EDQM Blood Conference
Innovation in Blood Establishment Processes

14-15 January 2025
Strasbourg, France

Session A4:
Recipient protection & blood safety
(10:30 – 12:00)

Moderators: **Jenny Mohseni Skoglund**, European Centre for Disease Prevention and Control, Sweden
Johannes Blümel, Paul Ehrlich Institute, Germany
Laurent Mallet, Head of ICND Department, EDQM

Speakers: **Johannes Blümel**, Paul Ehrlich Institute, Germany
Jenny Mohseni Skoglund, European Centre for Disease Prevention and Control, Sweden
Sandra Kurth, Swiss Transfusion SRC, Switzerland
Susan Galel, Roche Diagnostic Solutions, USA
Jeffrey Linnen, Grifols Diagnostic Solutions, USA

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European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

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Development of High-throughput Sequencing for Detection of Viruses in Blood.

EDQM Blood Conference
14-15 January 2025 I Strasbourg, France

Dr. Johannes Blümel
Head Viral Safety Section, Dept Infectiology

Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel
Federal Institute for Vaccines and Biomedicines

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BLOODVIR

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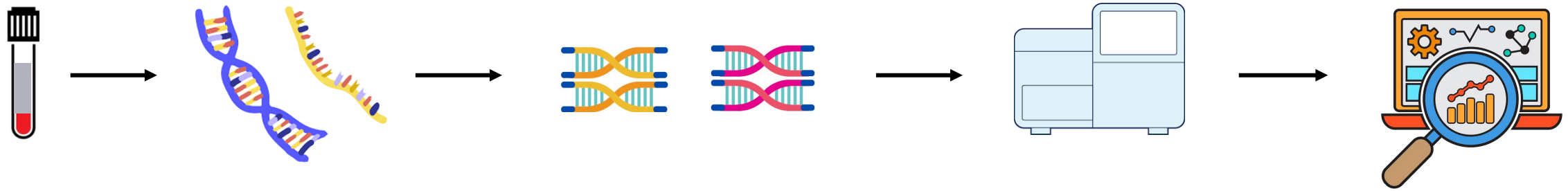
Surveillance system for novel viruses based on **next generation sequencing, NGS** (also termed **high throughput sequencing, HTS**) and artificial intelligence

Viral nucleic acid extraction

Sequencing

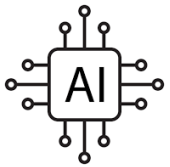


Blood plasma

Library preparation

Data Analysis

1. Known virus identification
2. Novel virus detection



Strategies for viral high throughput sequencing

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Non-targeted metagenomics

Host sequence depletion

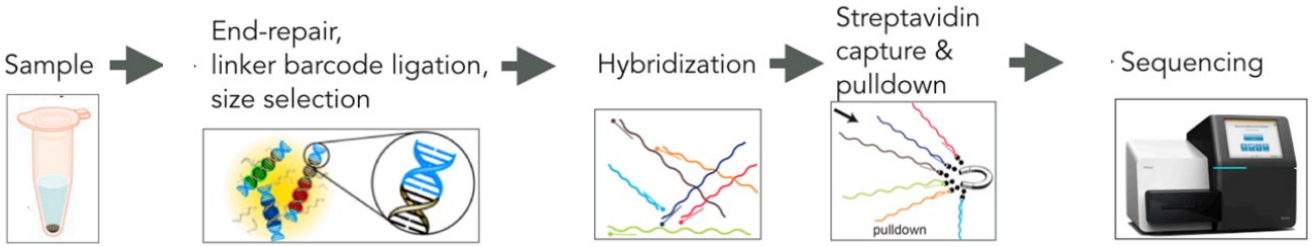
- No selection for virus type or genomic sequence
- Less virus material enrichment

Sample → Nuclease treatment → NEBNext Ultra II Library prep → Sequencing (Illumina)

Targeted virus enrichment

Virus enrichment with VirCapSeq-VERT (Ian Lipkin)

- 700,000 DNA probes against 207 known vertebrate viral taxons
- Enables hybridization-capture of genomes with as little as ca. 45% sequence identity



1st WHO International Reference Panel for Adventitious Virus Detection in Biological Products by High-throughput Sequencing

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CBER NGS Virus Reagents – *BEI NR-59622*

		Particle size (nm)	Envelope	Genome topology	Genome size (bp/b)	Physical chemical resistance	
Epstein-Barr virus type 1		122-180	YES	ds-DNA linear	172,281	Low to Medium	**Herpesvirus**
Feline leukemia virus		80-100	YES	ss-RNA dimeric	8,448	Low	**Retrovirus**
Human respiratory syncytial virus type A		150-300	YES	ss-RNA linear	15,158	Low to Medium	**Paramyxovirus**
Human reovirus type 1		60-80	NO	ds-RNA segmented	1,196 3,915	Medium to High	**Reovirus**
Porcine circovirus type 1		16-18	NO	ss-DNA Circular	1,758	High	**Circovirus**
Human coronavirus HCoV-OC43		80-120	YES	ss-RNA linear	30,741	Low	**Coronavirus**
Minute Virus of Mice		26	NO	ss-DNA linear	5,149	High	**Parvovirus**

1st WHO Intl Reference Panel *BEI NR-59630*

https://www.niaid.nih.gov/research/bei-resources-repository

WHO Panel for LoD comparison

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Epstein Barr Virus												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Replicate 1												
Replicate 2												
Replicate 3												
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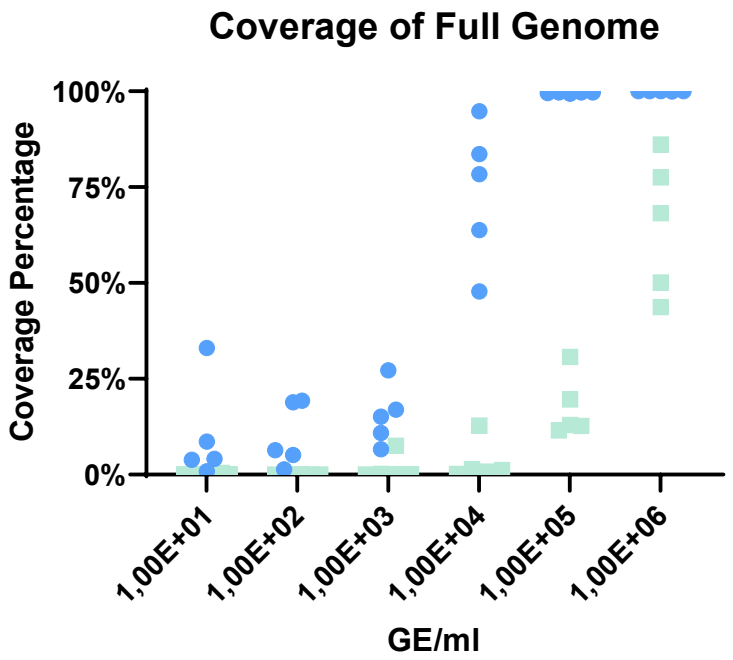

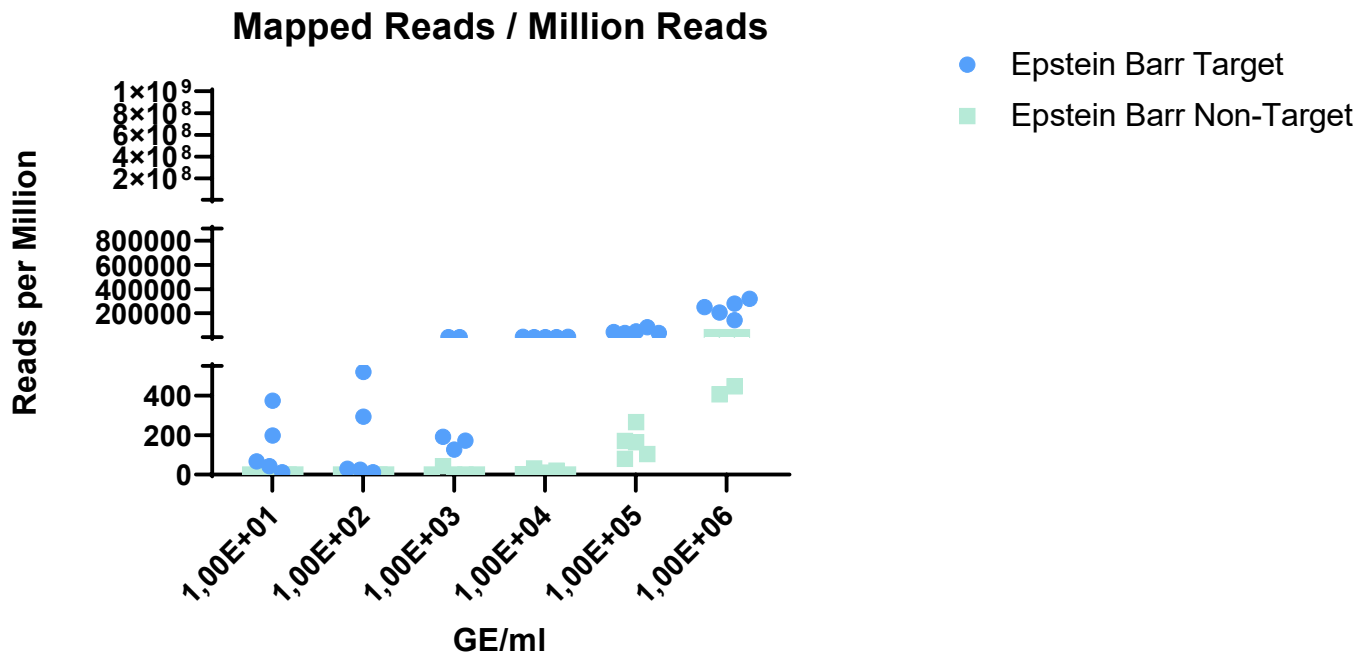

WHO Panel for LoD comparison

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Feline Leukemia Virus												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
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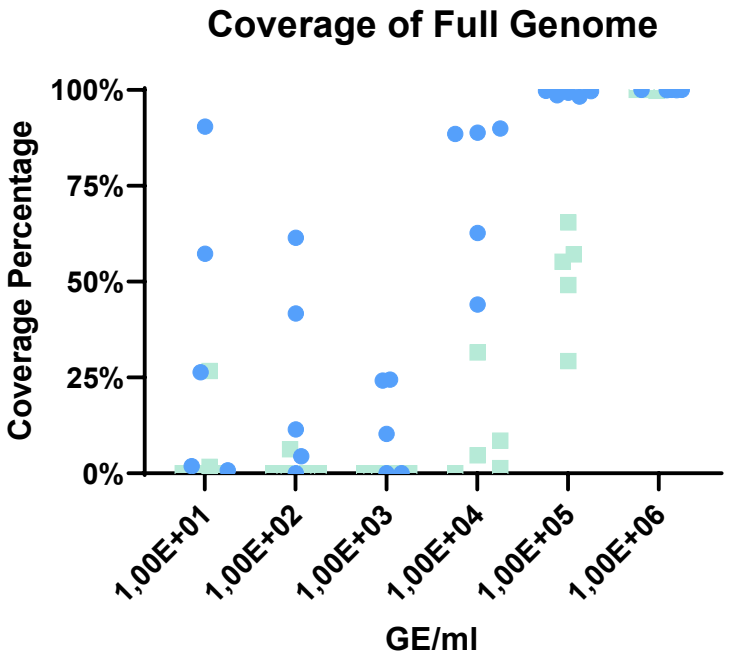

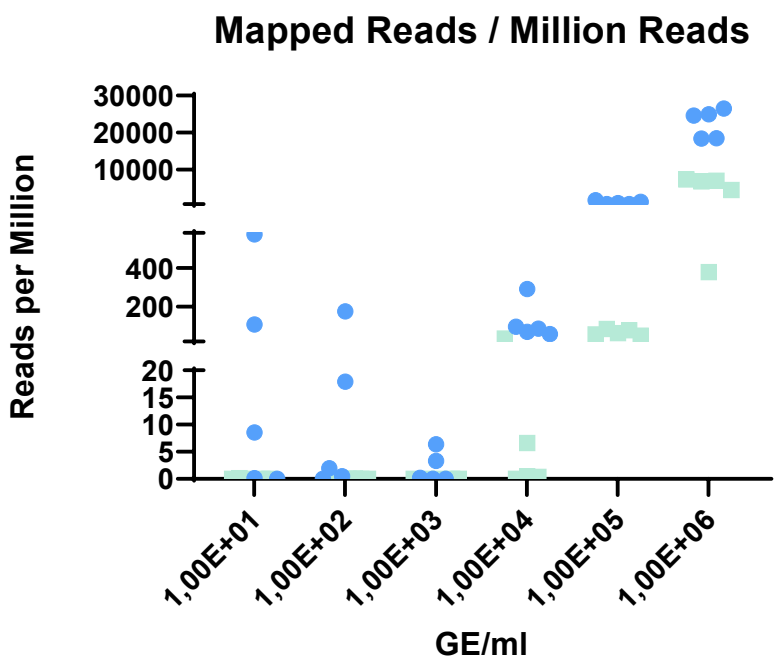

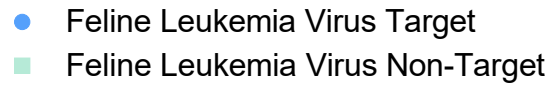

WHO Panel for LoD comparison

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RSV												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Replicate 1												
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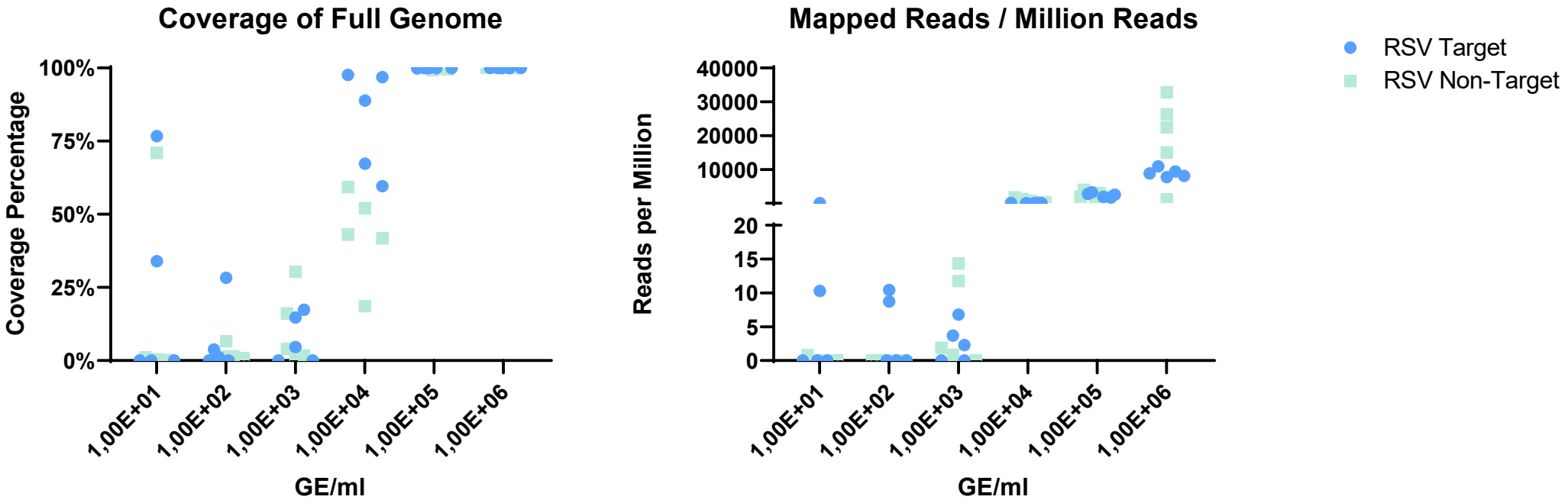

WHO Panel for LoD comparison

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Mammalian Orthoreovirus												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Replicate 1												
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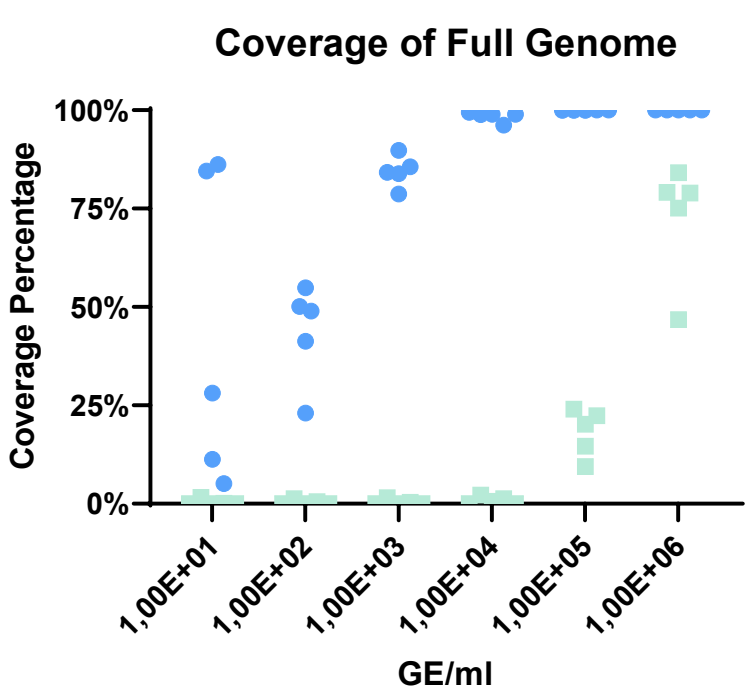

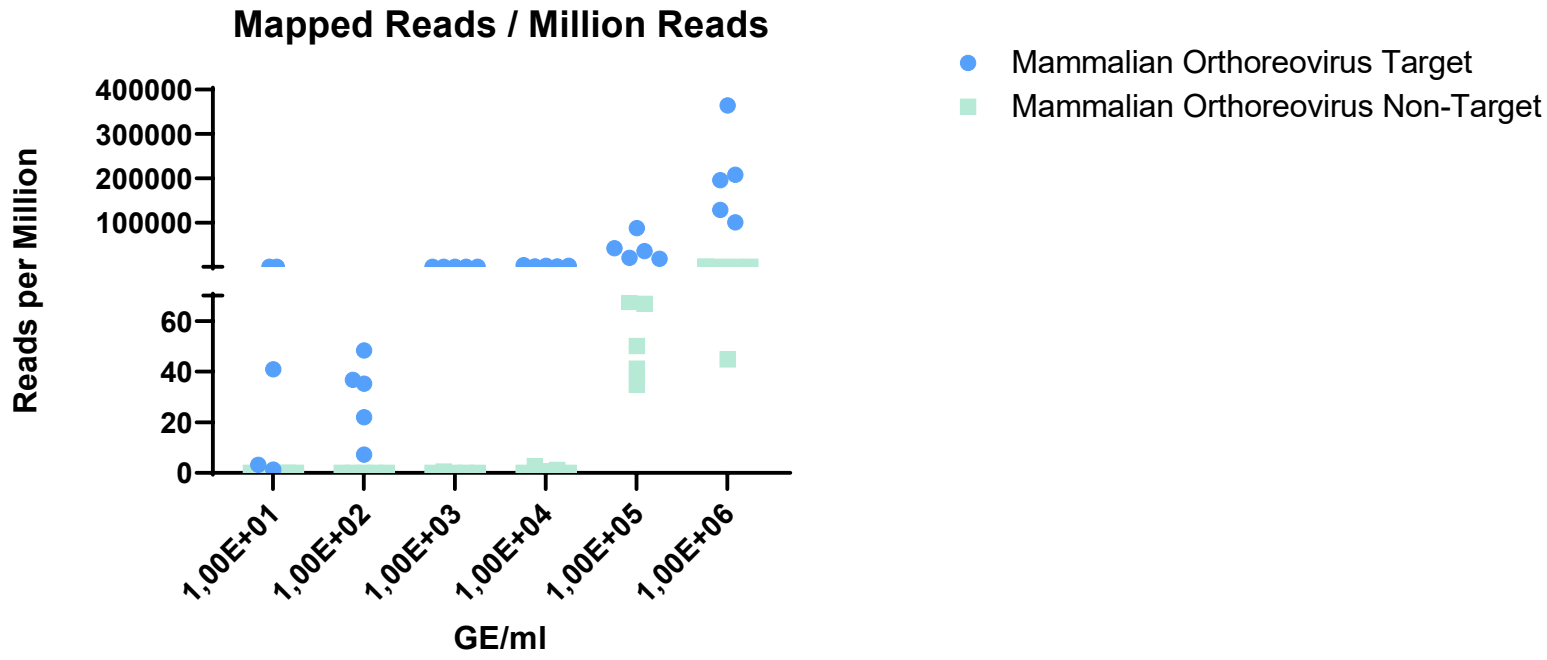

WHO Panel for LoD comparison

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Porcine Circovirus												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
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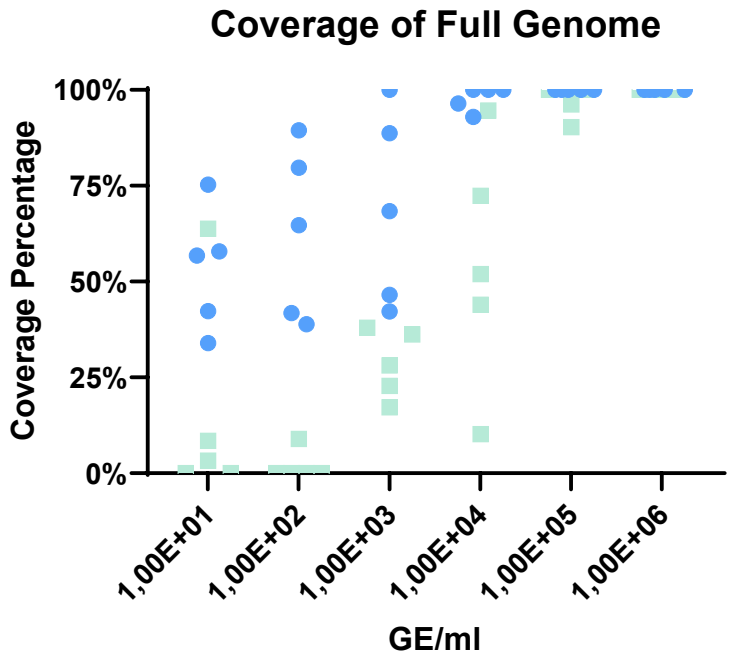

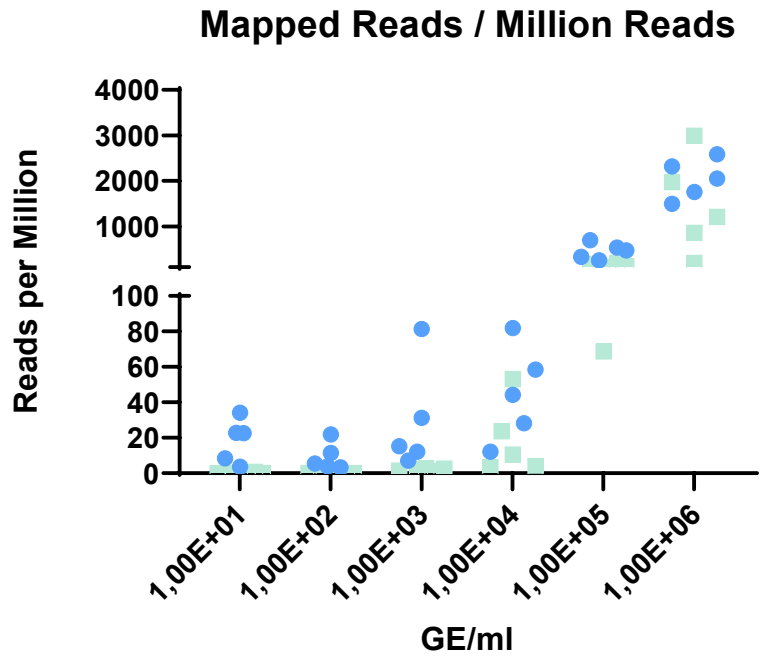

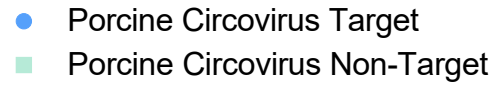

WHO Panel for LoD comparison

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Minute Virus of Mice												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Replicate 1												
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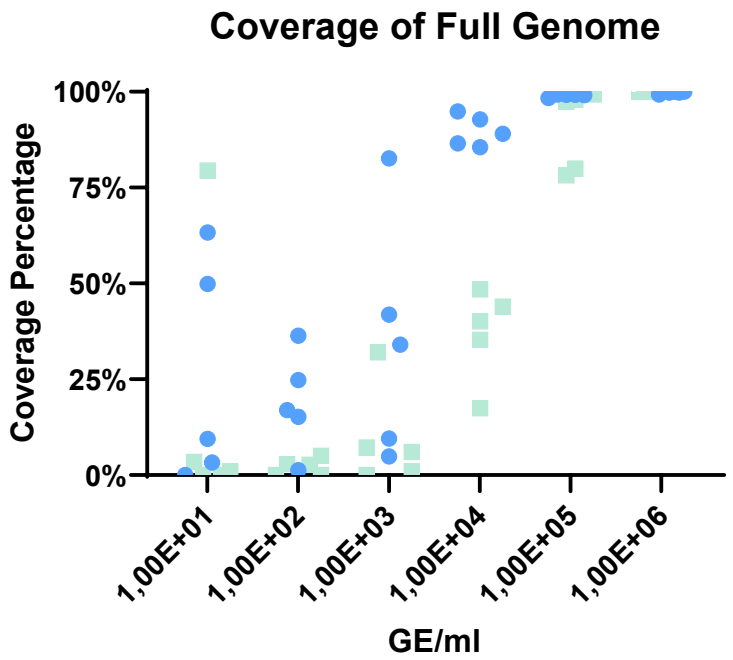

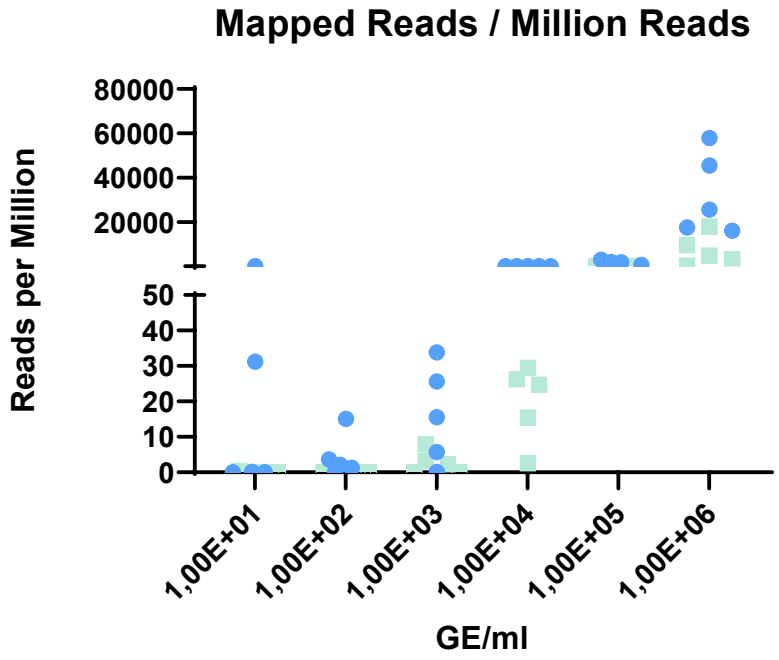

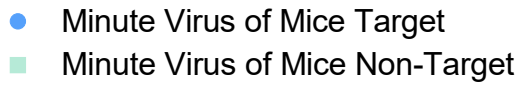

WHO Panel for LoD comparison

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Human Coronavirus OC43												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Replicate 1												
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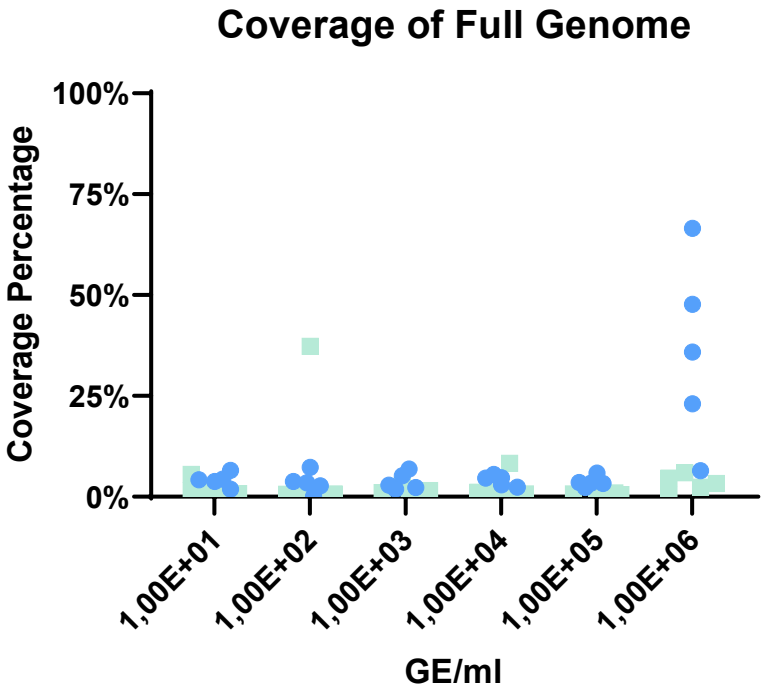

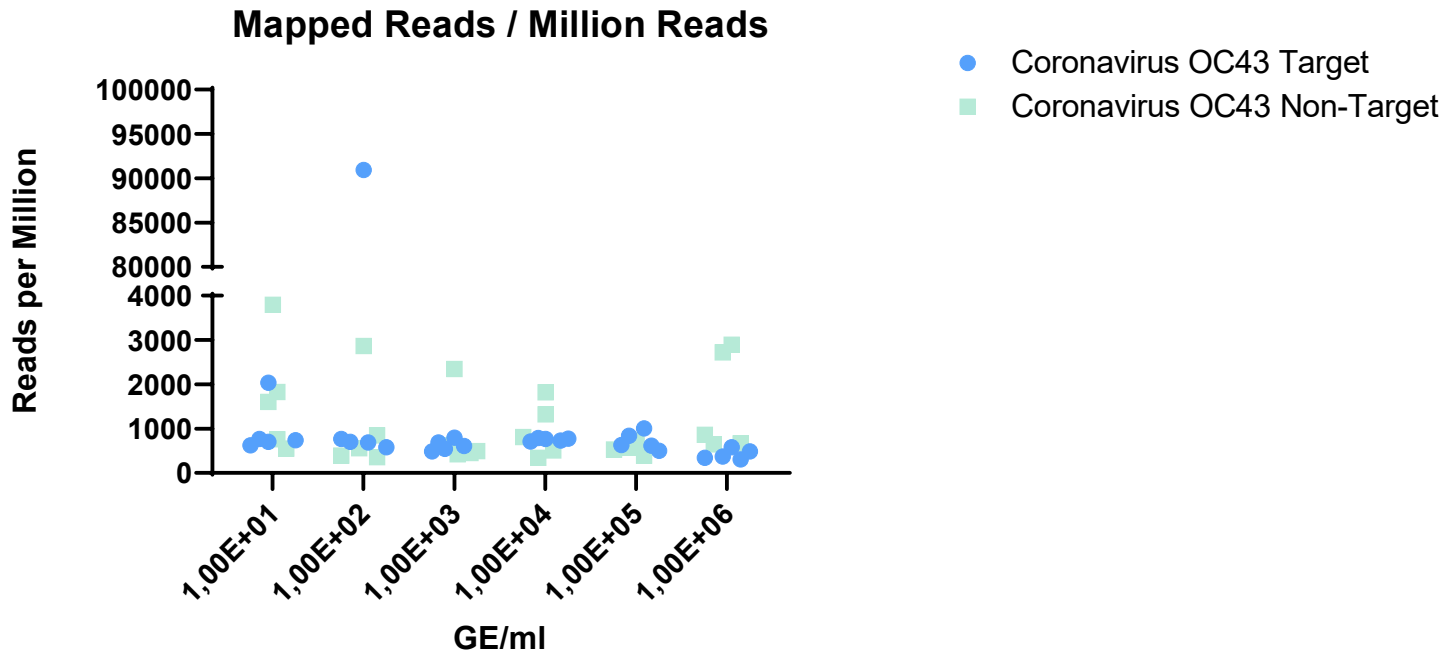

In-house Panel for Breadth of Virus Detection

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21-Virus Panel									
#	Virus	Family	Genus	Nucleic Acid	Segmented	Baltimore	Enveloped	Target	Non-Target
1	Hepatitis C genotype 1	Flaviviridae	Hepacivirus	ssRNA+	No	IV	Yes		
2	Usutu virus	Flaviviridae	Orthoflavivirus	ssRNA+	No	IV	Yes		
3	Zika virus; PF13/251013-18	Flaviviridae	Orthoflavivirus	ssRNA+	No	IV	Yes		
4	West Nile Virus	Flaviviridae	Orthoflavivirus	ssRNA+	No	IV	Yes		
5	Bovine viral diarrhea virus	Flaviviridae	Pestivirus	ssRNA +	No	IV	Yes		
6	Hepatitis B virus	Hepadnaviridae	Orthohepadnavirus	dsDNA-RT	No	VIII	Yes		
7	Hepatitis E virus	Hepeviridae	Orthohepevirus	ssRNA+	No	IV	No		
8	Herpes simplex virus type 1	Herpesviridae	Simplexvirus	dsDNA	No	I	Yes		
9	Suid herpesvirus 1 strain Kaplan	Herpesviridae	Varicellovirus	dsDNA	No	I	Yes		
10	Influenza A virus A/PR/8/34 (H1N1)	Orthomyxoviridae	Orthomyxovirus	ssRNA -	Yes	V	Yes		
11	Bovine parvovirus 1	Parvovirinae	Bocaparvovirus	ssDNA-	No	II	No		
12	Porcine parvovirus	Parvovirinae	Bocaparvovirus	ssDNA-	No	II	No		
13	Schmallenberg virus	Peribunyaviridae	Orthobunyavirus	ssRNA-	Yes	V	Yes		
14	Murine encephalomyelitis virus	Picornaviridae	Cardiovirus	ssRNA +	No	IV	No		
15	Bovine enterovirus 2	Picornaviridae	Enterovirus	ssRNA +	No	IV	No		
16	Human poliovirus strain Sabin 1	Picornaviridae	Enterovirus	ssRNA +	No	IV	No		
17	Hepatitis A virus	Picornaviridae	Hepatovirus	ssRNA +	No	IV	No		
18	Simian virus 40	Polyomaviridae	Polyomavirus	cdsDNA	No	I	No		
19	Vesicular stomatitis virus	Rhabdovirus	Vesiculovirus	ssRNA-	No	V	Yes		
20	Semliki forest virus	Togaviridae	Alphavirus	ssRNA+	No	IV	Yes		
21	Chikungunya	Togaviridae	Alphavirus	ssRNA+	No	IV	Yes		
22	Parainfluenza 5 virus	Paramyxoviridae	Orthorubulavirus	ssRNA-	No	V	Yes		
23	Human Pegivirus	Flaviviridae	Pegivirus	ssRNA+	No	IV	Yes		

Test (Challenge) our implemented system with real blood samples (pooled serum)

Mexican samples

Batch 1: Oct 2023-February 2024

- Mexico City
- Puebla
- Cancun
- Durango

Batch 2: March 2024 – October 2024

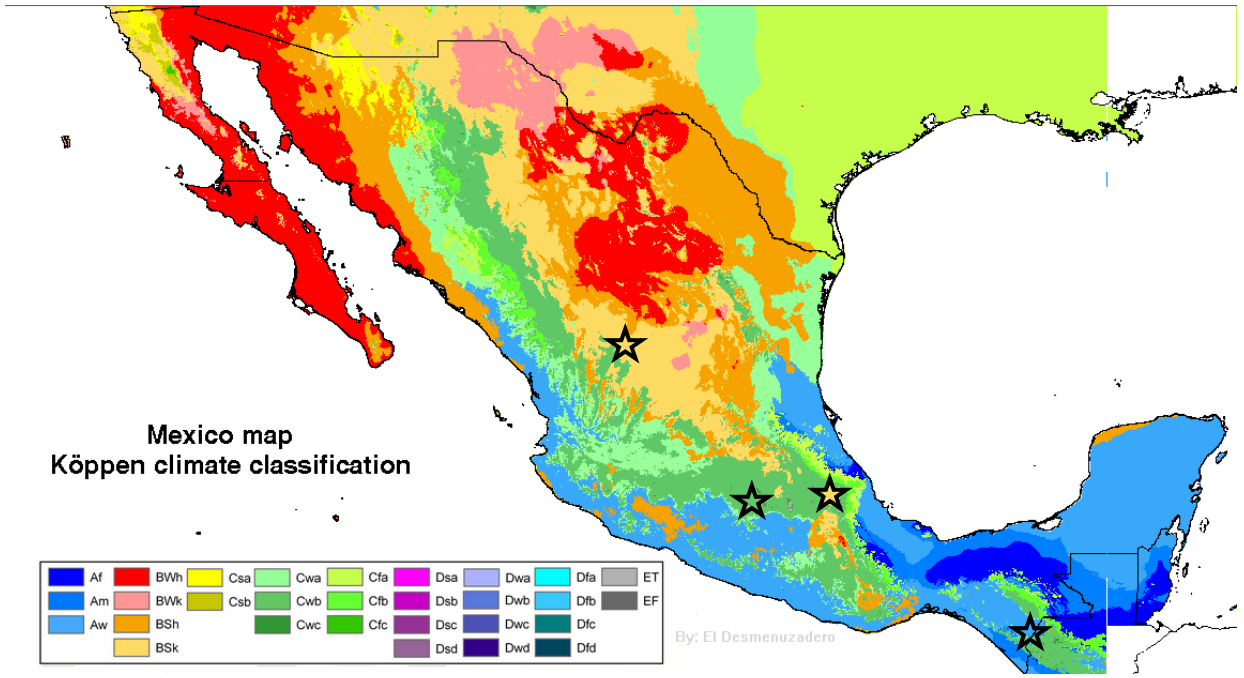
- Mexico City
- Puebla
- Cancun
- Durango
- Chiapas
- Baja California

Minipools from 100 donors
895 Pools = 89.500 Donors

	Pools (100)		Metapools (1.000)		
	Acepted Donors	Rejected Donors	Acepted Donors	Rejected Donors	Total
Mexico City	240	158	25	16	439
Durango	3	0	2	0	5
Cancun	19	3	3	1	26
Puebla	5	0	1	0	6
Total	267	161	31	17	476

Köppen climate classification scheme symbols description table[8][7][10]

1st	2nd	3rd
A (Tropical)	f (Rainforest) m (Monsoon) w (Savanna, dry winter) s (Savanna, dry summer)	
B (Dry)	W (Arid desert) S (Semi-arid steppe)	h (Hot) k (Cold)
C (Temperate)	w (Dry winter) f (No dry season) s (Dry summer)	a (Hot summer) b (Warm summer) c (Cold summer)
D (Continental)	w (Dry winter) f (No dry season) s (Dry summer)	a (Hot summer) b (Warm summer) c (Cold summer) d (Very cold winter)
E (Polar)	T (Tundra) F (Ice cap)	



Preliminary Results

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Pools	Origin	Pegivirus	TTV	HHV-6b	HHV-6a	HPV	EBV	HEV	ADV-11	Parvo B19	HHV-8	DNV-1	DNV-2	DNV-3
34	CDMX	31	33	7	1	1	2	2	1	1	1		1	
2	Puebla	2	2	1										1
3	Chiapas	3	3							1		2		
1	Cancun	1	1	1										
40	**Pools**	**37 (92,5%)**	**39 (97,5%)**	**9 (22,5%)**	**1 (2,5%)**	**1 (2,5%)**	**2 (5%)**	**2 (5%)**	**1 (2,5%)**	**2 (5%)**	**1 (2,5%)**	**2 (5%)**	**1 (2,5%)**	**1 (2,5%)**

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Ian Lipkin

Risk factors for carrying *Trypanosoma cruzi* Infection in Non-Endemic Countries: A Systematic Review

Anastasios Bastounis, University of Nottingham; Jenny Mohseni Skoglund, ECDC; François-Xavier Lamy, ECDC, Elisa Martello, University of Nottingham; Katerina Nikitara, University of Crete; Constantine Vardavas, University of Crete; Jo Leonardi-Bee, University of Nottingham

Jenny Mohseni Skoglund, Principal Expert of Microbial Safety of Substances of Human Origin, ECDC

On behalf of all contributors to the report

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- **ECDC:** Céline Gossner and Howard Needham

Background

Chagas disease

- Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*

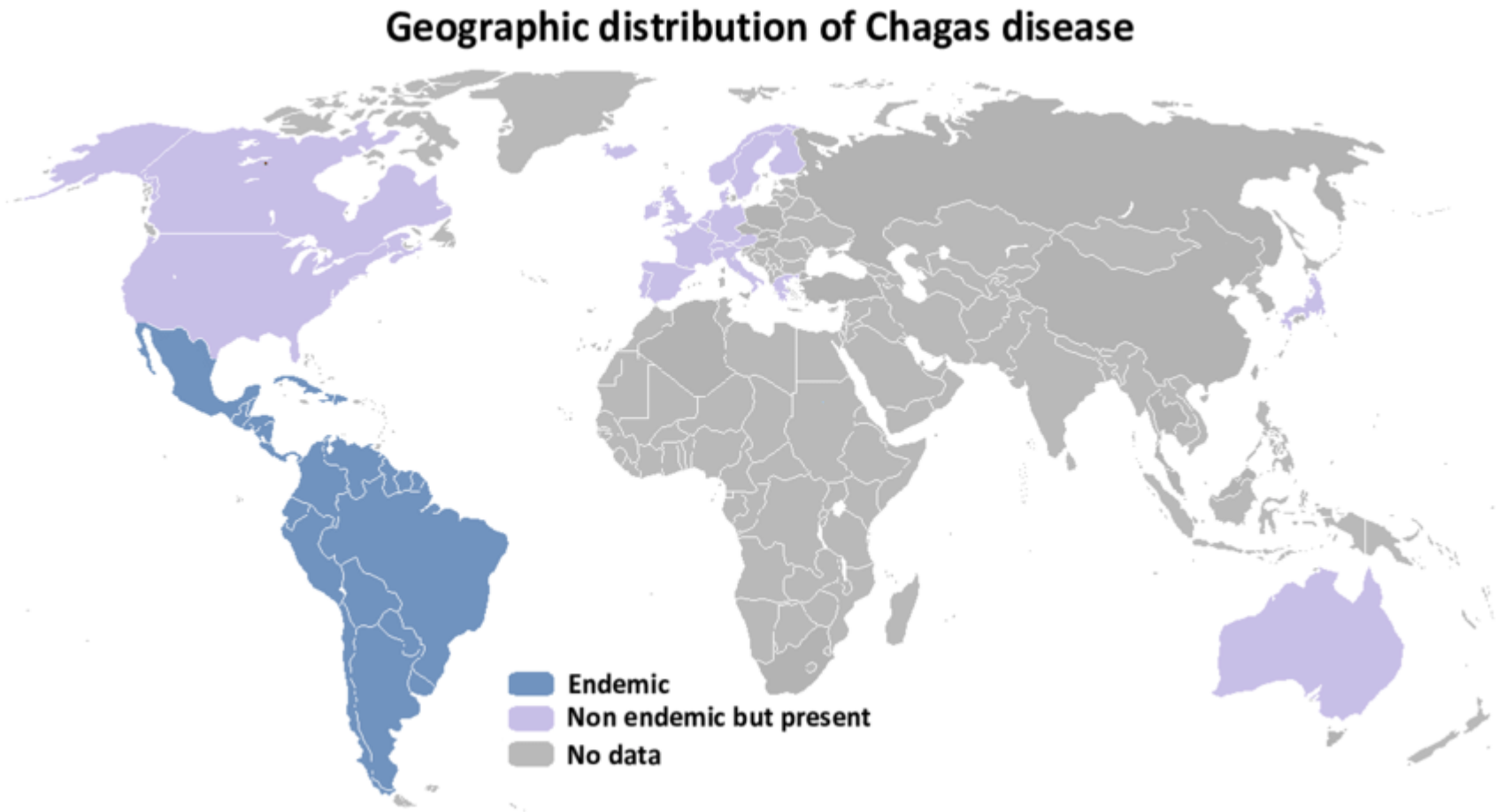
- Acute phase: often asymptomatic or mild symptoms
- Chronic phase: often asymptomatic; may last decades (or lifelong), up to a third of those infected develop serious clinical disease
  - Cardiac disorders
  - Digestive alterations
  - Neurological manifestations

Background

Transmission

- Contact with triatomine bugs in countries endemic for Chagas disease
- Ingesting food or drinks contaminated with the parasite
- Laboratory accidents
- Congenital
- Substances of human origin (SoHO)
  - Blood transfusions and organ transplanta

Chagas disease - epidemiology



Source: Sangenito LS, Branquinha MH, Santos ALS. Funding for Chagas Disease: A 10-Year (2009–2018) Survey Tropical Medicine and Infectious Disease 2020 Jun; 1;5(2):88. Available at https://pubmed.ncbi.nlm.nih.gov/32492834/

Background

Implications for blood safety

Challenges in the identification of donors infected with *T. cruzi*:

Newly infected donors, or donors with asymptomatic chronic *T. cruzi* infections

Measures to mitigate the risk of *T. cruzi* transmission by transfusion in non-endemic countries:

Selective testing or deferral of donors at risk

Objective

To provide evidence on the demographic, environmental, epidemiological, and other characteristics associated with carrying the *T. cruzi* parasite in at-risk individuals residing in countries non-endemic for Chagas disease.

Methods

Search question:

Which factors are associated with a higher risk of carrying the *T. cruzi* parasite in people residing in non-endemic countries?

Map produced on: 17 Dec 2024. Administrative boundaries: © EuroGeographics © UN–FAO © Turkstat.The boundaries and names shown on this map do not imply official endorsement or acceptance by the European Union.

Methods

Population:
Males and females of the general population of all ages residing in non-endemic countries

People infected via congenital transmission: eligible if not born in endemic countries*

Exposure:
Risk factors for *T. cruzi* infections

Outcome:
T. cruzi infection

* As outlined by WHO

Map produced on: 17 Dec 2024. Administrative boundaries: © EuroGeographics © UN–FAO © Turkstat.The boundaries and names shown on this map do not imply official endorsement or acceptance by the European Union.

Methods

Study design

Inclusion:

- Randomised controlled trials (including quasi), observational cohort, case-control and cross-sectional studies

Exclusion:

- Case reports, conference abstracts, and studies conducted in endemic countries.

Methods

Search strategy

- Time frame: from 1 January 2000 to 29 June 2022
- All languages
- Databases searched: MEDLINE(R) In-Process & Other Non-Indexed Citations and MEDLINE(R) (Ovid), including PubMed; EMBASE (Ovid)

Methods

Selection process: Two reviewers conducted screening of studies independently for relevance based on titles/abstracts and full texts, with disagreements resolved through discussion.

Quality assessment: JBI critical appraisal tools

Effect measures and synthesis method

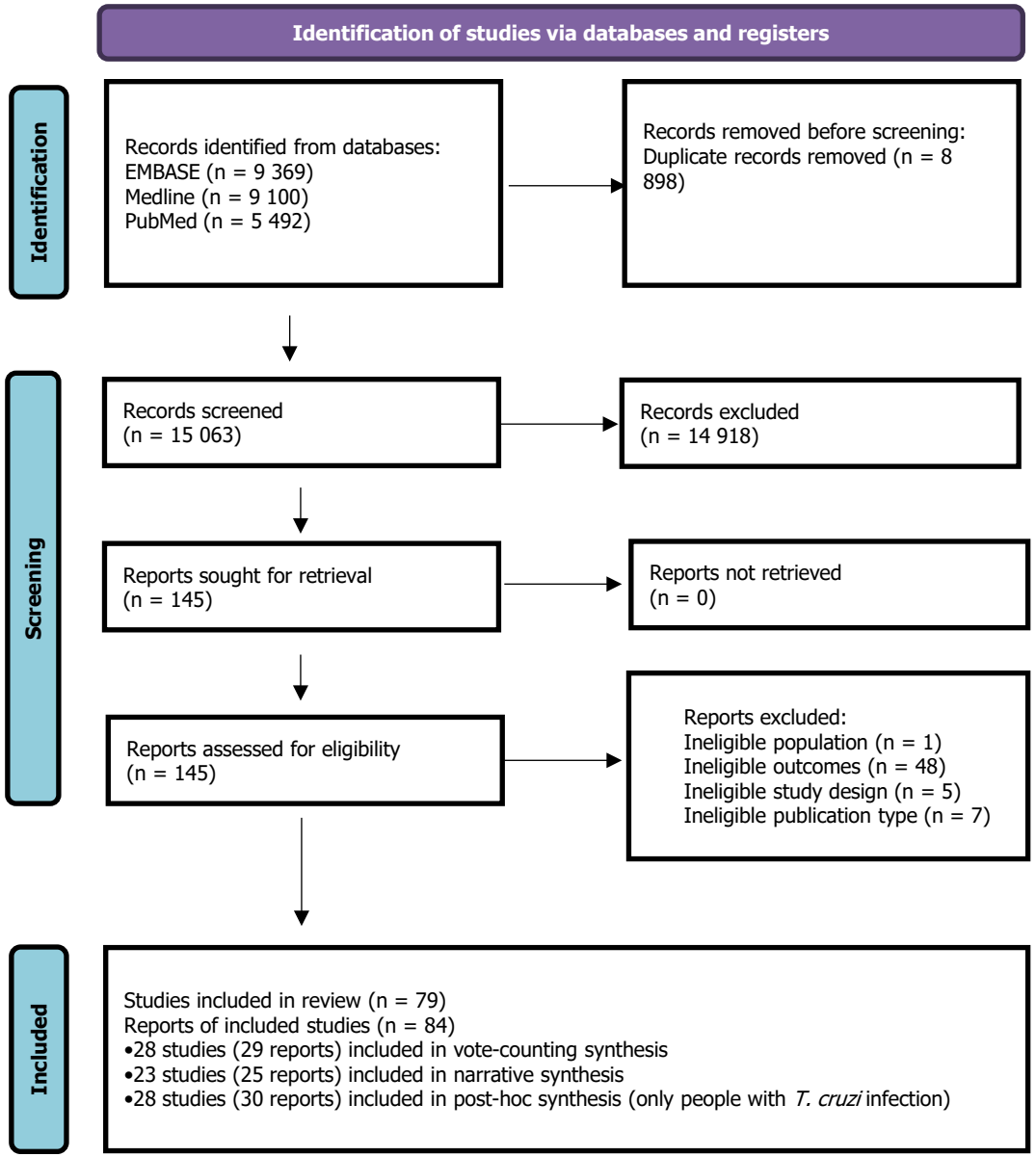
- Studies that reported measures of association: Vote counting synthesis
- Studies that did not report measures of association: Narrative synthesises
- Studies that focussed on patients infected with *T. cruzi* only without providing any comparison with healthy individuals: synthesised and reported separately

Results

- Title/Abstract = 15 063
- Full-text screening = 145

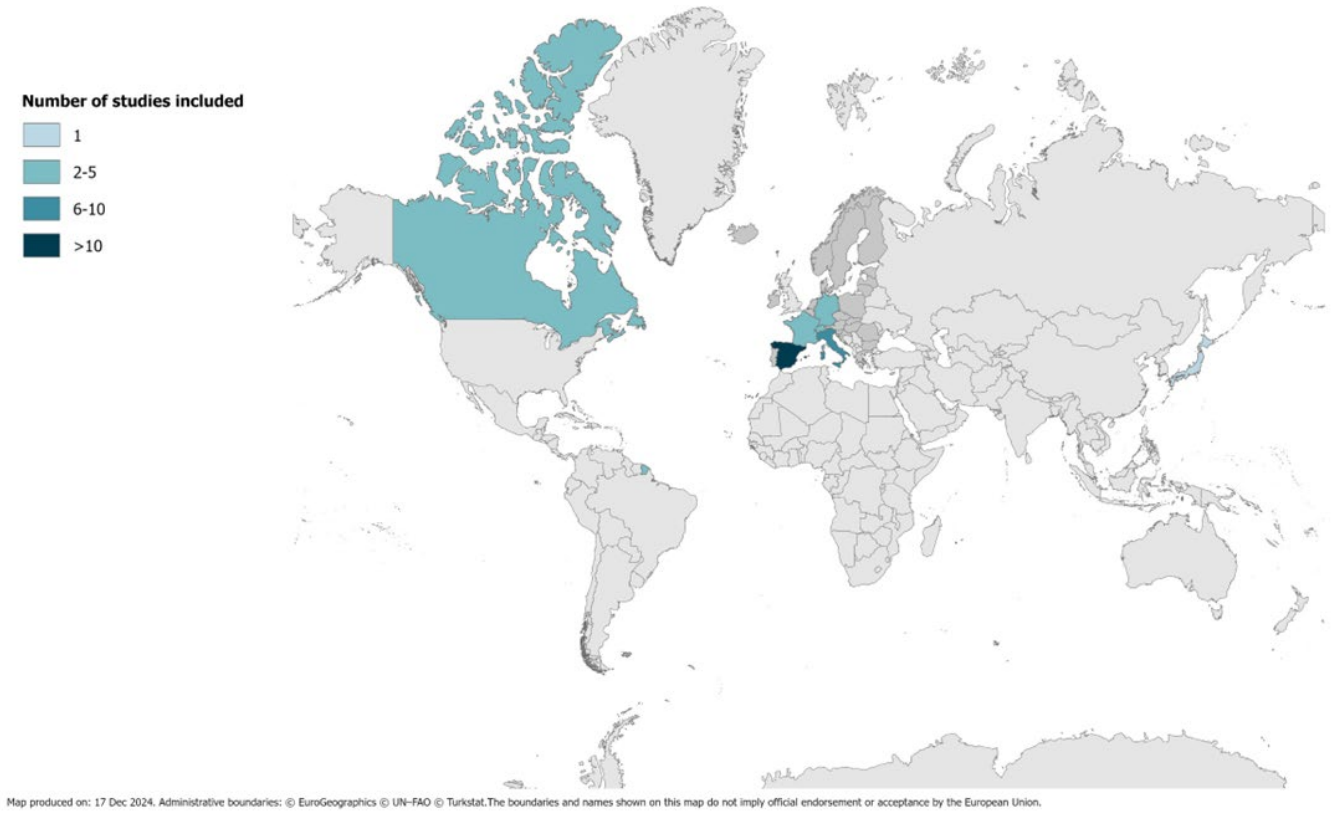
- Studies included in review: 79
- Studies included in synthesis: 51
  - Vote counting synthesis: 28
  - Narrative synthesis: 23

- Quality assessment: JBI critical appraisal tools



Results

Countries of studies included in the synthesis



- Studies conducted in EU/EEA and Switzerland: 42
- Studies conducted in the US: 6
- Studies conducted in Canada: 2
- Studies conducted in Japan: 1

- Studies where the population included blood donors: 9
  - Italy or Spain: 4; US, Canada or Japan: 5

Results

Illustration of graphical representation and synthesis of the data

Vote-counting synthesis

[Ref.] Study ID	Study design; Final sample (*T. cruzi* positive)*	Age[a]	Sex[b]	Country of origin[c]	Stay in endemic country	Mother/ grand-mother born in endemic country[d]	History of living in rural areas of endemic countries[e]	History of living in mud/adobe houses[f]	History of living in house(s) with thatched roof[g]	History of family/ relatives CD[h]	History of transfusions/ transplantation in endemic countries[i]	Contact with the vector (Inc. bites)[j]	Other infection(s)/ health issues[k]	Prior generic knowledge of CD
[53]Alcántara Román et al., 2018	Observational cohort; 192 (descendants of seropositive mothers) (23)	▲ [<14yr (ref.) vs >14yr]	▲	▲ [Born in EU (ref.) vs born outside EU]	-	-	-	-	-	-	-	-	-	-
[18]Antinori et al., 2018**	Cross-sectional; 501 (48)	▲	▲	▲	-	-	▲	▲	-	▲	▼	-	▲	-
		▲	-	▲	-	-	-	▲	-	▲	-	-	-	-
[19]Avila Arzanegui et al., 2013**	Cross-sectional; 158 (19)	-	-	▲	-	-	▲	▲	-	▲	-	▲	-	-
		-	-	-	-	-	-	▲	-	-	-	▲	-	-
[56]Cantey et al., 2012	Cross-sectional; 37 (15)	-	▲	-	-	-	-	-	-	-	-	-	-	-
[21]Custer et al., 2012	Cross-sectional; 221 (63)	-	-	-	▲	▲	▲	▲	▲	-	-	▲	▲	-
[22]Da Costa-Demaurex et al., 2019	Cross-sectional; 1010 (16)	▲ [<35yr (ref.) vs >35yr]	▲	▲	-	-	-	-	-	-	-	-	-	-
[23]Di Girolamo et al., 2016	Cross-sectional; 151 (12)	▲ [≤35yr (ref.) vs >35yr]	▲	▲	-	-	▲	-	-	▲	▲	-	-	-
[25]Favila Escobio et al., 2015	Cross-sectional; 251 (48)	▲	▲	-	-	-	▲	▲	-	▲	▲	▲	-	▲
		-	▲	-	-	-	-	-	-	▲	-	▲	-	-
[60]Guggenbühl Noller et al., 2020	Observational cohort; 1596 (NR)	▲	▲	▲	-	-	-	-	-	-	-	-	-	-
[29]Hernandez et al., 2019	Cross-sectional; 189 (14)	▲ [<40yr (ref.) vs >40yr]	▲	▲ [other(ref.) vs El Salvador]	-	-	-	-	-	▲	-	-	-	-
[62]Ikedionwu et al., 2020	Observational cohort (cross-sectional data);	▲ [15–24yr	-	-	-	-	-	-	-	-	-	-	-	-

Results

Summary of key risk factors assessed for their association with *T. cruzi* infection in non-endemic countries

Risk factor for *T. cruzi* infections	Number of studies
Being born in an endemic country (of which Bolivia)	19 (16)
Stay in endemic country	1
History of living in rural areas	13
History of living in poor housing conditions	12
Contact with the vector	7
History of blood transfusion in endemic countries	9
Being older	15
Mother or grandmother born in endemic country	1
Having a family history of Chagas disease	13
Prior generic knowledge of Chagas disease	8
Being female	17

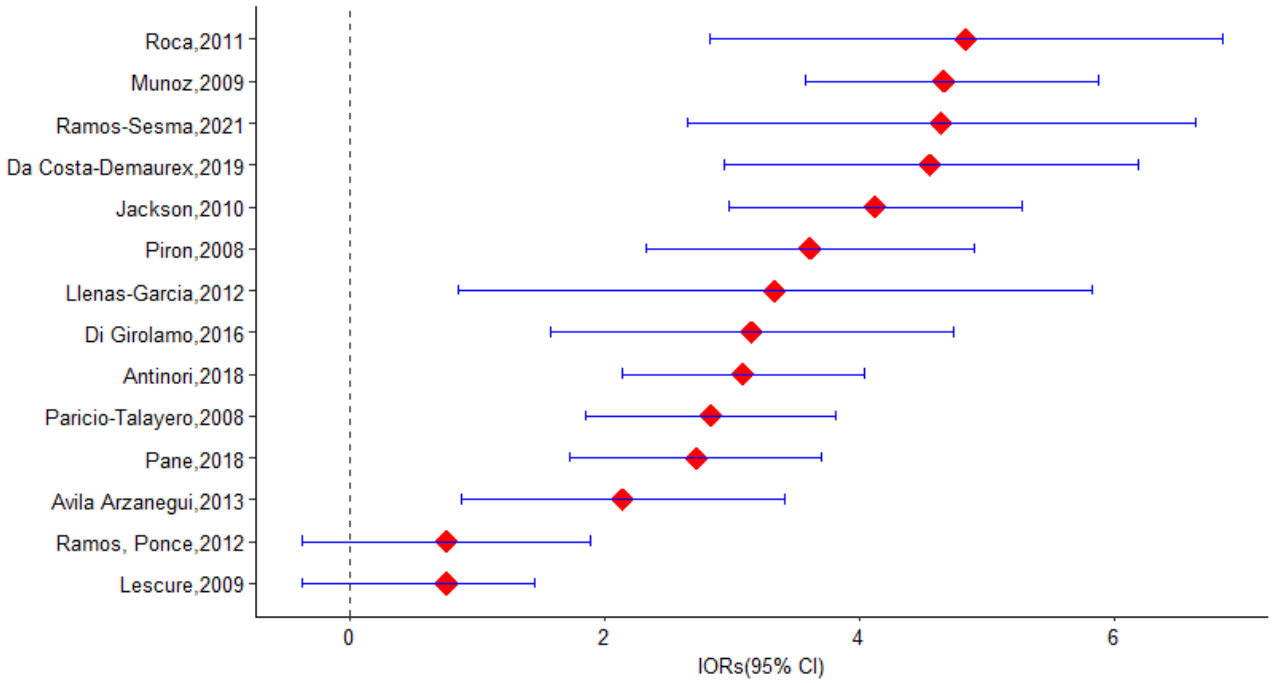
Results

Being born in an endemic country

Being born in an endemic country was always associated with *T. cruzi* infection when compared to non-endemic countries.

Bolivia most commonly assessed country in the included studies.

Associations between Bolivia as country of origin and *T. cruzi* infection



Results

Travelling to or having stayed in endemic countries

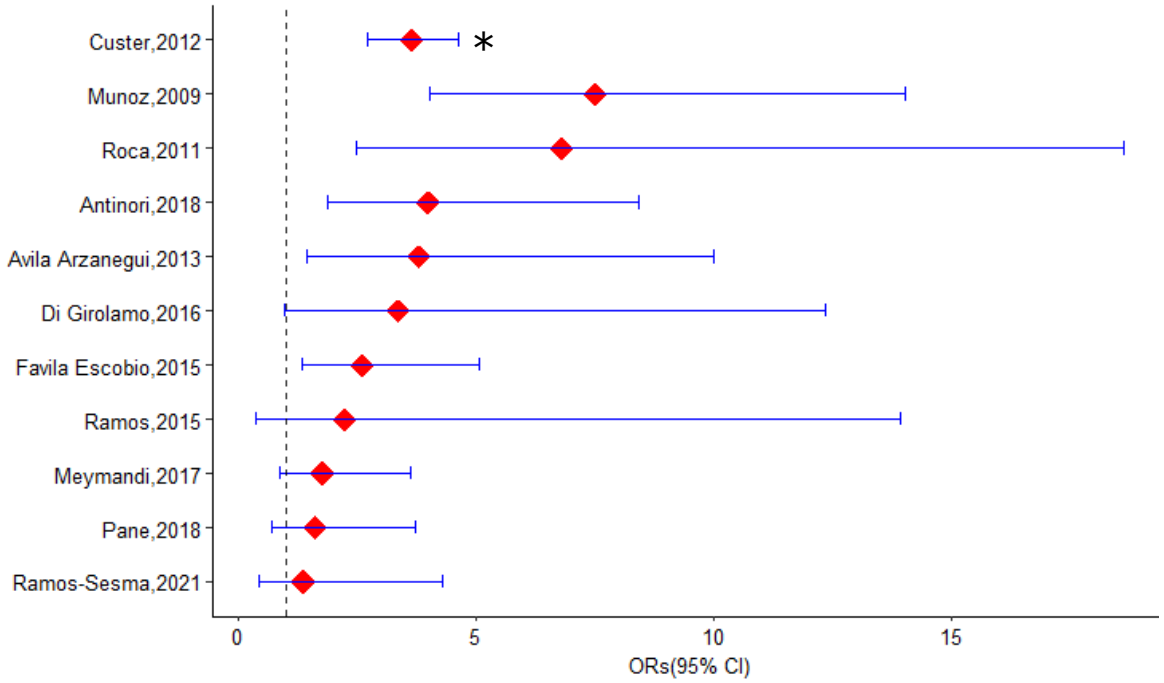
No studies in this review reported data regarding association between travelling to endemic area and carrying the *T. cruzi* parasite.

One study showed that having spent three or more months in an endemic country was associated with higher odds for being infected with *T. cruzi*.

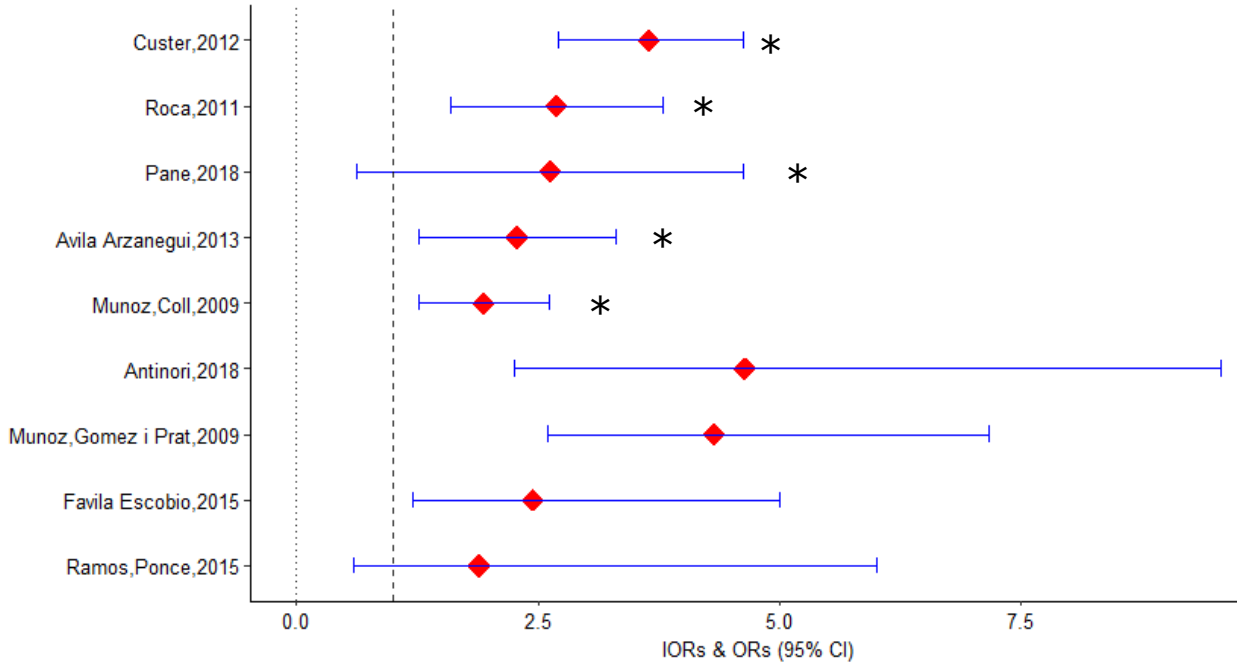
Studies included in the narrative syntheses: all positive cases in people born in non-endemic countries reported travelling to or residence in endemic countries.

Results

Associations between history of living in rural areas of endemic countries and *T. cruzi* infection



Associations between history of living in mud/adobe houses and *T. cruzi* infection



** Presented as log OR*

Results

History of blood transfusion in endemic countries

In six out of nine studies previous blood transfusion compared to no previous transfusion in endemic countries was associated with increased odds of being infected with *T. cruzi.*

None of these studies revealed the year for transfusion for the participants.

No possible to conclude whether *T. cruzi* infection was related to a transfusion received before the implementation of universal screening in Latin American countries or because of residual infectivity due to limited sensitivity of the screening tests.

Result

Being older

In all studies, older age was associated with *T. cruzi* infection, among individuals with other factors associated with *T. cruzi* infection, irrespective of the age categorisation used.

Could be attributed to two main factors:

- A longer exposure period to the *T. cruzi* parasite.
- Born during a time when there were limited control programs and screening initiatives in place in endemic countries.

Result

Mother or grandmother born in endemic country

A single study assessed the relationship between maternal origin and *T. cruzi* infection:

- Individuals with mothers' born in endemic countries have higher odds of carrying the *T. cruzi* parasite compared to individuals whose mothers did not originate from endemic countries.
- Individuals with grandmothers' born in endemic countries have higher odds of carrying the *T. cruzi* parasite compared to individuals whose grandmothers did not originate from endemic countries.

All the studies in this review included individuals with origins in endemic countries - lack of comparator in other studies.

Results

Having a family history of Chagas disease

In all 13 studies, a family history of CD was associated with carrying the *T. cruzi* parasite.

Includes:

- Maternal history of CD
- Siblings' history of CD
- Grandmaternal history of CD
- Relatives' history of CD

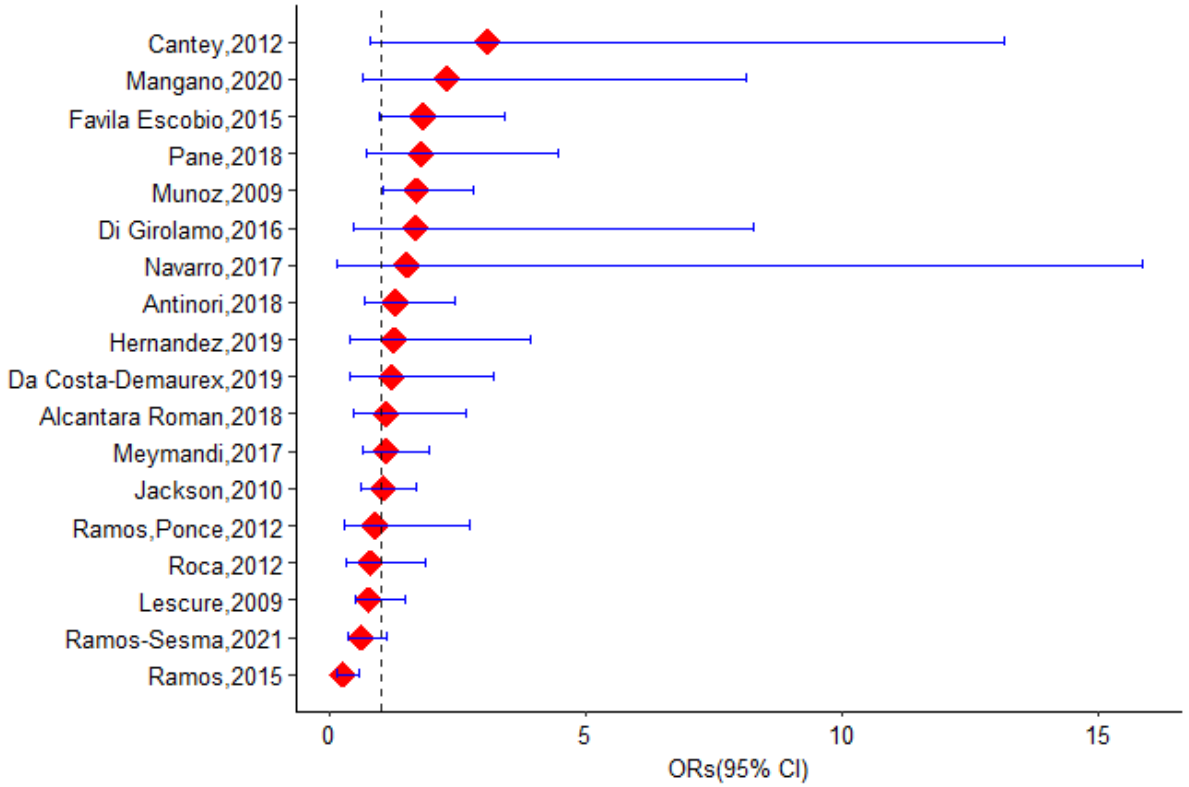
May reflect environment or congenital transmission of *T. cruzi.*

Results

Being female

Women overrepresented in the examined population (i.e. pregnant women from endemic countries).

Associations between sex (female) and *T. cruzi* infection



Subgroup analysis

Geographical location of the study (EU/EEA, Switzerland):

No differences in the reported factors associated with *T. cruzi* infection between individuals residing in EU/EEA countries and Switzerland and those residing in non-endemic countries outside the EU/EEA and Switzerland.

SoHO (blood donors) in general and within EU/EEA:

No differences in the risk factors for carrying the *T. cruzi* parasite between blood donors in general and the rest of the individuals.

In most studies on blood donors conducted in EU/EEA, *T. cruzi* -positive donors were from Latin American countries, while those born in EU/EEA had travelled to endemic countries.

Strength and limitations

Strength:

Conforming to the PRISMA statement.

Too the best of our knowledge, the largest systematic review that has synthesised data on factors associated with infection with *T. cruzi* in individuals residing in non-endemic countries.

Limitations:

Grey literature was not searched.

Due to the heterogeneity in populations and comparison groups included in this review, a meta-analysis was precluded.

Reporting bias cannot be excluded.

Some of the included studies were underpowered.

Conclusions

The following risk factors associated with carrying the *T. cruzi* parasite in non-endemic countries were identified:

i. being born or having stayed in Latin American countries;
ii. having a history of living in rural areas in endemic countries;
iii. having a history of living in poor housing conditions in endemic countries;
iv. having received blood transfusions in endemic countries;
v. older age among individuals with other factors associated with *T. cruzi* infection;
vi. maternal origin from endemic country;
vii. having a family history of CD;
viii. having a generic knowledge of CD prior to testing.

Conclusions and next step

Results from this systematic review could support the development of guidance for preventive measures, aiding in the identification of donors at risk to reduce SoHO-transmission of *T. cruzi* in non-endemic countries.

Acknowledgment

François-Xavier Lamy, Anastasios Bastounis, Jo Leonardi-Bee, Elisa Martello, Katerina Nikitara, Constantine Vardavas, Andrea Angheben, Ana Requena Méndez, Yves Carlier, Paolo Antonio Grossi, Maria Delmans Flores-Chavez, Evelin Lara Molina, Ryanne Lieshout-Krikke, Salvador Oyonarte, Maria del Pilar Fernandez, Marta Victoria Cardinal, Céline Gossner, Howard Needham and Flavia Cunha

Thank you!

Assessment of travel related donor eligibility in Switzerland using the online digital Tool “Travelcheck”

Sandra Kurth, Tiziana Janner-Jametti,
Anita Tschaggelar, Soraya Amar
Swiss Transfusion SRC, Berne

BLUTSPENDE SRK SCHWEIZ
TRANSFUSION CRS SUISSE
TRASFUSIONE CRS SVIZZERA

January 15, 2025

Conflicts of interest

- No Conflicts of interest

Content

- Introduction Swiss Transfusion SRC
- TTD screening tests in Switzerland
- Travel related TTD
- Monitoring of TTD
- Travelcheck Software & Demonstration
- Conclusion

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Swiss Transfusion SRC

- Umbrella organization of the 11 regional blood establishments

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TTD Screening tests

- Mandatory
  - HIV
  - Hepatitis B, C, E
  - Treponema pallidum (Syphilis)

  - *Parvovirus B19 & Hepatitis A*

- Selective tests 
  - West-Nile-virus
  - Chagas
  - Malaria

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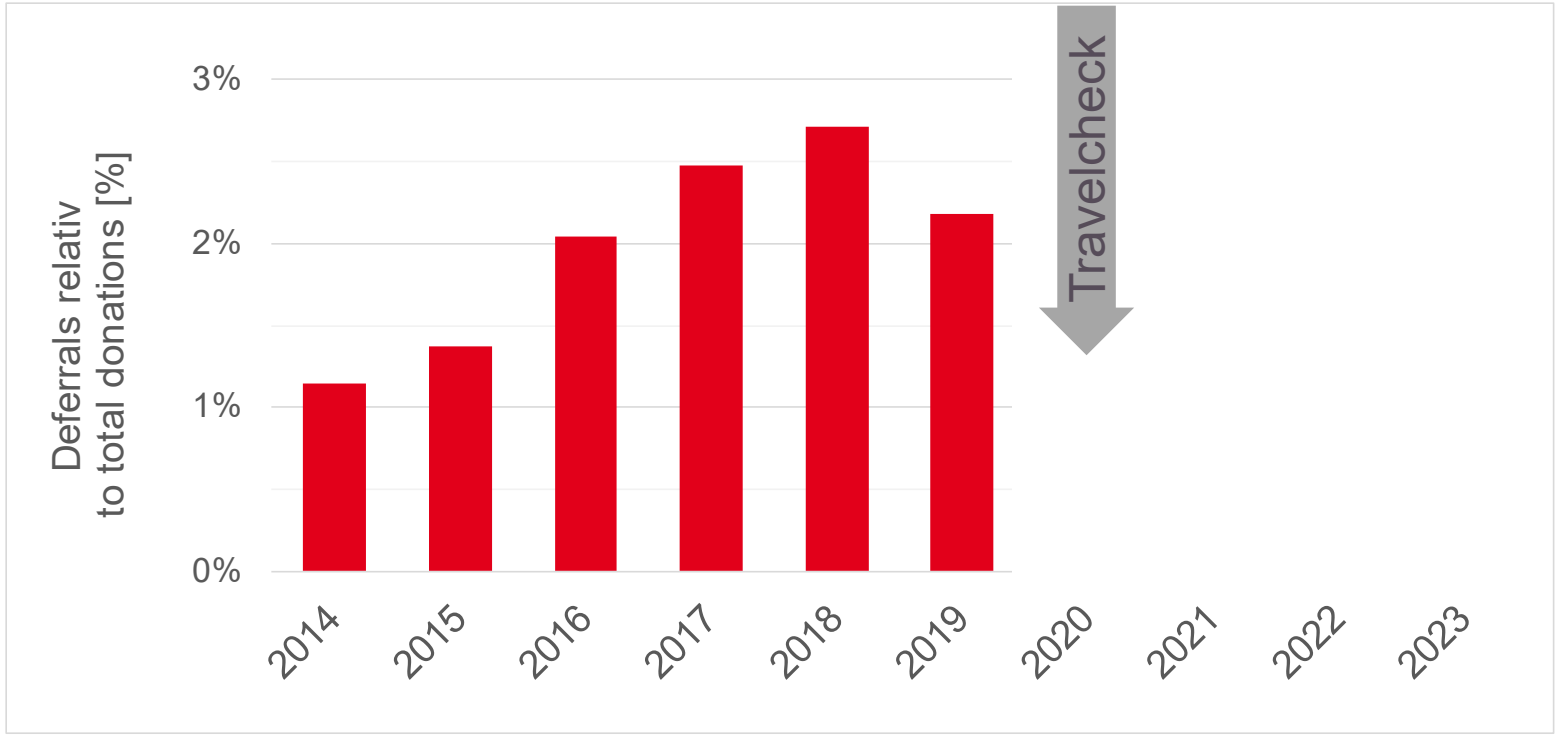
Travel related on-site deferrals

→ Deferrals hurt future donation behavior [1]



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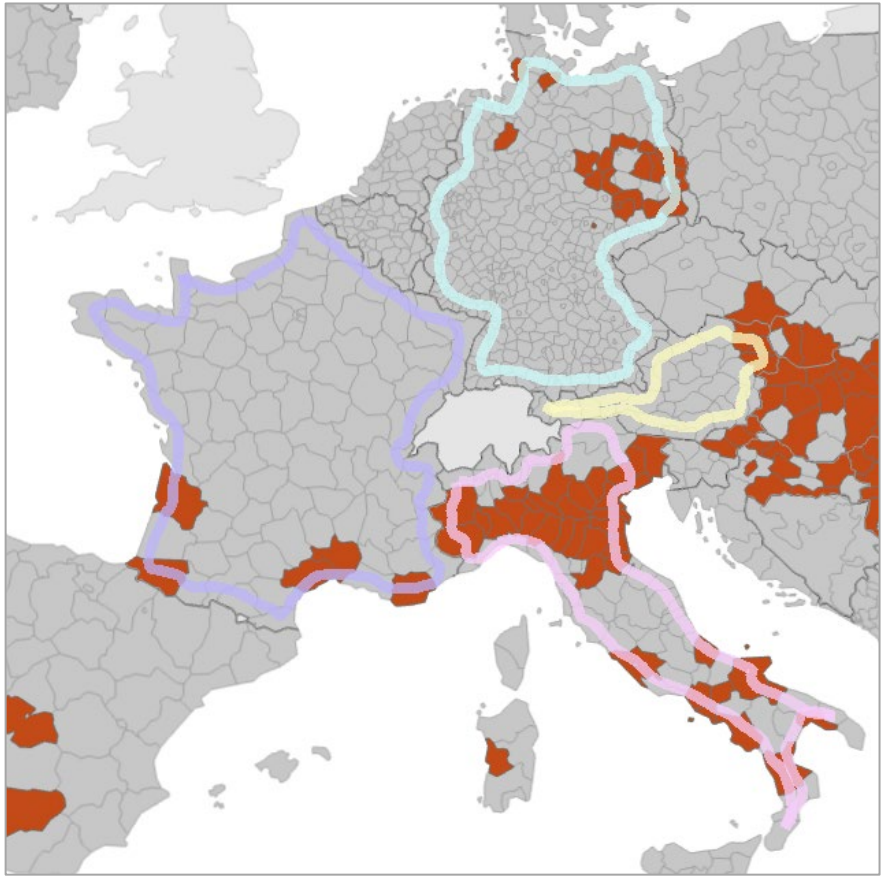
[1] Clement M, Shehu E, Chandler T. The impact of temporary deferrals on future blood donation behavior across the donor life cycle. Transfusion. 2021;61(6):1799-1808. doi:10.1111/trf.16387

Monitoring TTD – Arboviruses

- West Nile Virus, Dengue, Chikungunya, Zika*
- EU countries
  - Weekly Monitoring from June – November
  - ≥ 1 case for neighboring countries
  - ≥ 5 cases others
- Non-EU countries
  - Review every 2 years (≥ 5 cases)
- 30 Days deferral

** Weekly: only neighboring countries*



ECDC Homepage: Interactive dashboard: West Nile virus transmission

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Monitoring TTD - Others

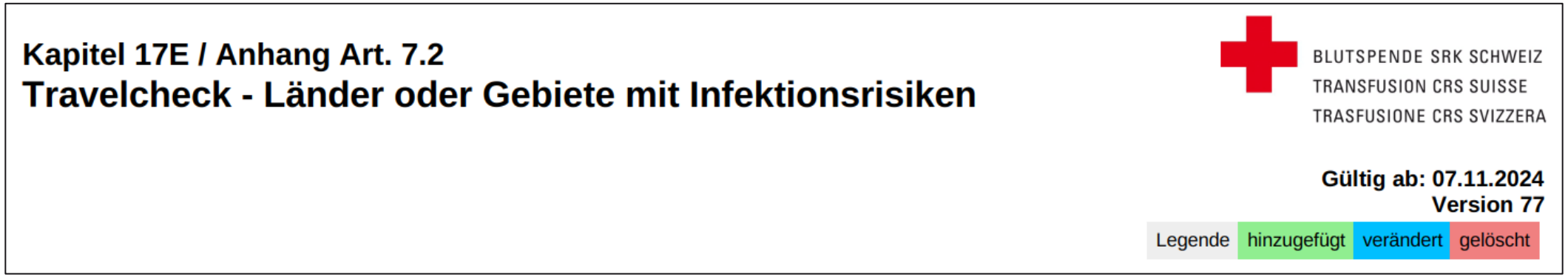
- Malaria
  - Deferral 6 months (4 months with test)
  - Based on country risk-classification
- HBV, HCV and HIV (every product is tested)
  - Only if the stay was > 6 months (4 months deferral)
  - Population prevalence (≥1%), updated every 2-3 years
- Chagas *(Trypanosoma cruzi)*
  - Relevant if born or mother from endemic region
  - Test mandatory

Monitoring TTD – Main data sources

- ECDC
  - Weekly threats reports
  - West Nile infections dashboard
- Country specific homepages
- WHO, CDC, EBA
- HealthyTravel (CH)

Travelcheck – Software backend

- Browser based, Login
- In 3 different languages 
- Versioning with change record
- PDF Export for offline use



Kapitel 17E / Anhang Art. 7.2
Travelcheck - Länder oder Gebiete mit Infektionsrisiken

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Gültig ab: 07.11.2024
Version 77

Legende | hinzugefügt | verändert | gelöscht

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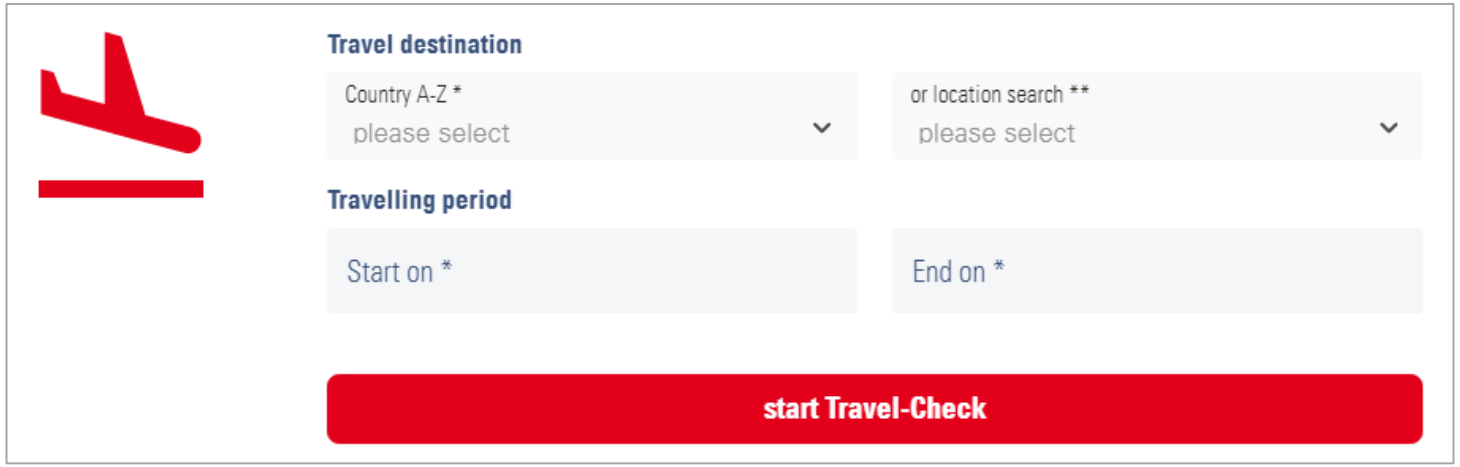
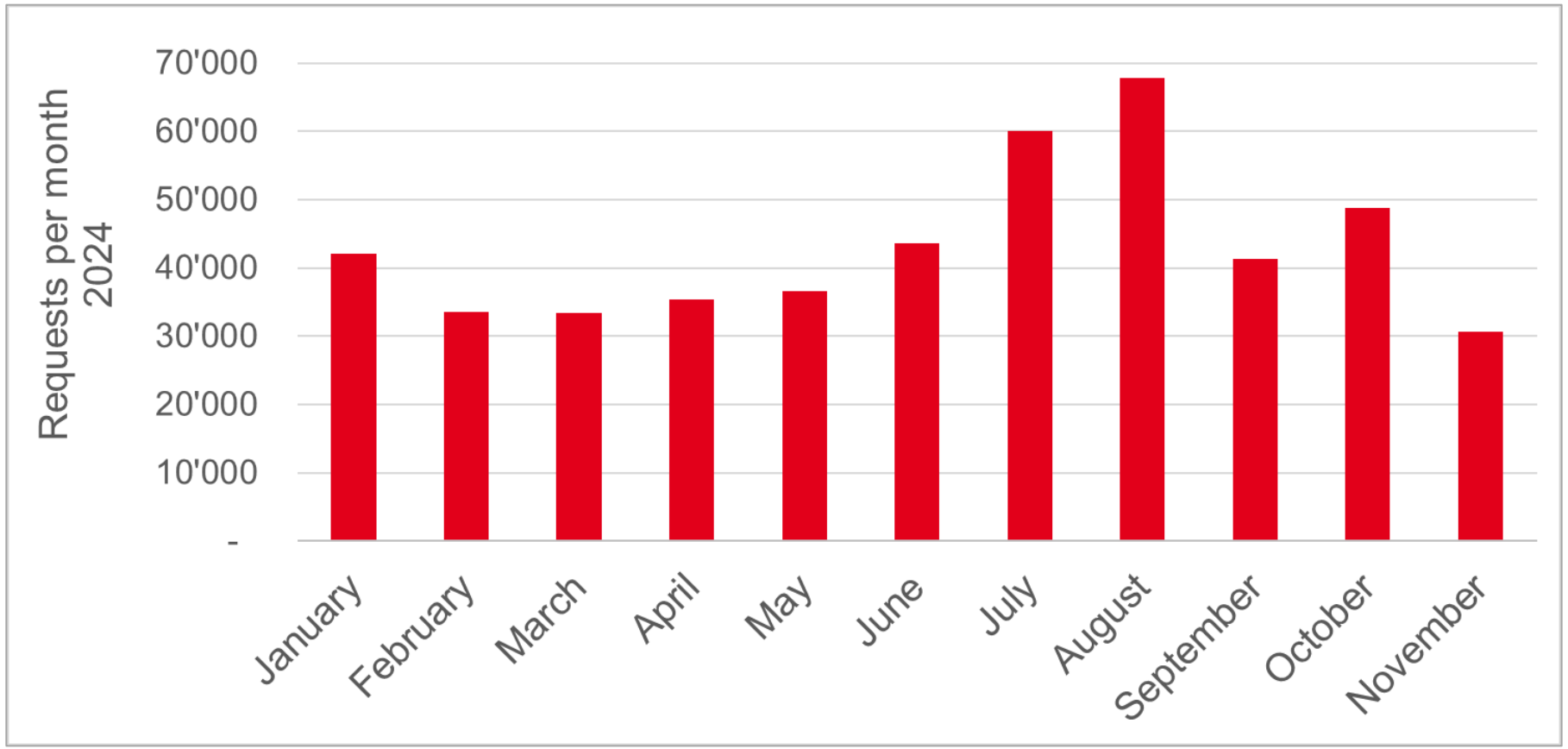
Travelcheck – Software frontend

- Iframe for homepages
- Mobile app
- Tool for donors (self-deferral) and blood donation staff



Travelcheck – Requests per month



Recording only if cookies have been accepted.

Total Blood donations: ≈250`000 / year

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Travel history of donors

- Donor questionnaire
  - During the past 12months, did you travel outside Switzerland?
  - Did you have any signs of illness there or since your return (e.g. fever)?
  - Were you born outside of Switzerland, did you grow up there or did you live there for 6 months or more? If yes, in which country?

→ Interview & **Travelcheck**

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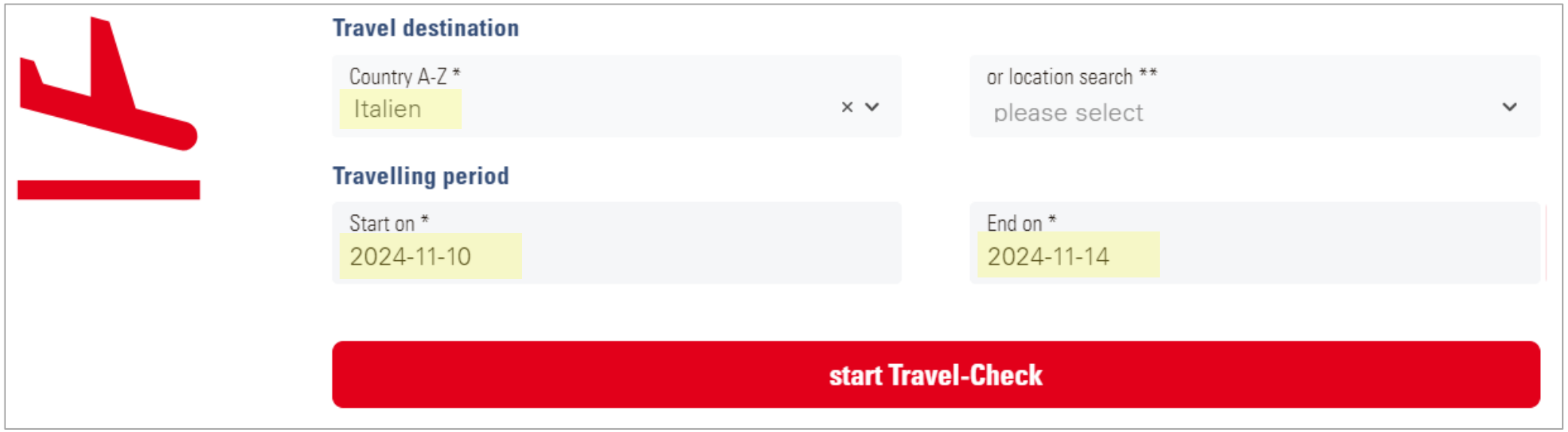
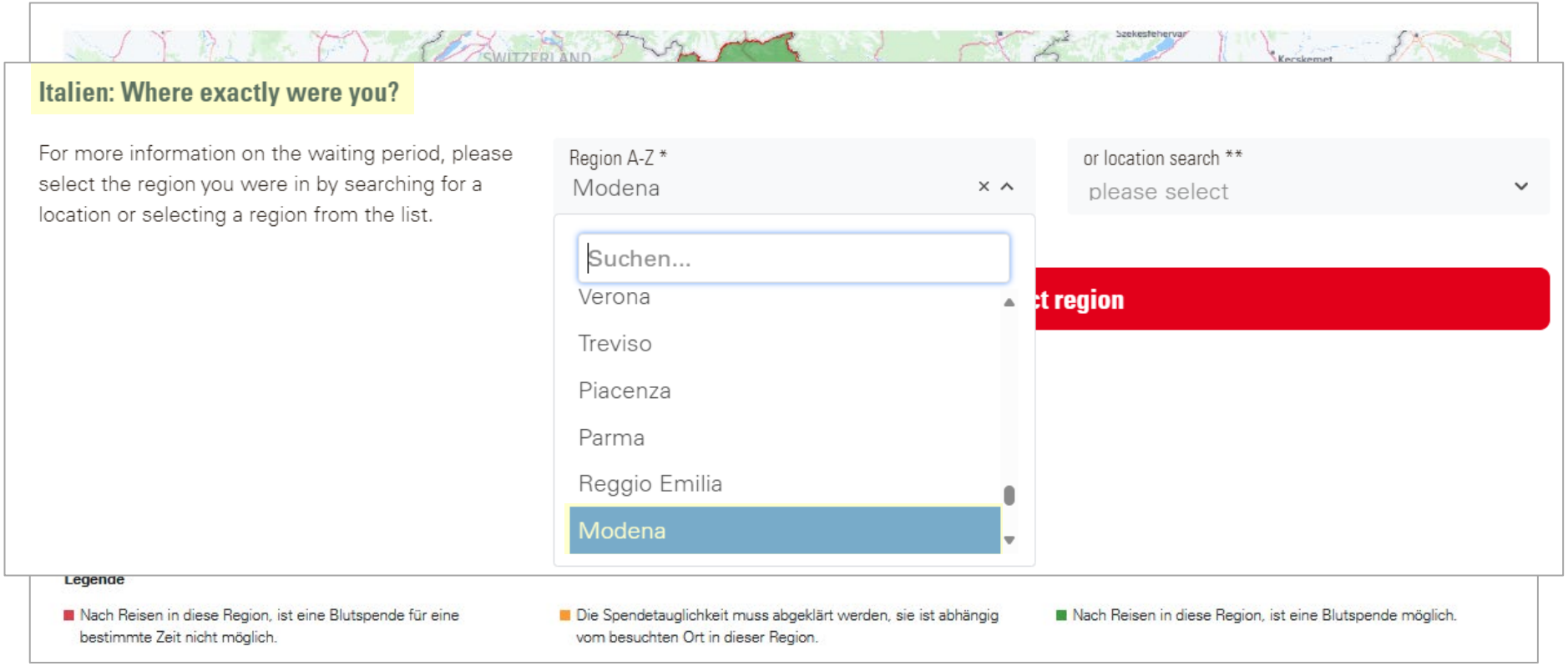
Travelcheck Demo

- Italy from November 10 to November 14
- Request date: December 12, 2024



Travelcheck Demo

- Region needs to be narrowed down



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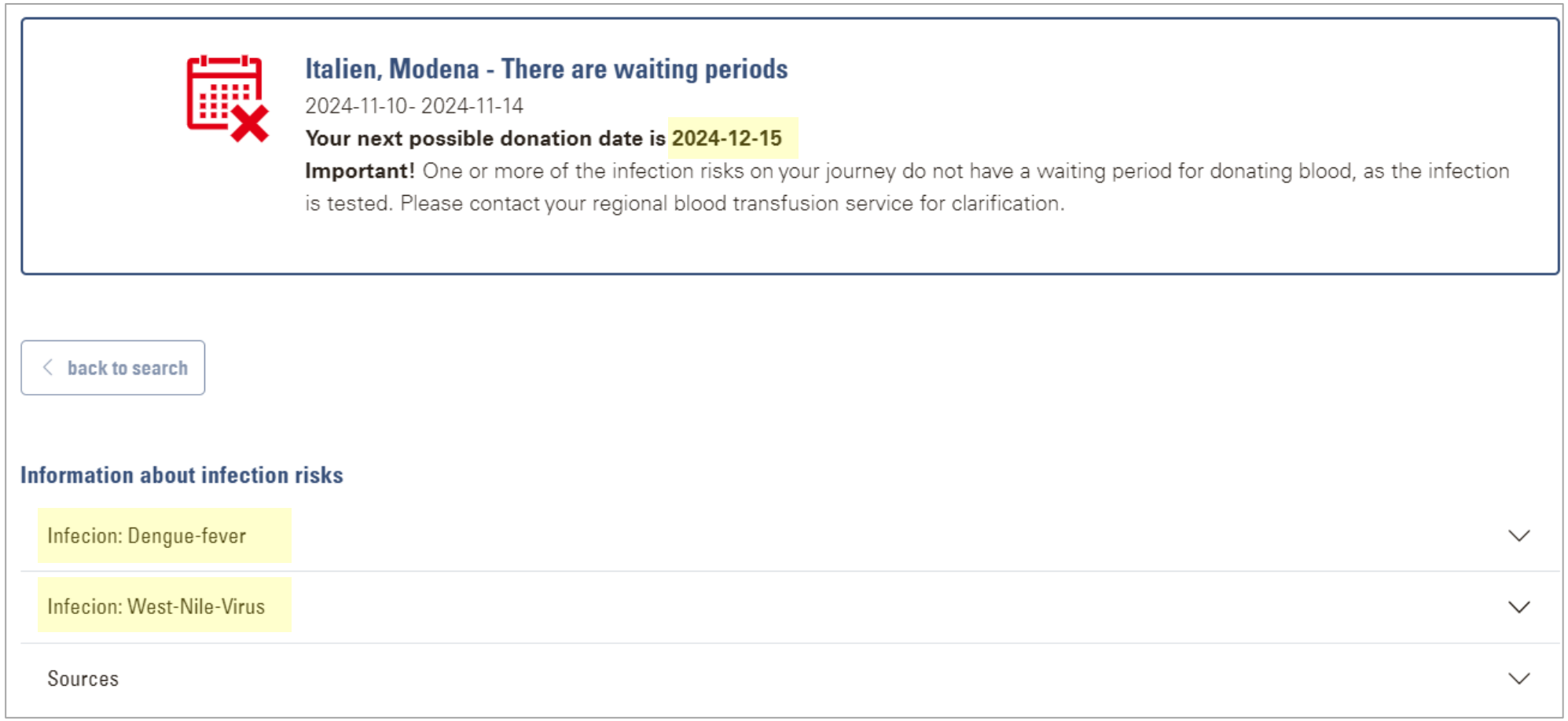
Travelcheck Demo



Travelcheck Demo

Infecion: West-Nile-Virus

Area:	
Period:	July, August, September, October, November
Risk of infection:	present
Waiting period:	30 days or testing The following regional blood donation services carry out testing for the infection so that you can donate blood without a waiting period due to this risk of infection: Transfusion Interregionale
Note:	For stays of more than 24 hours

Sources

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Feedback blood donation staff

- The Travelcheck tool was perceived as
  - simple
  - understandable
  - efficient
- It can quickly and reliably help find countries and unknown regions

Conclusion

- Simple, user-friendly tool for
  - donors **and**
  - donation staff
- Reduces on-site deferrals
- Same tool for **vaccinations** (vaccine check)
- National harmonization and digitalization
- Increased safety

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QR Codes

Travelcheck

Vaccine Check

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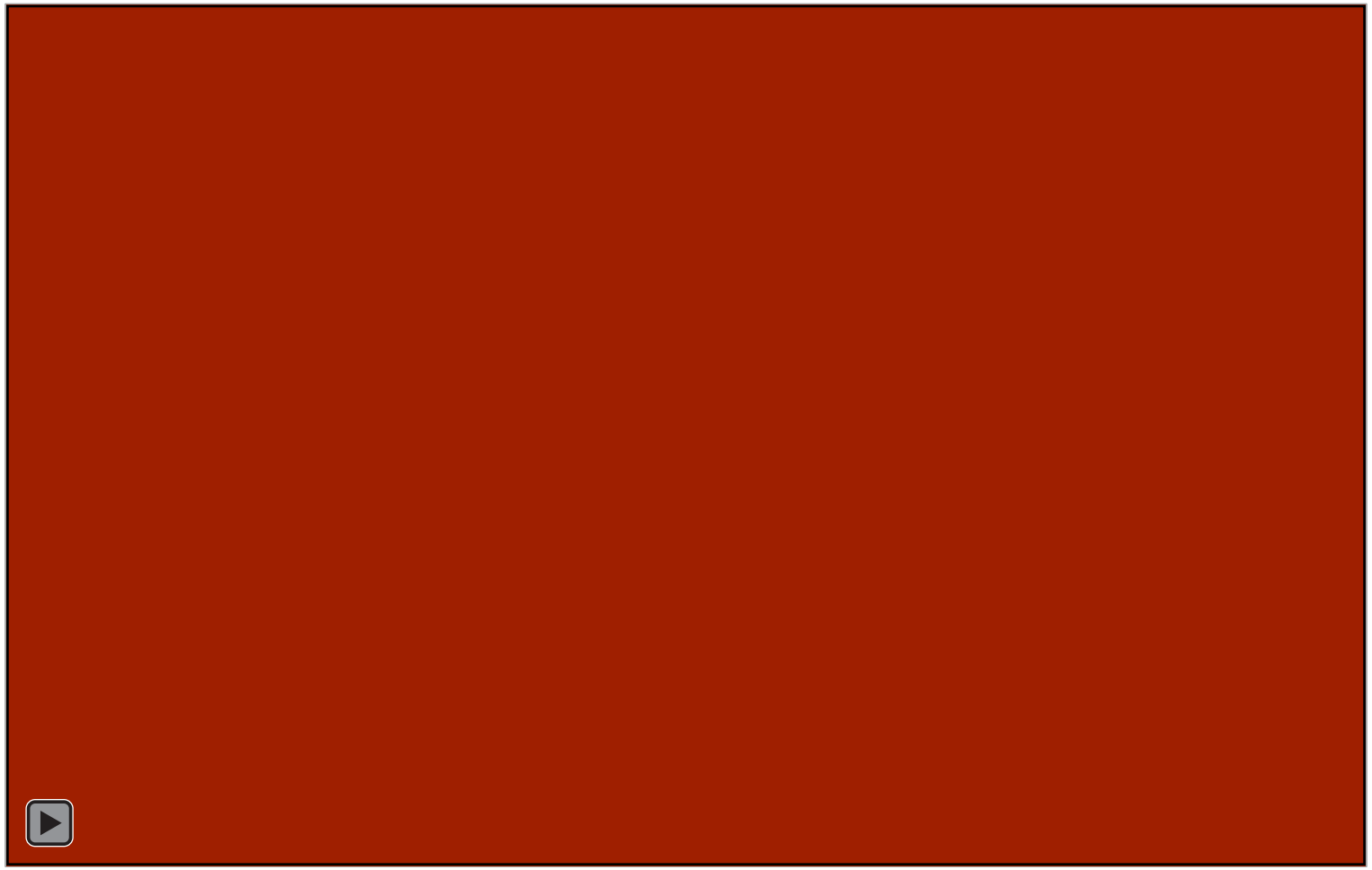
Travelcheck Demo – interactive map



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Defferals

- Chikungunya, West Nile Virus, Dengue, Zika: 30 days:
- Malaria: 6 months
- HIV, HBV, HCV: 4 months if the stay was longer than 6 months.
- Chagas: negative test if the stay was longer than 6 months.

Test strategien

Obligate, freigaberelevante Untersuchungen, systematisch bei jeder Spende		
Art der Analyse	Serologie	NAT (PCR)
HIV1/2	X	X
Hepatitis C (HCV)	X	X
Hepatitis B (HBV)	X	X
Hepatitis E (HEV)		X
Syphilis (*T. pallidum*)	X	

Selektive Untersuchungen (z.B. zur Abklärung der Spendetauglichkeit gemäss Spendetauglichkeitskriterien), freigaberelevant, falls Untersuchung angefordert		
Art der Analyse	Serologie	NAT (PCR)
Chagas (T.cruzi)	X	
Malaria (Plasmodium spp.)	X	
CMV	X	
WNV (saisonal)		X

Nicht freigaberelevante Untersuchungen, i.d.R. systematisch bei jeder Spende (freigaberelevant für Plasma zur Fraktionierung)		
Art der Analyse	Serologie	NAT (PCR)
Parvo B19		X
Hepatitis A (HAV)		X

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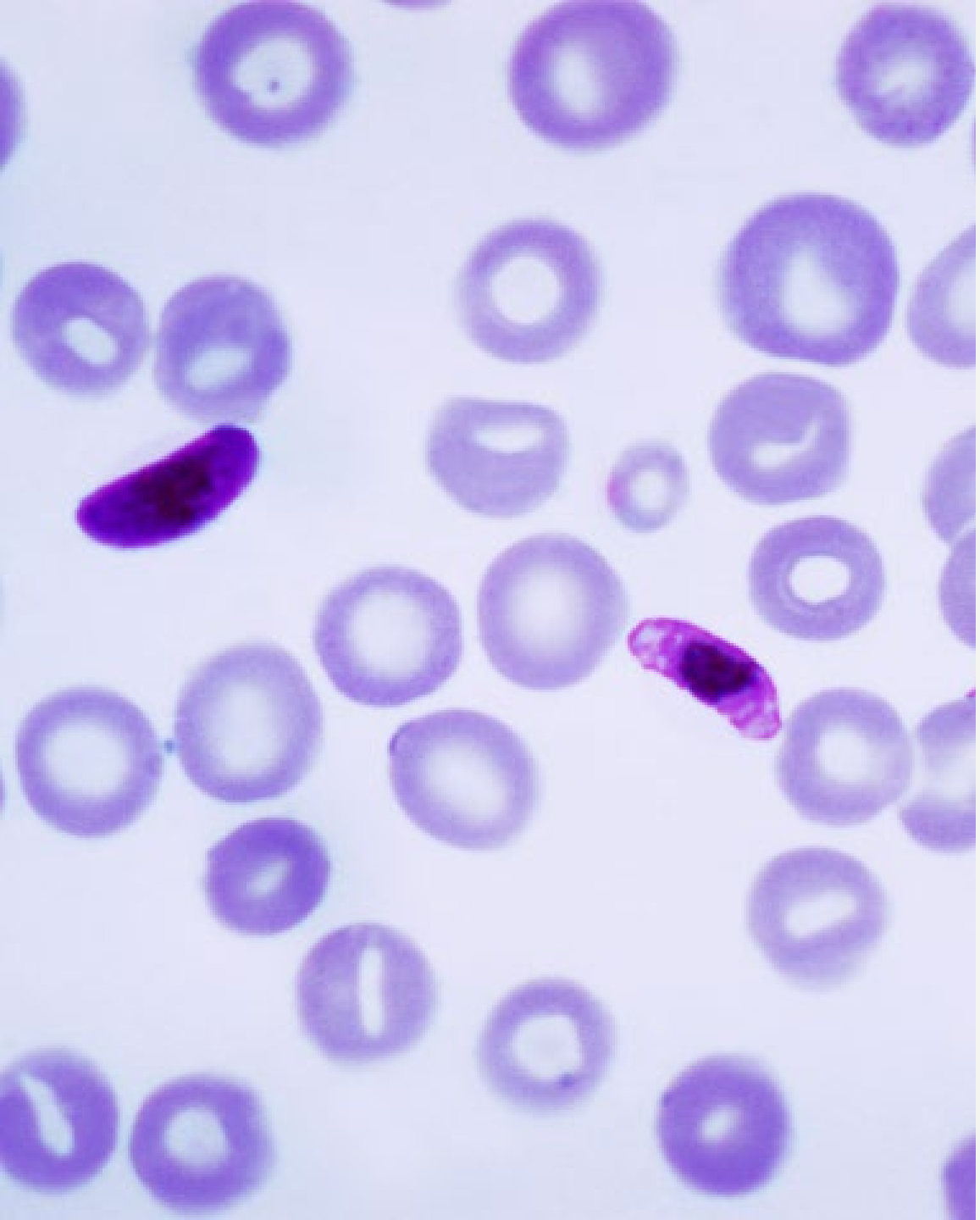


Is antibody testing enough to protect the blood supply from transfusion-transmitted malaria?

Susan A. Galel, MD
Global Medical Affairs Lead, Donor Screening
Roche Diagnostics Solutions

15 January 2025

Disclosures

Dr. Galel is a contract consultant to and shareholder of Roche Diagnostics

Malaria

Transmission

Infection caused by *Plasmodium* parasites

Transmitted to humans by *Anopheles* mosquitoes

Parasites infect red blood cells

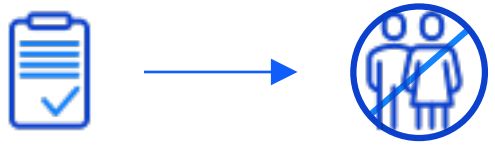
Side effects

- Infection can cause severe acute illness that can be fatal
- 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

Current strategies to prevent transfusion-transmitted malaria (TTM) in non-endemic areas

Donor Management



Questionnaire Donor exclusion

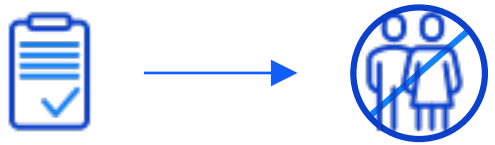
Questionnaire Donor exclusion Test for antibodies

Challenges:

- Imperfect reliability of donor information
- False negative antibody tests
- Exclusion 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 strategies to prevent transfusion-transmitted malaria (TTM) in non-endemic areas

Donor Management



Questionnaire — Donor exclusion

Questionnaire — Donor exclusion — Test for antibodies

Challenges:

- Imperfect reliability of donor information
- False negative antibody tests
- Exclusion 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

Additional screening tools are needed to ensure blood safety and expand donor diversity

Current diagnostic testing methods: *Plasmodium* detection

Microscopy, antigen, or nucleic acid

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

cobas® Malaria PCR assay
Licensed by the US FDA for donor screening, Class D CE certification under IVDR

Target

Ribosomal RNA (rRNA) and DNA

Detect

Detection to include the 5 main species known to infect humans:

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

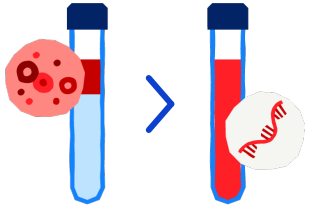
Identify

Plasmodium parasites are inside RBCs: sample type is whole blood, not plasma

Use Roche Whole Blood Collection Tube (also used for the cobas® Babesia test)

cobas® Malaria and **cobas®** 5800/6800/8800 Systems are not commercially available in all countries

cobas® Malaria workflow



Whole blood collection	Lysis of red blood cells	Fully automated sample preparation, NAT amplification/ detection/analysis
Approximately 1.1mL of whole blood is collected into tubes containing lysis buffer and preservatives	The red blood cells and any parasites are lysed and the nucleic acid is stabilized	The tube is placed on the cobas® 5800/6800/8800 Systems and tested using ready to use malaria-specific cobas® reagents

cobas® Malaria and **cobas®** 5800/6800/8800 Systems are not available in all countries

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

95% probability of detection by PROBIT:

2.9 iRBC/mL (95% CI 2.4–3.8 iRBC/mL)

This LOD is the same as the concentration needed to have a 95% probability of capturing one iRBC in the test sample, based on Poisson distribution

This confirms the prediction that if one iRBC is captured it would be detected

Galel et al, Vox Sanguinis 2024;119(Suppl. 1):344-5.

cobas® Malaria

Clinical specificity

Whole blood samples from volunteer donors in the US were collected in the Roche Whole Blood Collection tube. Lysates were tested individually or in pools of 6 lysates.

Results:

20,187 donations were tested by individual sample testing	No reactive donations	Specificity 100% in IDT (95% CI 99.982% to 100%)
67,612 donations were tested in pools of 6 lysates	No reactive pools and no reactive donations	Specificity 100% in pools (95% CI 99.995% 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)

199 samples evaluable

4 samples (2.0%) positive by microscopy and antigen

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

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

Zhu X et al., Vox Sanguinis 2023; 118(Suppl. s1):250

What tests can ensure blood safety in non-endemic areas?

Minimum infectious dose arguments

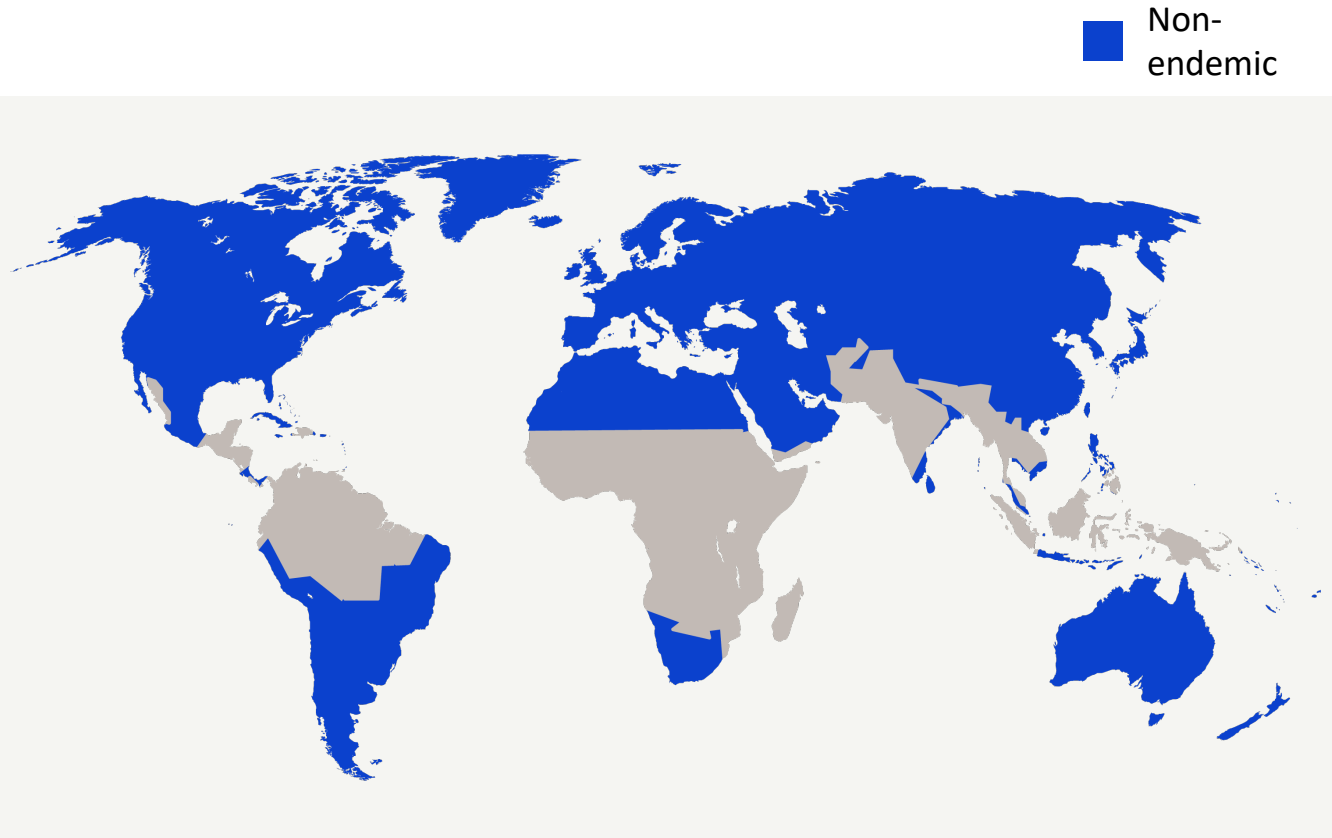
- A 1940's study suggested that *Plasmodium* infection could be transmitted by as few as 10 parasites.
- Some experts have assumed that a donor test would need to detect 10 parasites in a 500-mL unit of blood (i.e., sensitivity of one parasite per 50 mL).
- But: there is no evidence that people with *Plasmodium* infection would have a concentration that low!

More appropriate question:

What is the detectability of asymptomatic *Plasmodium* infections that occur in non-endemic areas?

- Are these infections detectable by molecular tests?
- How reliable are the antibody tests to detect these infections?

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



Asymptomatic *Plasmodium* infections are rarely identified in Europe 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

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

*Galel SA, Transfusion 2024; 64:2325-2331

Donors implicated in TTM: PCR results

Cases in US and Canada

- DNA-based PCRs
- Sensitivity 3,000–6,000 parasites/mL

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 travel 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 1.5 yr prior to donation; malaria- like sxs 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				

Donors implicated in TTM: PCR results

Cases in US and Canada

- DNA-based PCRs
- Sensitivity 3,000–6,000 parasites/mL

Case #	Country, year, species	Donor risk	Fresh f/u sample	Retained sample from index donation: Blood segment	Retained sample from index donation: Plasma	Retained sample from index donation: 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 travel 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 1.5 yr prior to donation; malaria- like sxs 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	+			

+ Positive – Negative

*Case 4: Retained segment had positive nested PCR, borderline PET-PCR ; f/u sample showed reactivity with late Ct past cutoff;. **Cases 5 and 7: Blood segments had been stored multiple weeks in the refrigerator

Donors implicated in TTM: PCR results

Cases in Europe

- DNA-based PCRs
- 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 4 yr prior to donation?)				
10	France, 2012, Pf	Former resident of Benin, 12 yr after departure				
11	France, 2015, Pm	Former resident of Comoro Islands, more than 3 yr after departure				
12	Italy, 2019, Pm	Missionary, more than 10 yr after departure from endemic areas				
13	Austria, 2019, Pf	Donor traveled to Uganda 2 wk prior to donation, became febrile 1 wk after donation and was diagnosed with malaria†				

†Case 13: test results not reported

Donors implicated in TTM: PCR results

Cases in Europe

- DNA-based PCRs
- 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 4 yr prior to donation?)	+		–	
10	France, 2012, Pf	Former resident of Benin, 12 yr after departure	+		+	
11	France, 2015, Pm	Former resident of Comoro Islands, more than 3 yr after departure	+		–	
12	Italy, 2019, Pm	Missionary, more than 10 yr after departure from endemic areas	+			
13	Austria, 2019, Pf	Donor traveled to Uganda 2 wk prior to donation, became febrile 1 wk after donation and was diagnosed with malaria†				

†Case 13: test results not reported

+ Positive – Negative

Donors implicated in TTM: DNA-based PCR results

Summary

The DNA-based 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 DNA-based PCR assays used for these cases

Abbrvs: TTM, transfusion-transmitted malaria

Donors implicated in TTM: *Antibody* results

EIA was negative in 4 of 7 donors tested

Case #	Country, year, species	Donor risk	Index or F/U sample	EIA	IFA
1	US, 2010, Pf	Former resident of Benin, 4 yr after departure	F/U	+	+
2	US, 2011, Pm	Former resident of Liberia, 15 yr after departure	F/U	—	+
3	US, 2016, Pf	Former resident of Democratic Rep of Congo, multiple travel back to Africa most recently 16 mo prior to donation	Index		+
4	US, 2017, Pf	Former resident of Togo, 2.8 yr after departure	Index and F/U		+
5	US, 2017, Po	Former resident of Cameroon, 2 yr after departure	Index		+
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	F/U	+	
7	US, 2020, Pf	Former resident of Nigeria, 4 yr after departure	Index	+	
8	Canada, 2022, Pf	Former resident of W. Africa, 12 yr after departure	F/U		+
9	Netherlands, 2011, Pm	Travel (more than 4 yr prior to donation?)	Index and F/U	—	+
10	France, 2012, Pf	Former resident of Benin, 12 yr after departure	Index and F/U	—	+
11	France, 2015, Pm	Former resident of Comoro Islands, more than 3 yr after departure	Index	—	
			F/U	Borderline	+

Donors implicated in TTM: *Antibody* results

EIA was negative in 4 of 7 donors tested

Case #	Country, year, species	Donor risk	Index or F/U sample	EIA	IFA
1	US, 2010, Pf	Former resident of Benin, 4 yr after departure	F/U	+	+
2	US, 2011, Pm	Former resident of Liberia, 15 yr after departure	EIA assay not stated	—	+
3	US, 2016, Pf	Former resident of Democratic Rep of Congo, multiple travel back to Africa most recently 16 mo prior to donation	Index		+
4	US, 2017, Pf	Former resident of Togo, 2.8 yr after departure	Index and F/U		+
5	US, 2017, Po	Former resident of Cameroon, 2 yr after departure	Index		+
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	F/U	+	
7	US, 2020, Pf	Former resident of Nigeria, 4 yr after departure	Index	+	
8	Canada, 2022, Pf	Former resident of W. Africa, 12 yr after departure	F/U		+
9	Netherlands, 2011, Pm	Travel (more than 4 yr prior to donation?)	Lab 21/Captia	—	+
10	France, 2012, Pf	Former resident of Benin, 12 yr after departure	Lab 21/Captia	—	+
11	France, 2015, Pm	Former resident of Comoro Islands, more than 3 yr after departure	Lab 21/Captia F/U	— Borderline	 +

Literature references to “Lab 21”, “Newmarket”, and “Captia” all refer to the same assay.

Unreliable detection by EIAs

These TTM cases indicate that the Lab 21/Captia assay is not perfect.

Other studies clearly demonstrate that variable detection by EIA is not limited to this assay

Evaluation of malaria antibody assays in France, 2017*

108 samples from patients with well-documented malaria

	Pan-malaria antibody CELISA (Cellabs)	Malaria Ab (DIA PRO)	ELISA anti-Plasmodium (EuroImmun)	Novalisa Malaria (Novatec)	Captia Malaria total antibody test (Trinity Biotech)	IFAT (malaria reference centre)
Sensitivity	50.00%	84.2%	71.1%	63.2%	71.1%	64.8%

All assays show incomplete sensitivity:
Sensitivity range 50-84%

* Data presented by Sophie Le Cam, EFS, at AABB October 2019

Evaluation of malaria antibody assays in Italy*

64 IFAT+ samples from patients with malaria or malaria history

	BioRad	DiaPro	Euroimmun	Novatec	DRG
Sensitivity	53.6%	64.2%	56.6%	54.5%	55.6%

Poor sensitivity and poor agreement between assays

* Mangano VD et al., Malaria Journal 2019; 18:17

UK: Results of other antibody tests on 14 DNA positive donations*

Donations initially detected by Lab21 antibody screen

Sample ID	Initial screen S/CO	Confirmatory serology assay S/CO			IFAT titer	Species
		DiaPro	Cellabs	Diamed		
009839	7.46	6.76/6.90	16.54/17.05	5·21/4·20	1/640	Pf
100255	7.51	**0.69/0.62**	19.09/18.79	11·39/13·26	1/640	Pf
208922	26.61	1.49/1.83	13.94/14.4	NT	1/640	Pf
204137	86.12	**0.44/0.48**	25.97/25.83	7·79/8·27	1/640	Pf/Pm
211908	7.14	**0.746/1.0**	13.71/13·80	NT	1/640	Pm
216512	2.75	**0.36/0.34**	9.89/8.41	NT	1/320	Pm
103461	1.82	2.39/2.48	**0.83/0.92**	4·07/3·55	1/80	Po
114294	19.36	**0.37/0.38**	9.12/9.64	2·77/3·07	1/160	Po
209306	4.65	**0.39/0.43**	1.88/2.04	NT	**Neg**	Po
102726	94.15	4.73/4.61	23.57/23.57	7·52/6·28	**Neg**	Pv
105435	96.89	2.30/2.65	12.37/12·84	4·00/4·46	**Neg**	Pv
205176	76.44	5.64/5.77	6.45/8.03	4·70/4·69	**Neg**	Pv
302327	99.79	5.05/5.95	9.11/9.32	NT	**Neg**	Pv
312209	76.797	10.5/10.5	5.418/5.976	NT	**Neg**	Pv

Note: All of these DNA+ samples tested negative for malaria antigen

(Binax-NOW and Cellabs Malaria Ag EIA)

* Kitchen AD et al, Vox Sanguinis 2014; 107:123-131

UK: Results of other antibody tests on 14 DNA positive donations*

Donations initially detected by Lab21 antibody screen

Sample ID	Initial screen S/CO	Confirmatory serology assay S/CO			IFAT titer	Species
		DiaPro	Cellabs	Diamed		
009839	7.46	6.76/6.90	16.54/17.05	5·21/4·20	1/640	Pf
100255	7.51	**0.69/0.62**	1	3·26	1/640	Pf
208922	26.61	1.49/1.83	1		1/640	Pf
204137	86.12	**0.44/0.48**		7	1/640	Pf/Pm
211908	7.14	**0.746/1.0**			1/640	Pm
216512	2.75	**0.36/0.34**			1/320	Pm
103461	1.82	2.39/2.48		5	1/80	Po
114294	19.36	**0.37/0.38**		07	1/160	Po
209306	4.65	**0.39/0.43**			**Neg**	Po
102726	94.15	4.73/4.61		8	**Neg**	Pv
105435	96.89	2.30/2.65		6	**Neg**	Pv
205176	76.44	5.64/5.77		9	**Neg**	Pv
302327	99.79	5.05/5.95	9		**Neg**	Pv
312209	76.797	10.5/10.5	5.418/5.976	NT	**Neg**	Pv

6 of 14 DNA positive samples were non-reactive on DiaPro

(Pf, Pm, Po)

Note: All of these DNA+ samples tested negative for malaria antigen

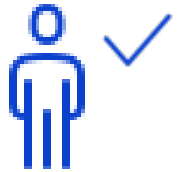
(Binax-NOW and Cellabs Malaria Ag EIA)

* Kitchen AD et al, Vox Sanguinis 2014; 107:123-131

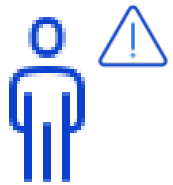
Plasmodium DNA positive donations in Germany, antibody results

A DNA-based NAT performed in IDT has been used at the DRK in Hagen to screen donors with malaria risk after a 4 year deferral.

Two DNA positive donors were recently reported.*



Donor 1: immigrated from Nigeria 5 yr prior to donation. Euroimmun EIA positive.



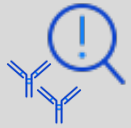
Donor 2: immigrated from Syria via Iraq 6 yr prior to donation. Euroimmun EIA negative.

* Pichl L. et al., Transfus Med Hemother 2024; 51:119–121

Why are the antibody EIAs failing?

EIAs

- May not contain antigens of all 5 species
- Contain selected antigens



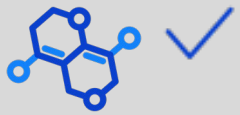
Potential causes of antibody EIA failure

- Donor may be infected with a species not represented in the assay
- Individual variation in antibody production to specific antigens:
  - Antibody responses to specific *Plasmodium* antigens have been shown to vary between individuals and within one individual over time*
- Immune suppression/tolerance associated with recurrent or chronic infection may alter antibody production

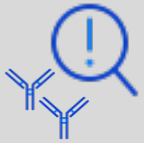
* White MT et al., J Infect Dis 2014;210(7):1115–22.

Testing for *Plasmodium* infection

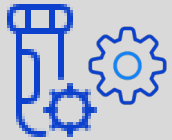
Summary



DNA-based molecular tests that were much less sensitive than the CE marked donor screening rRNA test were able to detect infection in the donors who caused transfusion-transmitted malaria in non-endemic countries



Commercial EIA tests for the detection of *Plasmodium* antibodies are not reliable



Donor screening with a highly sensitive, automated 5-species rRNA NAT can improve detection of asymptomatic *Plasmodium* infections and more safely enable expansion of RBC supply and diversity

Doing now what patients need next

Highly Sensitive Nucleic Acid Test for Detection of Plasmodium RNA

A potential tool to increase blood safety and availability

LINNEN, JM[1], Tonnetti, L[2], Groves, JA[2], Yadav, MC[1], Self, D[1], Livezey, K[1], Tayou Tagny, C[3], Stramer, SL[4]

[1] Grifols Diagnostic Solutions Inc., San Diego, CA USA; [2] American Red Cross, Rockville, MD USA; [3] University of Yaounde, Yaounde, Cameroon; [4] Infectious Disease Consultant, North Potomac, MD USA

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FR-PLMD-2400001

Disclaimers

- Procleix Plasmodium Assay is CE marked (not approved in the US)
- Procleix Babesia Assay is US licensed, and CE marked
- Procleix and Bloodstream are trademarks of Grifols Worldwide Operations Limited
- Panther is a trademark of Hologic, Inc.

For CE marked products, product registration and availability vary by country

Procleix Plasmodium Assay on Panther System

CE-marked nucleic acid test (NAT) for red blood cell parasite that causes malaria

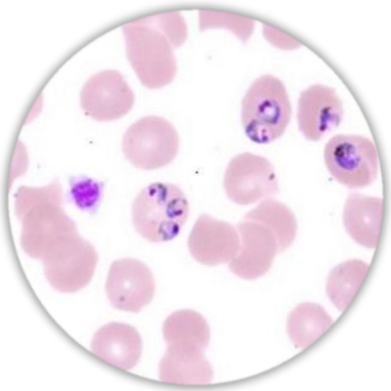
Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Vector-Borne Diseases (DVBD)

Malaria is caused by protozoa of the genus *Plasmodium* and transmitted by *Anopheles* mosquitos

- *Plasmodium* also transmitted from mother to fetus and from blood products from infected donors

To reduce transfusion-transmitted malaria (TTM) risk many countries defer at-risk individuals, negatively impacting blood availability

- Selective or universal donation screening with a sensitive *Plasmodium* nucleic acid test (NAT) could reduce the number of deferrals



Procleix Plasmodium Assay on Procleix Panther system:

- Qualitatively detects at least 5 species of *Plasmodium* **18S ribosomal RNA*** (*P. falciparum*, *P. ovale*, *P. vivax*, *P. malariae*, *P. knowlesi*) in human whole blood specimen
- Intended for screening blood donations in individual whole blood lysates and in lysate pools of up to 16, similar to FDA licensed and CE-marked Procleix Babesia Assay

* ~ 10,000 copies of 18S rRNA per 12-hr ring-stage parasite in cultured Plasmodium falciparum 3D7: Murphy SC, et al. Am J Trop Med Hyg. 2012 Mar;86(3):383-94

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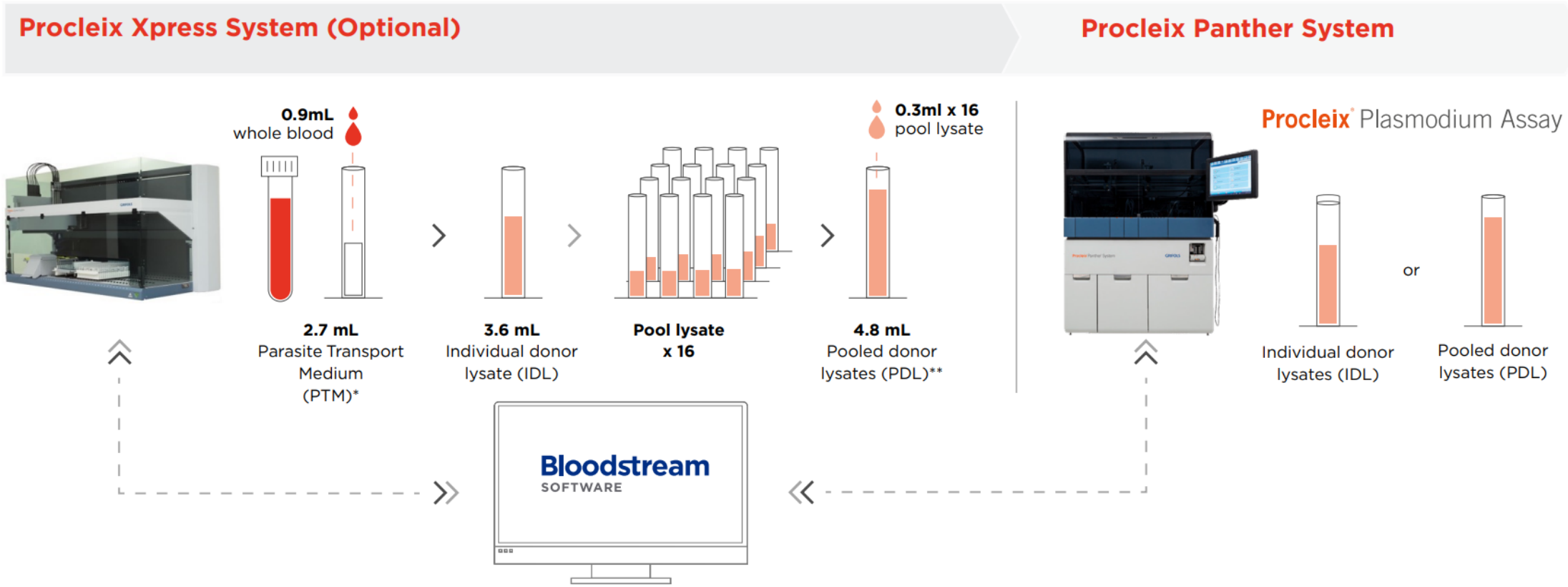
Workflow for the Procleix Plasmodium Assay (CE marked)

Identical to that used for licensed Procleix Babesia Assay screening in US



*PTM is pre-filled on the Procleix Xpress System
**Option for lysate pools of 8

BLOODSTREAM SOFTWARE

- Complete sample traceability
- Deconvolution of reactive PDLs by testing constituent IDLs

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Analytical Sensitivity of Procleix Plasmodium Assay

Limit of Detection (LOD) determined for both RNA and infected red blood cells

- Serially diluted *in vitro* transcripts corresponding to 18S ribosomal RNA of *P. falciparum*, *P. ovale*, *P. malariae*, *P. vivax*, and *P. knowlesi*
- Infected RBCs (*P. falciparum*, *P. ovale*, *P. malariae*, *P. vivax*, and *P. knowlesi*) grown in culture or from clinical specimens were serially diluted in human whole blood, prior to lysis in PTM
- 95% detection probabilities were determined by probit analysis (3 lots; 60 replicates at each concentration)

***Plasmodium* Species**	**95% LOD Estimates, RNA Copies/mL (Fiducial Limits)**	**95% LOD Estimates, Infected RBCs/mL (Fiducial Limits)**
P. vivax	11.89 (9.04– 17.74)	2.85 (1.66–16.75)
P. ovale	11.16 (8.15–18.01)	6.82 (5.63–8.75)
P. malariae	8.47 (6.80–11.45)	2.39 (1.85–3.59)
P. knowlesi	9.08 (7.21–12.58)	2.10 (1.72–2.87)
P. falciparum	11.37 (8.88–16.19)	3.50 (2.85–4.62)

Data source: Procleix Plasmodium Assay IFU, GDSS-IFU-000069-EN v. 3.0

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Clinical Sensitivity & Specificity

Individual and pooled lysate formats evaluated

- **Clinical Specificity**: Fresh whole blood specimens from voluntary US donors were tested individually and in 16-sample pools at Grifols Diagnostic Solutions Inc. R&D (San Diego, CA) and at American Red Cross (Gaithersburg, MD)

Sample Type	n	% Specificity	95% CI
Individual Donations	12,800	99.99	99.96 - 100
16-Sample Pools	283	100	98.71 - 100

- **Clinical Sensitivity**: 50 unique specimens including *P. falciparum*, *P. ovale*, *P. malariae*, and *P. vivax* naturally infected whole blood specimens and *P. knowlesi* cultured infected RBCs were tested neat and pooled 1:16
  - For each of 2 reagent lots used, individual lysate samples tested in singlet for a total of 2 replicates (2x50); pooled samples tested in triplicate for a total of 6 replicates (6x50)

Sample Type	n	True Positive	False Negative	% Sensitivity	95% CI
Neat	100	100	0	100	96.38 - 100
Diluted (1:16)	300	300	0	100	98.78 -100

P. falciparum, *P. ovale*, *P. malariae*, and *P. vivax*) obtained from the Wadsworth Center (New York State Department of Health); *P. knowlesi* obtained from the University of Georgia (Athens, GA)
Data source: Procleix Plasmodium Assay IFU, GDSS-IFU-000069-EN v. 3.0

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Reproducibility of the Procleix Plasmodium Assay

- Reproducibility was evaluated by testing a panel consisting of *P. falciparum in vitro* RNA transcript panel members (positive and negative) along with whole blood lysates from positive and negative samples
- Panel tested by 3 operators, 3 different reagent lots and 3 Procleix Panther instruments over 3 days

Panel	C/mL	n	Percent Agreement	Analyte S/CO Mean	Inter-Operator		Inter-Instrument		Inter-Day		Inter-Lot		Intra-Run	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasmodium High QC Panel Member (100 c/mL)	100	405	100%	12.41	0.09	1%	0.10	1%	0.13	1%	0.08	1%	0.46	4%
Plasmodium Low QC Panel Member (30 c/mL)	30	405	100%	12.25	0.09	1%	0.03	0%	0.10	1%	0.03	0%	0.46	4%
Plasmodium Positive Lysate	9.6**	405	100%	12.14	0.17	1%	0.11	1%	0.11	1%	0.08	1%	0.60	5%
Negative QC Panel Member	0	405	100%	0.00	0.00	48%	0.00	61%	0.00	46%	0.00	57%	0.03	639%
Negative Lysate*	0	411	99.8%	0.01	0.00	31%	0.01	102%	0.00	16%	0.00	44%	0.01	232%
Plasmodium Negative Calibrator	0	80	100%	0.00	0.00	93%	0.00	94%	0.05	80%	0.00	74%	0.00	525%
Plasmodium Positive Calibrator	500	81	100%	12.47	0.05	0%	0.05	0%	0.05	0%	0.05	0%	0.34	3%

n = number of reactions, C/mL = copies per milliliter, S/CO = Signal to Cutoff Ratio, SD = Standard Deviation, CV = Coefficient of variation, IC = Internal Control * Data for only nonreactive tests included in the final analysis ** Infected RBCs/mL
Data source: Procleix Plasmodium Assay IFU, GDSS-IFU-000069-EN v. 3.0

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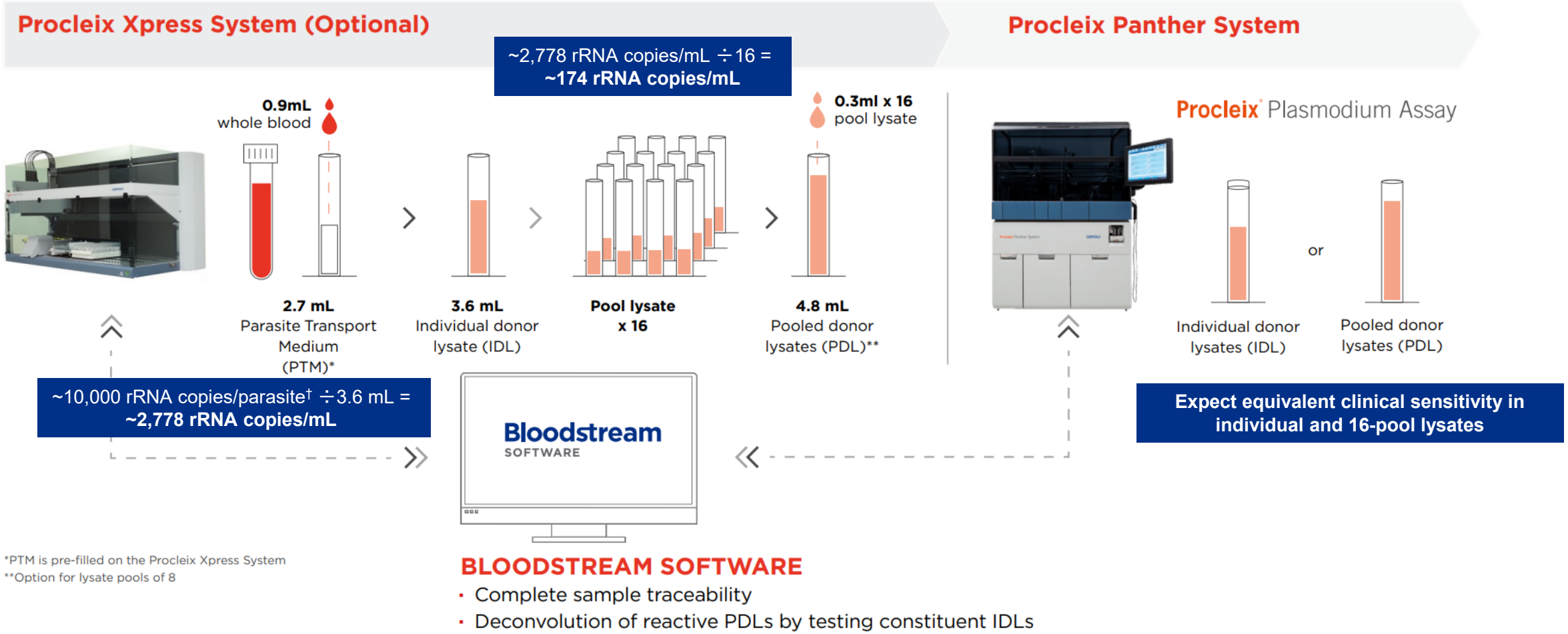
Workflow for the Procleix Plasmodium Assay (CE marked)

Identical to that used for licensed Procleix Babesia Assay screening in US



†18S rRNA per 12-hr ring-stage parasite in cultured *Plasmodium falciparum* 3D7: Murphy SC, et al. Am J Trop Med Hyg. 2012 Mar;86(3):383-94.

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ORIGINAL RESEARCH

TRANSFUSION

A novel mitigation strategy for the prevention of transfusion-transmitted malaria

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- Describes performance characteristics and research study with CE-marked Procleix Plasmodium Assay by the American Red Cross
- **US Deferred donor screening: 862** deferred donor samples (under 3-year deferrals) yielded one confirmed positive (0.12%)*
  - Infected donor was prior resident of malaria endemic area in West Africa; confirmed positive individually and in all pooled lysate testing; remained NAT positive for 13 months, antibody** positive, and antigen† negative

*Current results: 1,030 deferred donors screened, with 1 confirmed positive (~0.10%), as of 22 April 2024
**Captia™ Malaria Total Antibody EIA (Trinity Biotech Plc, Bray, Co Wicklow, Ireland); †BinaxNOW™ Malaria Antigen Test (Abbott Laboratories, Abbott Park, IL, USA)

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Detection of 18S rRNA in Malaria Endemic Countries in Africa*

Procleix Plasmodium assay performance in high-risk asymptomatic blood donors

- In collaboration with Prof. Claude Tayou Tagny, Africa Society for Blood Transfusion (AfSBT); samples collected in **Cameroon, Madagascar, and Mali**
- ~250 whole blood samples collected from **routine, asymptomatic donors** at each site with paired serum or plasma aliquots (not all samples were suitable for testing)
- Specimens shipped to American Red Cross (ARC) Gaithersburg, MD USA and **tested as individual whole blood lysates with Procleix Plasmodium Assay**
  - When available, matching plasma samples were tested for *Plasmodium* antibodies by enzyme immunoassay (EIA)

*Tonnetti L, Groves JA, Self D, Yadav MC, Tayou Tagny C, Rakoto Alson OA, Livezey K, Linnen JM, Stramer SL. Estimated Plasmodium 18S ribosomal RNA prevalence in asymptomatic blood donors from three African countries. Vox Sang. 2024 Oct 30.

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Routine Asymptomatic Blood Donor Samples from Africa*

Screened with Procleix Plasmodium Assay

Country	# Whole Blood Specimens Tested*	TMA RR / Total Tested (%)	# Matched Plasma Samples	EIA Positive / Matched Plasma (%)	TMA RR / EIA Positive	TMA RR / EIA Negative (%)
Cameroon	223	91/223 (41%)	131	113/131 (86%)	44/113 (39%)	5/18 (28%)
Madagascar	249	3/249 (1%)	248	68/248 (27%)	3/68 (4%)	0/180 (0%)
Mali	216	26/216 (12%)	17	10/17 (59%)	0/10 (0)	0/7 (0%)

TMA: transcription-mediated amplification; RR: repeat reactive; EIA: enzyme immunoassay, Captia™ Malaria Total Antibody EIA (Trinity Biotech, Wicklow, Ireland)
*numbers correspond to samples considered suitable for testing

- Plasmodium NAT repeat reactivity ranged from 41% (91/223 tested) in Cameroon to 12% (26/216) in Mali and 1% (3/249) in Madagascar
- Matched-plasma subgroup EIA reactivity ranged from 86% (113/131 tested) in Cameroon to 59% (10/17) in Mali and 27% (68/248) in Madagascar
- Antibody detection not seen in 28% (5/18) of matching TMA RR samples from Cameroon, indicating a possible limitation of antibody testing (consistent with previous observations**)

*Tonnetti L, Groves JA, Self D, Yadav MC, Tayou Tagny C, Rakoto Alson OA, Livezey K, Linnen JM, Stramer SL. Estimated Plasmodium 18S ribosomal RNA prevalence in asymptomatic blood donors from three African countries. Vox Sang. 2024 Oct 30.
**Galel SA. Laboratory detection of donors implicated in transfusion-transmitted malaria. Transfusion. 2024 Dec;64(12):2325-2331.

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Summary

- Procleix Plasmodium Assay is currently CE-marked
- Detection of 18S ribosomal RNA (rRNA) is a critical aspect of the assay's design and is expected to substantially enhance clinical sensitivity and may allow screening in whole blood lysate pools
- Assay uses same workflow as licensed Procleix Babesia assay, which has proven to play a key role in the successful blood safety intervention for *Babesia** in the US
  - Procleix Babesia Assay (also detects 18S rRNA) is predominantly used with 16-lysate pools
- Procleix Plasmodium Assay has high sensitivity and results here demonstrated high reproducibility
- Assay detected *Plasmodium* 18S rRNA in asymptomatic blood donors from Cameroon, Madagascar and Mali, showing a range of RNA prevalence in these endemic countries
- Highly sensitive *Plasmodium* NAT can play an important role in blood safety and availability

*Eder AF, O'Callaghan S, Kumar S. Reduced Risk of Transfusion-Transmitted Babesiosis With Blood Donor Testing. Clin Infect Dis. 2024 Jan 25;78(1):228-230

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THANK YOU!