Mass General Brigham

Malaria: A Clinical and Diagnostic Discussion

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Center for Disaster Medicine – Region 1 Disaster Health Response System and Regional Emerging Special Pathogens Treatment Center

Malaria:
A clinical and diagnostic discussion

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Moderators & Speakers

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Learning Objectives

1. Identify the clinical presentation of and epidemiologic risk factors for malaria infection
2. Understand the diagnostic tools available to diagnose malaria and best practices for implementation
3. Recognize severe malaria and understand the treatment guidelines for severe disease
4. Describe malaria in non-travelers (locally transmitted malaria)

Case 1

A 44-year-old female presents with fatigue, chills, anorexia, and subjective fevers for 4 to 5 days

Born in Guinea and migrated to U.S. 10 years ago
She traveled to Guinea for one month

- Presents 5 days after return to the U.S.

She denies taking any prophylaxis for malaria

Exam and laboratory values

Physical Exam

BP 131/77 **HR 123** **37.3°C** RR 20 0_2 sat 99% RA

Non focal exam

Test	Result	Normal range
Creatinine	**1.2 mg/dL**	0.5-0.9 mg/dL
WBC	**11.0 x 10^3/ul**	4-10.8 x 10^3/ul
HCT	**29 %**	37-47%
Platelets	**24 x 10^3/ul**	150-450 x10^3/ul

Rings seen on blood smear

Likely diagnosis?

How is malaria diagnosed?

Malaria is a MEDICAL EMERGENCY

Clinical suspicion is key:

- Symptoms can be nonspecific
- Fever in a returning traveler should raise suspicion
  - Obtain a travel history with emphasis on region of travel
  - Obtain time elapsed between travel and return to U.S.

Table 1. Characteristics of Malaria Species Infections

Plasmodium species	Incubation period, d	No. of days between arrival to US and onset of symptoms (%)[a]		Hypnozoite stage	Geographic distribution
		<30 d	≥365 d		
P falciparum	9-14	96.4	0.1	No	Sub-Saharan Africa, South and Southeast Asia, Eastern Mediterranean, Western Pacific, South America
P vivax	12-17 Relapse: 6-12 mo (>2 y in some cases)	59.5	1.4	Yes	Similar to *P falciparum* and also present in the Korean Peninsula
P ovale	16-18 Relapse: 8-45 mo	47.5	7.5	Yes	Sub-Saharan Africa, Southeast Asia, Western Pacific
P malariae	18-40 Persistence for decades	54.8	0	No	South America, Asia, Africa
P knowlesi	9-12	No data	No data	No	Southeast Asia

90% of infections are acquired in sub-Saharan Africa
>95% of travelers will have infection within 1-month of return

70% of infections are acquired in Asia/Latin America
60% of travelers will have infection within 1-month of return

JAMA. 2022;328(5):460-471. doi:10.1001/jama.2022.12366

How is malaria diagnosed?

Laboratory methods:

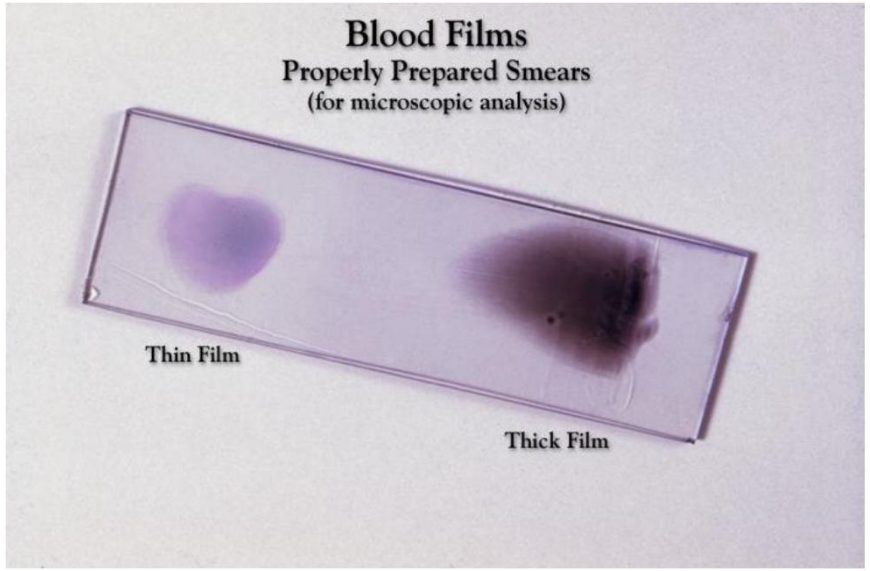
- Blood smear microscopy
- Rapid Malaria Antigen Detection
- Nucleic Acid Detection

Blood smear microscopy

- Direct visualization of blood parasites in blood smear
  - ***Plasmodium* spp. (RBC's)**
  - ***Babesia* spp. (RBC's)***
  - *Trypanosoma* spp. (outside of RBC's)
  - Some filaria (outside of RBC's)
  - *Leishmania* (monocytes)

* Cases predominantly seen in the northeastern and midwestern U.S.

Blood smear microscopy



https://phil.cdc.gov/Details.aspx?pid=5905

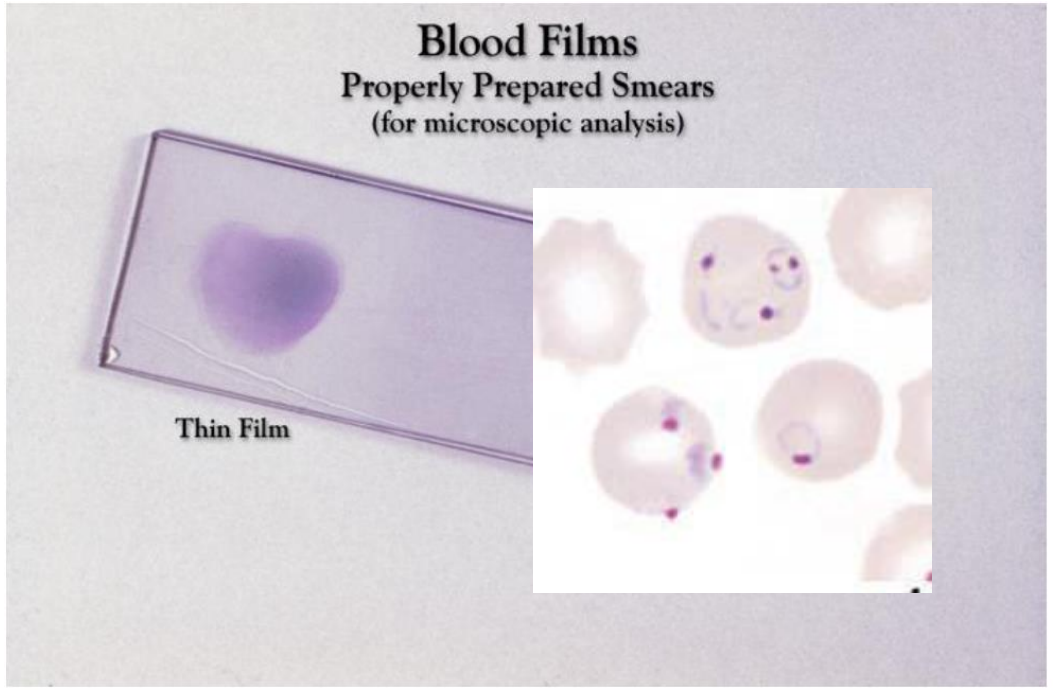
Thick smear

- 1-2 drops of lysed blood
- Air dried (no methanol or heat)
- Giemsa stain

- Advantages:
  - Highly sensitive
  - Detects multiple parasite stages

- Limitations:
  - Suboptimal detection of parasite morphology
  - Suboptimal quantification of parasitemia
  - Interpretation requires expertise

Blood smear microscopy



https://phil.cdc.gov/Details.aspx?pid=5905

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Thin smear

- 1 drop of blood w/feathered edge
- Air dried
- Methanol fixation
- Giemsa stain

- Advantages:
  - Sensitive
  - Determine (most) parasite species
  - Quantify parasitemia

- Limitations:
  - Not as sensitive as thick smear
  - Interpretation requires expertise

Blood smear microscopy: species identification

TABLE 2 Comparative morphology of *Plasmodium* spp. in Giemsa-stained thin films[a]

Diagnostic criterion	*P. falciparum*	*P. malariae*[b]	*P. vivax*	*P. ovale*	*P. knowlesi*
Size and shape of infected erythrocytes	Normal size and shape	Normal or slightly smaller size, normal shape	Normal or enlarged size, may appear molded against neighboring erythrocytes	Normal or enlarged size, frequently oval, may be fimbriated	Normal size and shape
Cytoplasmic inclusions	Occasional Maurer's clefts; larger (comma-shaped) and less numerous than Schüffner's dots	Ziemann's dots rarely seen; requires deliberate over-staining	Schüffner's dots/ stippling; may not be present in early trophozoites	Dark Schüffner's/ James' dots/ stippling; may not be present in early trophozoites	Irregular stippling in late trophozoites and schizonts
Parasite stages in peripheral blood	Early trophozoites and gametocytes	All stages	All stages	All stages	All stages
Multiply infected erythrocytes	Common	Rare	Occasional	Occasional	Common
Early trophozoite characteristics	Delicate rings, <1/3 diameter of the erythrocyte, frequently with double chromatin dots ("headphone" form); often at edge of erythrocyte ("appliqué/accolé form")	Rings ≤1/3 diameter of the erythrocyte; chromatin dot may appear unattached in center of ring ("bird's eye" form)	Rings ≥1/3 diameter of the erythrocyte; larger chromatin dot than *P. falciparum*	Rings ≥1/3 diameter of the erythrocyte; similar to *P. vivax*	Rings ≤1/3 diameter of the erythrocyte; double chromatin dots, rare appliqué forms; resembles *P. falciparum* early trophozoites
Mature trophozoites	Not typically seen in peripheral blood, compact thick rings	Compact cytoplasm, round, oval, basket or band-shaped, dark brown pigment	Amoeboid trophozoites, fine golden-brown pigment	More compact and less amoeboid than *P. vivax*, dark brown pigment	Slightly amoeboid; band forms common; scattered clumps of golden-brown pigment; resembles *P. malariae* mature trophozoites
Schizont characteristics	Not typically seen in peripheral blood, 8–24 merozoites	6–12 merozoites, often radially arranged around central pigment ("rosette" or "daisy head" schizont)	12–24 merozoites	6–14 merozoites	10–16 merozoites
Gametocyte characteristics	Crescent- or banana-shaped; distorting the shape of the erythrocyte	Round to oval; filling most of the erythrocyte	Round to oval; filling most of the erythrocyte	Round to oval; filling most of the erythrocyte	Round to oval; filling most of the erythrocyte

[a]Adapted from references 7, 21, and 31.
[b]Identification of *P. malariae* in patients with recent travel to Southeast Asia should raise the possibility of *P. knowlesi* infection, given the morphologic similarities of these two parasites. In this situation, severe clinical disease and a high parasite burden are consistent with *P. knowlesi* infection.

Challenges:

- Low level parasitemia
- Organisms with similar morphology
  - *P. vivax* vs. *P. ovale* vs. *Babesia*
- Mixed infection

Carroll et al. *Manual of Clinical Microbiology, 13th ed.* 2023. ASM Press.

Blood smear microscopy

Best Uses:

- Routine screening (thick smear)
- Determine infecting species (thin smear)
- Determination of degree of infection (thin smear)
- Monitor response to treatment (thin smear)

Carroll et al. *Manual of Clinical Microbiology, 13th ed.* 2023. ASM Press.

How is malaria diagnosed?

Laboratory methods:

- Blood smear microscopy
- Rapid Malaria Antigen Detection
- Nucleic Acid Detection

Rapid Malaria antigen testing

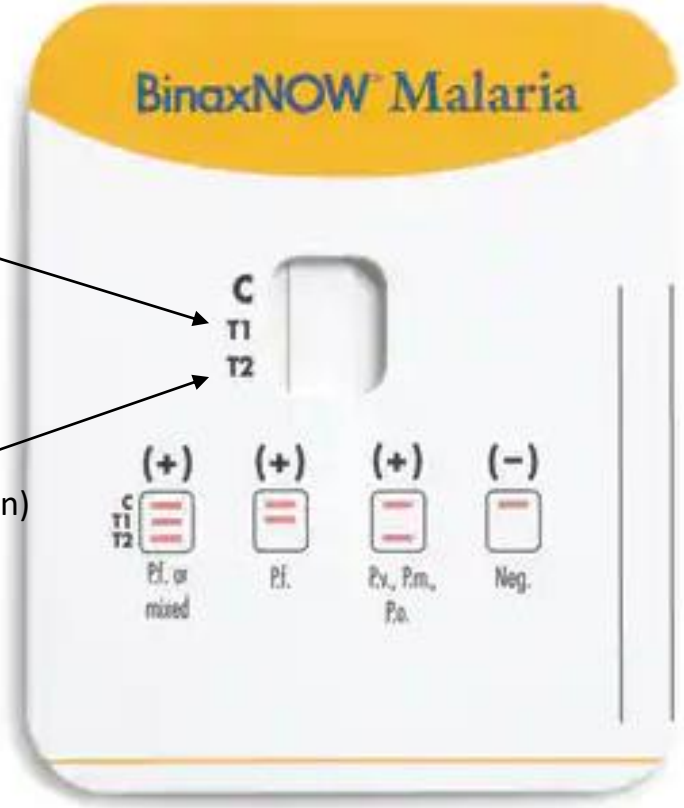
Only 1 FDA approved rapid malaria antigen test: BinaxNow Malaria

T1: HRPII

- Specific for *Plasmodium falciparum*
- Highly sensitive (~0.02% parasitemia)

T2: Aldolase

- Detects any *Plasmodium spp.* (pan malarial antigen)
- Slightly less sensitive (~0.1% parasitemia)



https://www.globalpointofcare.abbott/us/en/product-details/binaxnow-malaria.html

Performance characteristics of BinaxNOW Malaria Assay

Detection of parasitemia level (per µl)	*P falciparum* % Sensitivity	*P falciparum* 95% confidence interval	*P vivax* % Sensitivity	*P vivax* 95% confidence interval
>5,000 (> 0.1%)	99.7	98-100	93.5	91-96
1,000-5,000 (0.02 – 0.1%)	99.2	96-100	81.0	76-85
500-1,000 (0.01 – 0.02%)	92.6	76-99	47.4	36-59
100-500 (0.002 – 0.01%)	89.2	75-97	23.6	17-31
0-100 (0 – 0.002%)	53.9	37-70	6.2	3-12
Overall	95.3	93-97	68.9	66-72
Specificity	94.2	93-95	99.8	99-100

*Thick smear can detect parasite densities as low as 5-10 parasites per µl of blood (0.0001 – 0.0002%)

Adapted from Murray et al. Clin Micro Reviews 2008; 21 (1): 97-108.

Rapid Malaria antigen testing: BinaxNow Malaria

Advantages	Limitations
Rapid and easy to perform	Lower sensitivity at lower parasitemia levels
Requires less expertise for interpretation	Lower sensitivity for non-*P. falciparum* infections
Good sensitivity for *P. falciparum*	False positive results can occur
Relatively inexpensive	False negative results reported with HRP2 deletion strains
Can help differentiate *P. falciparum* from *Babesia* spp.	Can detect residual, nonviable antigen
Provide some idea about mixed infection	Will not detect other blood parasites or differentiate among non-*falciparum* species
	Does not replace blood smear microscopy

Carroll et al. *Manual of Clinical Microbiology, 13th ed.* 2023. ASM Press.

Rapid Malaria antigen testing: BinaxNow Malaria

Best Uses:

- Rapid diagnosis:
  - Overnight when trained microscopists are not available
- Aid in diagnosis in laboratories without expertise in interpreting blood microscopy
- Aid in the diagnosis when smear cannot safely be performed

How is malaria diagnosed?

Laboratory methods:

- Blood smear microscopy
- Rapid Malaria Antigen Detection
- Nucleic Acid Detection

Nucleic acid detection

Direct detection of *Plasmodium* spp. DNA

Often targets the 18srRNA gene

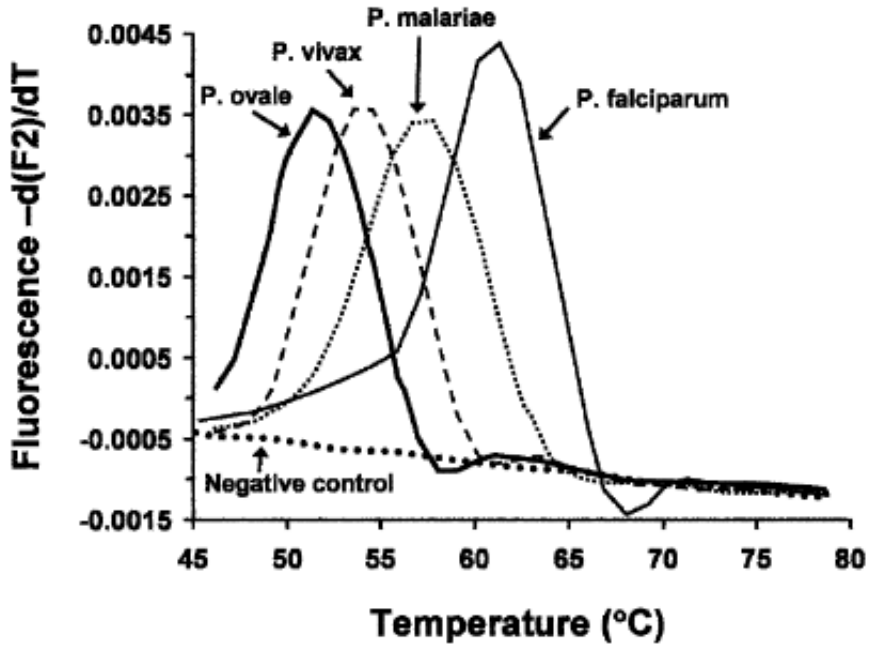
- Allows for species identification and differentiation through melting curve analysis

Performed on whole blood specimens

Highly sensitive and specific

Assays do not have FDA clearance

- Only available at reference laboratories



Am. J. Trop. Med. Hyg., 73(5), 2005, pp. 850–854

Nucleic acid detection

Advantages	Limitations
Objective and reproducible results	No commercially available FDA cleared assay
More sensitive than blood smear microscopy	Prolonged turnaround time (days)
Highly specific-allows for species determination	Can detect residual, nonviable DNA
Improved detection of mixed infections	Will not detect other blood parasites (*Babesia, Trypanosoma*)
	Does not replace blood smear microscopy
	Higher cost compared to other methods

Best Uses:

- Confirmation of suspected infection
- Species identification
  - Mixed infection
  - Low parasitemia

Carroll et al. *Manual of Clinical Microbiology, 13th ed.* 2023. ASM Press.

Malaria diagnostics

Frequently asked questions

If making blood smears, how many? Should I make both thick and thin smears?

Ideally, both thick and thin smears should made

- Thick: Most sensitive screening method
- Thin:
  - Identify species
  - Determine degree of infection
    - % parasitemia should be reported

Standard preparation: 2 thick smears, 2 thin smears

- If thin smear positive, no need to read thick smears
- If thin smears are negative, review thick smear

How many negative smears are needed to “rule out” malaria?

In general:

- If 3 smears collected at 12-hour intervals are negative, another diagnosis should be considered.

JAMA. 2022;328(5):460-471. doi:10.1001/jama.2022.12366

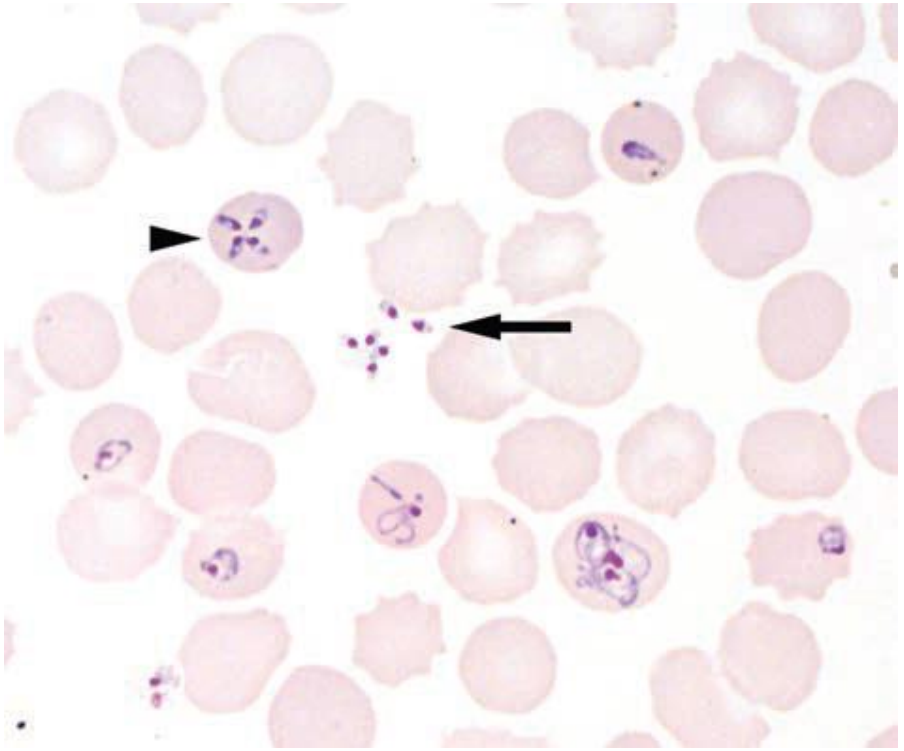
Can I use a rapid malaria antigen test to “rule out” malaria?

No

Due to its reduced sensitivity compared to blood smear microscopy, all negative antigen tests must be confirmed by microscopy

Serial antigen tests 6-12 hours apart can be performed while microscopy results are pending if clinical suspicion persists

Can you differentiate *Babesia* infection from malaria using blood smear microscopy?



Jorgensen et al. *Manual of Clinical Microbiology, 11th ed.* 2015. ASM Press.

Answer: Most of the time

Babesia spp.

- Only ring forms seen
- Extracellular rings more common
- Intraerythrocytic tetrads pathognomonic
- Rings with pleomorphic size and shape

- Travel history helpful

My micro lab cannot make a smear overnight-can I use the hematology smear for review?

Yes, if rapid results are needed

Hematology smears: Wright (Wright-Giemsa) stain

- Some microscopic features will be lost
  - Lose some differentiating ability
  - Should be adequate to determine presence of parasites and rough parasite estimate

Giemsa-stained smear should be performed for confirmation when able

Blood microscopy is not available overnight-what other tests can be performed?

Laboratory could perform a rapid malaria antigen test

Not perfect but can be life saving

Should blood smears be used to monitor response to treatment?

Yes

Blood smears should be performed every 12-24 hours to monitor response to treatment

- Also helps determine duration of treatment or transition to oral therapy in severe cases

Documentation of a negative smear at end of treatment recommended

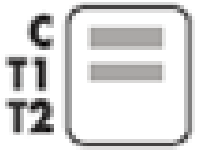
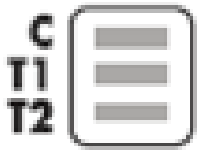
Should rapid malaria antigens be used to monitor response to treatment?

No

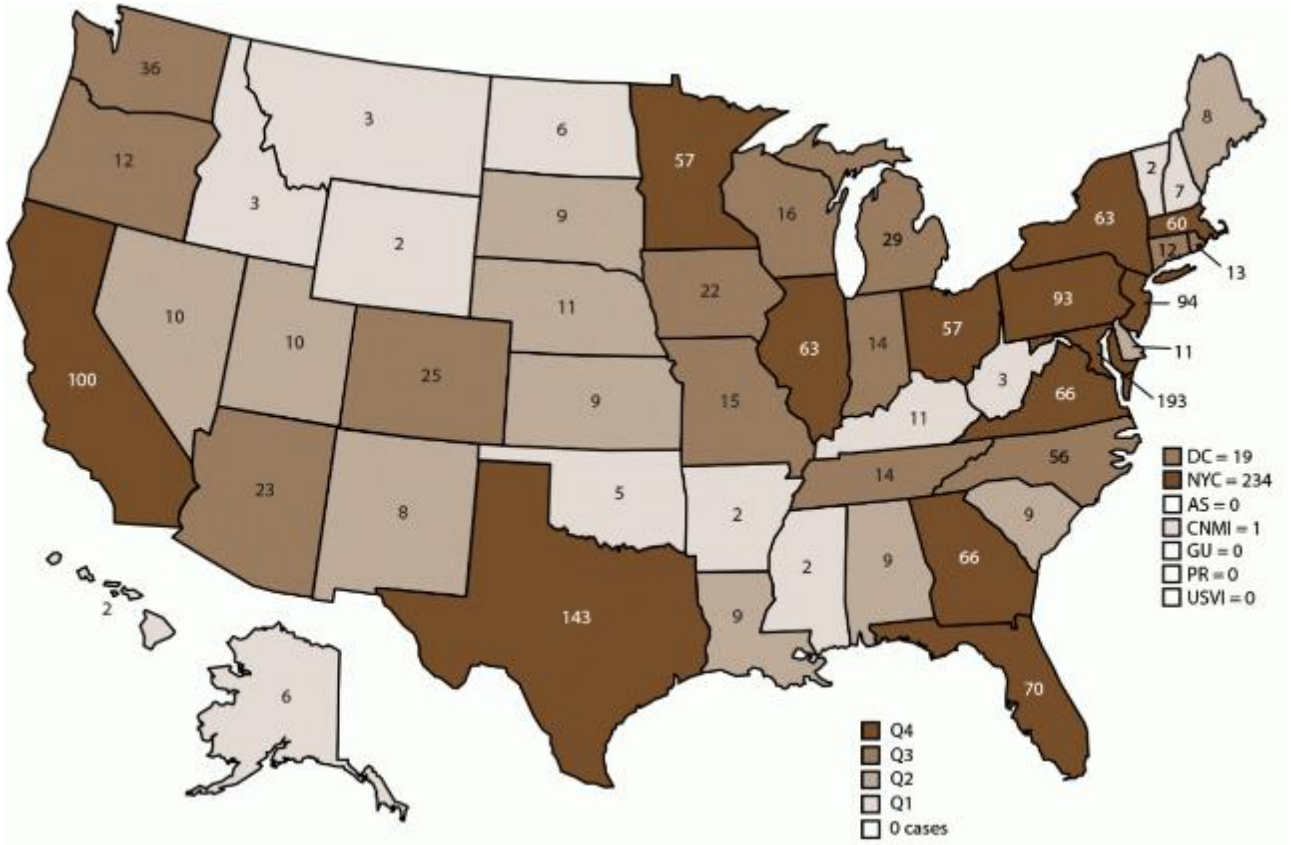
Antigen tests are not quantitative and do not correlate well with degree of parasitemia

Antigen can persist after successful treatment

Her RDT + *P.f.* **and** + 2nd species

TEST	RESULTS	
T1 POSITIVE		Positive for *P. falciparum (P.f)*
T2 POSITIVE		Positive for *P. vivax (P.v), P. ovale (P.o) or P. malariae (P.m)*
T1 + T2 POSITIVE		Positive for *P.f* AND *P. vivax (P.v), P. ovale (P.o) or P. malariae (P.m)*
NO T1 OR T2 LINES		Negative for malaria antigen

Malaria Surveillance - United States, 2018



N=1,823 cases (77% VFR)

1 from bone marrow
1 cryptic
251 severe (7 deaths)

70% *P. falciparum*
95% no chemoprophylaxis

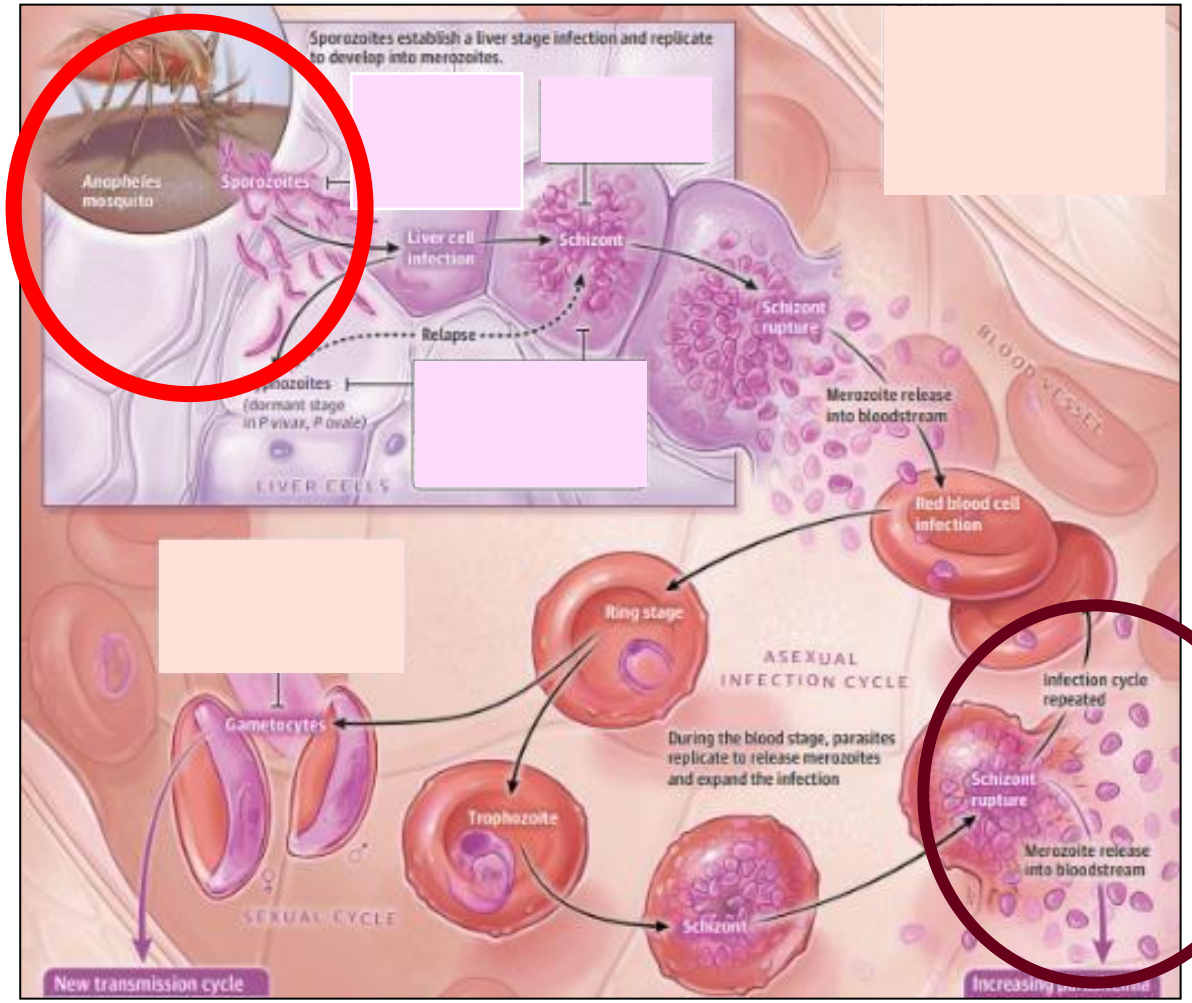
85% acquired in Africa

Mace et al MMWR 2022 71(8);1-29

Malaria life cycle

Vaccines: target the "Bottleneck"

- pre-erythrocytic stage



Clinical symptoms

Daily JP.,*et al.* Review: Diagnosis, Treatment, and Prevention of Malaria in the US JAMA 2022

Always review severe disease criteria

Clinical manifestation:

- Prostration
- Impaired consciousness
- Respiratory distress (acidotic breathing)
- Multiple convulsions
- Circulatory collapse
- Pulmonary oedema (radiological)
- Abnormal bleeding
- Jaundice
- Haemoglobinuria

<92% on room air with a respiratory rate >30/min

Laboratory test:

- Severe anaemia — hgb < 7g/dL
- Hypoglycaemia — glucose <40 mg/dL
- Acidosis — plasma bicarbonate <15 mEq/L
- Renal impairment — creatinine >3 mg/dL
- Hyperlactataemia
- Hyperparasitaemia ≥ 5%

WHO Guidelines for Treatment of Malaria

Treatment for *P.f.*

AL

artemisinin combination therapy (ACT)

- Artemether Lumefantrine (2 tabs po BID)
- Malarone can be used (atovaquone-proguanil)
- Quinine is not available
- NOT Chloroquine (most P.f. is Resistant)

If second species is *P. vivax* or *P. ovale* will need to treat hypnozoite stage

- In New York the NYS Public Health Dept automatically provides species by PCR analysis

Overnight her blood pressure decreases to 70/50

Unable to take PO

Overnight her blood pressure decreases to 70/50

Unable to take PO

Patients can deteriorate on therapy, **continue close observation** to identify development of severe disease

update

Artesunate commercially available

Consider stocking in pharmacy

Artesunate: Drug of choice for severe malaria,

- Quinidine is no longer available.

Some hospitals are sharing ART stocks

- 30K for 3 days
- Consignment program available

CDC will supply it if you cannot get it within 24 hours

--treat with ACT oral awaiting ART

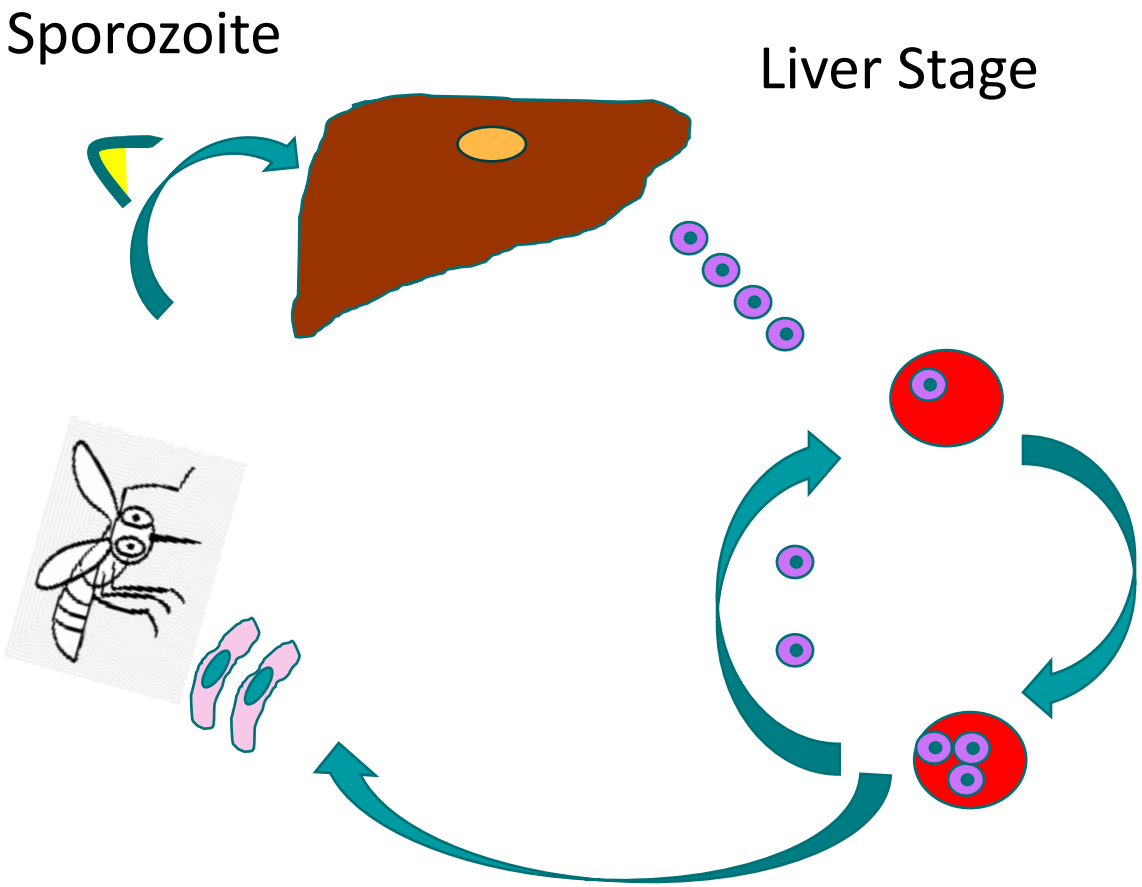
Follow for post artesunate hemolysis

She recovers and is confirmed to have *P. ovale* co-infection

She completes full po AL course after IV ART

How will you manage her?

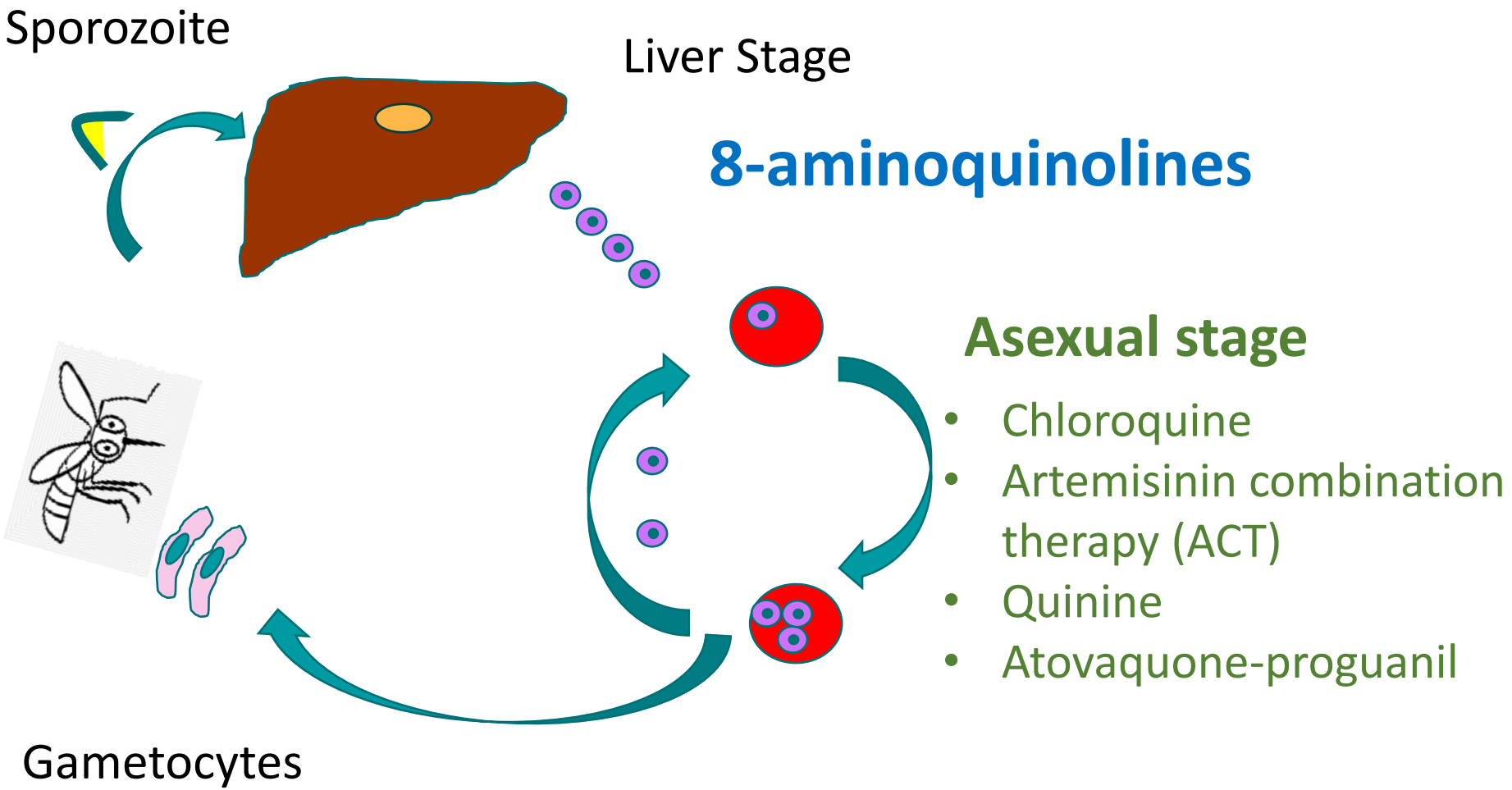
Standard anti-malarials do not kill hypnozoites



Asexual stage

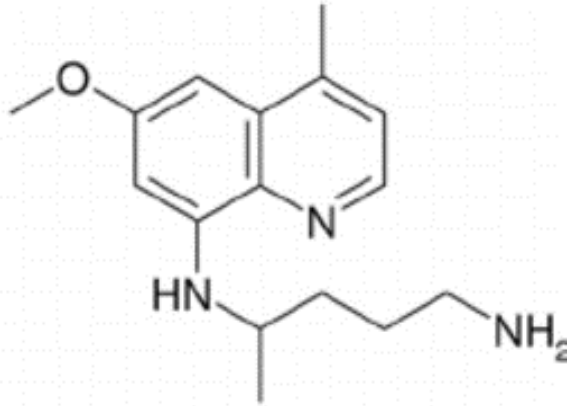
- Chloroquine
- Artemisinin combination therapy (ACT)ie AL
- Quinine
- Atovaquone-proguanil

8 aminoquinolines kill hypnozoites



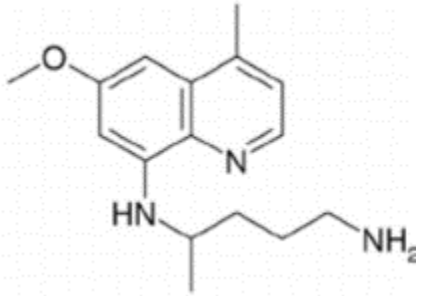
	PRIMAQUINE	TAFENOQUINE
FDA approved	1952	
Indications	• Radical cure • Gametocytes • Prophylaxis	
Contraindicated	• G6PD deficiency • Children • Pregnancy/lactation	
Dosing	QD x 2 weeks	

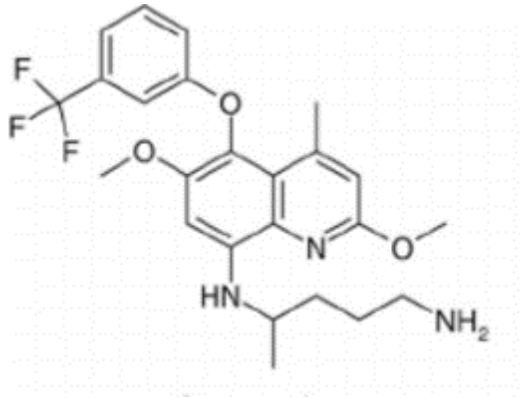
8-aminoquinolines



	PRIMAQUINE	TAFENOQUINE
FDA approved	1952	2018
Indications	• Radical cure • Gametocytes • Prophylaxis	same
Contraindicated	• G6PD deficiency • children • Pregnancy/lactating	same
Dosing	QD x 2 weeks	Single Dose

8-aminoquinolines





The patient recovers completely, was treated with IV artesunate, the **drug of choice for severe malaria of any type**.

Completed a course of CQ, then Tafenoquine (SD) or Primaquine (2 wks) for radical cure

- Was G6PD normal

P. vivax causes severe disease

Increasingly recognized
Evidence for large biomass of intact asexual stage parasites **in spleen** of asymptomatic patients

PLOS MEDICINE 2021

RESEARCH ARTICLE

Evaluation of splenic accumulation and colocalization of immature reticulocytes and *Plasmodium vivax* in asymptomatic malaria: A prospective human splenectomy study

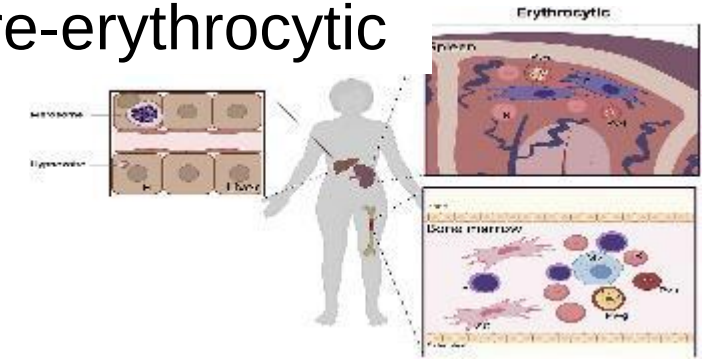
Steven Kho[1], Labibah Qotrunnada[2], Leo Leonardo[3], Benediktus Andries[3], Putu A. I. Wardani[4], Aurelie Fricot[5], Benoit Henry[5], David Hardy[6], Nur I. Margyaningsih[2], Dwi Apriyanti[2], Agatha M. Puspitasari[2], Pak Prayoga[3], Leily Trianty[2], Enny Kenangalem[3,4], Fabrice Chretien[6], Valentine Brousse[5], Innocent Safeukui[7], Hernando A. del Portillo[8,9,10], Carmen Fernandez-Becerra[8,9], Elamaran Meibalan[11,12], Matthias Marti[11,13], Ric N. Price[1,14,15], Tonia Woodberry[1], Papa A. Ndour[5], Bruce M. Russell[16], Tsin W. Yeo[1], Gabriela Minigo[1], Rintis Noviyanti[2], Jeanne R. Poespoprodjo[3,4,17], Nurjati C. Siregar[2,18], Pierre A. Buffet[5*], Nicholas M. Anstey[1*]

Parasitology International 2022

Cryptic erythrocytic infections in *Plasmodium vivax*, another challenge to its elimination

Carmen Fernandez-Becerra [a, b], Iris Aparici-Herraiz [a], Hernando A. del Portillo [a, b, c]

Pre-erythrocytic



erythrocytic

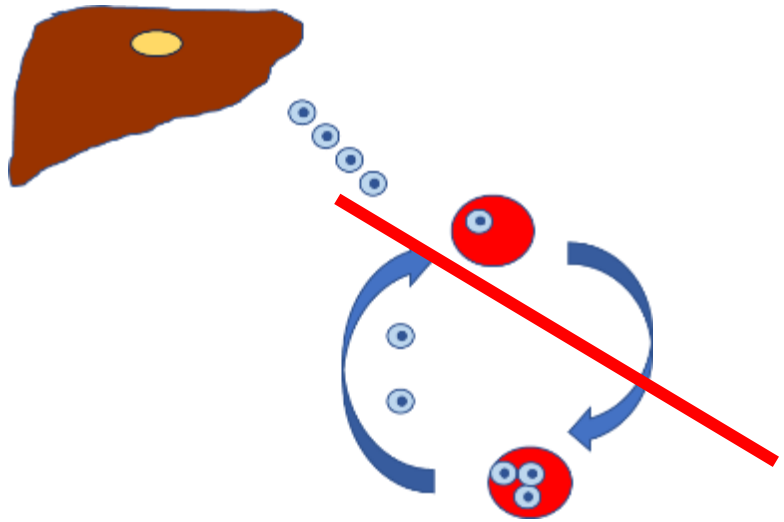
Review itinerary: malaria transmission and drug sensitivity status

Country	Areas with Malaria	Drug Resistance[2]	Malaria Species[3]	Recommended Chemoprophylaxis[4]	Needed and Helpful Links to Assess Need for Prophylaxis for Select Countries
India	All areas throughout country, including cities of Bombay (Mumbai) and New Delhi, except none in areas > 2,000 m (6,562 ft) in Himachal Pradesh, Jammu and Kashmir, and Sikkim.	Chloroquine	*P. vivax* 50%, *P. falciparum* >40%, *P. malariae* and *P. ovale* rare	Atovaquone-proguanil, doxycycline, mefloquine, or tafenoquine[5]	1) City(ies) of travel 2) Altitude of city(ies) of travel 3) Province(s) of travel Altitude information and to determine if city is within a certain province Map of provinces in India

CDC https://www.cdc.gov/malaria/travelers/country_table/i.html

Malaria prophylaxis

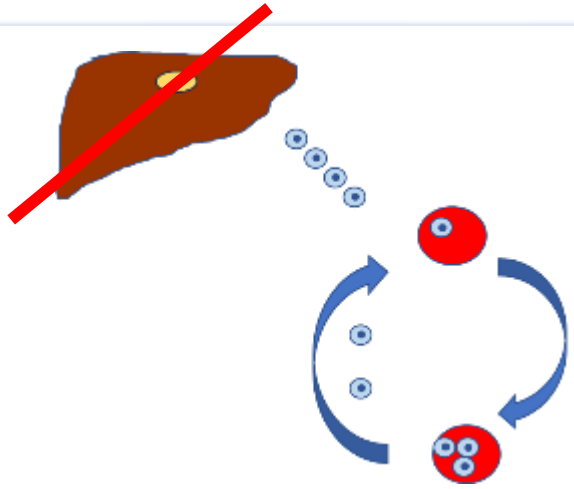
Drug	Pretravel		Travel		Posttravel		Adverse events
	Daily, *n*	Weekly, *n*	Daily	Weekly	Daily, *n*	Weekly, *n*	
Mefloquine		✔ 1–2		✔		✔ 4	Neuropsychiatric
Atovaquone/Proguanil	✔ 1–2		✔		✔ 7		Nausea/vomiting
Doxycycline	✔ 1–2		✔		✔ 30		Oral ulcers, sunburn



Chen V et al. Current Opinion Infect Dis 2019 32:407-412

P vivax endemic regions-tafenoquine is ideal

Drug	Pretravel		Travel		Posttravel		Adverse events
	Daily, *n*	Weekly, *n*	Daily	Weekly	Daily, *n*	Weekly, *n*	
Tafenoquine	✔ 3			✔		✔ 1	G6PD deficiency-associated anemia



For adults, if not G6PD deficient, pregnant, breastfeeding or hx of psychiatric illness

Tafenoquine: Two formulations with two indications

Radical cure

- **Single Dose**

≥ 16 years of age

Prophylaxis

- **load**
- **weekly** during travel
- **one dose** post travel

≥ 18 years of age

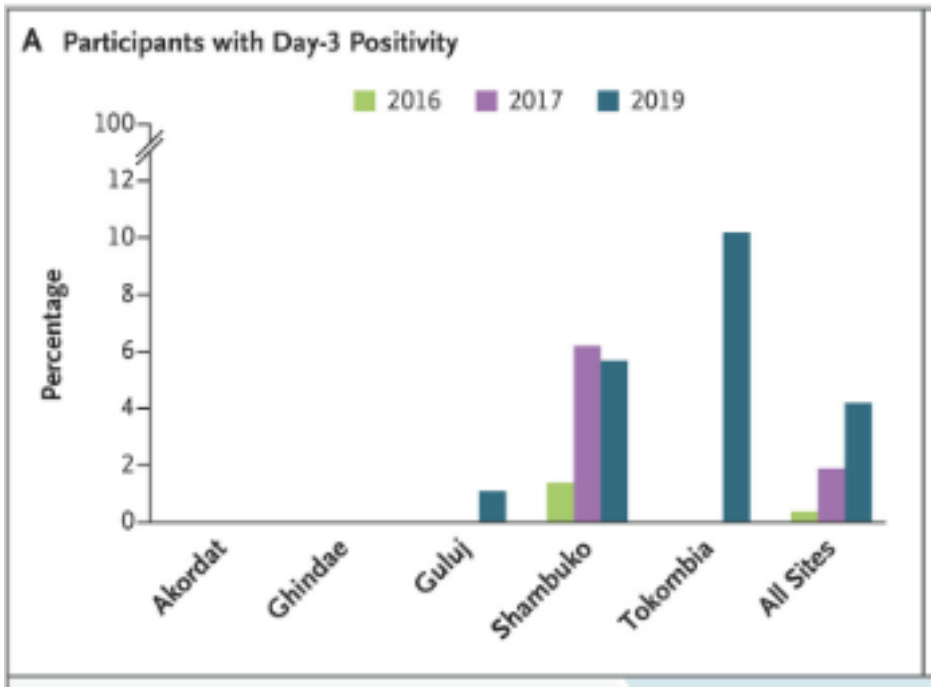
- Both contraindicated in psychiatric illness

update

Emergence of *P.f.* partial resistance to Co-Artem

Eritrea (n=841)

Patients with parasites seen Day 3 of AL



Mihreteab, B et al Sept 28th 2023 NEJM

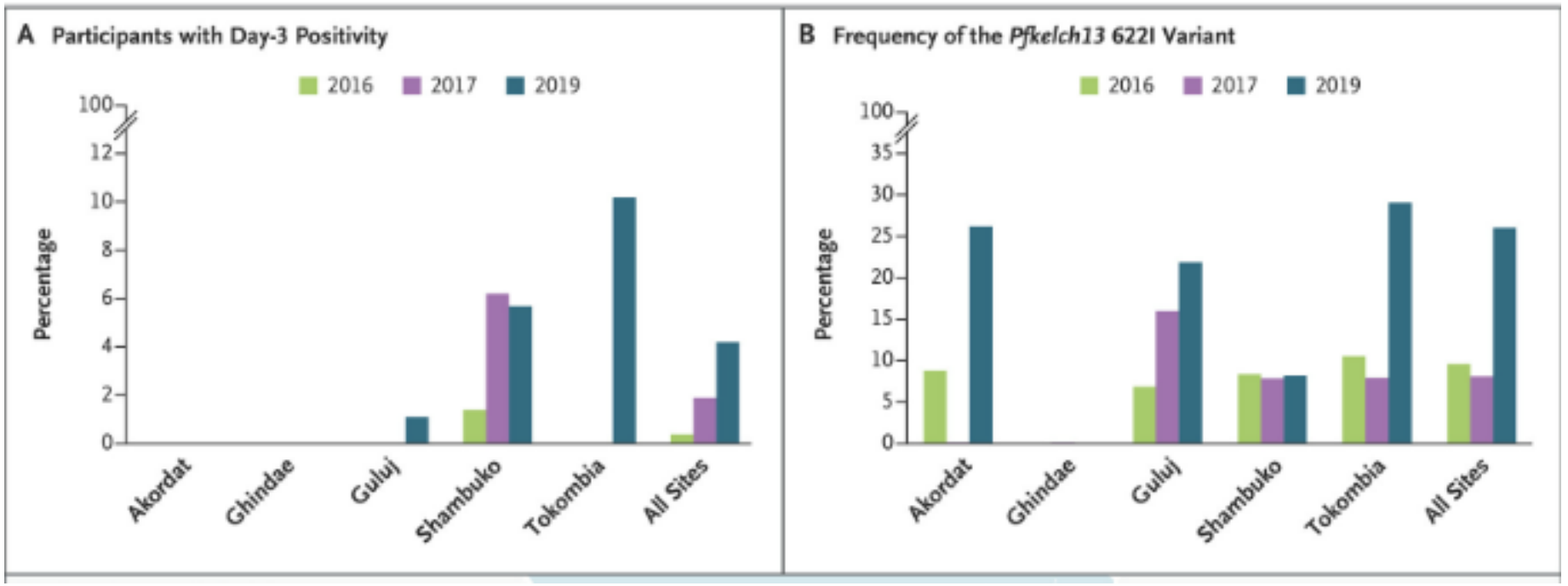
update

Emergence of *P.f.* partial resistance to Co-Artem

Eritrea (n=841)

Day 3 positivity

ART resistant genotypes



Mihreteab, B et al Sept 28th 2023 NEJM

update

Autothonous malaria in the US

Location	Species
Florida	*P vivax n-7*
Texas	*P vivax n-1*
Maryland	*P. falciparum n-1*
Arkansas	*P. vivax n-1*

update

Maryland *P. falciparum* case

August 6, :resident of the Maryland National Capital Region

7-days of fever, malaise, and myalgias.

No international travel.

- patient reported daily walks near home and an occurrence of a tick attachment

LABS: anemia, thrombocytopenia, hyperbilirubinemia, and 3.2

% intraerythrocytic parasites

update

Maryland *P. falciparum* case

August 6, :resident of the Maryland National Capital Region
7-days of fever, malaise, and myalgias.
No international travel.

- patient reported daily walks near home and an occurrence of a tick attachment

LABS: anemia, thrombocytopenia, hyperbilirubinemia, and 3.2
% intraerythrocytic parasites
CDC telediagnosis could not distinguish if rings were babesia or Plasmodium:
treated for Babesia with: atovaquone, azithromycin and doxycycline
(reduction in parasites)
August 15th: Routine MDH identified P.f. species
re treated for malaria with AL

update

When to consider locally transmitted malaria

clinicians should consider a malaria diagnosis in any person with

1) an unexplained cause of fever, regardless of their travel history,

2) particularly in patients with new anemia or thrombocytopenia.

in areas of locally acquired malaria cases follow guidance from your state and local health departments

CDC: 24 hours 7 days a week for ANY questions:

They can also perform **drug resistant genotyping** on your recurrent malaria or if patient slow to clear parasites

Hours: 8am–8pm ET / Monday–Friday (closed on weekends and holidays)

Contact Information: For Healthcare Providers Only

Note: *Healthcare providers needing assistance with diagnosis or management of suspected cases of malaria or other parasitic diseases should call one of the hotlines below. Guidance for diagnosis and treatment of malaria is also available here. The following hotlines are for healthcare providers only; healthcare providers should not direct patients to these hotlines for information.*

Malaria Hotline for Healthcare Providers

Via telephone: 1-770-488-7788 or 1-855-856-4713 (toll free)
Via telephone (after hours): 1-770-488-7100
Via e-mail: malaria@cdc.gov
Hours: 9am–5pm ET / Monday–Friday

Parasitic Diseases Hotline for Healthcare Providers (for all non-malaria parasitic diseases)

Via telephone: 1-404-718-4745
Via telephone (after hours): 1-770-488-7100
Via e-mail: parasites@cdc.gov
Hours: 8am–4pm ET / Monday–Friday

Page last reviewed: October 9, 2019
Content source: Global Health, Division of Parasitic Diseases and Malaria

Questions

Thank you!

REGION 1
Regional Disaster Health Response System

RESPTC
REGION 1 EMERGING SPECIAL PATHOGENS TREATMENT CENTER

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