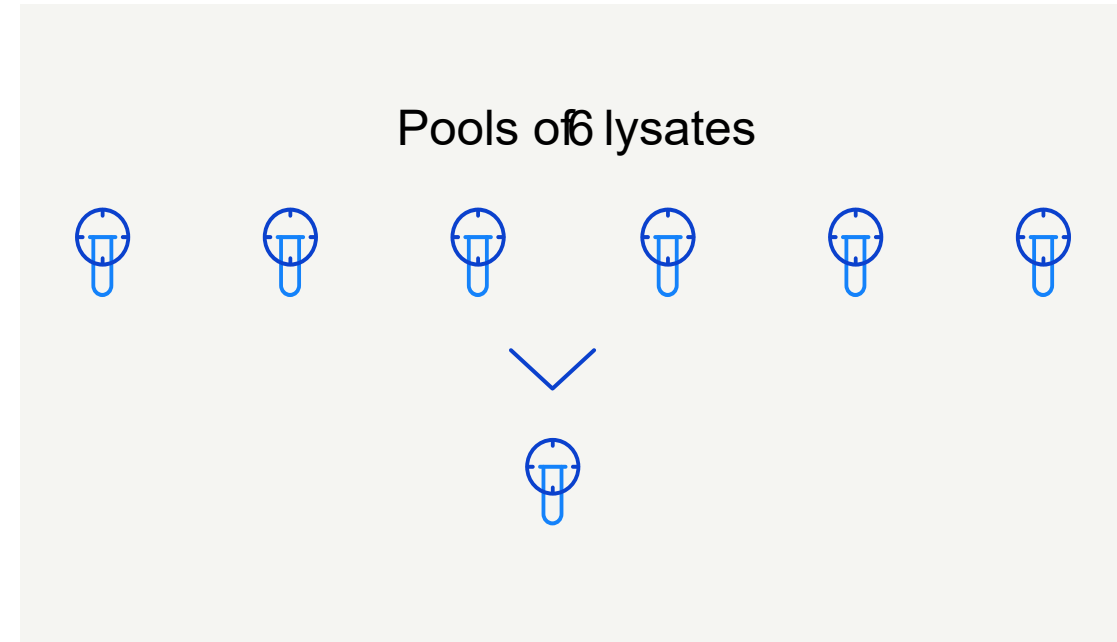
**cobas®Malaria** is approximately **1,000-fold more sensitive** than the assays used for these cases

# Potential for testing lysates in pools



We have performed studies using cobas® Malaria in pools or simulated pools.

We plan one additional study to further support a pooling claim.





# Potential for testing lysates in pools

- Testing of pooled lysates appears to be sufficient for *Babesia*
- Babesiosis, like malaria, is caused by parasites that infect red blood cells
- FDA guidance May 2019 requires testing of donations collected in regions of the US where *Babesia*s endemic
- Workflows similar to that described for malaria except that testing is permitted on pools of lysates

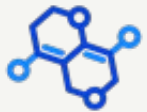


No transfusion-transmitted *Babesia* has been identified from donations that were tested in pools



# Molecular testing for malaria

## Summary



### Molecular methods

More sensitive than antigen or microscopy testing for the detection of asymptomatic *Plasmodium* infections



### Molecular tests

Able to detect infection in donors implicated in transfusion-transmitted malaria in non-endemic countries



Highly sensitive automated 5-species NAT that detects ribosomal RNA and DNA

May provide a useful tool for further reducing the risk of transfusion-transmitted malaria

Doing now what patients need next