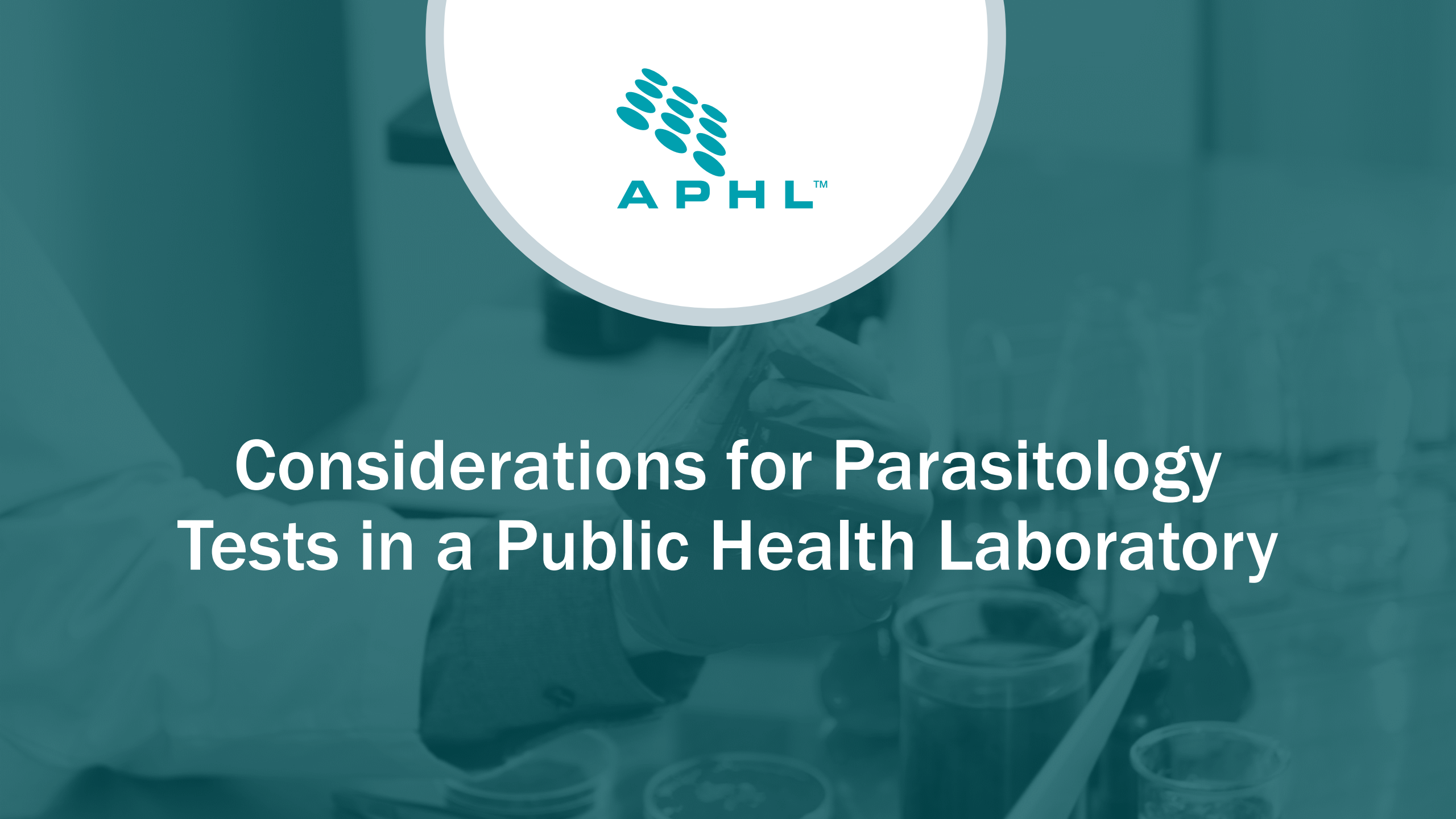


Considerations for Parasitology Tests in a Public Health Laboratory

Overview

- Availability of Testing
- Relevance
- Test Volume
- Turn-around Time
- Resources
- Case Examples

Availability of testing

- Review test directories of clinical laboratories including those in the hospital systems, academic medical centers, large commercial laboratories.

  Online access including submission criteria; TAT; Specimen types accepted

- Consider the quality of the tests available.

  Accreditation: CAP; ISO 17025; CLIA; A2LA

- Consider recommended algorithms for testing and is the test confirmatory.

  Do available Point of Care tests require confirmatory testing; how to rule/out other pathogens

Available tests (CDC): morphology

Test Order CDC-10563

Parasites: Telediagnosis

Images, videos

Preferred starting point if possible!

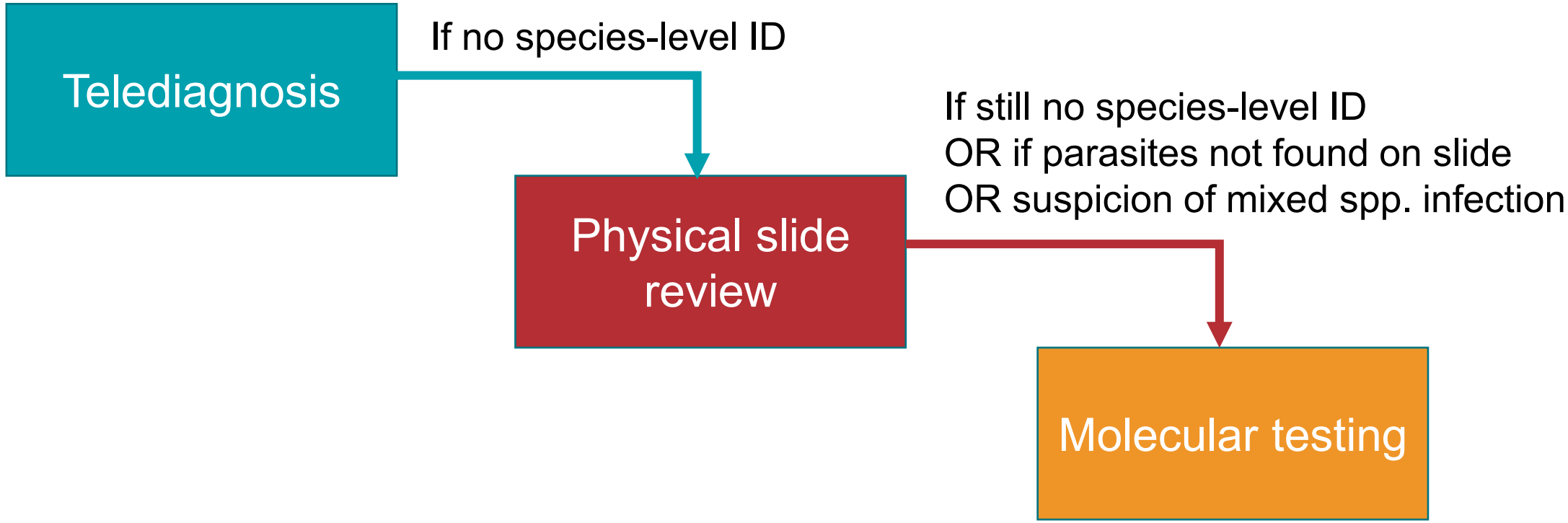
Test Order CDC-10234

Parasites: Morphologic Identification

Appropriate physical specimens

AKA Ova & Parasite “O&P”

Malaria testing algorithm (CDC)



Relevance

- Clinical validity: does the test reliably reflect the patient's clinical status? Can the test be used to drive appropriate patient management such as accurate diagnosis and treatment decisions?

  Patient has cyclic fever and chills with travel hx to malaria endemic area

- Does a test result have implications beyond the individual patient result?

  Reportable disease; potential for outbreak; potential for transfusion/transplant transmission

- Do populations that a laboratory serves have greater numbers of cases of specific diseases due to a regional significance of a parasite or significant travel to/from endemic regions?

  STH (soil-transmitted-helminths) in rural poverty settings; pinworm in children <10

Test Volume

- How often is the test requested (too few/too many requests)?

  Large number of children diagnosed with hookworm in certain rural areas which prompted f/u surveillance testing.

- What is the positivity rate?

  High number of positive test results (e.g. *Cyclospora* or *Cryptosporidium*) can trigger outbreak investigations.

Turn-Around-Time (TAT)

- Are alternative sources of testing faster?

  POC RDTs (Point Of Care Rapid Diagnostic Tests); **Telediagnosis**

- Can results be returned in a timeframe useful to aid in patient management?

  If PCR is the confirmatory test, how long for results? Is culture of the organism required to confirm and/or for PCR?

Resources

- Are specialized methods such as lab developed tests (LDTs), reagents, or highly specialized personnel required?

  FDA “Final Rule” must be considered for LDTs; cost of specialized reagents and/or equipment; disposal stream of used reagents

- Reference laboratories

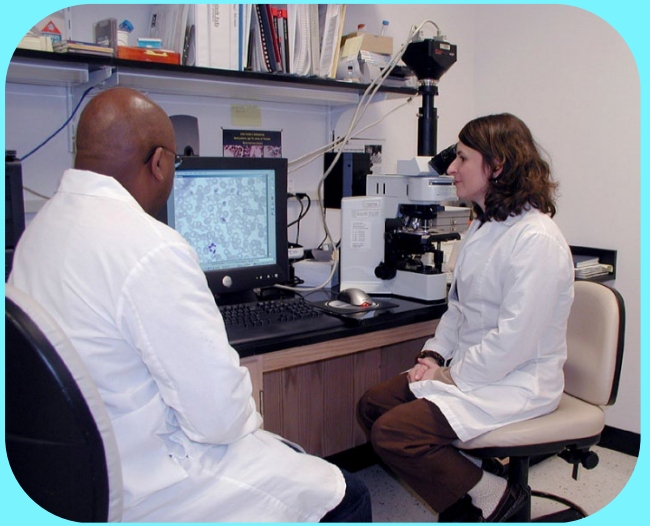
  State Public Health Laboratories; CDC (dpdx@cdc.gob)

Telediagnosis Process

SUBMITTER
(SPHL or other HCP)

1. Request for telediagnosis sent to dpdx@cdc.gov
2. Response with Sharefile upload link, instructions for submission
3. **Transmission of images and 50.34 form via secure Sharefile link**
4. **Email message with dx based on images* (no PII) and recommended follow-up submission or testing**
5. **Official test report generated in ELIMS and forwarded automatically to SPHL (w/in 7 days)**

DPDx TEAM
(Morphology Section)



* Usually within same or next business day!

Specimen Types for Morphological Dx

Slides

- **Blood films, stool smears, touch preparations**: dried and/or fixed, stained or unstained
- **Histopath slides:** H&E; other stains such as PAS, GMS may be helpful in some cases.
- Acceptable up to 1 yr post-collection.
- Ship ambient or refrigerated.

Whole Blood (EDTA)

- NOT preferred for morphology submissions – submit smears if at all possible!
- Must be received **≤3 d post collection** for preparation of acceptable smears
- Ship refrigerated (2-8 C) only

Stools (& other bodily fluids)

- Must be preserved in a fixative appropriate for desired use or stain (e.g. formalin, PVA, TotalFix, etc.)
- Acceptable up to 1 yr post-collection.
- Ship ambient or refrigerated.

Whole Organisms (Gross Specimens)

- Worms, proglottids, arthropods
- Must be preserved in a fixative (e.g. 70% EtOH, 5-10% Formalin, TotalFix)
- Acceptable up to 1 yr post-collection.
- Ship ambient or refrigerated.

Chagas Disease (American Trypanosomiasis)

- Caused by ***Trypanosoma cruzi***
- **Route of transmission:**
  - Vector-borne; introduction of “Kissing Bug” (Reduviidae) feces into bite wounds or mucous membranes
  - Transfusion / transplantation and congenitally- acquired infections also occur
- **Geographic occurrence**: Central and South America; limited transmission within southern United States
- **Typical presentation**:
  - **Acute phase:** non-specific somatic symptoms; may present with peri-orbital swelling, mild hepatosplenomegaly
  - **Chronic phase:** associated with potentially fatal cardiac complications, megaesophagus, megacolon, possible disseminated infection

Int. Assoc. of Medical Assistance to Travelers

Trypanosoma cruzi

DPDx

Triatomine Bug Stages

1. Triatomine bug takes a blood meal
(passes metacyclic trypomastigotes in feces, trypomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)

8. Metacyclic trypomastigotes in hindgut

7. Multiply in midgut

6. Epimastigotes in midgut

5. Triatomine bug takes a blood meal
(trypomastigotes ingested)

Mammalian Stages

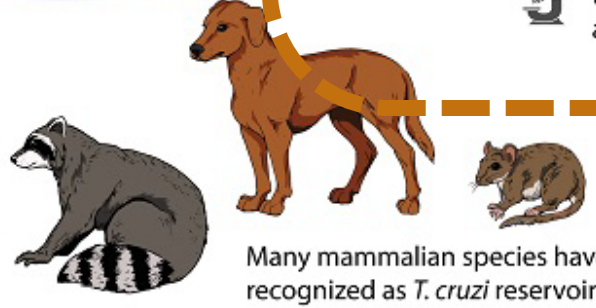
2. Metacyclic trypomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes.

3. Amastigotes multiply by binary fission in cells of infected tissues.

4. Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream.

Trypomastigotes can infect other cells and transform into intracellular amastigotes in new infection sites.

DIAGNOSTIC FORMS



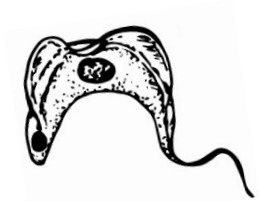
Many mammalian species have been recognized as *T. cruzi* reservoir hosts.

CDC

Infective stage

Diagnostic stage

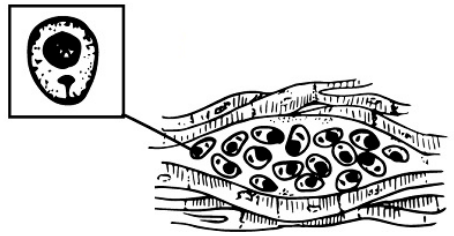
Diagnostic Strategies: Chagas Disease



ACUTE PHASE

★ Molecular diagnostic assays

Microscopic examination of blood smears



CHRONIC PHASE

★ Antibody detection

Microscopic examination of tissue sections

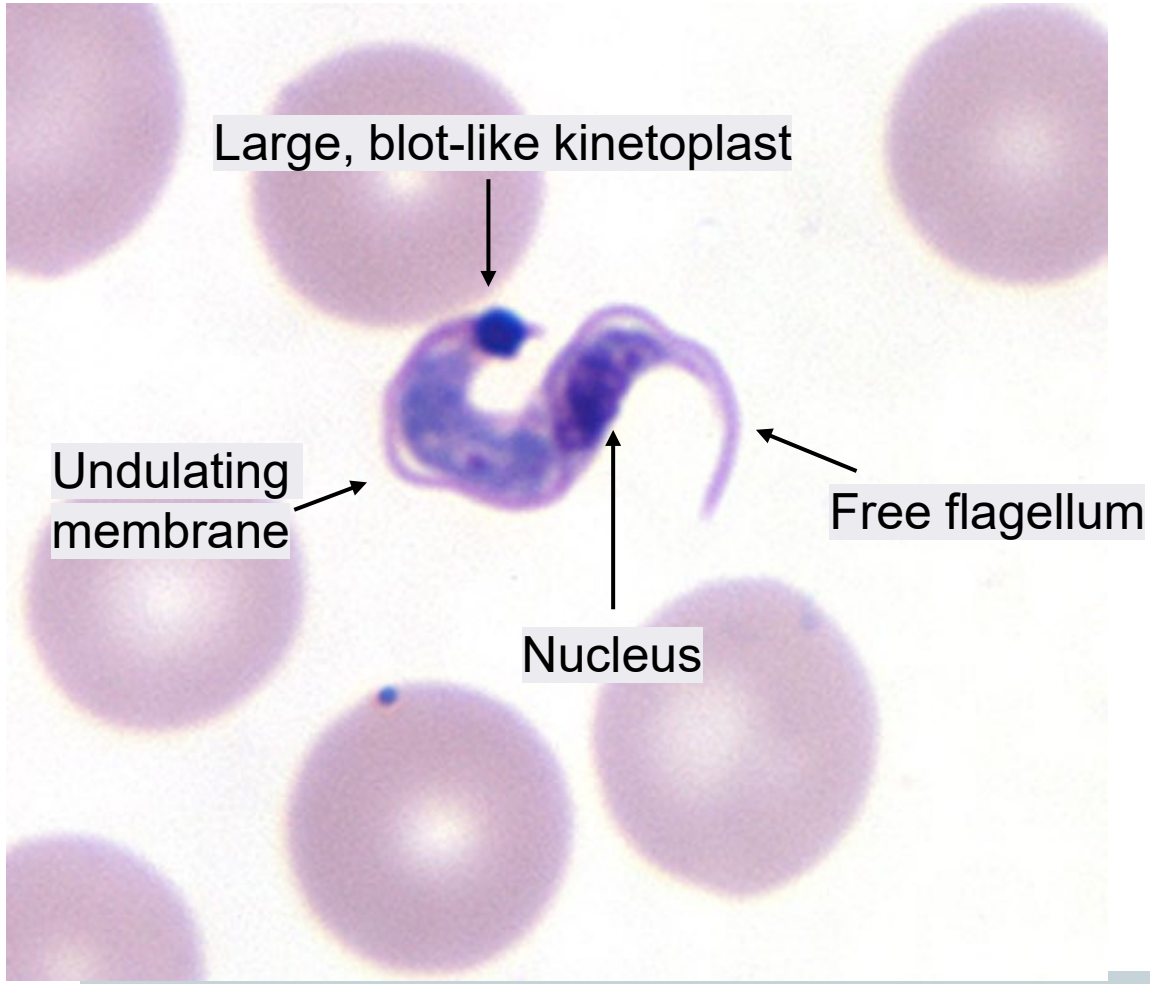
★ Primary / preferred diagnostic method

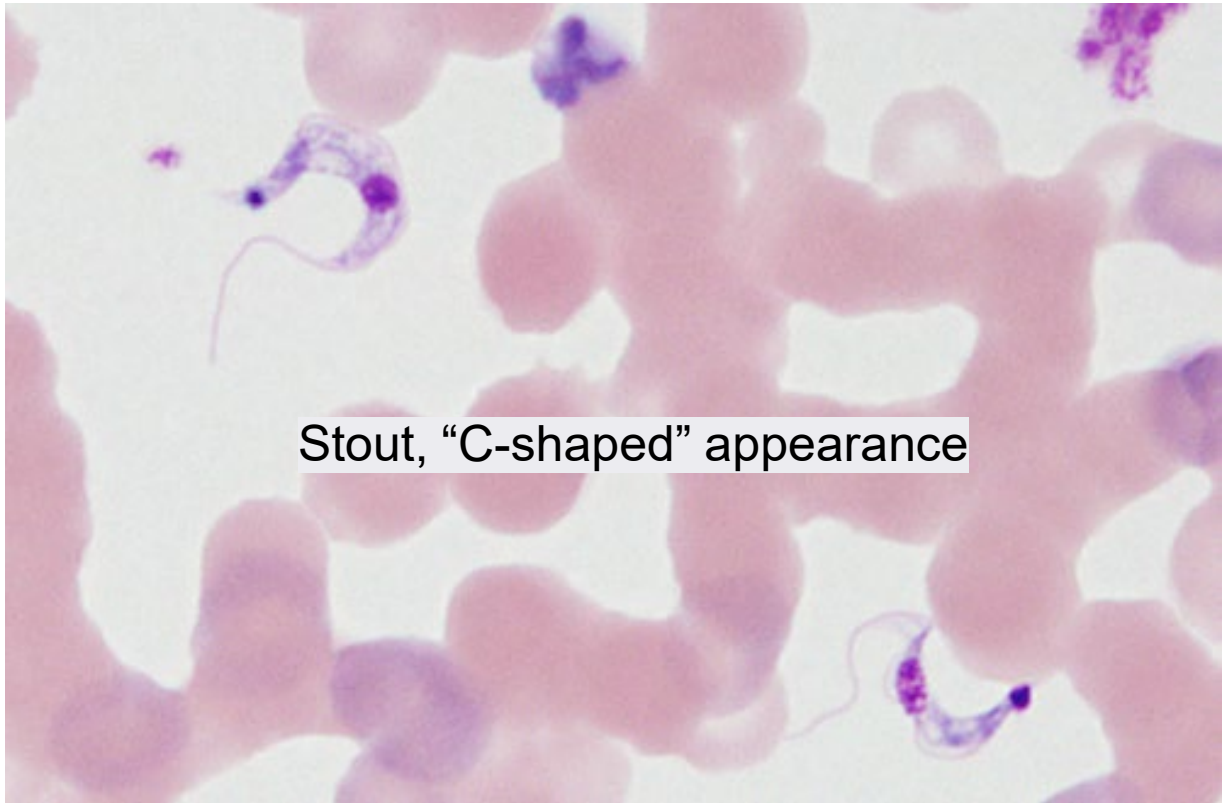
Chagas Disease

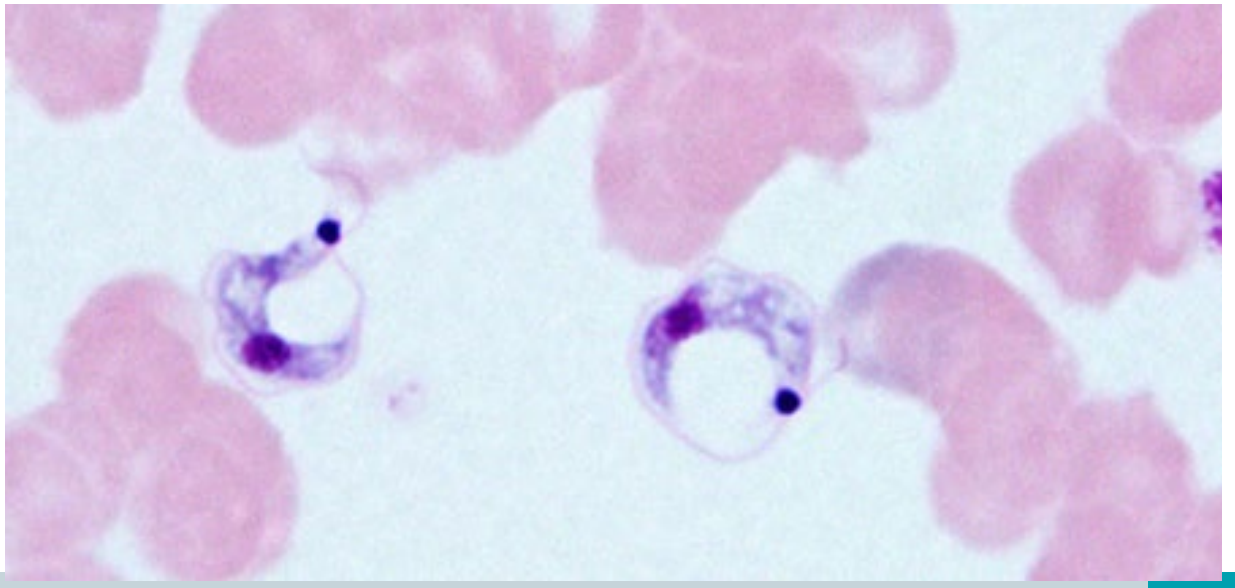
- In the **acute** phase of infection, healthcare providers (labs) might detect parasites circulating in the blood. They can diagnose Chagas disease by detecting these parasites in a blood smear through microscopic examination. This involves preparing and staining both thick and thin blood smears to visualize the parasites.
- To diagnose **chronic** Chagas disease, healthcare providers should assess the patient's clinical findings and infection likelihood, including factors like a history of residence in a Chagas-prevalent area. Typically, this diagnosis requires testing for parasite-specific antibodies.

T. cruzi: trypomastigotes

- Essentially seen only in **acute phase** infection
- May be seen in CSF in neural infections
- 12-30 µm long

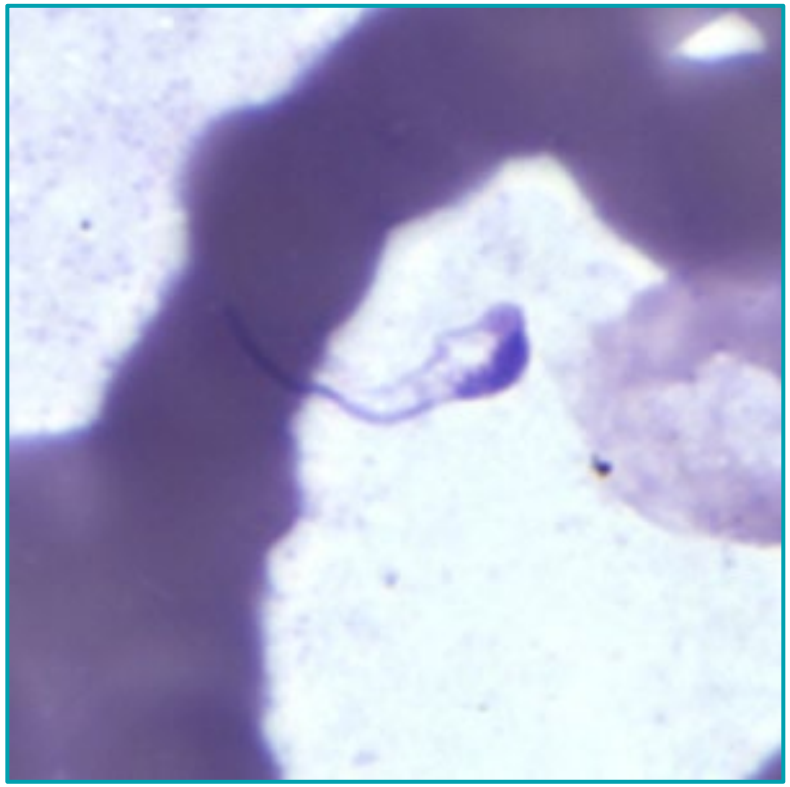


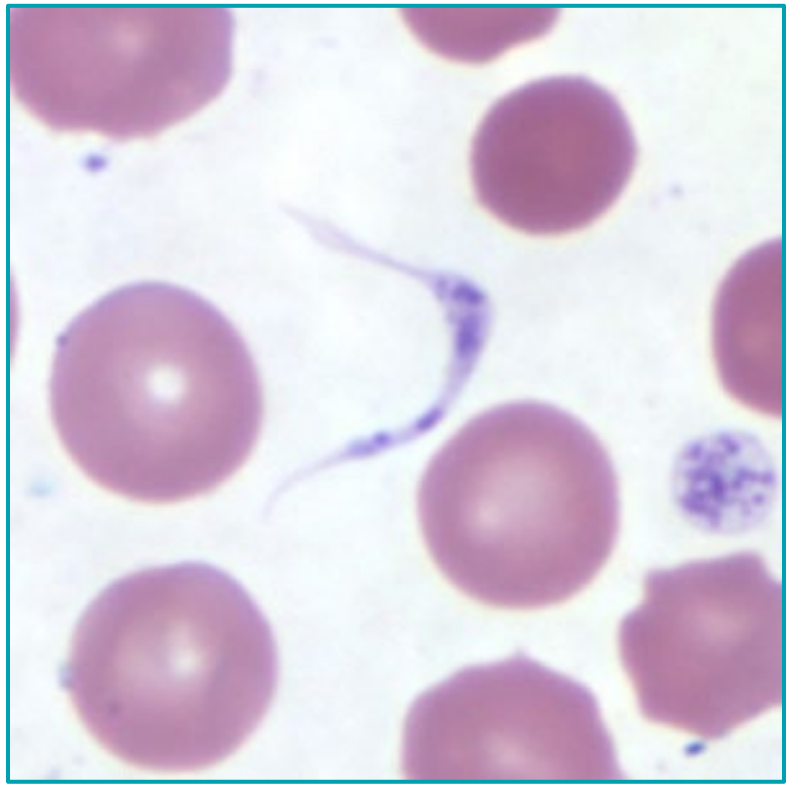




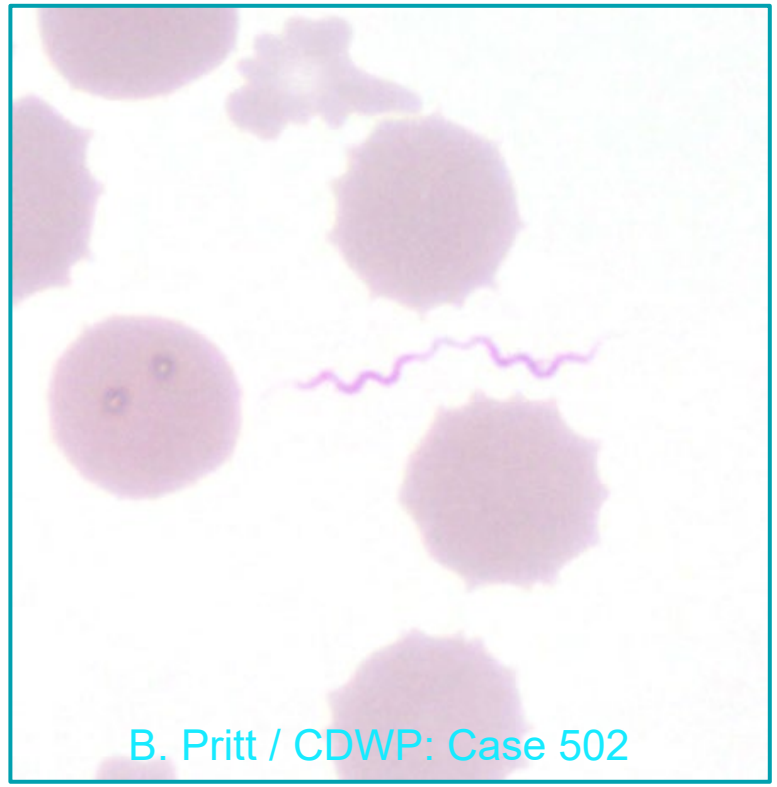
Impostors and artifacts

Elongated platelets





Spirochetes (*Borrelia* spp.)



Chagas Disease

- For **chronic** Chagas disease, healthcare providers detect antibodies against the parasite using serologic tests.
- Testing protocol:
  - **No single test is sufficiently sensitive and specific enough for accurate diagnosis**.
  - Use **two** or more tests that detect antibodies to different antigens.
  - Common techniques include enzyme-linked immunosorbent assay (**ELISA**) and immunofluorescent antibody test (**IFA**).
- Assessing the patient's history for infection risks is also helpful.
- Consult with a Chagas disease specialist for guidance (chagas@cdc.gov)

Malaria

- Tests results urgently needed for patient care, however capacity and proficiency to review blood smears in hospital laboratories 24/7 may be limited.
- In complicated cases (unable to distinguish *Plasmodium* spp, mixed infections, etc.) a rapid test (RDT, PCR) may be able to deliver results rapidly.
- Public health implications are especially critical in locally-acquired cases and may prompt epi-investigation (e.g. vector control, case distribution, etc.)

Malaria: Fast Facts

IMPACT

2020: estimated **241 million** clinical episodes, **627,000** deaths

Half of the world's population lives within endemic area

Domestic cases in US nearly always travel related; isolated transmission incidents in 2023

DISEASE

Most common diagnosis for febrile patients in low-resource healthcare settings globally

Severe complications can arise including cerebral malaria and more

AGENT

Plasmodium spp., an Apicomplexan protozoan parasite vectored by female *Anopheles* spp. mosquitoes…

Malaria: The Key Players

Four major causative agents of human malaria

Plasmodium falciparum

Deadliest; found worldwide in tropical, subtropical areas

Plasmodium vivax

Predominant species outside of sub-Saharan Africa

Plasmodium ovale

Restricted to West Africa and some islands of Western Pacific

Plasmodium malariae

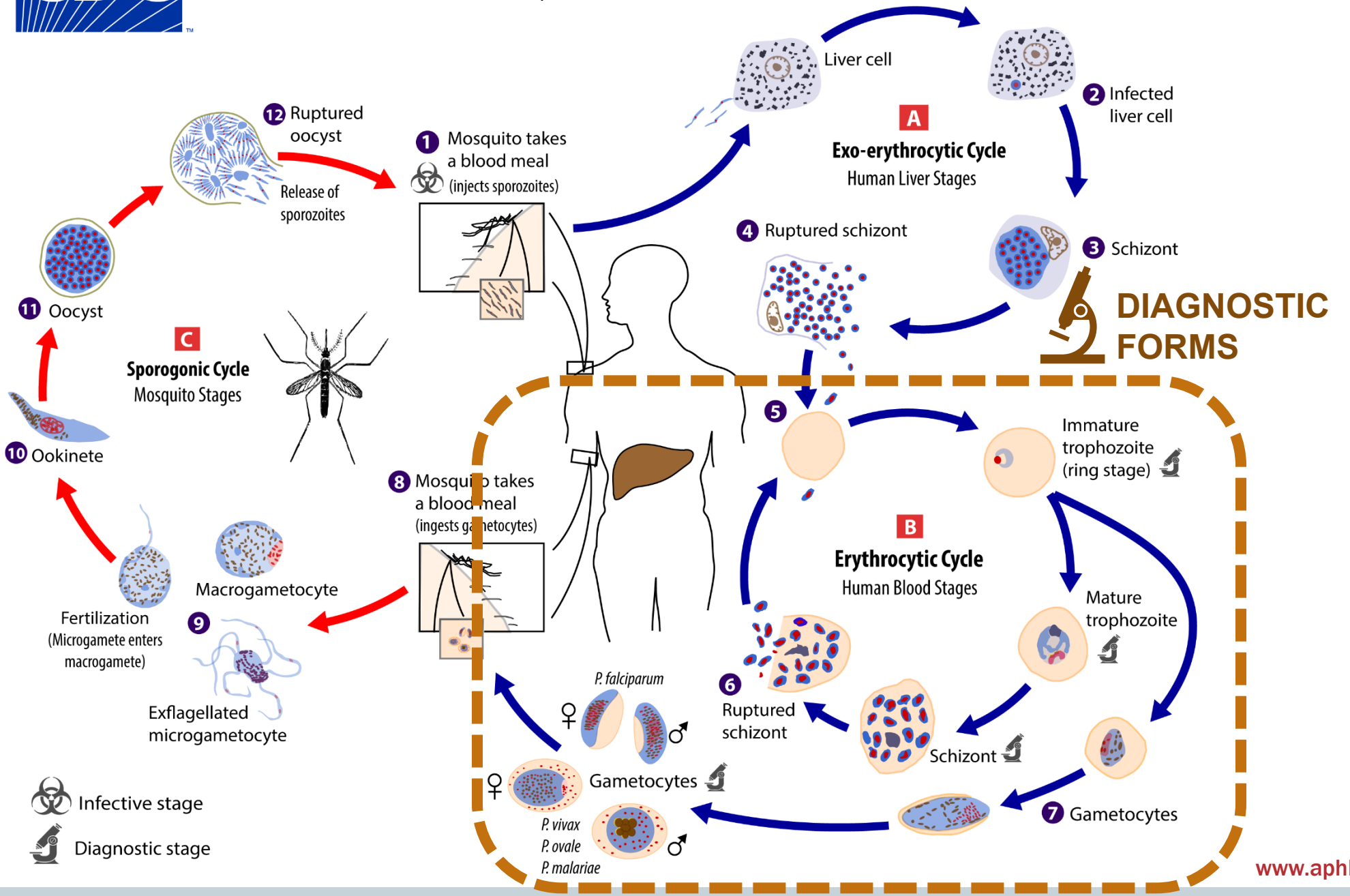
"Broad distribution in tropical, subtropical areas

Zoonotic agents of malaria (uncommon)

- ***Plasmodium knowlesi***
- *Rare others*

Malaria

Plasmodium falciparum, P. ovale, P. vivax, P. malariae



Diagnostic Strategies

- Microscopic examination of blood smears
- Molecular diagnostic assays
- Antigen detection - rapid diagnostic tests (RDTs): Malaria
- Antibody detection: Babesiosis

Why microscopy?

- Fast turnaround
- Very low cost; minimal equipment, reagents
- Distinction between parasite stages and/or other blood parasites; can quantify parasitemia
- Can reduce the need for follow-up PCR if a species ID is achieved

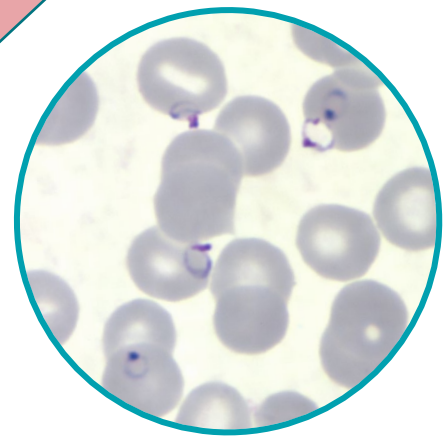
The two most urgent dx questions…

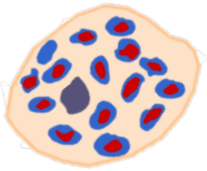
Is it malaria?

(Yes?)

Is it *Plasmodium falciparum*?

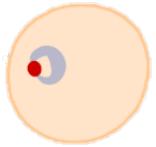
Malaria is a medical emergency! *P.f.* can rapidly lead to death.

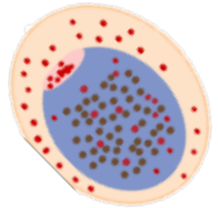




TROPHOZOITE

SCHIZONT

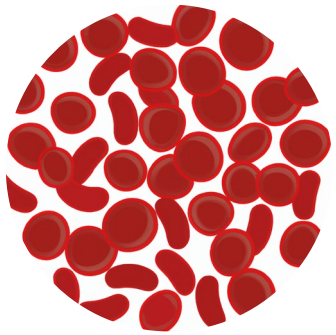




RING STAGE

GAMETOCYTE

Appearance of parasite



Appearance of infected RBCs

Host factors: travel history, transfusions, etc.

Species-level ID via microscopy

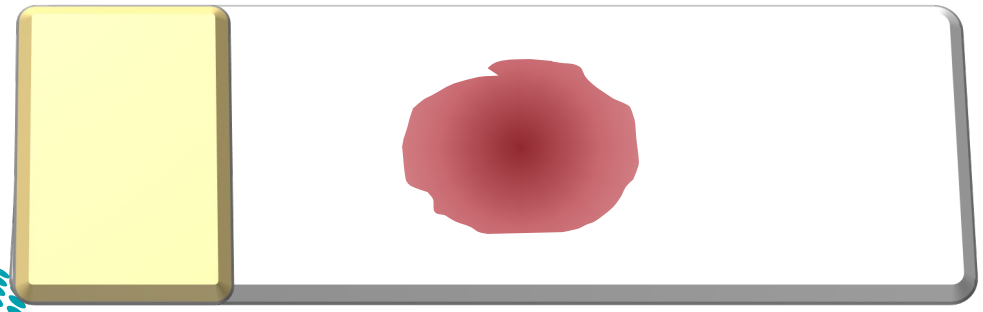
(i.e.) Gold standard!

Types of smears (films)

THICK SMEAR

Greater amount of blood, lysed RBCs (smear never fixed in methanol) - ~30x more concentrated than a thin smear

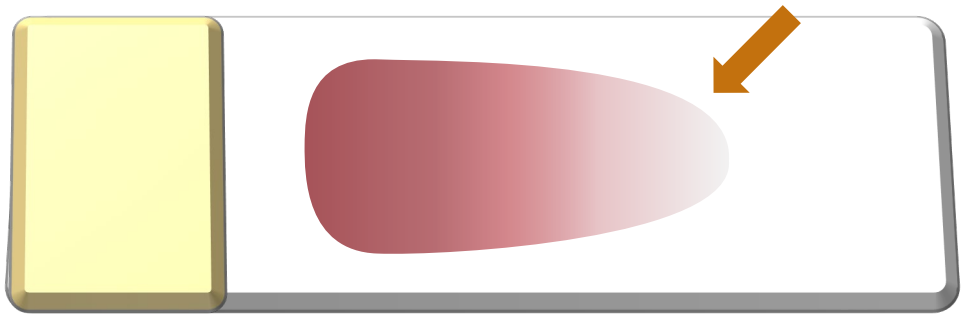
- \+ Efficient and sensitive detection (SCREENING)
- − Usually requires follow-up exam of thin smear for species ID



THIN SMEAR

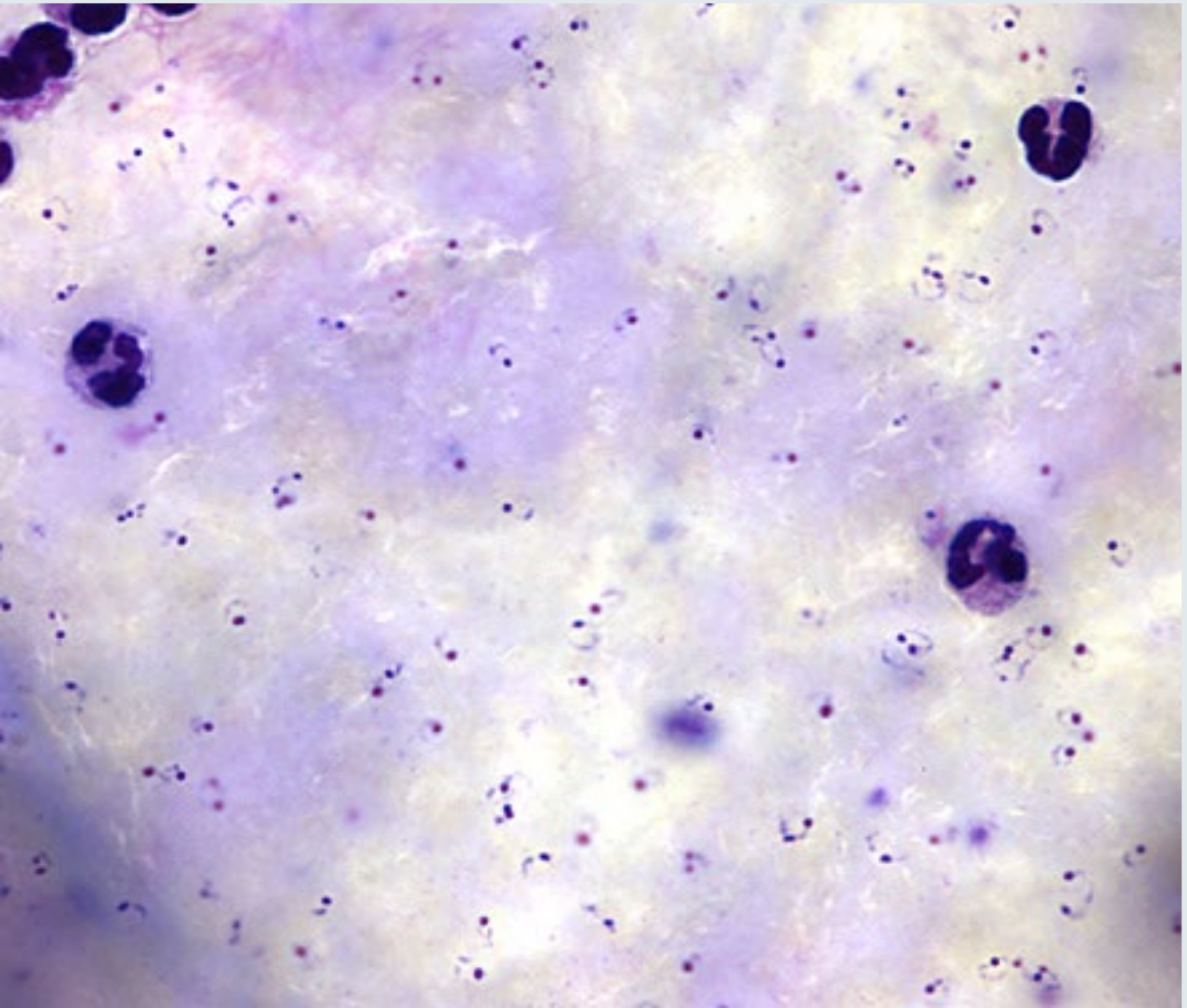
Small amount of blood spread thin, RBC monolayer at the "feathered edge". Must be fixed (methanol) to prevent lysis.

- \+ Morphology of infected RBCs preserved, useful for species ID
- − Less sensitive than thick smears

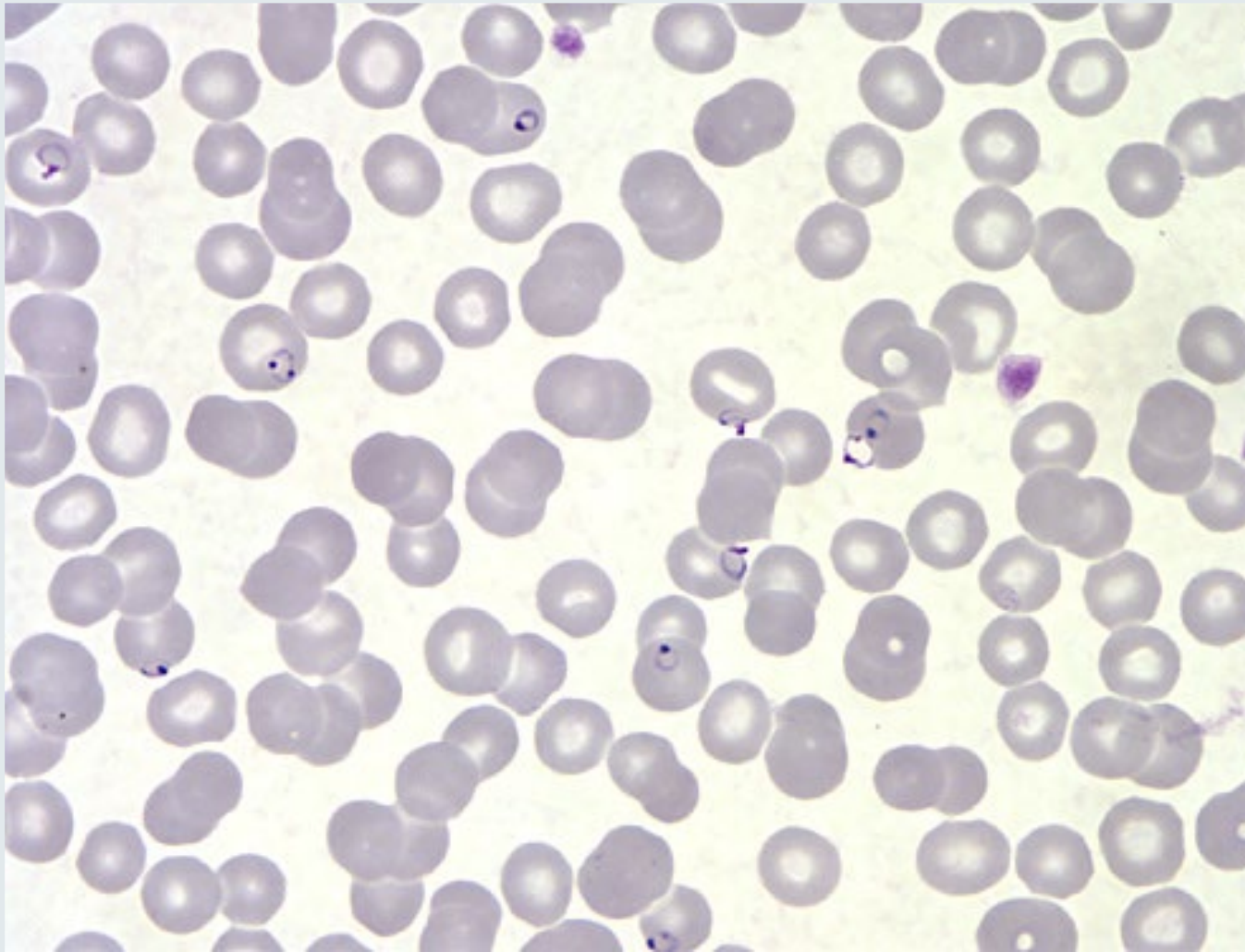


Types of smears (films)

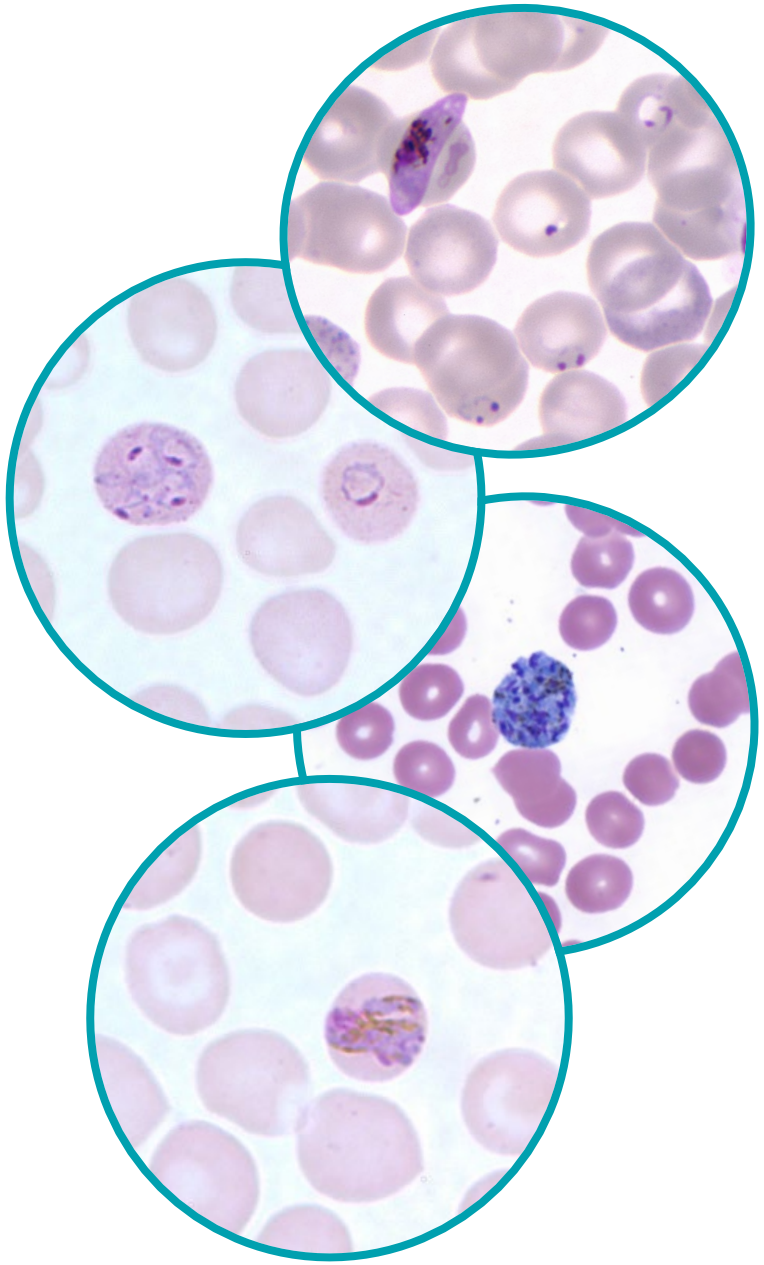
THICK SMEAR



THIN SMEAR



Morphologic Analysis



Requires extensive training, study, and practice to develop and maintain the "eye"!

How we can help…

Bench aids, guides on DPDx website

Telediagnosis service

Physical slide review, if needed

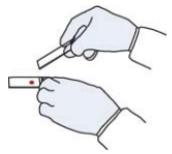
DPDx Bench Aids

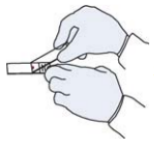
- Technical procedures: smear prep and staining
- Comparative morphology charts

Laboratory diagnosis of malaria

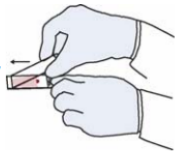
Making thick and thin blood smears

1. Whenever possible, use separate slides for thick and thin smears.
2. Thin film (a): Bring a clean spreader slide, held at a 45° angle, toward the drop of blood on the specimen slide.





3. Thin film (b): Wait until the blood spreads along the entire width of the spreader slide.
4. Thin film (c): While holding the spreader slide at the same angle, push it forward rapidly and smoothly.



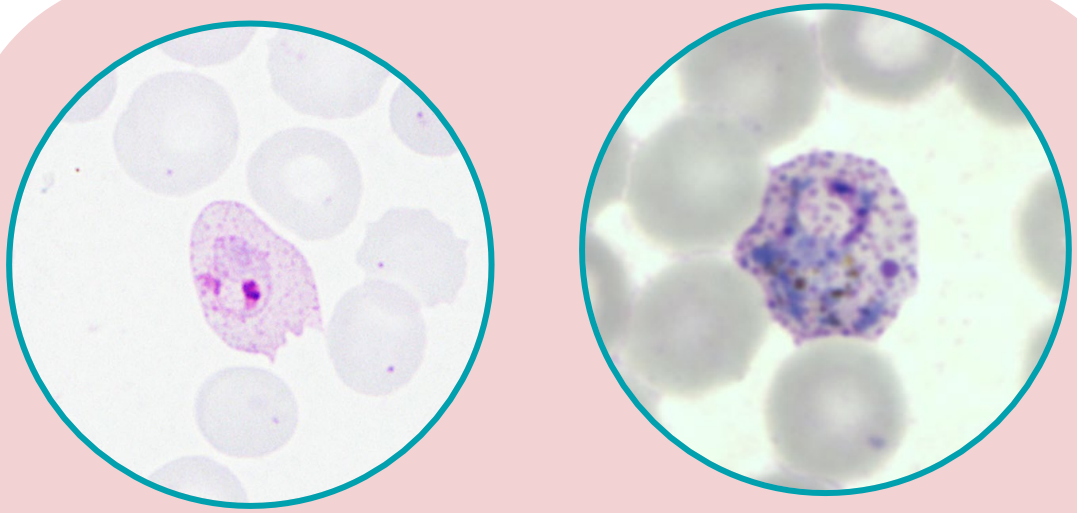
5. Thick film: Using the corner of a clean slide, spread the drop of blood in a circle the size of a dime (diameter 1-2 cm). Do not make the smear too thick or it will fall off the slide. (You should be able to read newsprint through it.)

Plasmodium species	Stages found in blood	Appearance of Erythrocyte (RBC)	Appearance of Parasite
P. falciparum	Ring	normal; multiple infection of RBC more common than in other species	delicate cytoplasm; 1-2 small chromatin dots; occasional appliqué (accollé) forms
	Trophozoite	normal; rarely, Maurer's clefts (under certain staining conditions)	seldom seen in peripheral blood; compact cytoplasm; dark pigment
	Schizont	normal; rarely, Maurer's clefts (under certain staining conditions)	seldom seen in peripheral blood; mature = 8-24 small merozoites; dark pigment, clumped in one mass
	Gametocyte	distorted by parasite	crescent or sausage shape; chromatin in a single mass (macrogametocyte) or diffuse (microgametocyte); dark pigment mass
P. vivax	Ring	normal to 1-1/4 X, round; occasionally fine Schüffner's dots; multiple infection of RBC not uncommon	large cytoplasm with occasional pseudopods; large chromatin dot
	Trophozoite	enlarged 1-1/2–2 X; may be distorted; fine Schüffner's dots	large ameboid cytoplasm; large chromatin; fine, yellowish-brown pigment
	Schizont	enlarged 1-1/2–2 X; may be distorted; fine Schüffner's dots	large, may almost fill RBC; mature = 12-24 merozoites; yellowish-brown, coalesced pigment
	Gametocyte	enlarged 1-1/2–2 X; may be distorted; fine Schüffner's dots	round to oval; compact; may almost fill RBC; chromatin compact, eccentric (macrogametocyte) or diffuse (microgametocyte); scattered brown pigment
P. ovale	Ring	normal to 1-1/4 X, round to oval; occasionally Schüffner's dots; occasionally fimbriated; multiple infection of RBC not uncommon	sturdy cytoplasm; large chromatin
	Trophozoite	normal to 1-1/4 X; round to oval; some fimbriated; Schüffner's dots	compact with large chromatin; dark-brown pigment
	Schizont	normal to 1-1/4 X; round to oval; some fimbriated; Schüffner's dots	mature = 6-14 merozoites with large nuclei, clustered around mass of dark-brown pigment
	Gametocyte	normal to 1-1/4 X; round to oval; some fimbriated; Schüffner's dots	round to oval; compact; may almost fill RBC; chromatin compact, eccentric (macrogametocyte) or more diffuse (microgametocyte); scattered brown pigment
P. malariae	Ring	normal to 3/4 X	sturdy cytoplasm; large chromatin
	Trophozoite	normal to 3/4 X; rarely, Ziemann's stippling (under certain staining conditions)	compact cytoplasm; large chromatin; occasional band forms; coarse, dark-brown pigment
	Schizont	normal to 3/4 X; rarely, Ziemann's stippling (under certain staining conditions)	mature = 6-12 merozoites with large nuclei, clustered around mass of coarse, dark-brown pigment; occasional rosettes
	Gametocyte	normal to 3/4 X; rarely, Ziemann's stippling (under certain staining conditions)	round to oval; compact; may almost fill RBC; chromatin compact, eccentric (macrogametocyte) or more diffuse (microgametocyte); scattered brown pigment

https://www.cdc.gov/dpdx/diagnosticprocedures/index.html

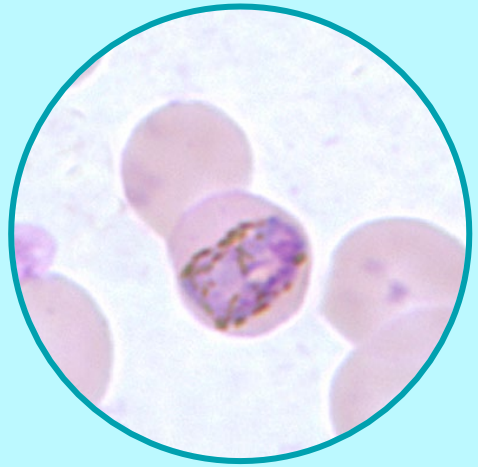
What if it isn't *P. falciparum* malaria?

There are still special considerations for other species, so it's important to resolve the identity!



P. vivax & P. ovale

Latent stages can sequester in liver cells; requires specific treatment to prevent relapses

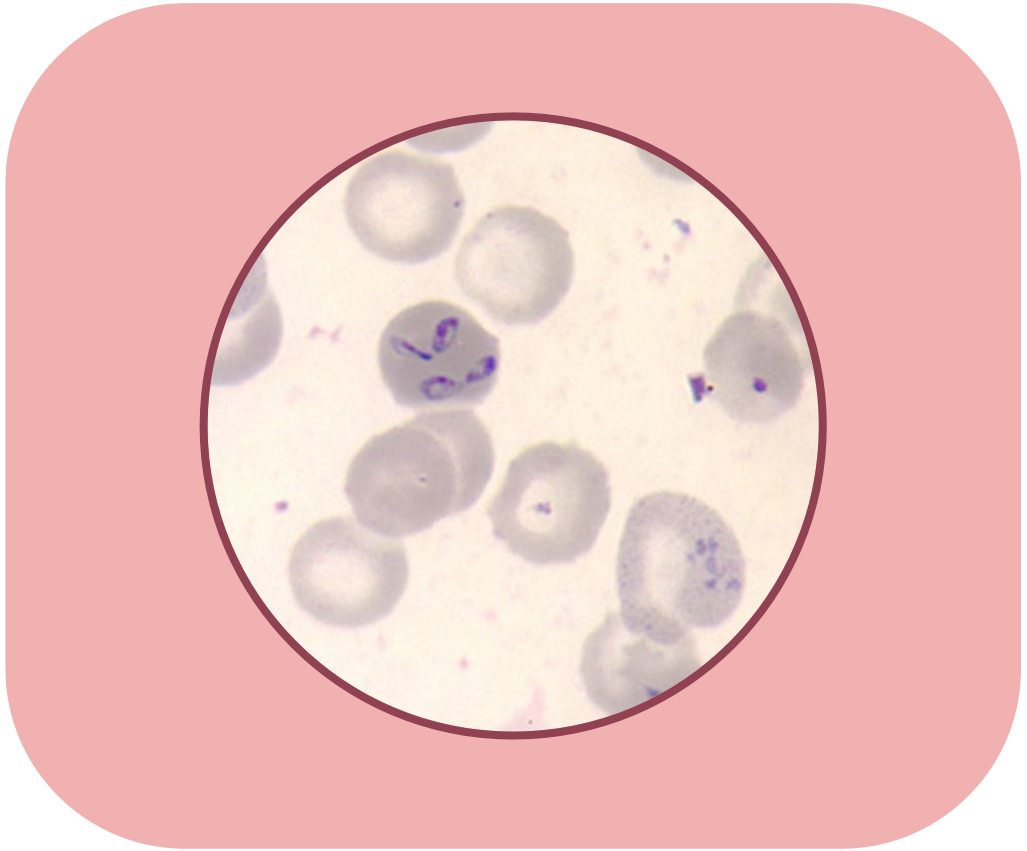


P. malariae

Can sometimes clear spontaneously, or there may be a recrudescence or a series over many years

What if it is actually *Babesia*?

This is still an important point to distinguish…



Treatment strategies are different from malaria.

Babesiosis in asplenic individuals can be very dangerous.

Babesiosis: Fast Facts

IMPACT

2020: **1,827** cases reported to CDC. True incidence likely higher.

Endemic in many parts of the United States (particularly Northeast, Upper Midwest)

Reportable disease in 40 states plus Washington DC.

DISEASE

Febrile illness which may be asymptomatic or mild in many; however can cause severe, life-threatening disease in certain populations (elderly, immunosuppressed, asplenic, etc.)

AGENT

Babesia spp., an Apicomplexan protozoan parasite vectored by hard ticks (primarily *Ixodes* spp.)

“Piroplasms”

Babesiosis: The Key Players

Primary causative agents of human babesiosis
+ General geographic distribution and animal reservoirs

BABESIA MICROTI

Northeastern and Midwestern USA

Babesia divergens

Europe

Babesia duncani

Western USA

***Babesia* MO-1**
(*"divergens-like"*)

Central USA

Uncommon or understudied species/strains

Babesia venatorum

Babesia motasi

Babesia odocoeilei

Babesia crassa-like

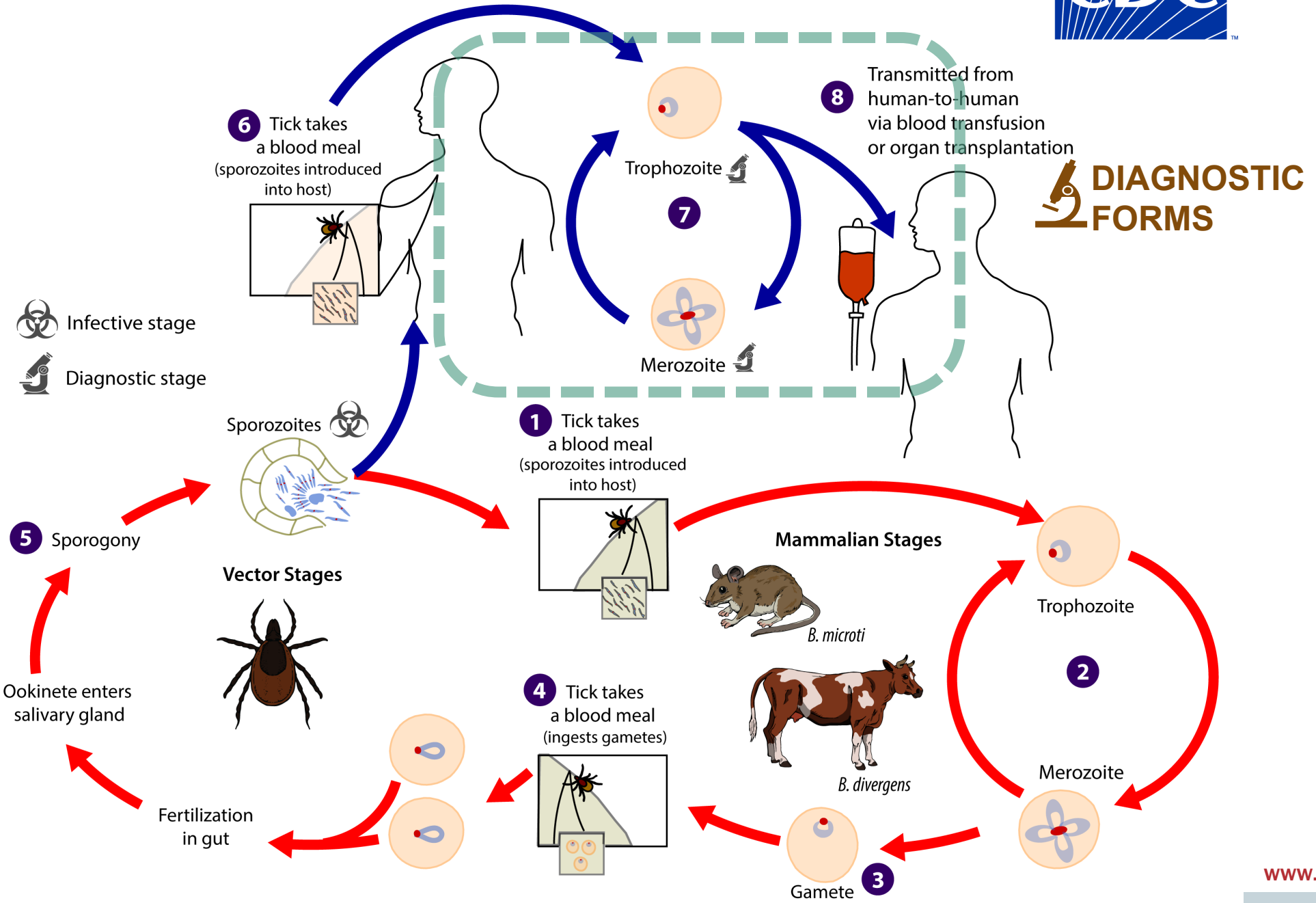
Babesia microti-like

Babesia FR-1

DPDx

Babesia spp.

CDC



DIAGNOSTIC FORMS

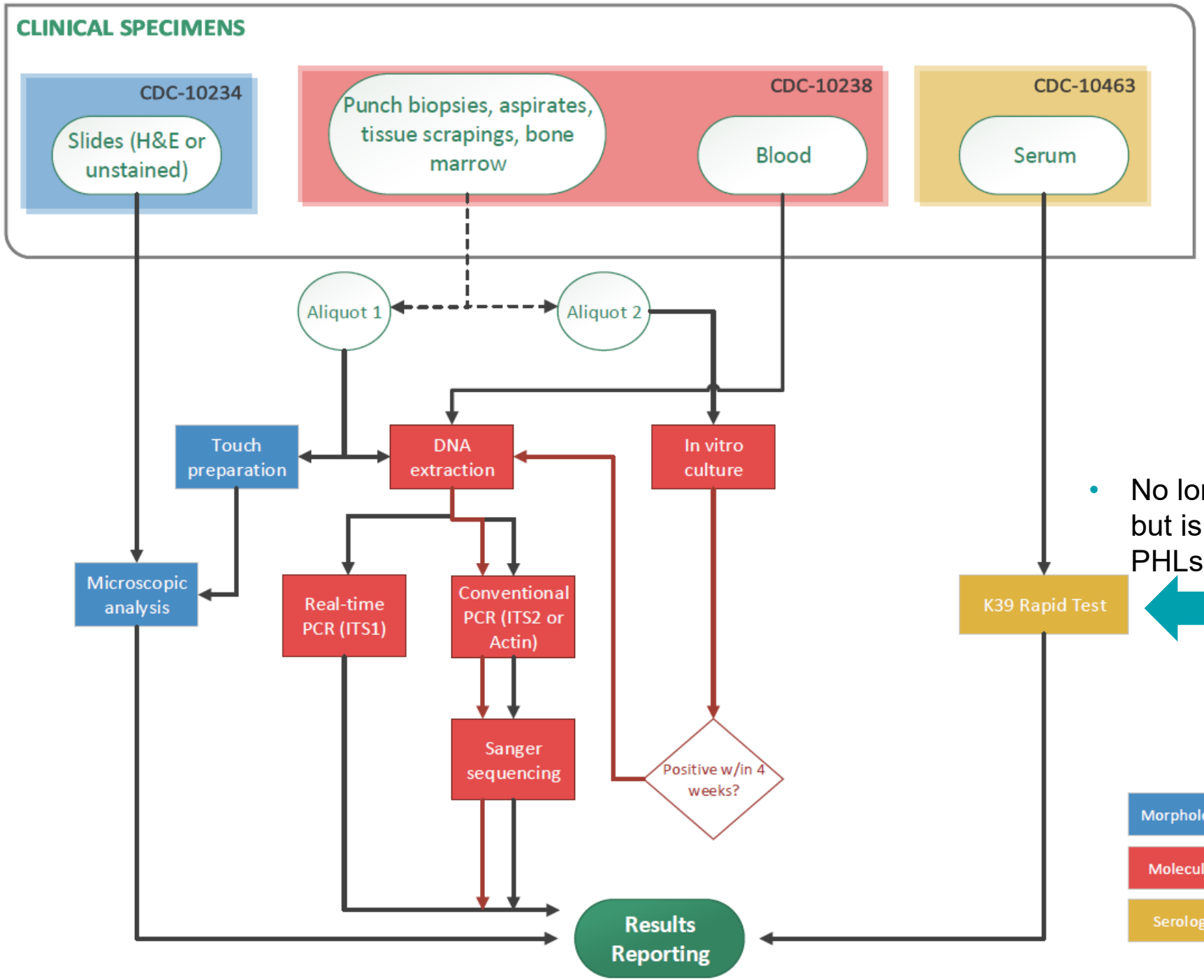
APHL

www.aphl.org

Leishmania Diagnostic Approach

Complex taxon, complex disease, complex approach!

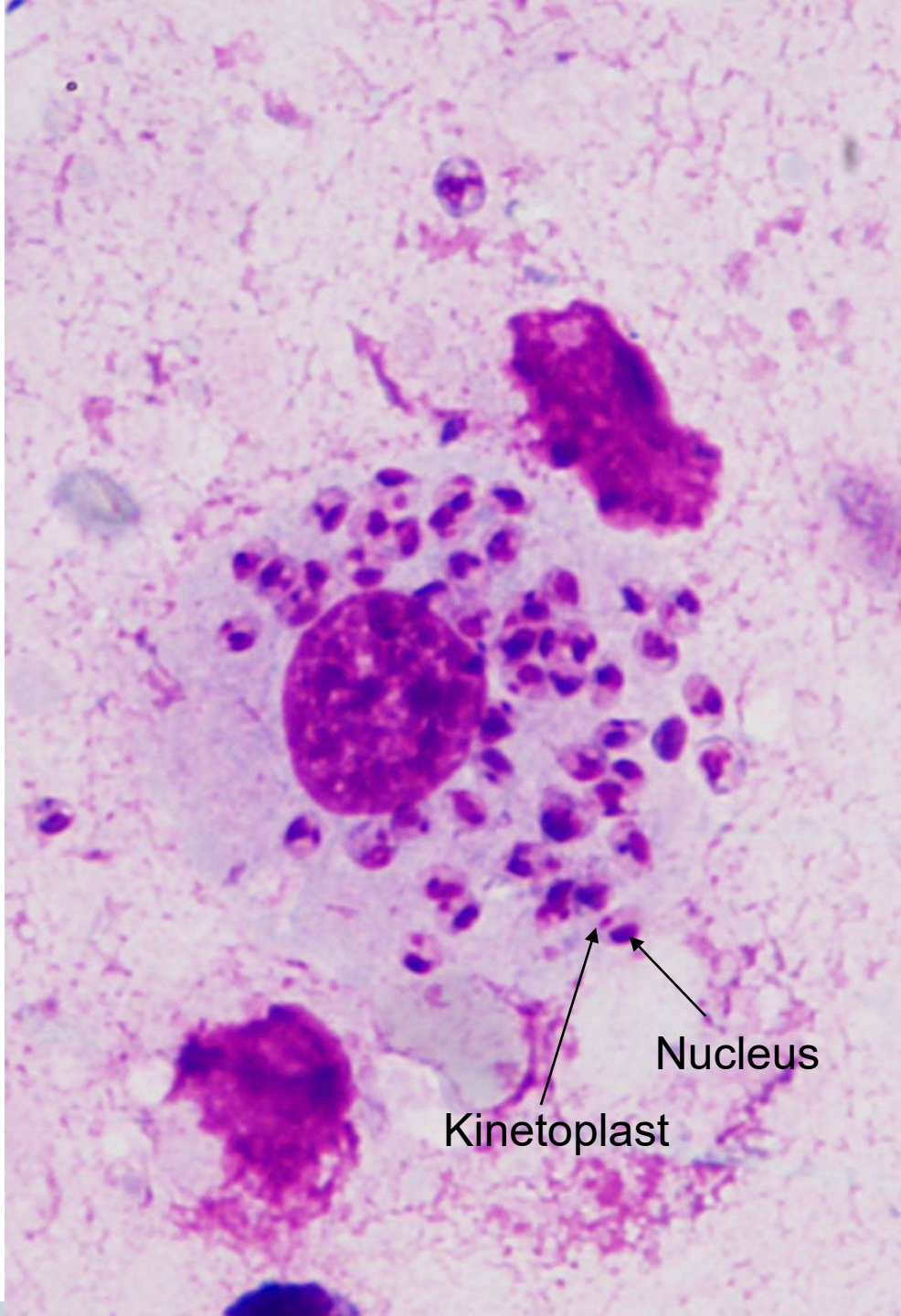
- Combination of:
  - Epidemiologic evidence
  - Clinical findings
  - Microscopy
  - Molecular detection, sequencing
  - In vitro culture
  - Serology



- No longer offered at CDC but is available in some state PHLs

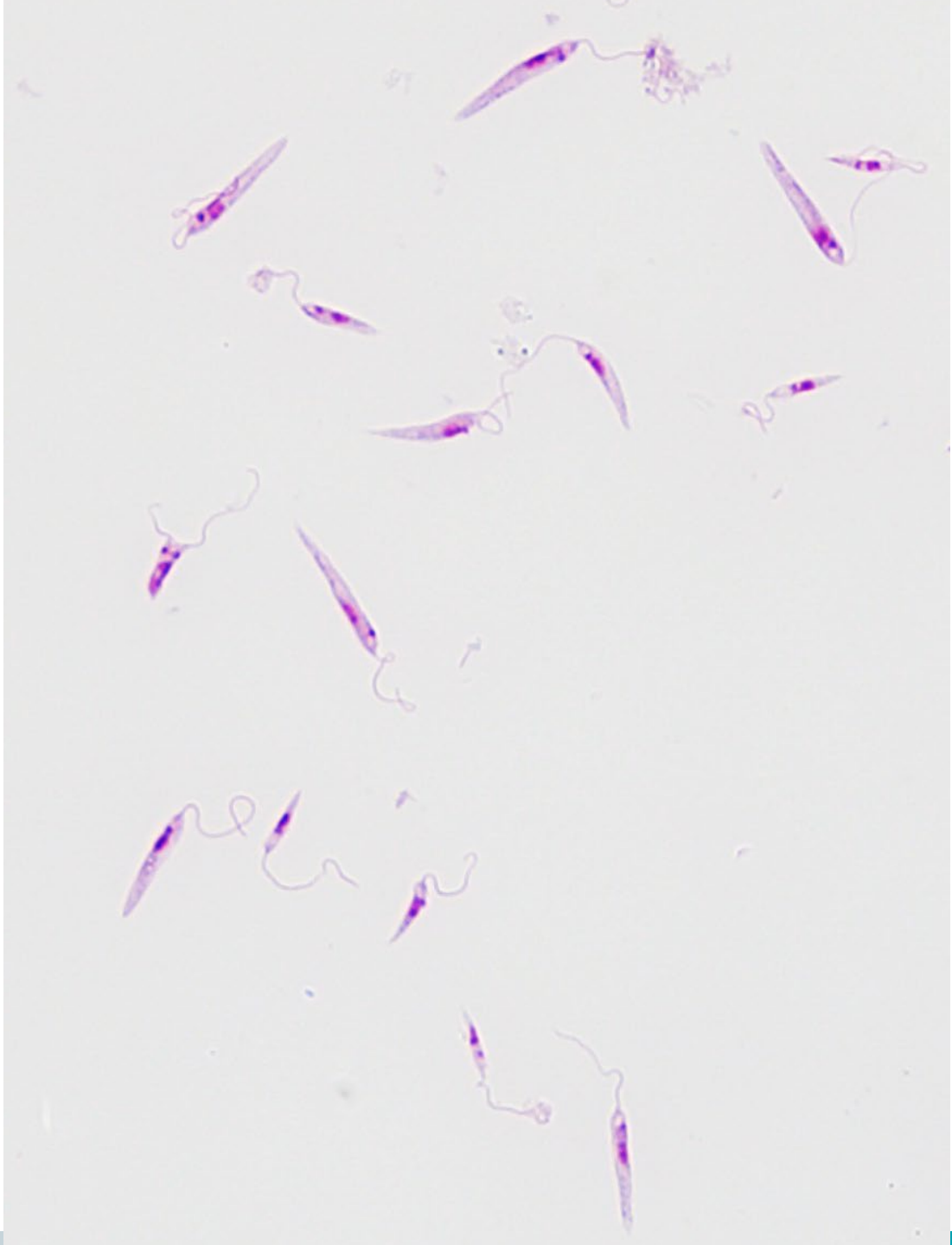
Microscopy

- Touch-preps
  - AKA “Impression Smears”
  - Biopsy smeared onto glass slide, fixed, stained with Giemsa
  - Examined for amastigotes
  - Insensitive – but provides a quick answer while PCR pending
  - No species-level ID possible
- Histopathology slides
  - Accepted as submissions, not usually made in-house
  - Giemsa or H&E stain
  - Examined for amastigotes
  - Similar drawbacks to touch-preps



In Vitro Culture

- Not a primary diagnostic test but a “backup”
  - Cultures propagated concurrently with other testing, subject to PCR/sequencing if positive
  - Results only reported if conflicting with molecular results (e.g. positive culture, negative PCR; different species)
- Performed on punch biopsies, aspirates, bone marrow
- Promastigotes grown in E-6 (Vero) cell flasks @ 26 C; previously on NNN agar overlay
- Allowed to grow for 4 weeks
- Not always successful
  - Certain species harder to culture, also dependent on quality of biopsy, etc.



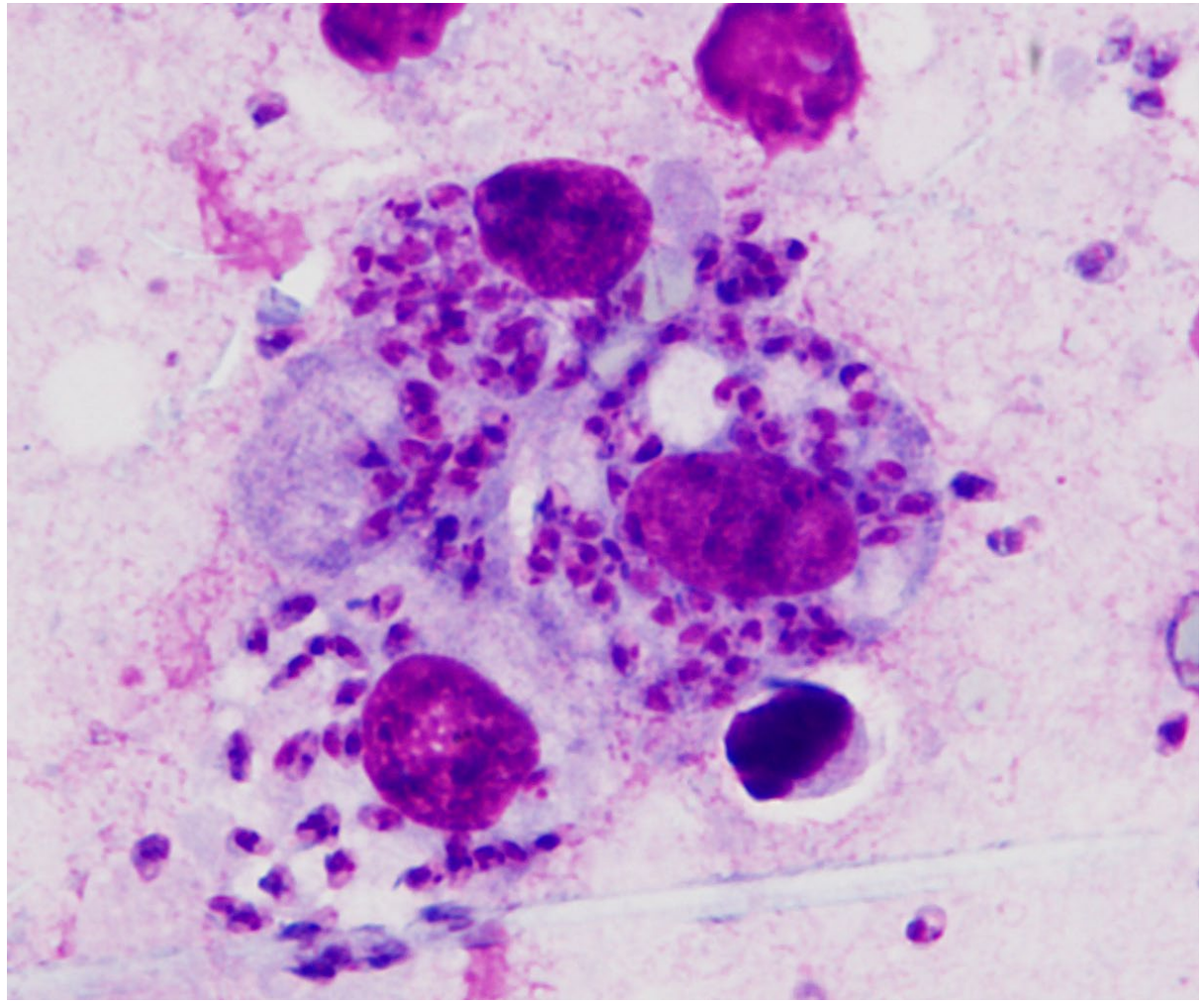
FFPE Tissue Blocks

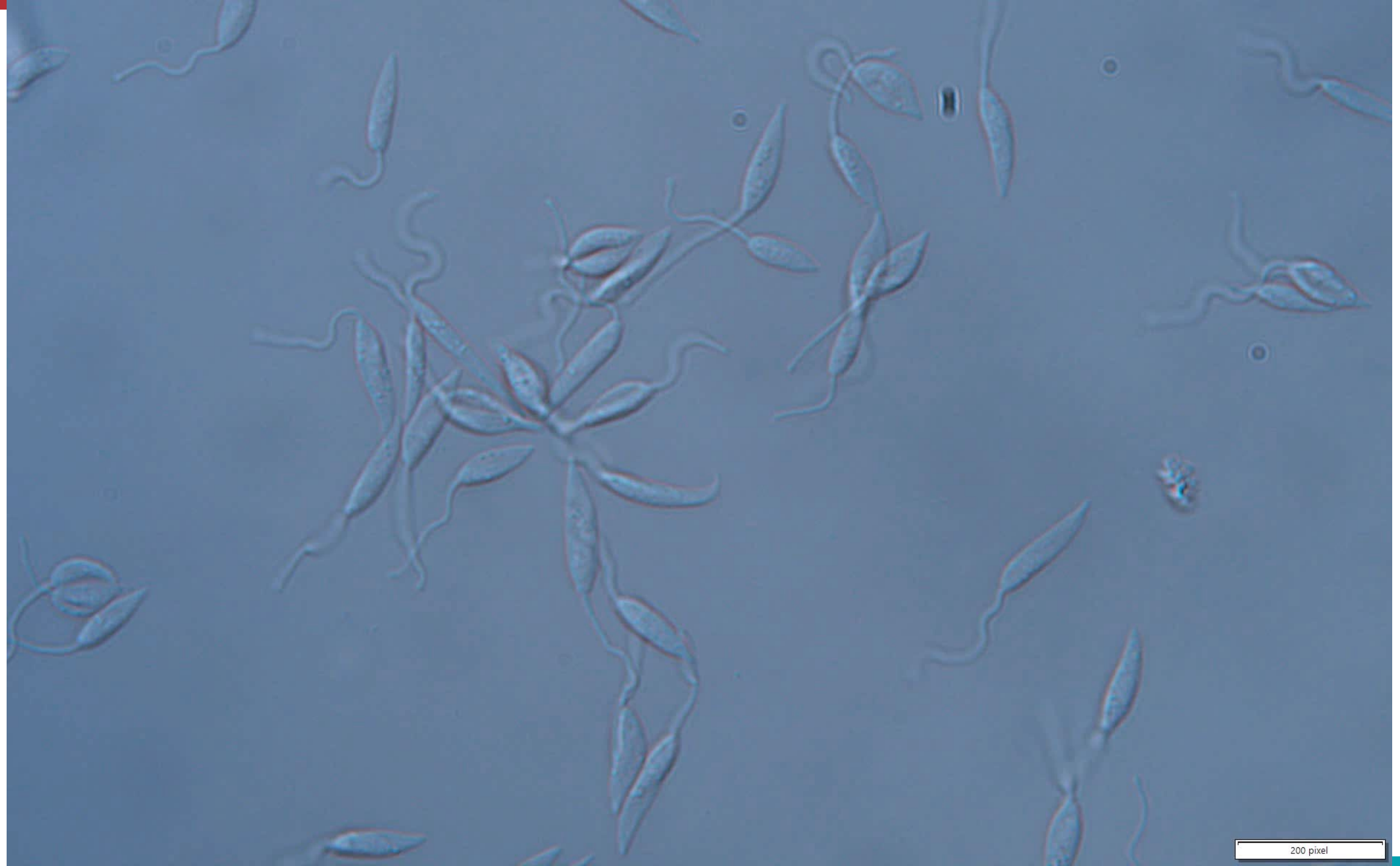
- **Not processed by D&B Labs**
- Must be submitted directly to CDC Infectious Diseases Pathology Branch (IDPB) for production of stained slides and/or DNA extraction
  - IDPB also performs *Leishmania* immunohistochemistry (IHC)
- DNA then transferred back to PDB for molecular species-level identification (Test Code CDC-10238)

Case Study

- 2018: 72-year-old Caucasian female living in Arizona (no international travel history)
  - History of granulomatosis with polyangiitis, chronic sinusitis, chronic kidney disease and pulmonary coccidioidomycosis.
- Developed lower back papular lesions described as discrete, progressive, edematous, violaceous
- Pathologic examination suspicious for amastigotes; tissue biopsy sent to CDC

- Touch-preparation revealed abundant amastigotes
- Real Time PCR (ITS1):
  - Positive for G2 group (incl. *L. donovani, L. infantum, L. tropica*)
- Conventional PCR (ITS2) and sequencing:
  - *L.* sp.; low match to *L. donovani* (90.1% identity)
- In vitro culture yielded promastigotes…

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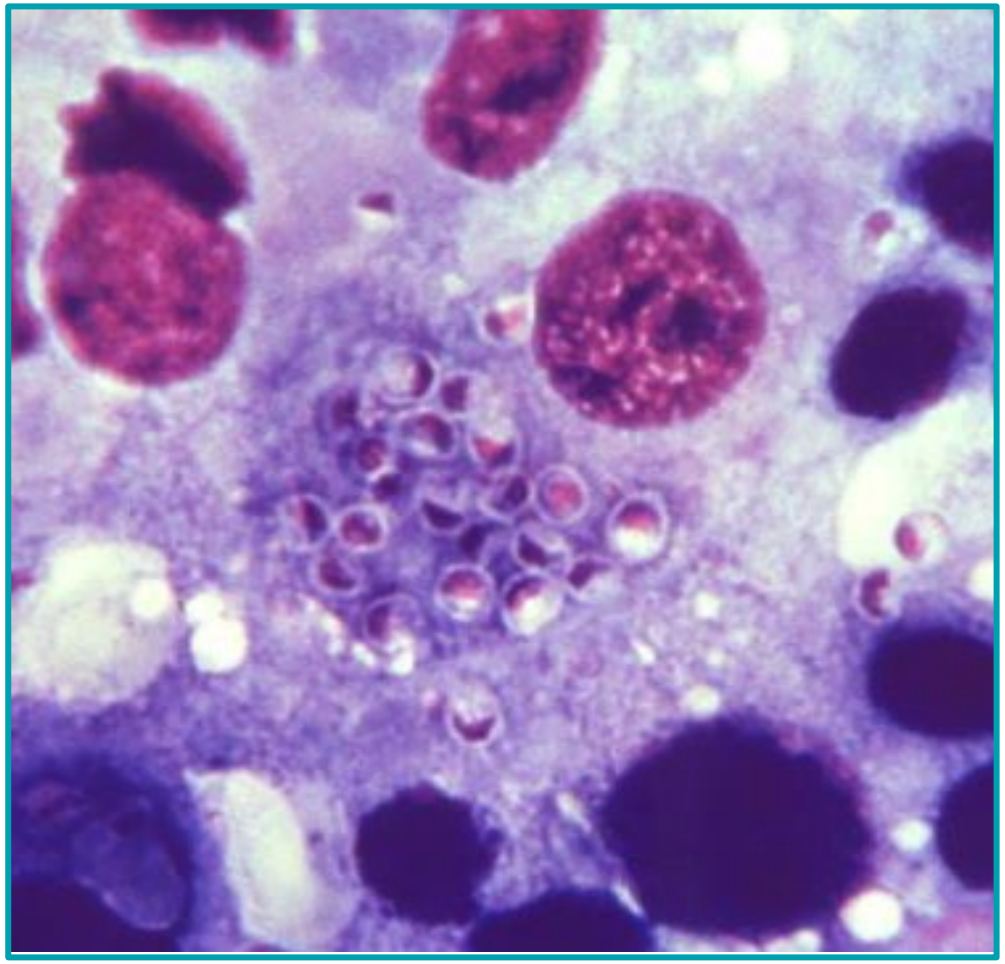
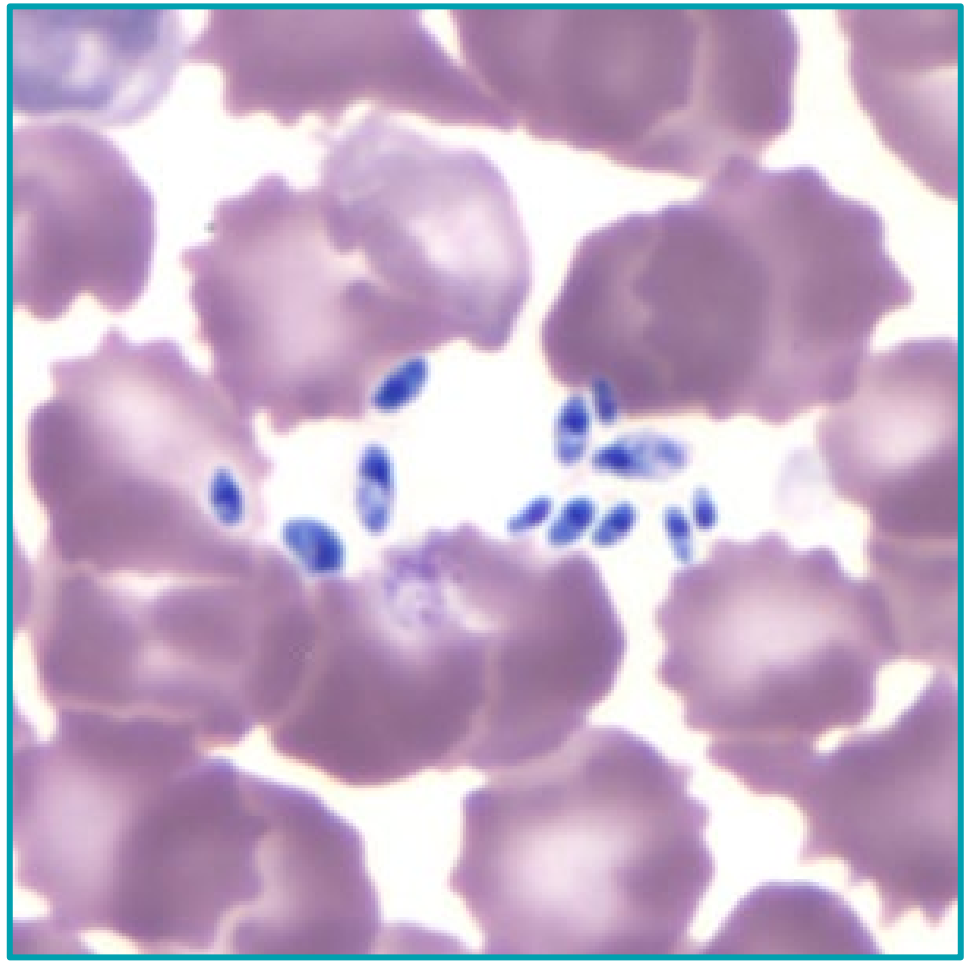
- Patient continued to receive trimethoprim-sulfamethoxazole for sinusitis, but did not receive specific leishmaniasis treatment due to small size of lesions and spontaneous shrinkage
- Lesions have resolved and she continues to be asymptomatic
- Currently working to characterize this novel US-endemic isolate
  - New species?
- Genomics, proteomics ongoing
- Possible future work in characterizing growth, ultrastructure

Impostors and artifacts

Histoplasma capsulatum



Yeasts, contaminant fungal spores



Thank you!

Contacts:

- dpdx@cdc.gov -- telediagnosis, microscopy, general parasitology inquiries
- parasiteslab@cdc.gov – molecular testing, serology, other submission questions
- malarialab@cdc.gov – surveillance and resistance testing
- chagas@cdc.gov – diagnostic considerations

Test Directory

- **CDC-10563 Parasites: Telediagnosis**
- **CDC-10234 Parasites: Morphologic Identification**
- **CDC-10480 Malaria Molecular Identification**
- **CDC-10473 Babesia Molecular Detection**
- **CDC-10475 Chagas Disease Molecular Detection**
- **CDC-10458 Chagas Disease Serology**