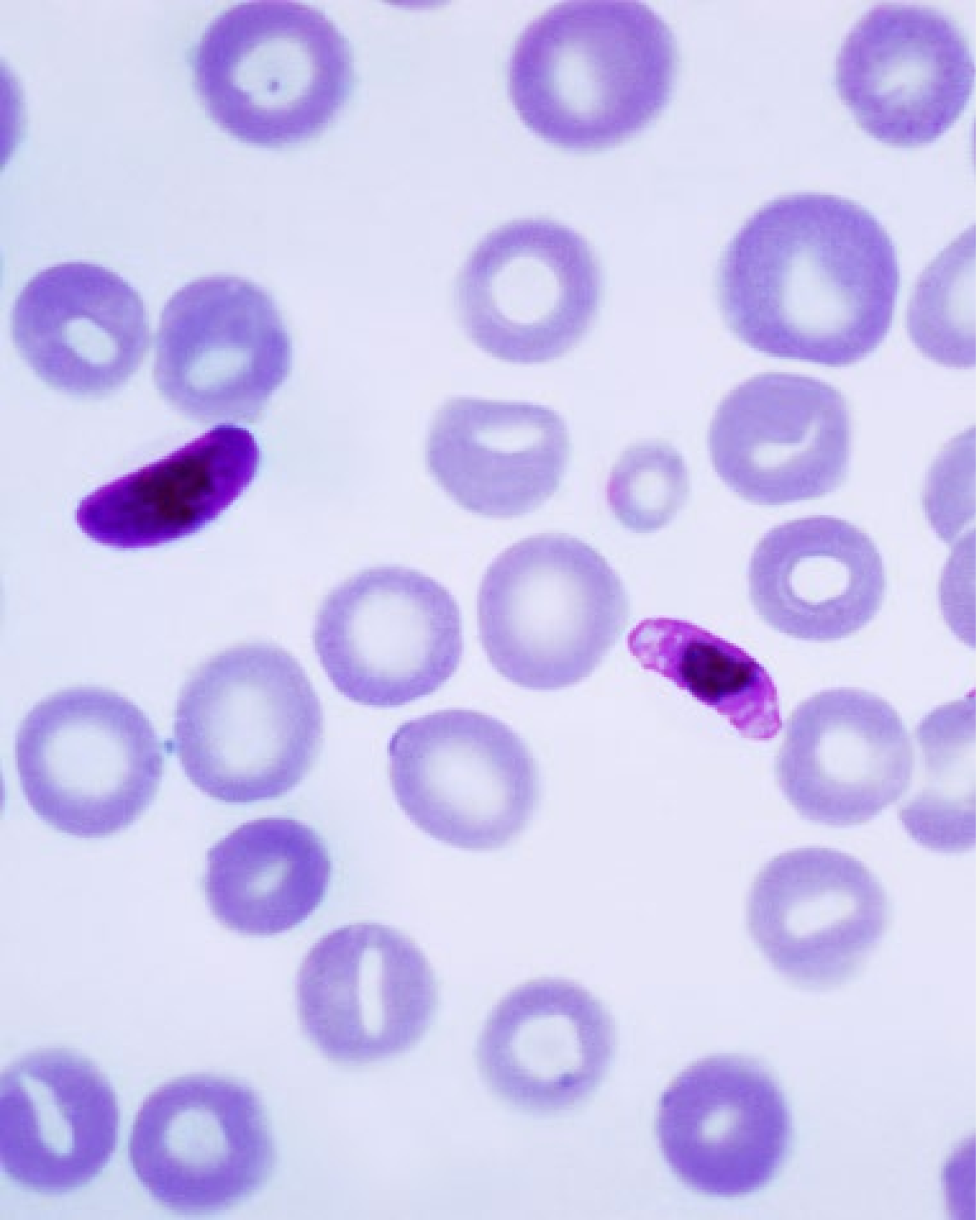

Molecular testing for detection of asymptomatic *Plasmodium* infections

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Disclosures

- Dr. Galel is 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”)

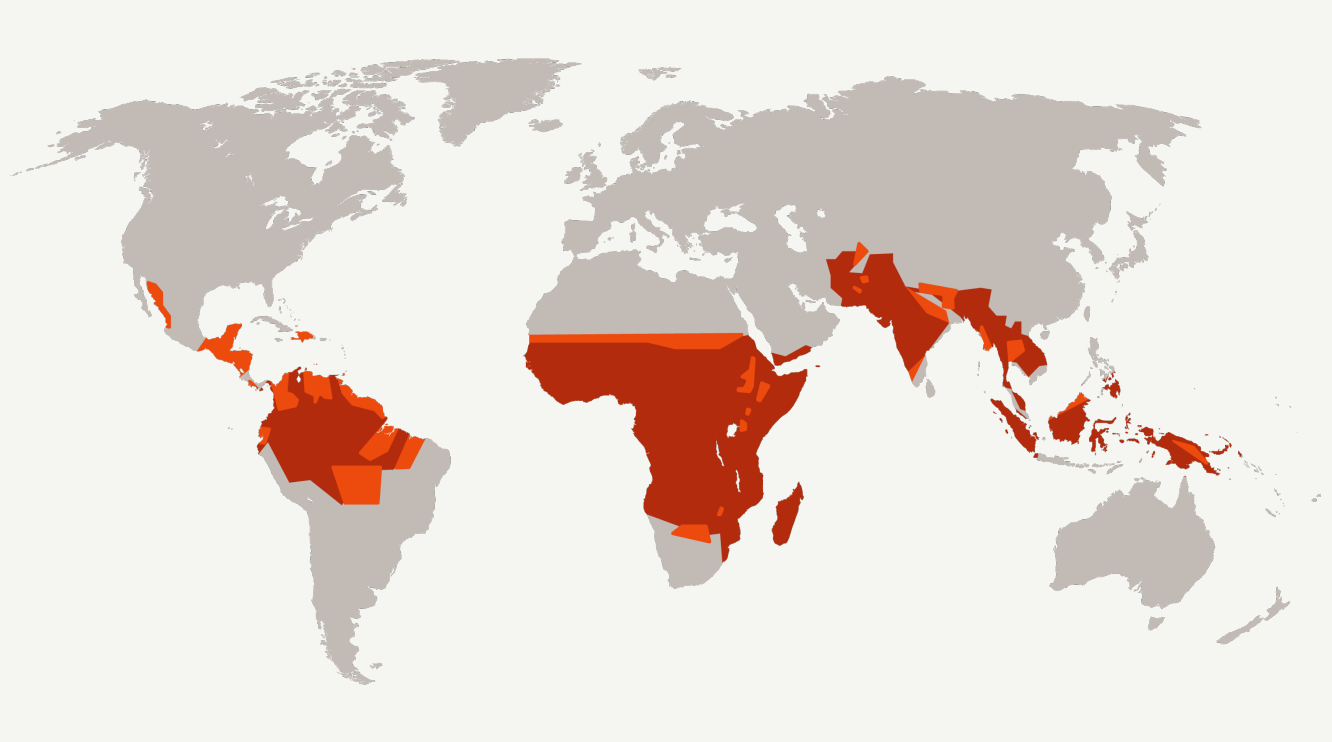
There are many *Plasmodium* species. Most human infections are due to 5 species:
P. falciparum, P. vivax, P. malariae, P. ovale, and P. knowlesi

Transfusion-transmitted malaria (TTM)

Worldwide risk

Global rates of malaria[1]

High risk Limited risk



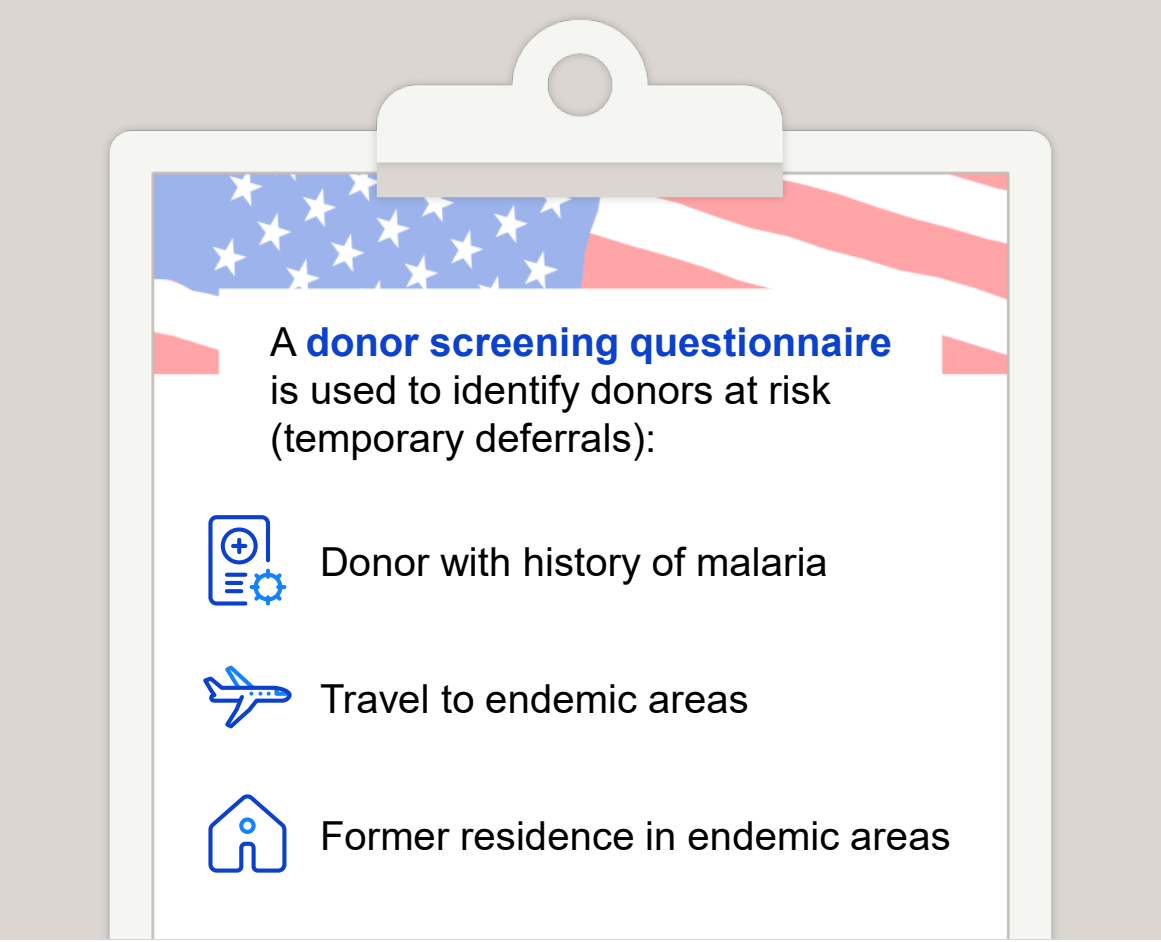
Transfusion-transmission of malaria can occur in both endemic and non-endemic areas. In non-endemic areas, transfusion-transmission is due to:

- 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



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[1])
- **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):1466-76

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[1]
- Brazil: testing of donor plasma samples in pools of 6 with locally produced assay[2]

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!**

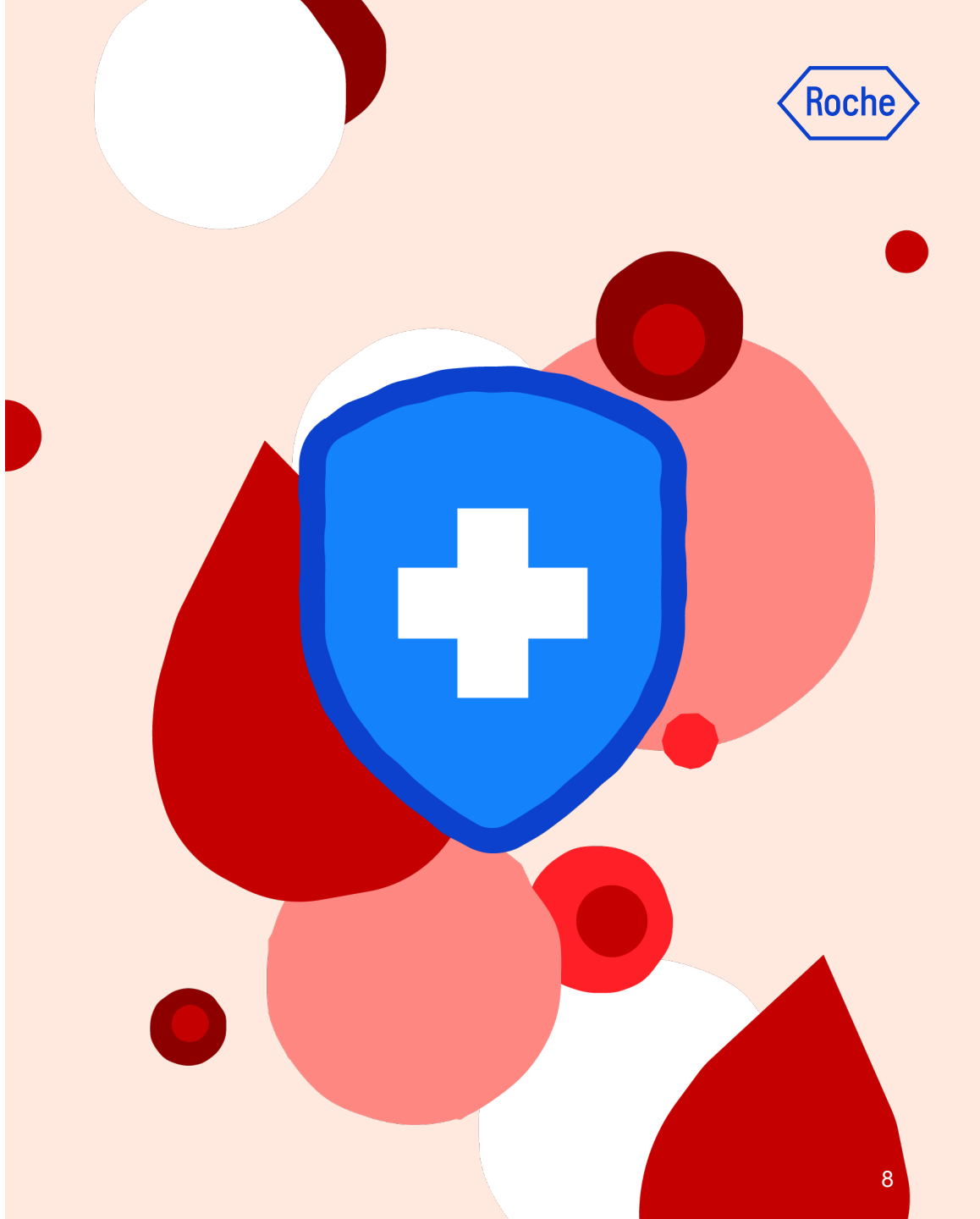
1. Bharti AR, et al., Am J Trop Med Hyg 2007;77:444-446.
2. Costa E., et al., Transfusion 2024;64:501-509

cobas® Malaria design goal

High sensitivity 5-species *Plasmodium* NAT

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**[1]

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

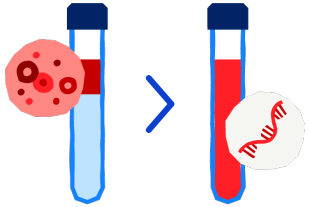
Identify

***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):1466-76

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 (iRBC)

***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 **lysate 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 armored 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

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)**

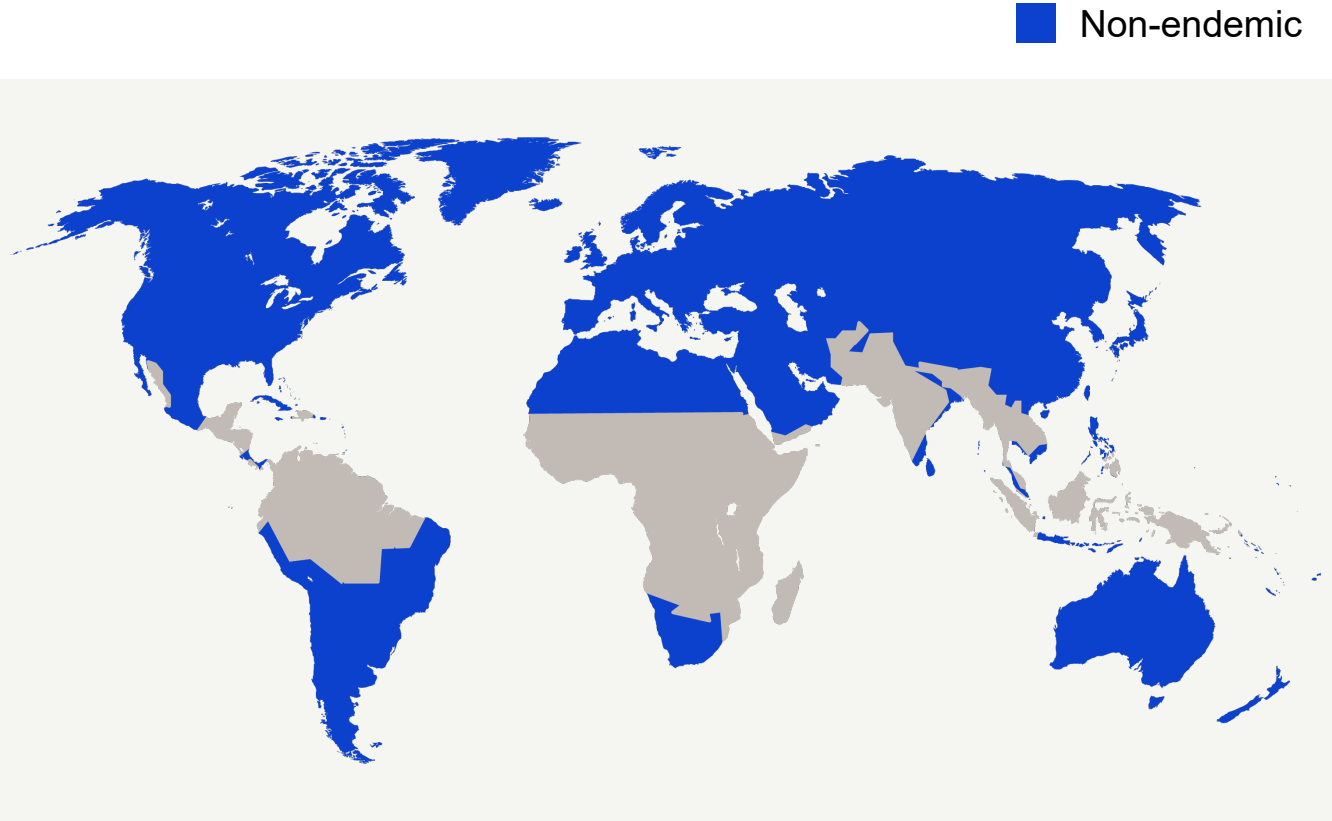
199 samples evaluable

4 samples (2.0%) positive by microscopy and antigen

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

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

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



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 non-endemic 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

12 cases of TTM

1 case of BMT

7 cases

1 case

5 cases

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

Abbrvs: BMT, bone marrow transplant; TTM, transfusion-transmitted malaria

Donors implicated in TTM: PCR results

Cases in US and Canada

- PCR for Cases 1–7 performed at US CDC; Case 8 at Natl Ref Center for Parasitology, McGill
- Laboratory-developed PCR assays
- **Sensitivity 3,000–6,000 parasites/mL**

Case #	Country, year, species	Donor risk	Fresh f/u sample	Retained blood segment from index donation	Retained plasma from index donation	Retained unknown sample type from index donation
1	US, 2010, Pf	Former resident of Benin, 4 yr after departure	Positive	No data	No data	No data
2	US, 2011, Pm	Former resident of Liberia, 15 yr after departure	Positive	No data	No data	Negative
3	US, 2016, Pf	Former resident of Democratic Rep of Congo, multiple travel back to Africa most recently 16 mo prior to donation	Positive	No data	No data	No data
4	US, 2017, Pf	Former resident of Togo, 2.8 yr after departure	Negative	Positive*	No data	No data
5	US, 2017, Po	Former resident of Cameroon, 2 yr after departure	No data	Negative**	No data	No data
6	(BMT) US, 2018, Pf	BMT donor traveled to Ghana 1.5 yr prior to donation; malaria- like sxs on return, microscopy neg, not treated	Positive	No data	No data	No data
7	US, 2020, Pf	Former resident of Nigeria, 4 yr after departure	No data	Negative**	No data	No data
8	Canada, 2022, Pf	Former resident of W. Africa, 12 yr after departure	Positive	No data	No data	No data

*Positive nested PCR, borderline PET-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 blood segment from index donation	Retained plasma from index donation
9	Netherlands, 2011, Pm	Travel (more than 4 yr prior to donation?)	Positive	No data	Negative
10	France, 2012, Pf	Former resident of Benin, 12 yr after departure	Positive	No data	Positive
11	France, 2015, Pm	Former resident of Comoro Islands, more than 3 yr after departure	Positive	No data	Negative
12	Italy, 2019, Pm	Missionary, more than 10 yr after departure from endemic areas	Positive	No data	No data
13	Austria, 2019, Pf	Donor traveled to Uganda 2 wk prior to donation, became febrile 1 wk after donation and was diagnosed with malaria†	No data	No data	No data

†Case 13: test results not reported

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

TTM, transfusion-transmitted malaria

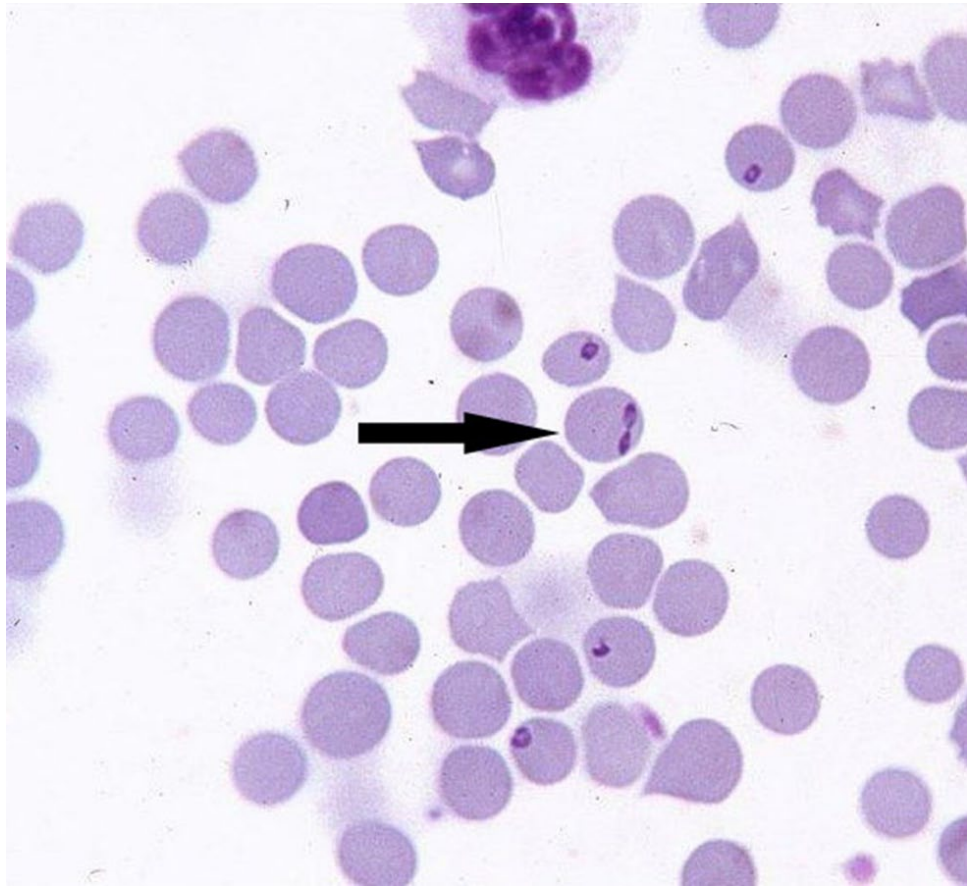
Potential for testing lysates in pools

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

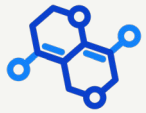
- Testing of pooled lysates appears to be sufficient for *Babesia*
- Babesiosis, like malaria, is caused by parasites that infect red blood cells
- FDA guidance May 2019 requires testing of donations collected in regions of the US where *Babesia* is endemic
- Workflow similar to that described for malaria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



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

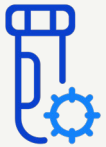
Molecular testing for malaria

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