Wisconsin State Laboratory of Hygiene

UNIVERSITY OF WISCONSIN–MADISON

Bloodborne Parasites: A New Perspective on Some Old Nemeses

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WCLN Audioconference- Jan 14, 2015

Objectives

- Discuss blood parasites most often seen in WI
  - Clinical presentation and geographic distribution (state data)
- Update on diagnostic methods available for the detection of blood parasites
  - Travel/ treatment history critical
  - Conventional and rapid methods in use
- Become aware of available diagnostic references and telediagnostic aid
  - WSLH and CDC- Division of Parasitic Diseases

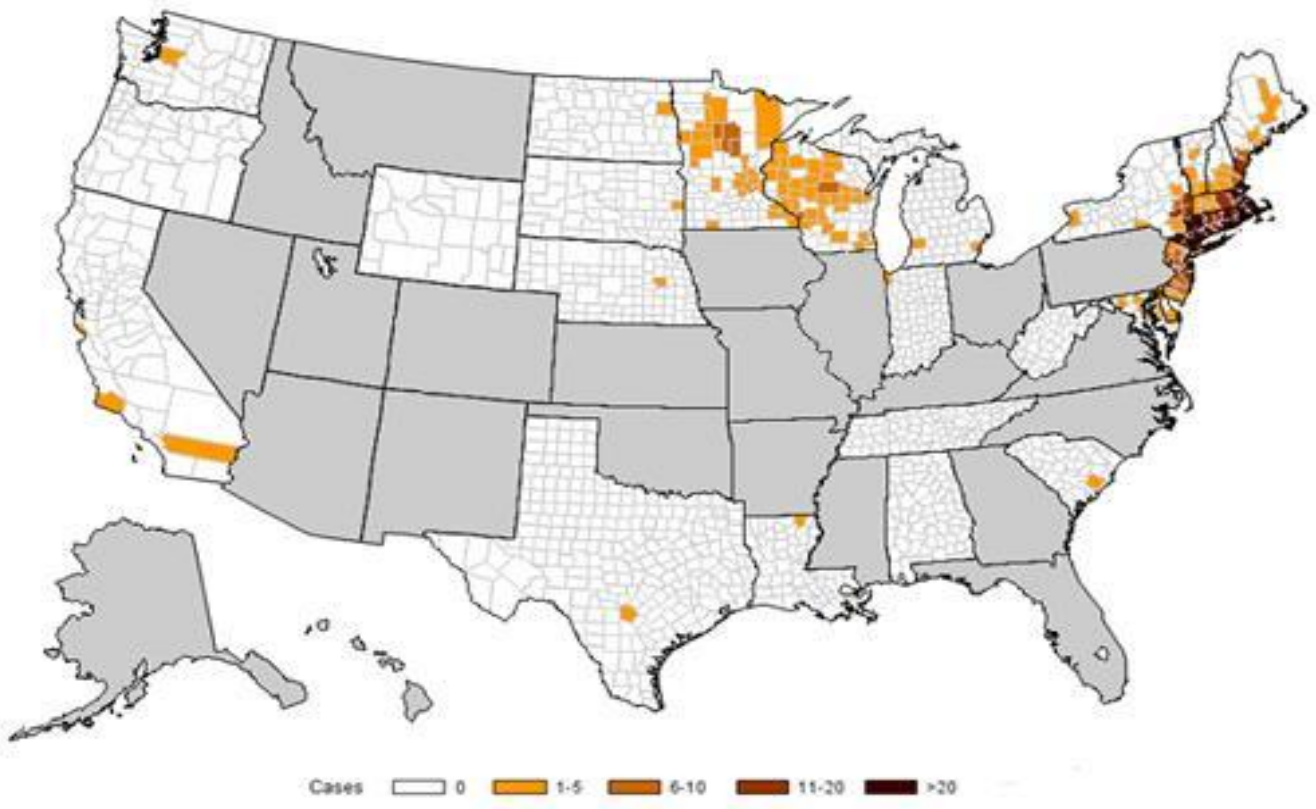
Our home grown adversary: *Babesia microti*

- Transmission
  - Tick bite
    - Nymphal stage of Ixodes scapularis
  - Transfusion
    - (Annals of Internal Medicine-Sept 2011)
  - Congenital transmission

Babesia microti

- Notifiable disease since 2011
  - Surveillance conducted in 27 states
  - 2013: 1762
    - 22/27 states report cases
    - Most cases reported in the northeast and upper midwest
    - Tickborne transmission well established in 7 states (CT, MA, MN, NJ, NY, RI WI)

Babesia microti



Number of reported cases of babesiosis, by county of residence* — 27 states,† 2013

Babesia microti in Wisconsin

Babesia microti

asymptomatic

Flu-like symptoms

- Fever, chills
- Body aches
- fatigue

Babesia microti

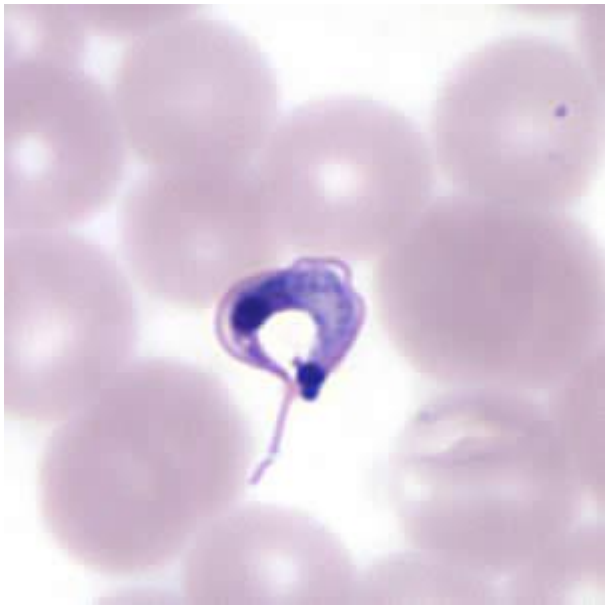
Clinical manifestations in acutely ill patients

- Hemolytic anemia
- Thrombocytopenia
- Proteinuria
- Hemoglobinuria
- Elevated liver enzymes, BUN, creatinine

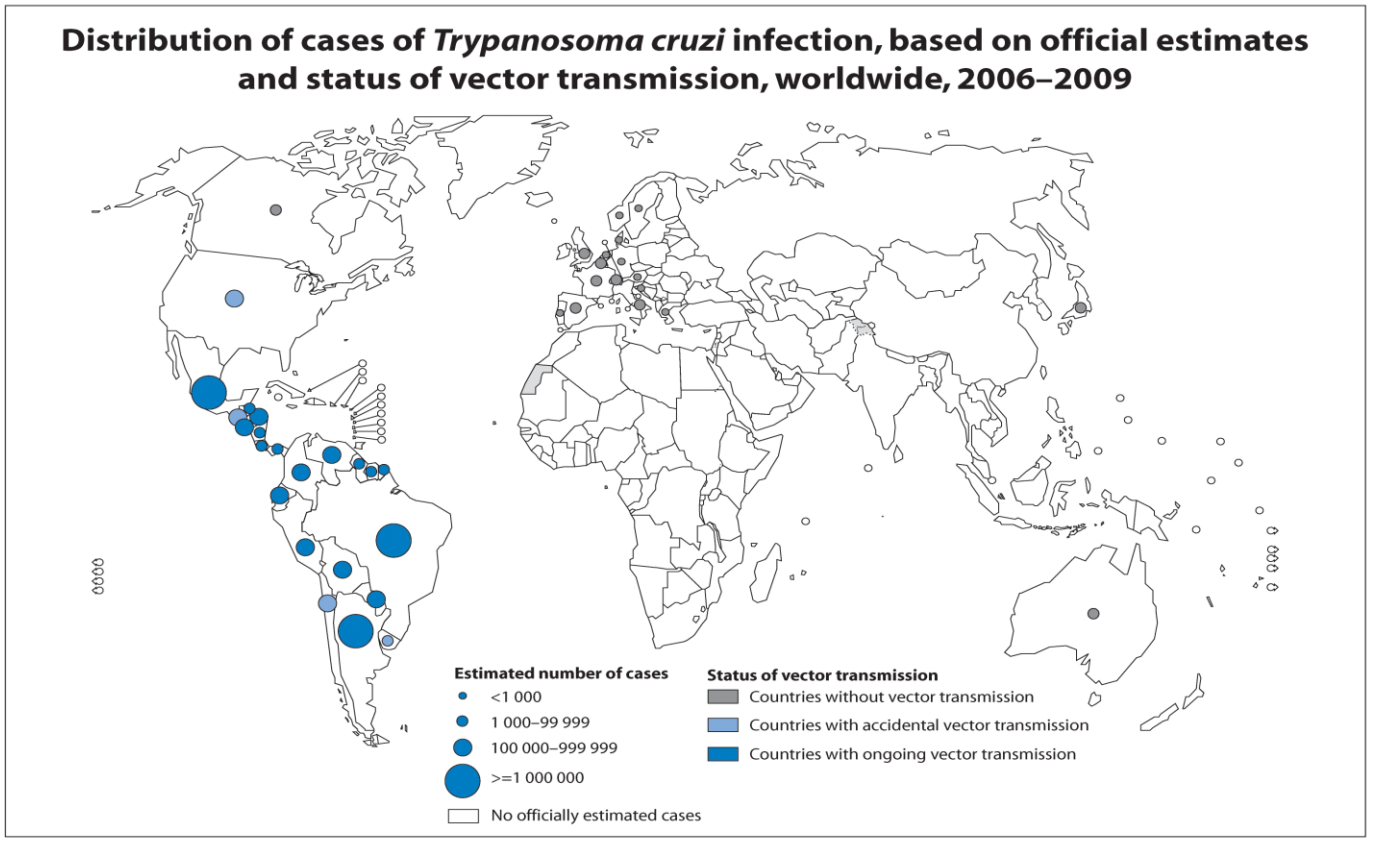
Populations at risk of complications

- Asplenic patients
- Immunocompromised
- Elderly

The enemy at the border
Trypanasoma cruzi



Trypanasoma cruzi



Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

World Health Organization

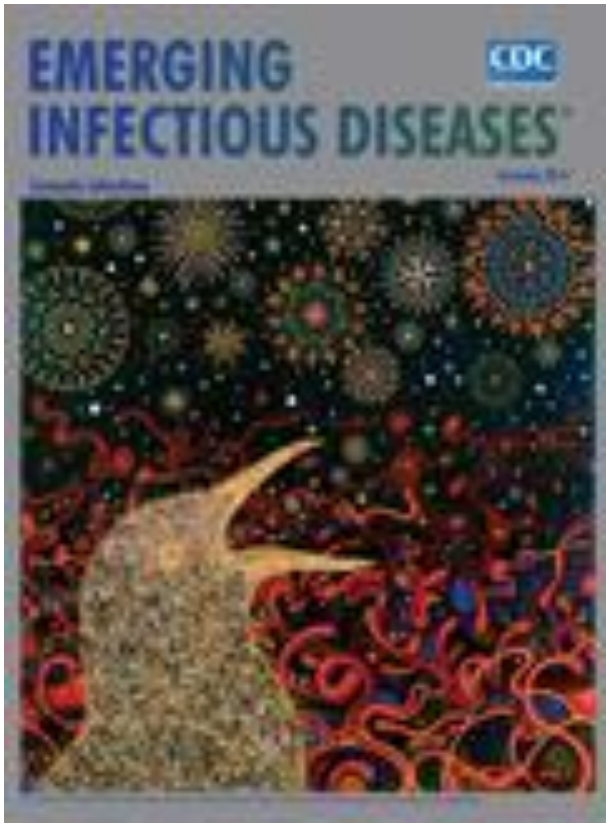
Trypanasoma cruzi

Trypanasoma cruzi

United States

- CDC Estimates that 300,000 persons in US are infected with T. cruzi (*and most don't know it*)
  - Acquired in endemic areas
  - Transfusion
  - Organ transplant
  - Rare cases of vectorborne transmission

Trypanasoma cruzi



***Triatoma sanguisuga* Blood Meals and Potential for Chagas Disease, Louisiana, USA**

Etienne Waleckx,1 Julianne Suarez, Bethany Richards, and Patricia L. Dorn

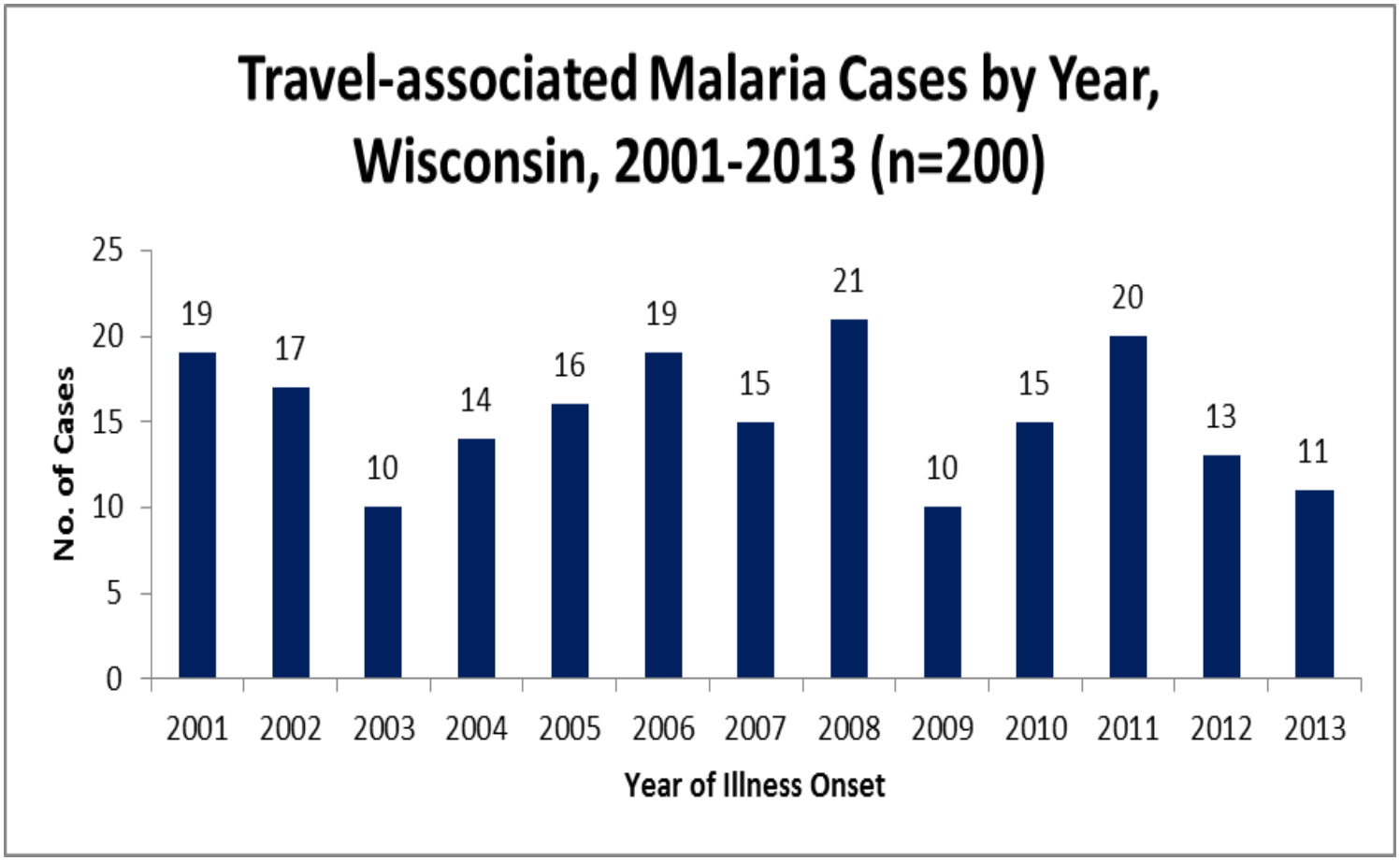
Emerging Infectious Disease. Volume 20, Number 12-December 2014

Trypanasoma cruzi

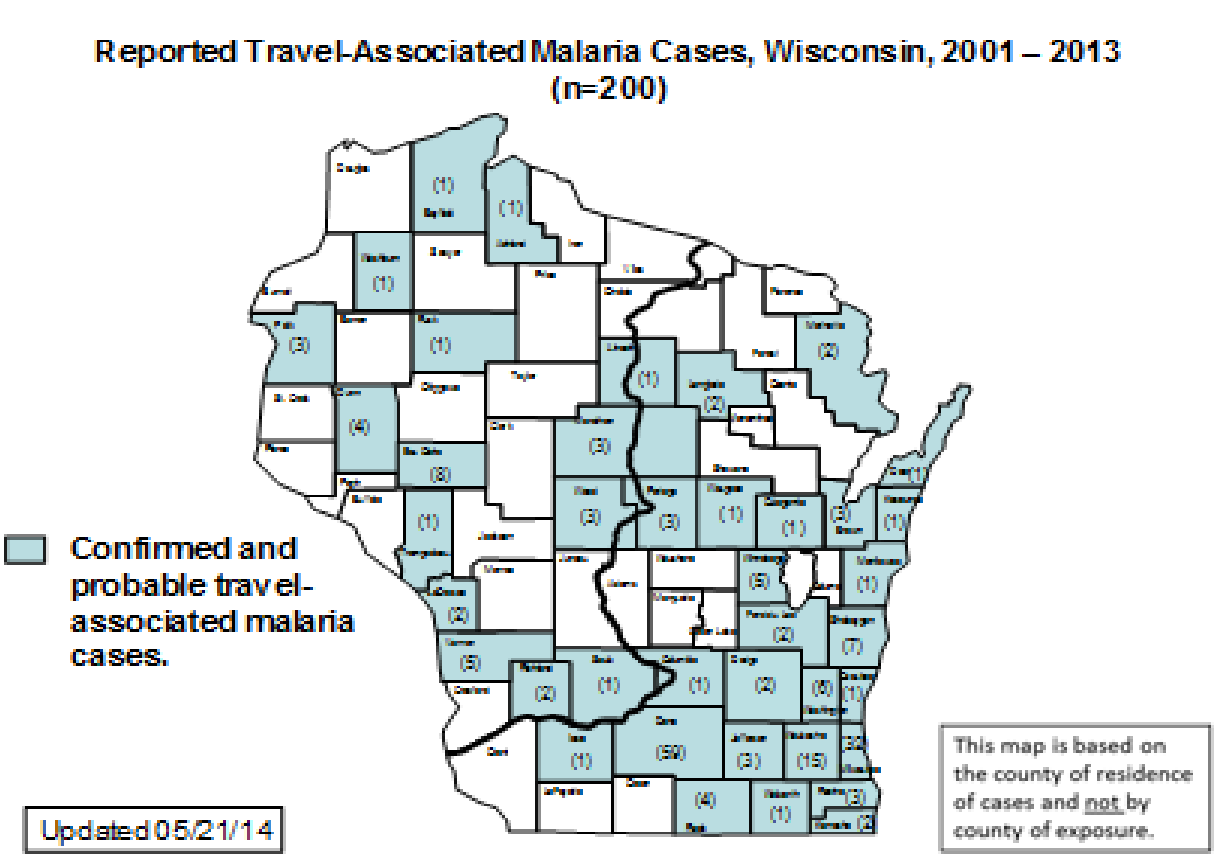
- Acute stage
  - Fever
  - Headache
  - Swollen lymph nodes
  - Muscle pain
- Chronic phase
  - 30% of patients have cardiac disorders
  - 10% of patients have digestive disorders
- Reactivation

The adversaries abroad

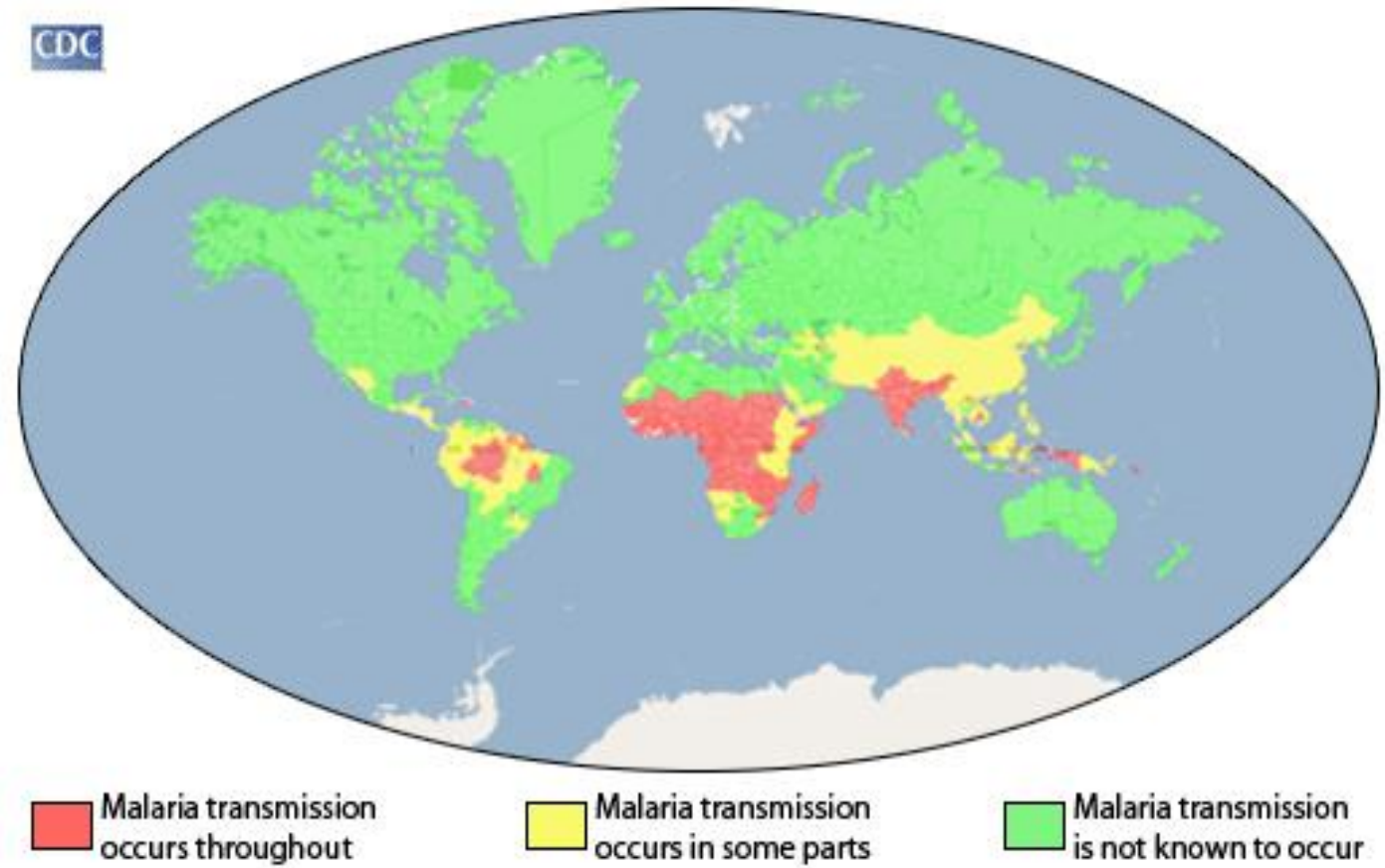
Plasmodium spp.



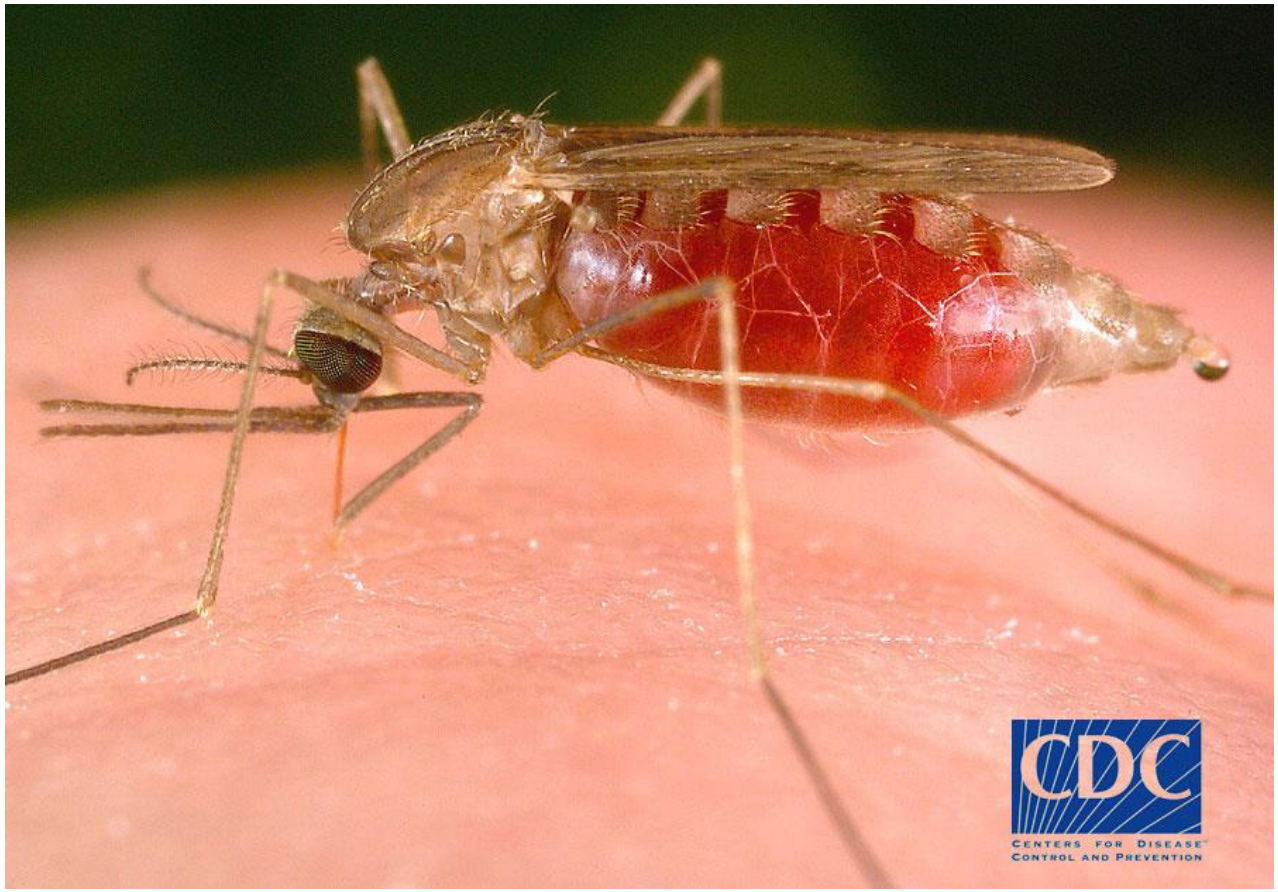
Plasmodium spp.



Plasmodium spp.



Plasmodium spp.



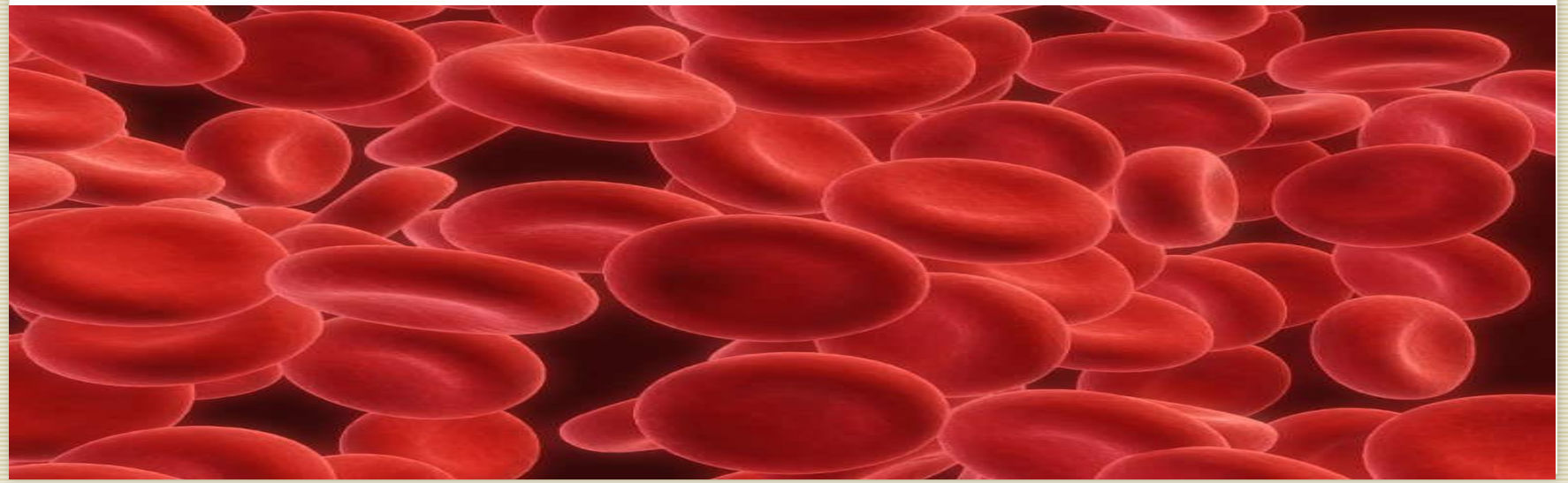
Plasmodium ssp.

where it lives

Plasmodium spp.

Malaria in the United States

- Travel
- Local mosquito-borne
- Transfusion

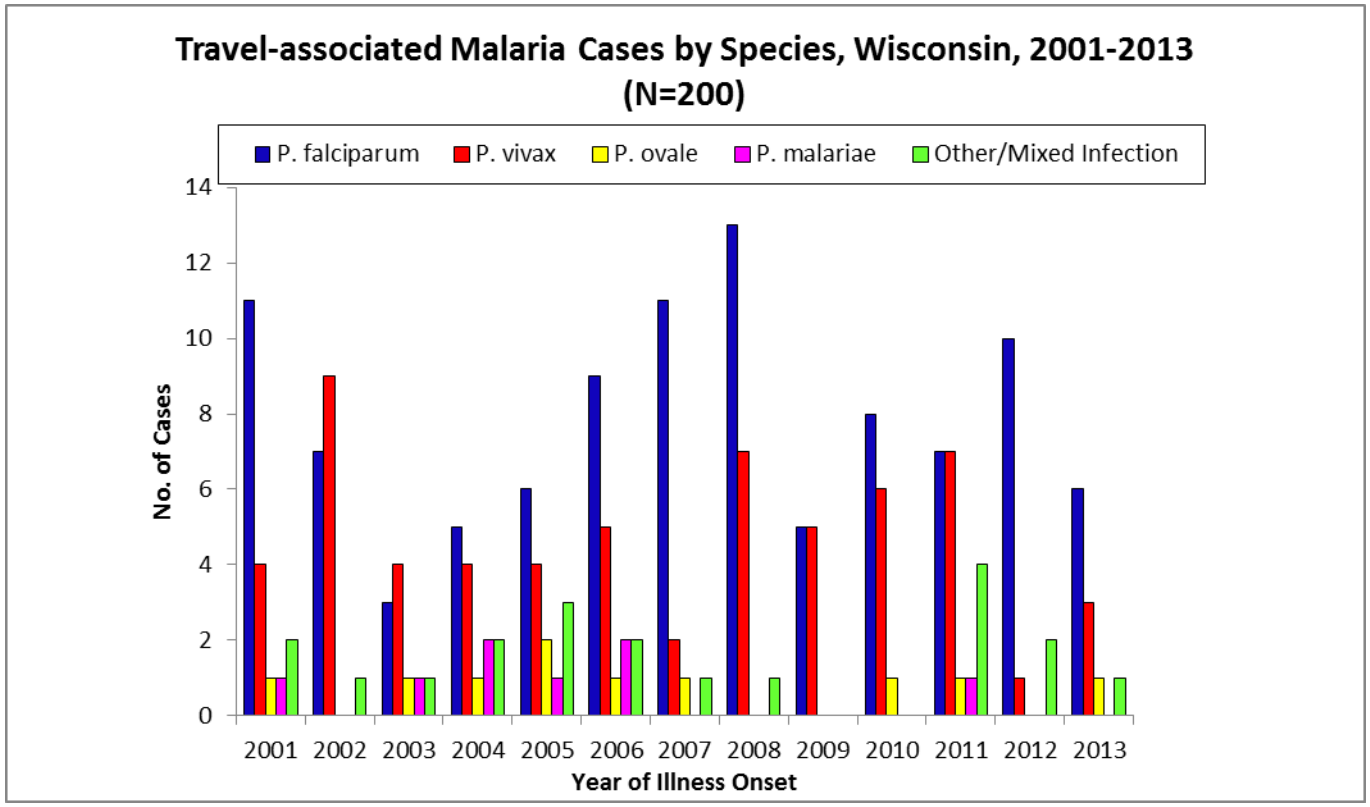


Plasmodium spp.

- *P. falciparum*
- *P. malariae*
- *P. ovale*
- *P. vivax*

http://cdc-malaria.ncsa.edu/

Plasmodium spp.



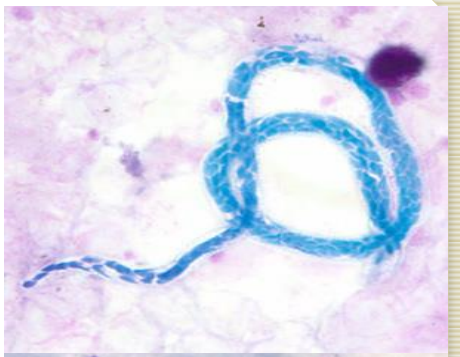
Plasmodium spp.

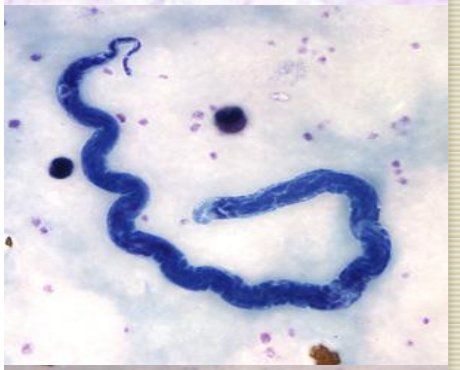
- Incubation
  - 7-30 days
- Disease
  - Periodic fever, chills, body aches, nausea
  - Splenomegaly, hepatomegaly, jaundice
  - Anemia, thrombocytopenia
- Complications
  - Neurological
  - Hemolysis, blood coagulation, kidney failure

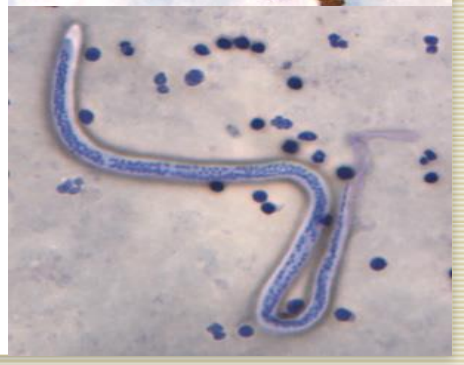
Rarely seen invaders from abroad

Microfilariae

- *Loa Loa*
- *Brugia*
- *Wuchereria*
- *Mansonella*







Something Old and Something New

- Conventional diagnostic testing
  - Microscopy
  - Serology
    - Antigen/ Antibody
- Molecular diagnostic assays implemented at WSLH
  - *Babesia* PCR
  - Malaria PCR
  - *Ehrlichia/ Anaplasma* PCR*

Microscopy- Blood Smears

- Still a tried and true diagnostic method for the detection of blood parasites
- Should be performed by an experienced laboratorian; hematologist or parasitologist
- Important to make blood smears within one hour of blood collection
- Recommended that multiple collections every are made to account for periodicity (8-12 hr)
- See CDC DPDx instructions for the proper creation of thick and thin blood smears

Microscopy

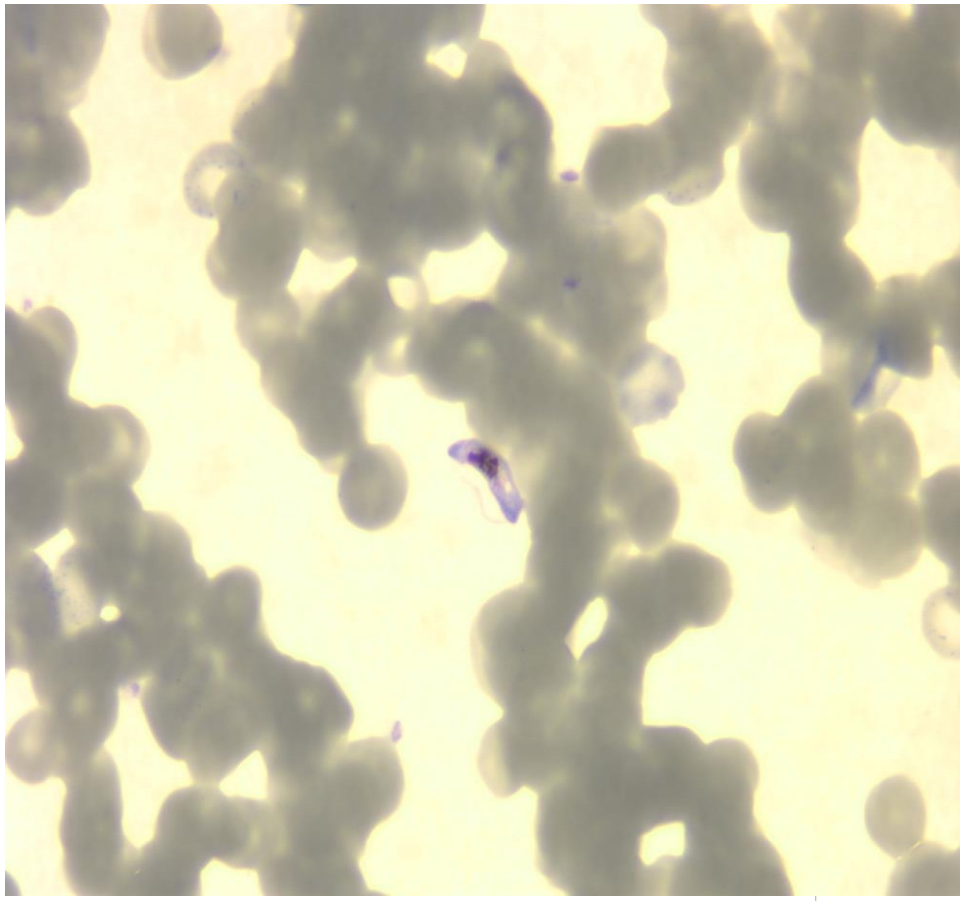
- Important notes about microscopic diagnosis
  - Assess the quality of the smears upon arrival
  - Ensure thick and thin smears are examined
  - If slides were received pre-stained, know what stain was used
  - Examine under both low (10x, for large filarial larvae) and high power (100x, oil) for optimal blood parasite detection
  - Recommended to read a minimum of 300 fields before determining a set of smears parasite free
  - Parasitemia required for malaria and *Babesia*

Malaria- Microscopic Diagnosis

Key characteristics to look for:

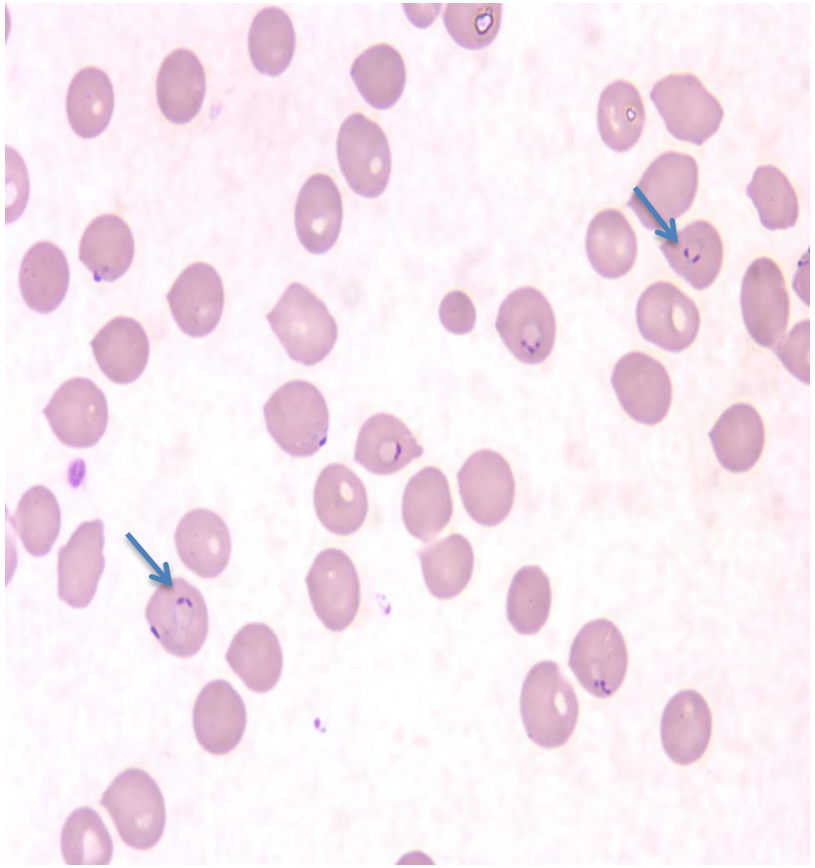
- Stages seen in circulating blood
- Stippling/ Schüffner's dots present
- Size and shape of the red blood cells
  - Normal, enlarged or slightly smaller size
  - Fimbriation present
  - Amoeboid/ misshapen
- Number of merozoites seen in schizont
- Key diagnostic presentations
- Parasitemia (if positive)

Malaria- *P. falciparum*



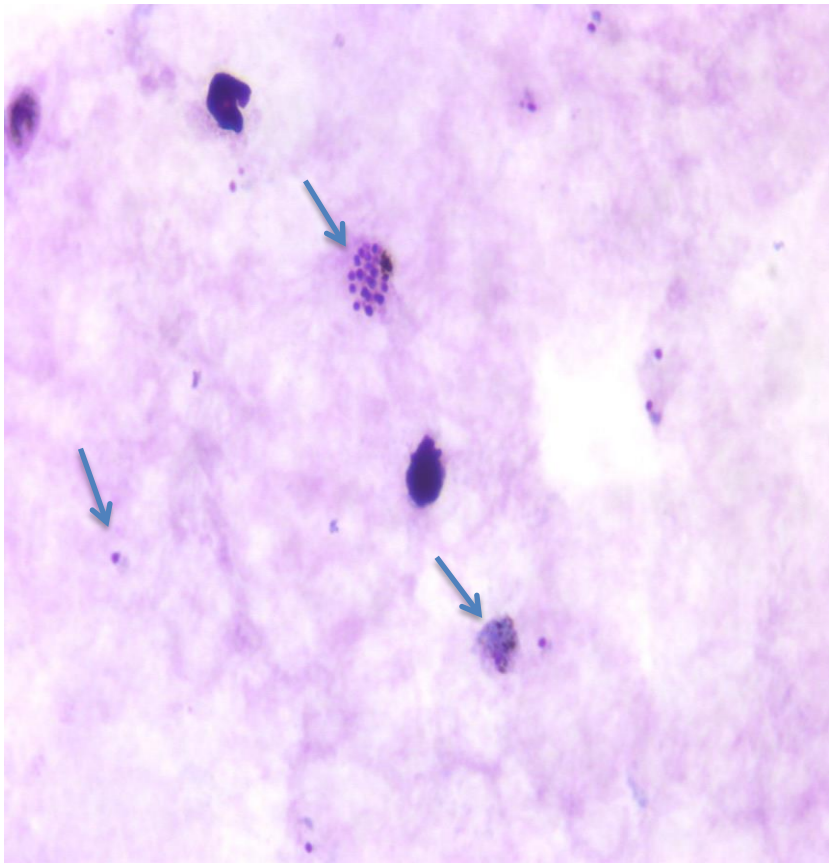
- Gametocyte (Banana-Shaped)
  - If you are fortunate enough to stumble upon these in the course of a blood smear examination, time to buy a lottery ticket!
  - Rarely seen in blood smear examinations
  - Diagnostic for *P. falciparum*

Malaria- *P. falciparum*



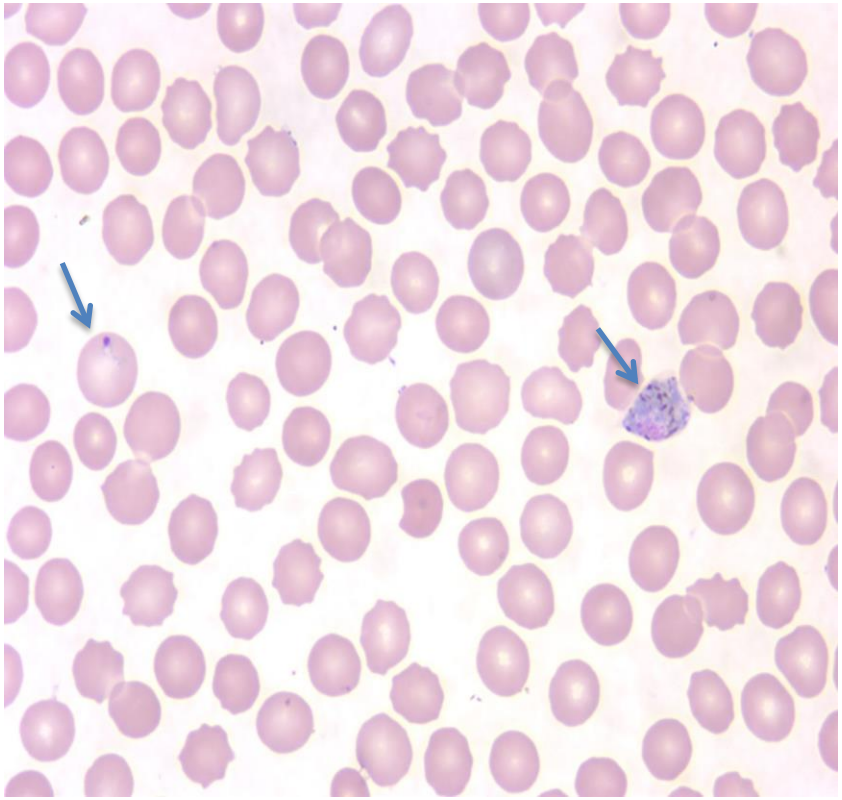
- Only ring forms seen
  - Double chromatin dots common (“headphone” forms- arrows)
  - Delicate and thin normally
- Applique ring forms and multiply-infected RBC’s not uncommon
- Infected RBC’s normal size and shape
- No stippling/ Schüffner’s dots present

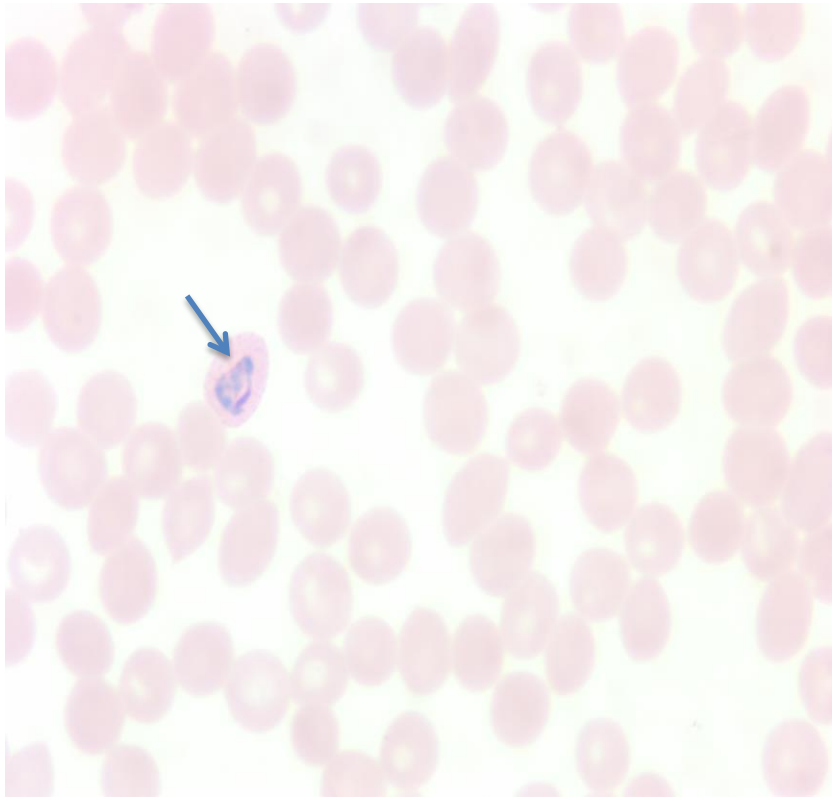
Malaria- *P. vivax*



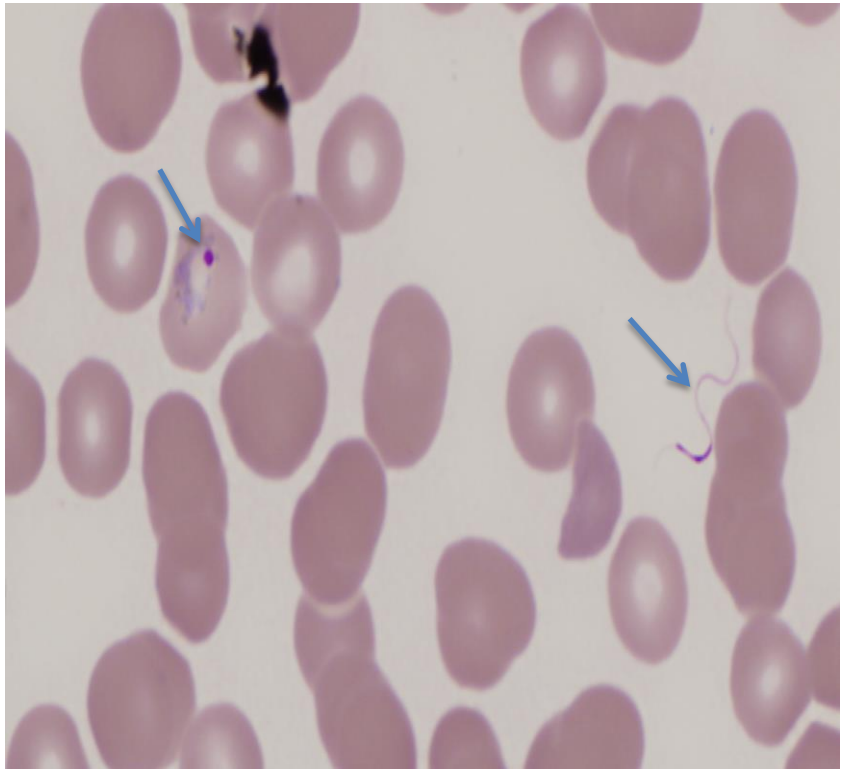
- All stages may be seen in peripheral blood
- Enlarged, amoeboid infected RBC's
- Stippling/ Schüffner's dots usually present
- Thick, heavy rings
- Amoeboid trophozoites
- 12-24 merozoites seen in schizont (16-20 normally; differentiates from *P. ovale*)
- Gametocyte may fill RBC

Malaria- *P. vivax*



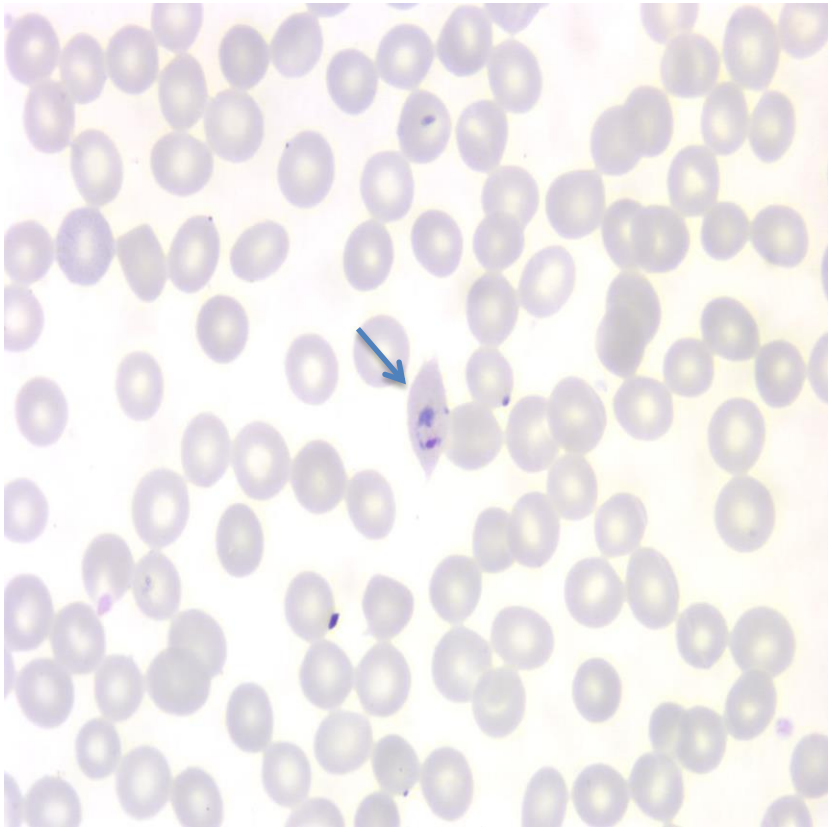


Malaria- *P. vivax* (Atypical)



- Amoeboid trophozoite (L) and an exflagellated microgametocyte (R)
- Exflagellate forms are normally only seen in the gut of the mosquito
- Presence in peripheral blood may indicate significant delay between blood collection and smear preparation
- May be confused with *Borrelia* or *Trypanosoma*

Malaria- *P. ovale*



- May appear very similar to *P. vivax* morphologically
- All stages may be seen in peripheral blood
- Enlarged, amoeboid infected RBC's
- Stippling/ Schüffner's dots usually present
- Thick, heavy rings
- Fimbrionated RBC's common (arrow)
- Schizonts with 6-14 merozoites (usually 6-10)

Malaria- *P. ovale*

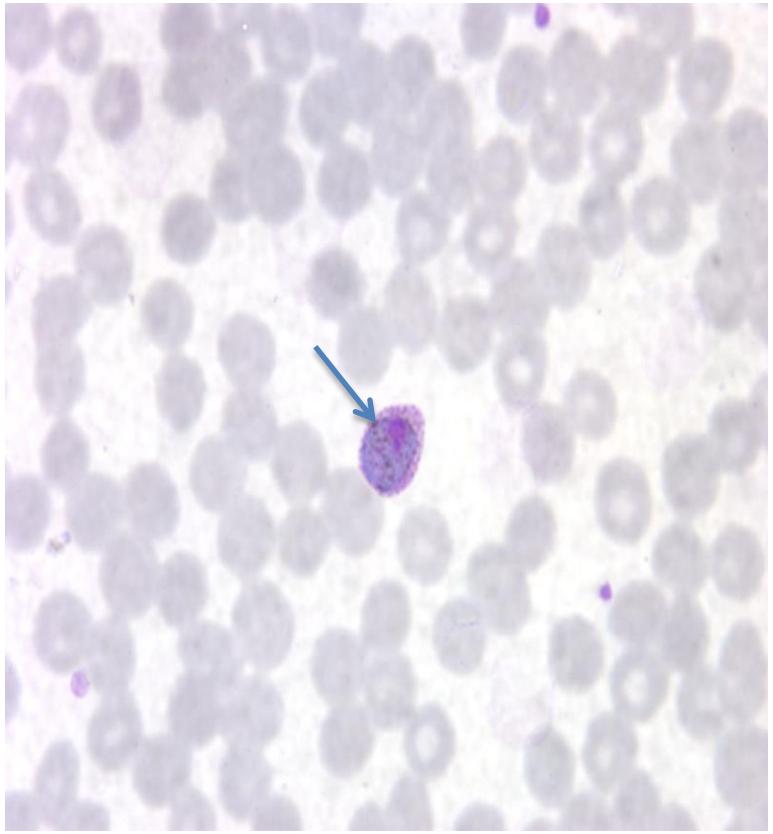
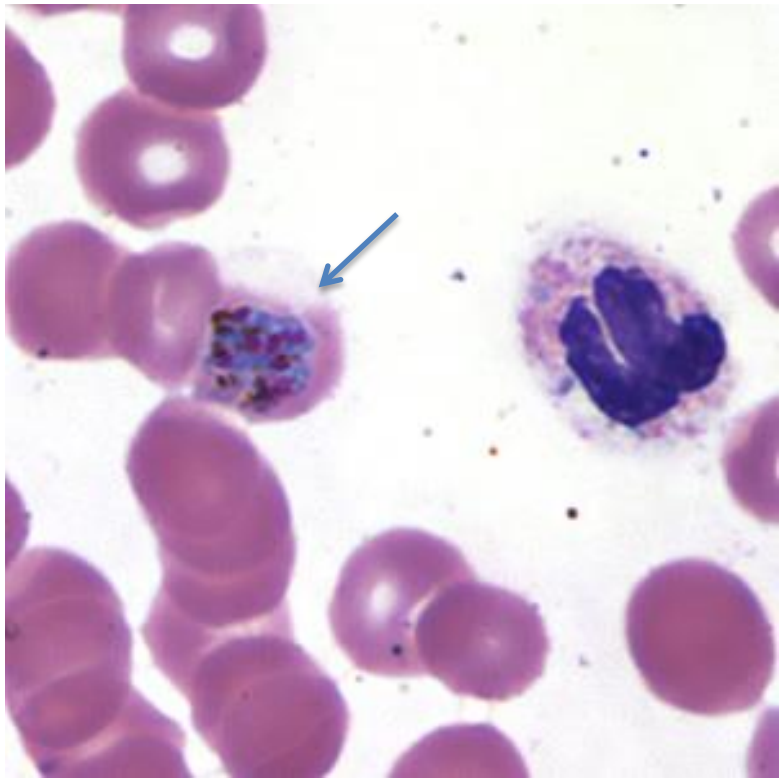
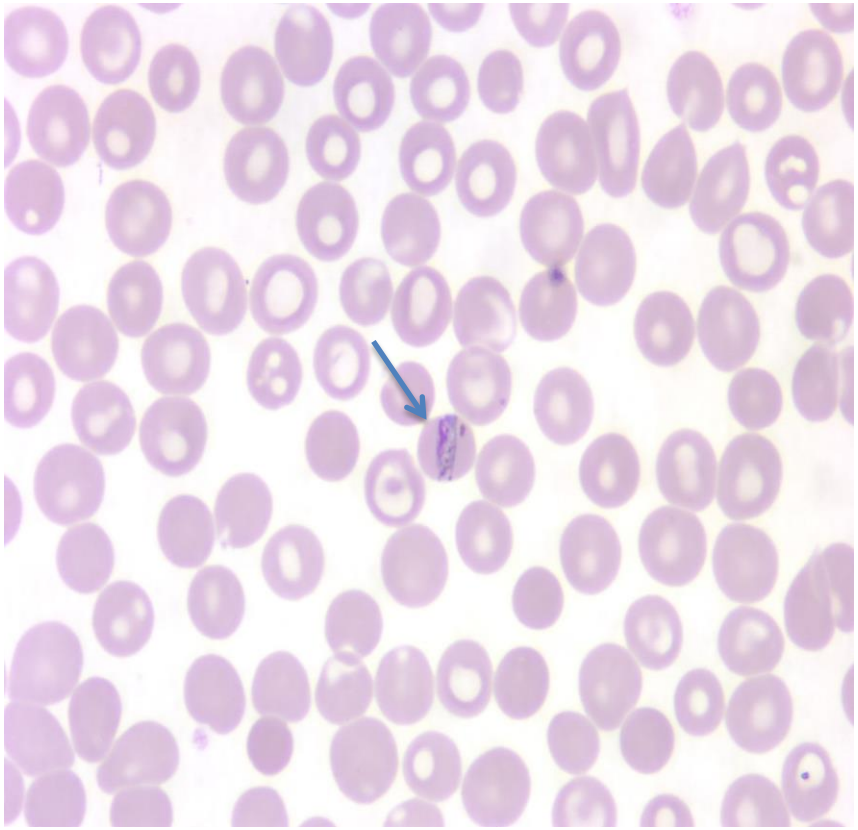


Image from CDC

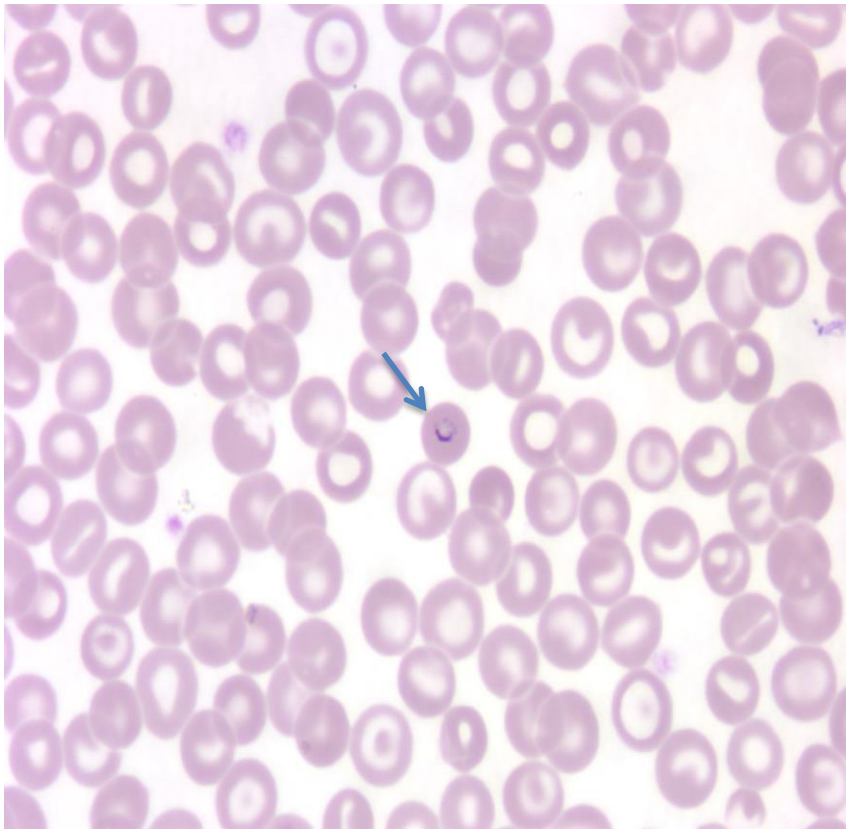


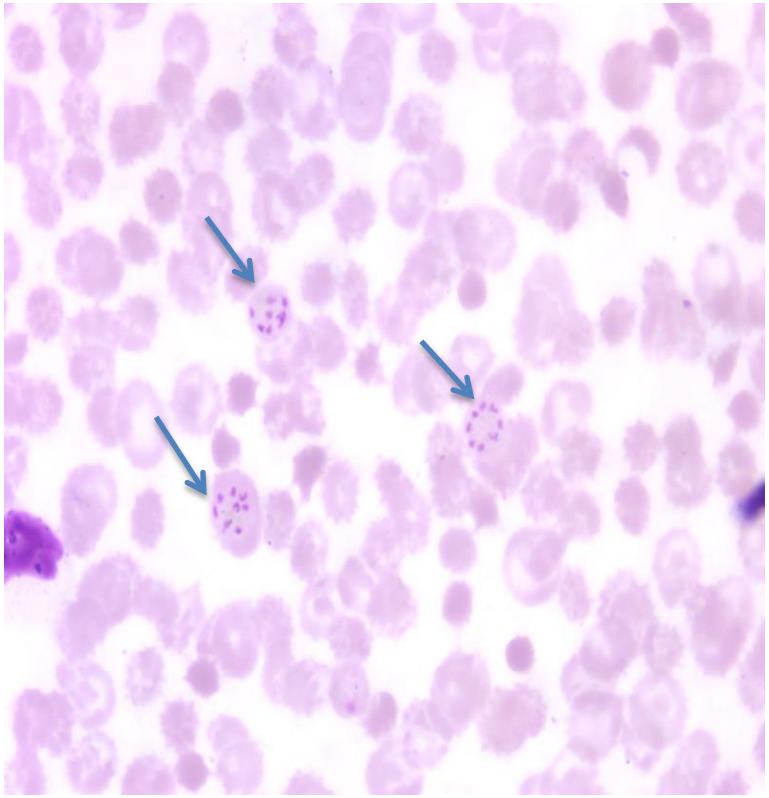
Malaria- *P. malariae*



- All stages may be seen in peripheral blood
- Infected RBC's are normal size to slightly smaller
- Stippling/ Schüffner's dots only found in very early ring stages (rare)
- Band form trophozoite (see arrow left) may aid diagnosis if present
- Scizont with 6-12 merozoites; may form rosette pattern

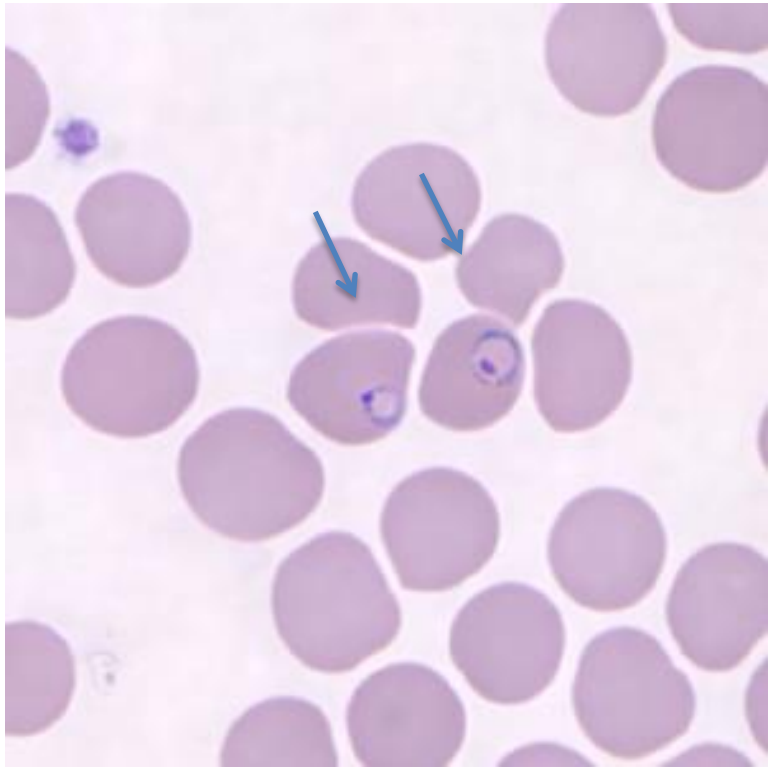
Malaria- *P. malariae*





Malaria- *P. knowlesi*

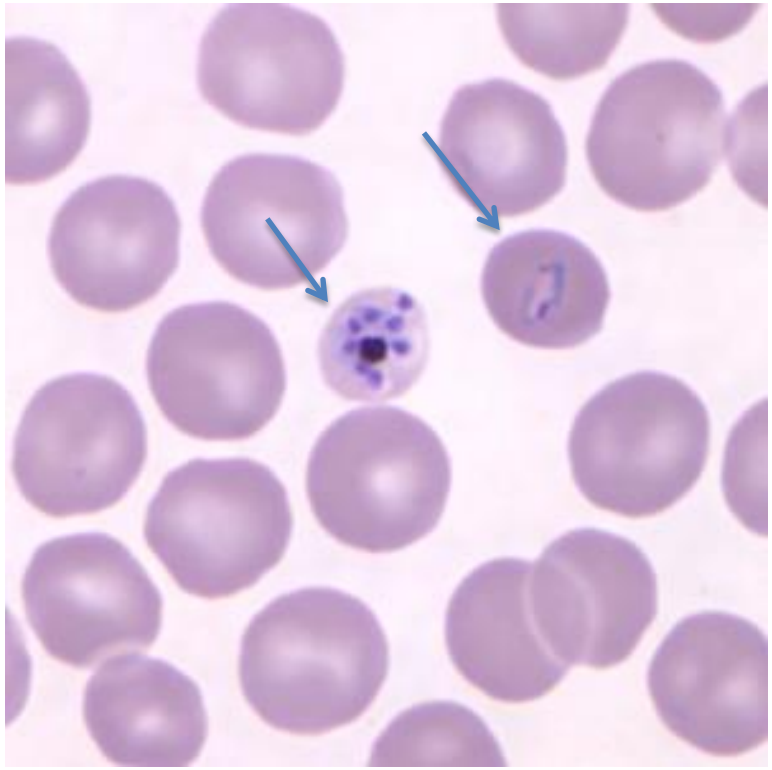
Image from CDC



- Morphologically similar to both *P. falciparum* and *P. malariae*
  - Normal to slightly smaller size infected RBC's
  - Multiply-infected RBC's, double chromatin dot ring forms and occasional applique forms present (like *P. falciparum*)
  - Occasional band form trophs and schizonts with 6-16 merozoites (like *P. malariae*)

Malaria- *P. knowlesi*

Image from CDC



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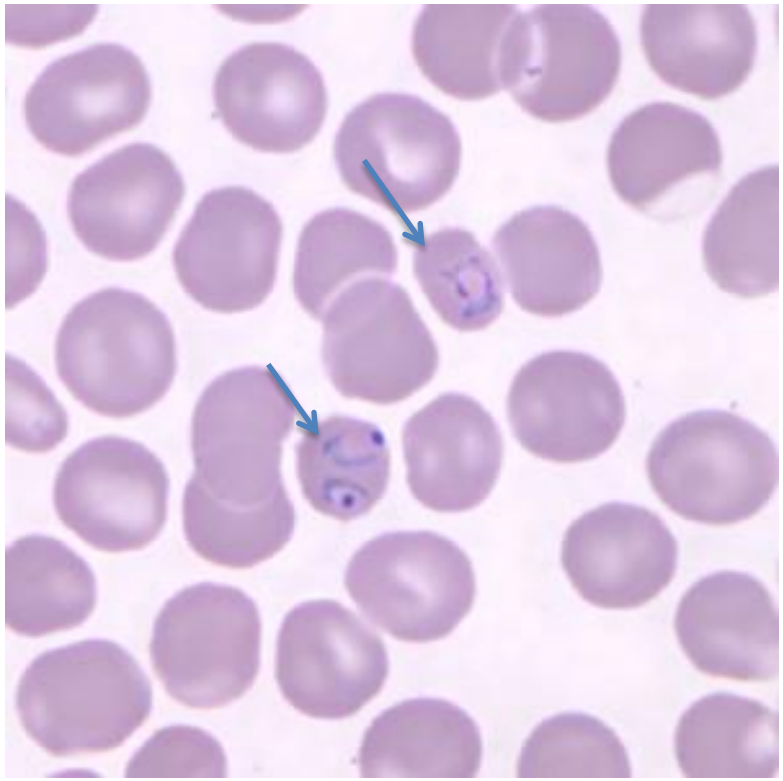
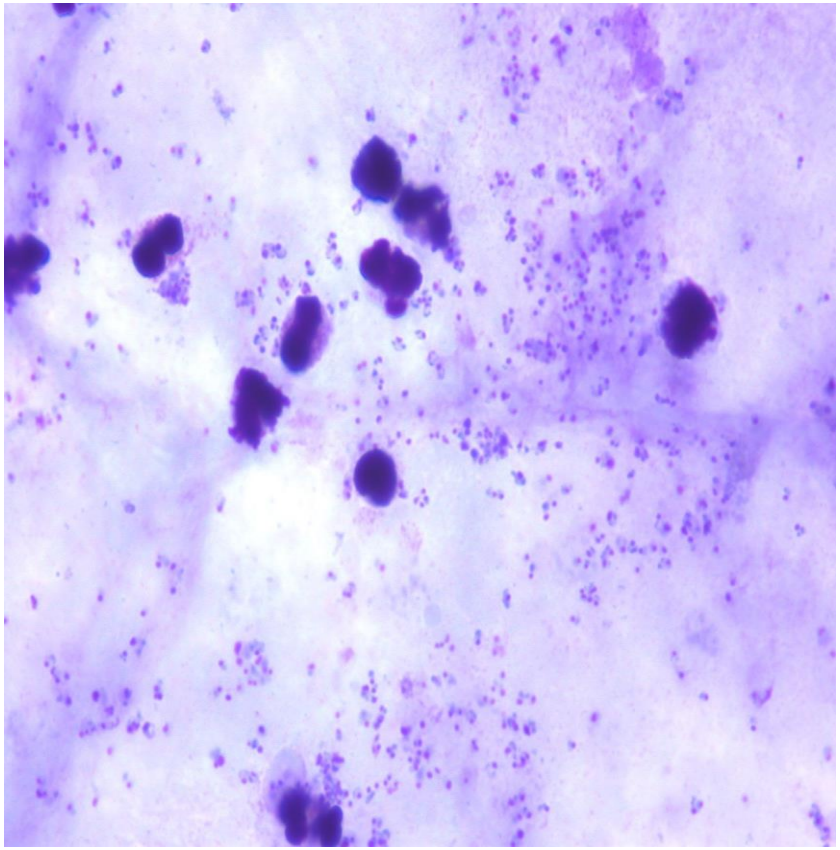


Babesia Morphology



- Only ring forms seen in peripheral blood
- Rings normally very small and delicate
- Multiply-infected (up to 4) RBC's common
  - "Maltese Cross" diagnostic
- Extracellular rings often found
- May be very difficult to differentiate from *P. falciparum* without travel history

Babesia Morphology

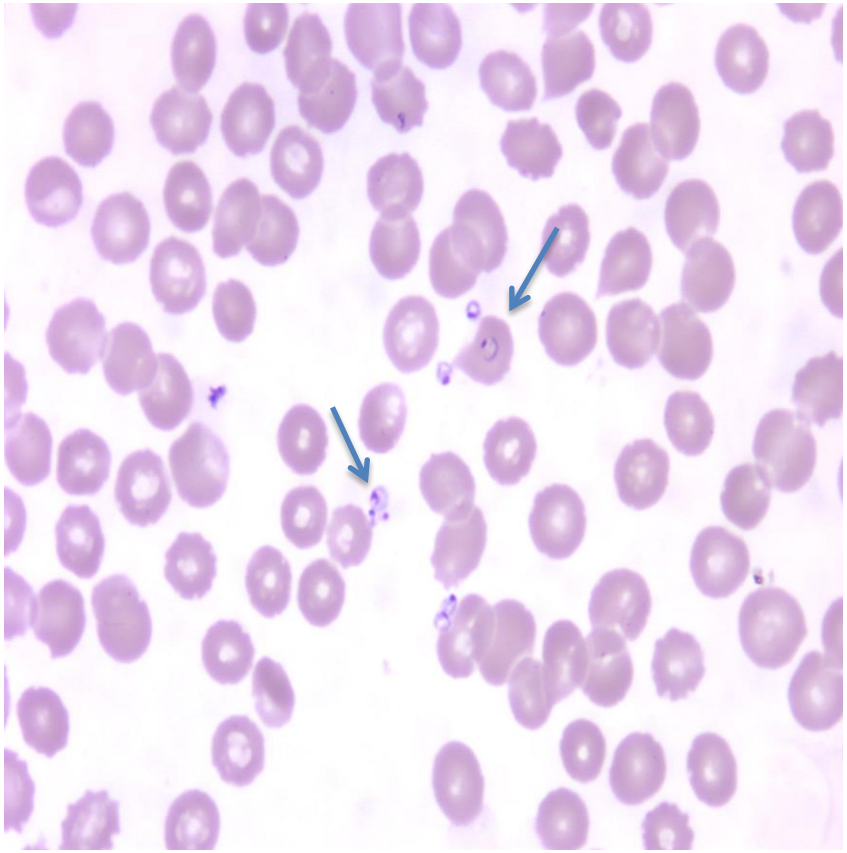
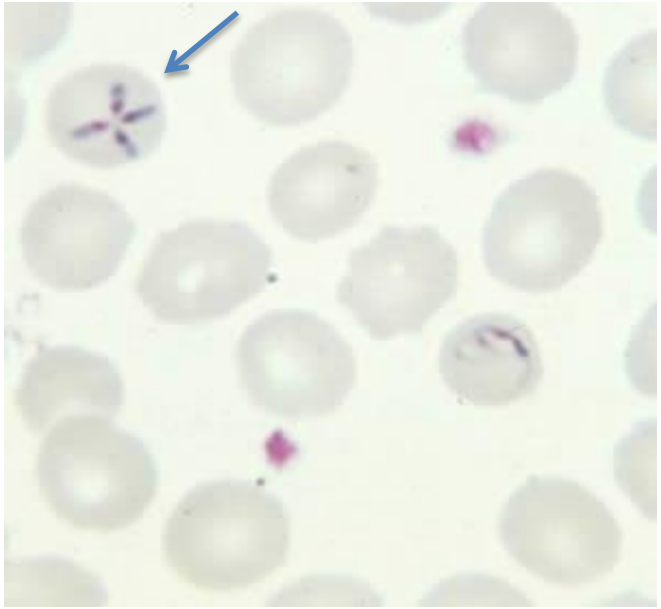
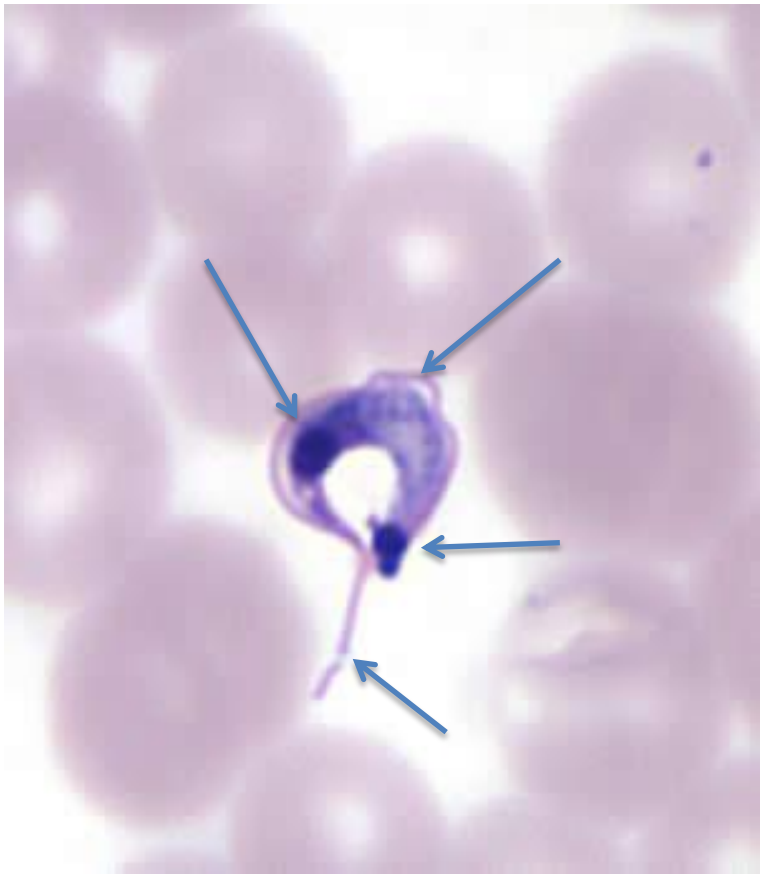


Image from CDC



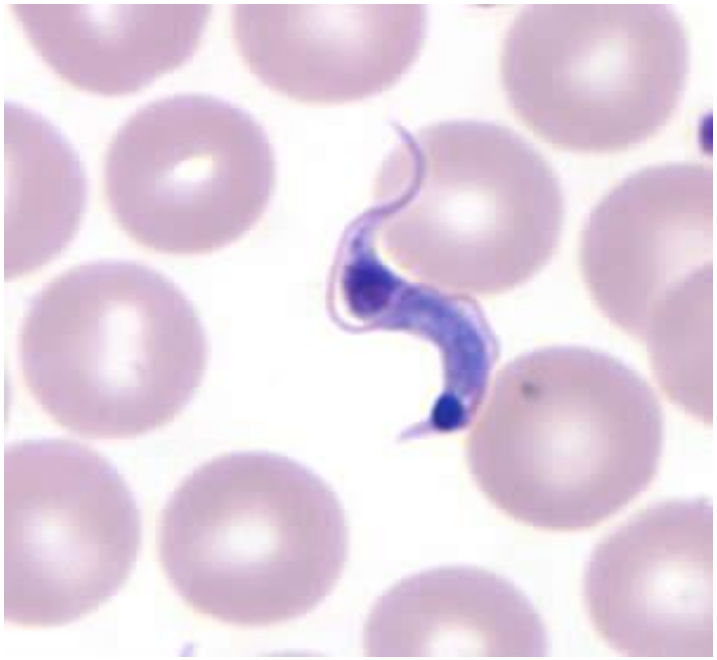
T. cruzi Morphology



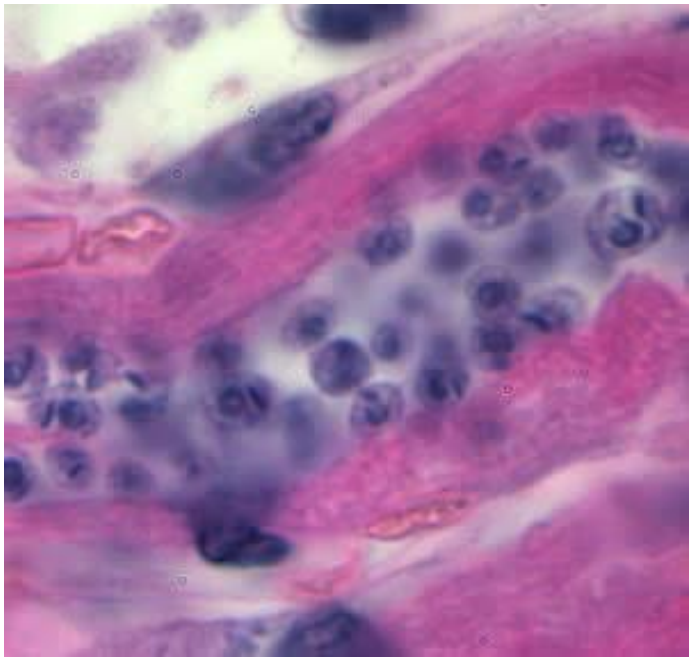
- Typical “C” form trypomastigote
- Notice heavy central kinetoplast and terminal nucleus
- Undulating membrane along length and anterior flagellum present
- Size of trypomastigote is 12-30 µm (differentiate from *T. rangeli*)

T. cruzi Morphology

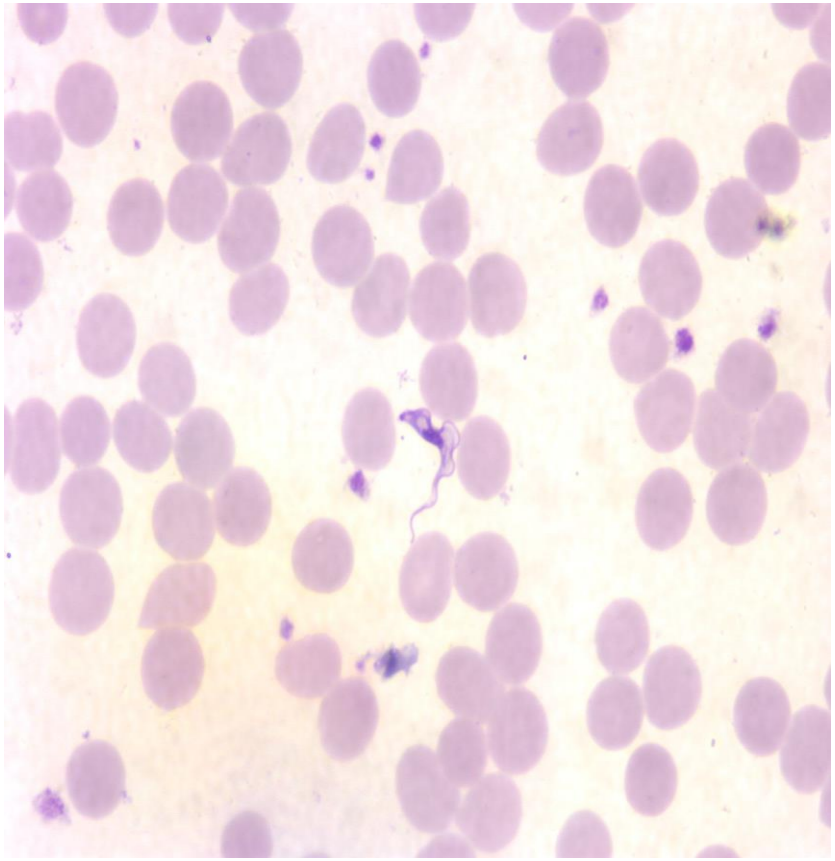
T. cruzi trypomastigote in peripheral blood (image from CDC)



T. cruzi amastigotes in heart tissue (Image from CDC)

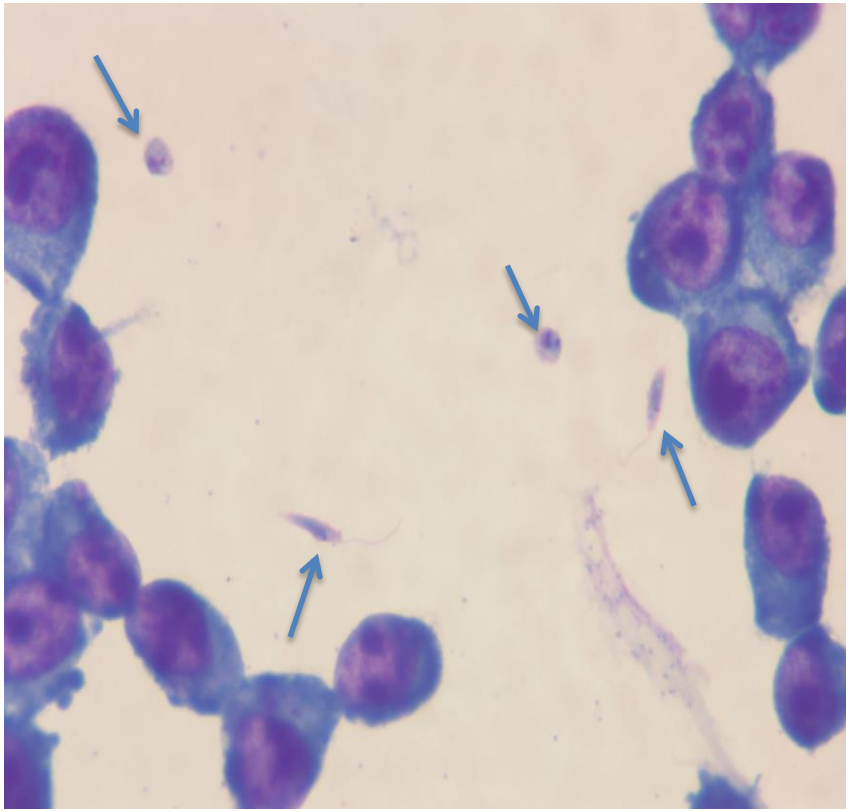


African Trypanosomes Morphology



- *T. brucei rhodesiense* (East Africa) and *T. brucei gambiense* (West Africa) are morphologically identical to one another
- Dividing forms may be seen in peripheral blood (not seen with *T. cruzi*)
- Have undulating membrane and flagellum like *T. cruzi* but much smaller terminal nuclei

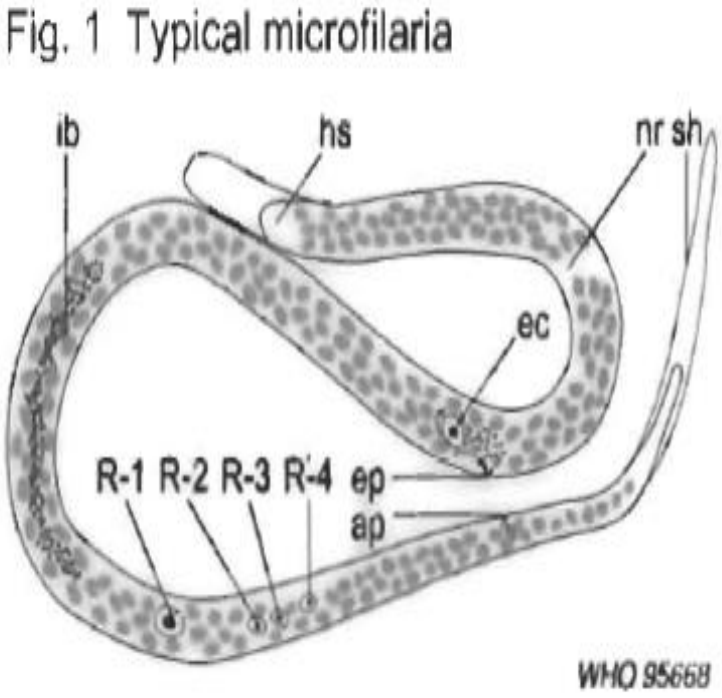
Leishmania Morphology



- Normally only seen in tissue smears
- Amastigote stage seen almost exclusively (downward-pointing arrows to the left)
- Rare, flagellated promastigote stage forms seen (upward-pointing arrows)
- Differentiated at CDC by PCR, antibody detection or isoenzyme analysis

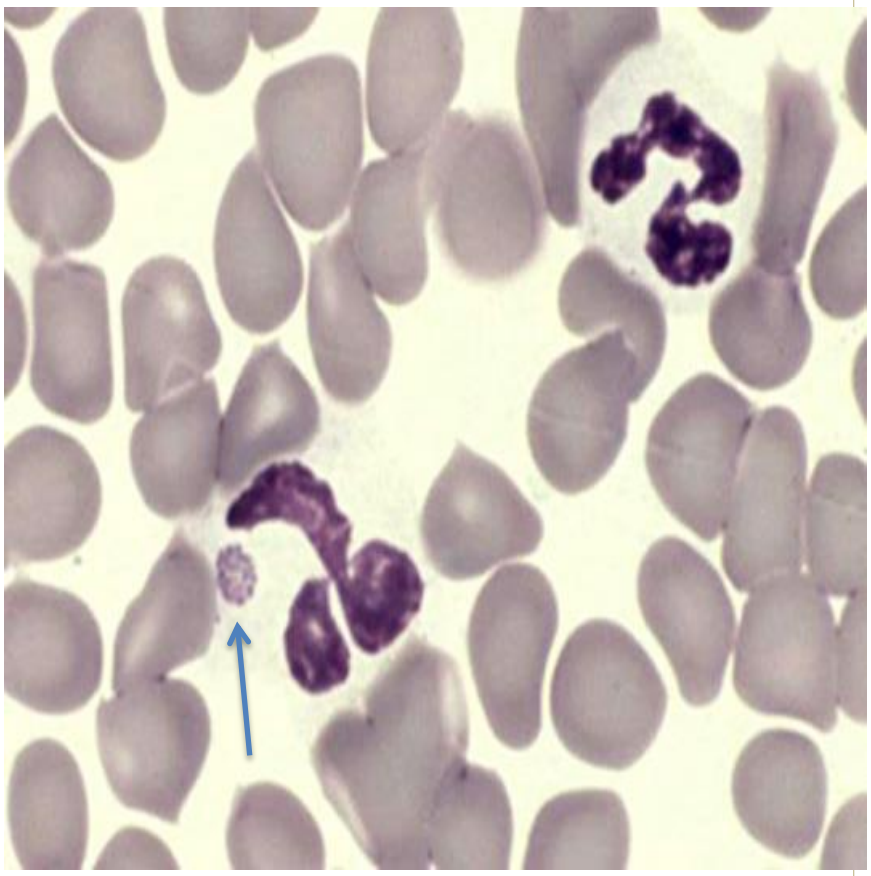
Microfilaria Morphology

Image from CDC



- Key morpological features to note when identifying:
  - Amount of cephalic headspace (hs)
  - Presence of sheath (sh); *visibility stain dependent
  - Posterior nuclei
    - To tip of tail, column, subterminal/ terminal?
  - Size
  - Shape of tail

Ehrlichia/ Anaplasma



- Not officially a blood "parasite"
- May come across these pathogens in blood smear examinations
- Endemic to WI
- Travel/vector history key to diagnosis

Microscopy- Digital Imagery

- Extremely beneficial to utilize a microscope with a digital camera mounted on it
  - Save images of parasites (or artifacts) encountered in rare numbers on a smear
  - Create an image library for in-house reference and training
  - Able to send digital images out for telediagnosis
- Available software allows for measurement, annotation and stamping

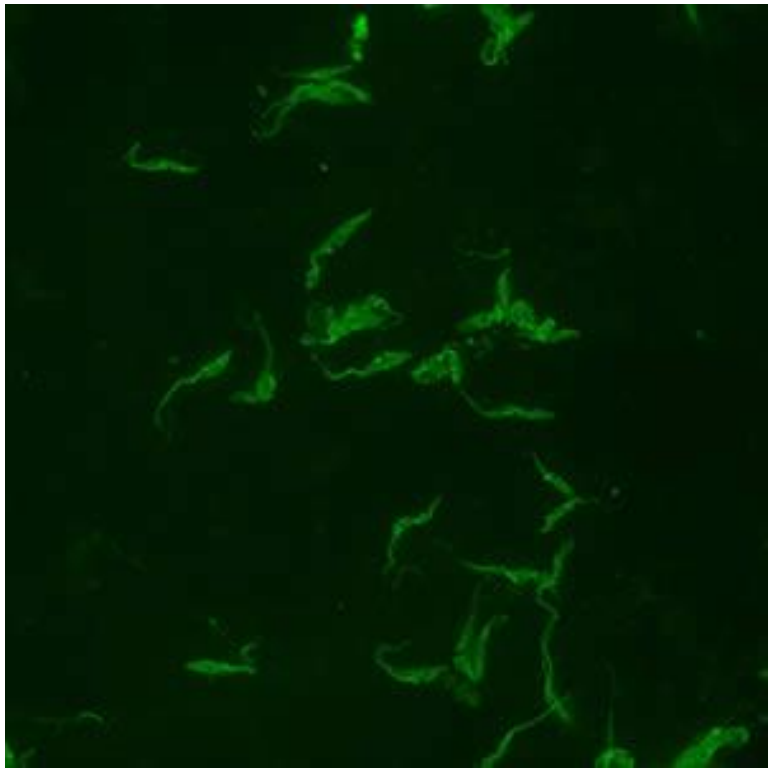
Serology

- No serology tests available at WSLH for diagnosis of blood parasite infections
- CDC offers a limited menu of test options for blood parasites
  - Antigen detection
  - Antibody detection
- Reference laboratories might offer testing for the more common (to the U.S.) parasites

Antibody Detection

- CDC offers a limited menu of antibody tests for blood parasite diagnosis
  - *Babesia* IFA
  - *T. cruzi* IFA
  - *W. bancrofti* and *Brugia malayi* (filariasis) EIA
  - *Leishmania* IFA
  - Malaria IFA
- Call/ email CDC for questions and test requirements
  - (404) 718-4100/ dpd@cdc.gov

T. cruzi Serology- IFA (CDC)



- IFA test available at CDC
- Can cross-react with *Leishmania* species; confirmation recommended by CDC
- CDC also performs FDA-cleared EIA for *T. cruzi*

Antigen Detection- Limited

Organism	Kit name	Manufacturer - distributor[a]	Type of Test[b]
Plasmodium	BinaxNOW® Malaria Test	Inverness Medical	Rapid (HRP2 and aldolase)
	Malaria-Ag	Cellabs	EIA
	OptiMal	Flow	Rapid (LDH)
	MAKROmed malaria test	MAKROmed Manufacturing, LTD	Rapid (HRP2)
	Paracheck Pf	Orchid	Rapid (HRP2)
	Visitect Malaria Pf	Omega Diagnostics LTD	Rapid (HRP2)
Wuchereria bancrofti	ICT Filariasis	Binax	Rapid
	Filariasis Ag-CELISA	Cellabs	EIA

1.Cellabs, P O Box 421, Brookvale, NSW 2100, Australia
Flow, Inc., 6127 SW Corbett, Portland, OR 97201
MAKROmed Manufacturing, LTD, P O Box 28928, Kensington 2101, South Africa
Orchid, 4390 US Route One North, Princeton, NJ 08540
Binax, Inc., 217 Read Street, Portland, ME 04103
2.bEIA = enzyme immunoassay; Rapid = rapid immunochromatographic test
3.From CDC web site

Antigen Detection- BinaxNOW®

- **BinaxNOW® Malaria Test**
  - Rapid immunochromatographic membrane antigen test for the detection of *Plasmodium* species and *P. falciparum* from EDTA blood
  - Results within one hour
  - Should be followed up with thick and thin blood smear examination
  - Not indicated as a screen for asymptomatic populations
  - Antigen may be detected for several days post-malaria elimination (post-treatment)
  - The only available RDT for Malaria in the U.S.; gathered publicity during Ebola preparedness

Antigen Detection

P. falciparum-

99.7% sensitive/94.2% specific

Plasmodium species-

93.5% sensitive/99.8% specific

BinaxNOW® Malaria Test

Molecular Detection- Malaria

JOURNAL OF CLINICAL MICROBIOLOGY, May 2005, p. 2435–2440 Vol. 43, No. 5
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.5.2435–2440.2005

Real-Time PCR for Detection and Identification of *Plasmodium* spp.

Kathy A. Mangold,[1,2] Rebecca U. Manson,[2] Evelyn S. C. Koay,[3,4] Lindsey Stephens,[1] MaryAnn Regner,[1] Richard B. Thomson, Jr.,[1,2] Lance R. Peterson,[1,2] and Karen L. Kaul[1,2]*

Department of Pathology and Laboratory Medicine, Evanston Northwestern Healthcare, Evanston,[1] and Northwestern University Feinberg School of Medicine, Chicago,[2] Illinois; Molecular Diagnosis Centre, National University Hospital, Singapore[3]; and Department of Pathology, National University of Singapore, Singapore[4]

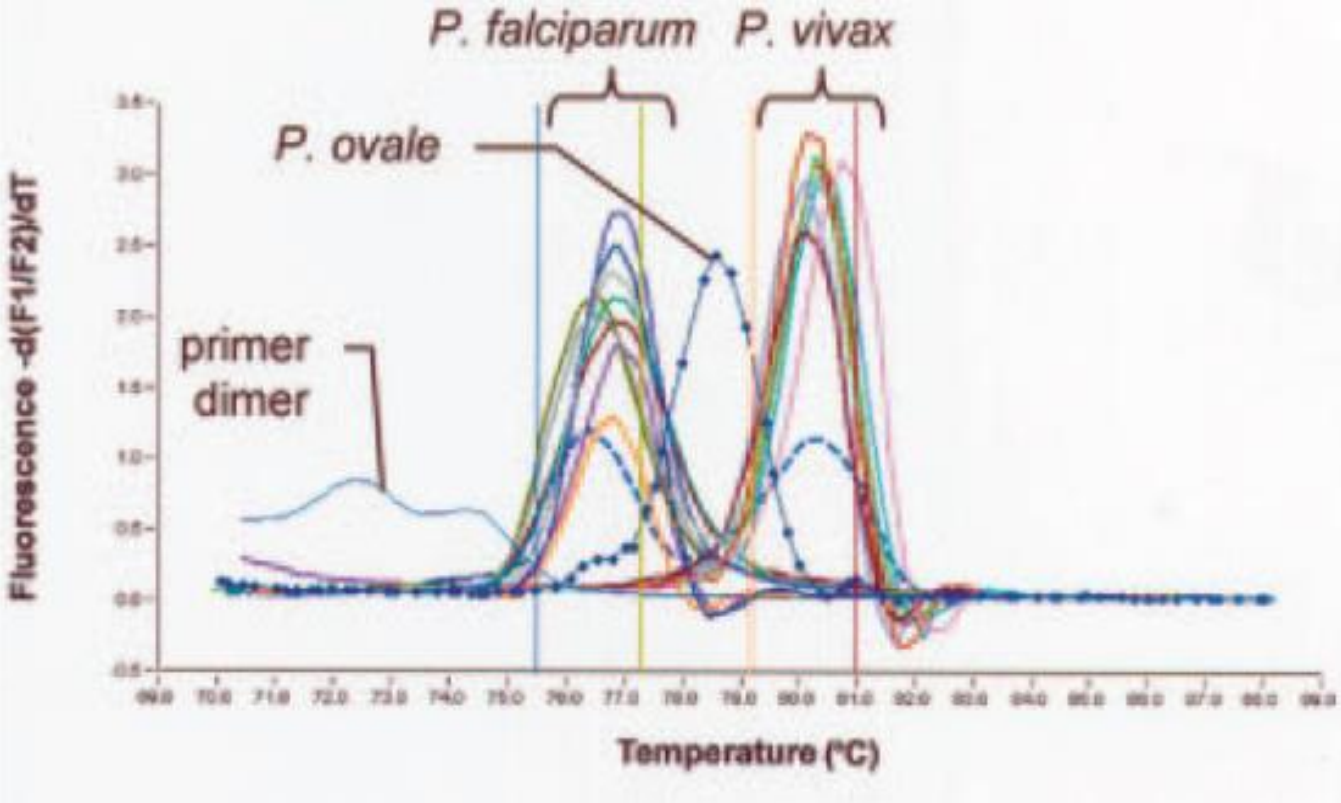
Received 16 September 2004/Returned for modification 9 November 2004/Accepted 6 January 2005

Rapid and accurate detection of malaria parasites in blood is needed to institute proper therapy. We developed and used a real-time PCR assay to detect and distinguish four *Plasmodium* spp. that cause human disease by using a single amplification reaction and melting curve analysis. Consensus primers were used to amplify a species-specific region of the multicopy 18S rRNA gene, and SYBR Green was used for detection in a LightCycler instrument. Patient specimens infected at 0.01 to 0.02% parasitemia densities were detected, and analytical sensitivity was estimated to be 0.2 genome equivalent per reaction. Melting curve analysis based on nucleotide variations within the amplicons provided a basis for accurate differentiation of *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. For assay validation, 358 patient blood samples from the National University Hospital in Singapore and Evanston Northwestern Healthcare in Illinois were analyzed. Of 76 blinded patient samples with a microscopic diagnosis of *P. falciparum*, *P. vivax*, or *P. ovale* infection, 74 (97.4%) were detected by real-time PCR, including three specimens containing mixed *P. falciparum*-*P. vivax* infections. No *Plasmodium* DNA was amplified in any of the 82 specimens sent for malaria testing but that were microscopically negative for *Plasmodium* infection. In addition, 200 blood samples from patients whose blood was collected for reasons other than malaria testing were also determined to be negative by real-time PCR. Real-time PCR with melting curve analysis could be a rapid and objective supplement to the examination of Giemsa-stained blood smears and may replace microscopy following further validation.

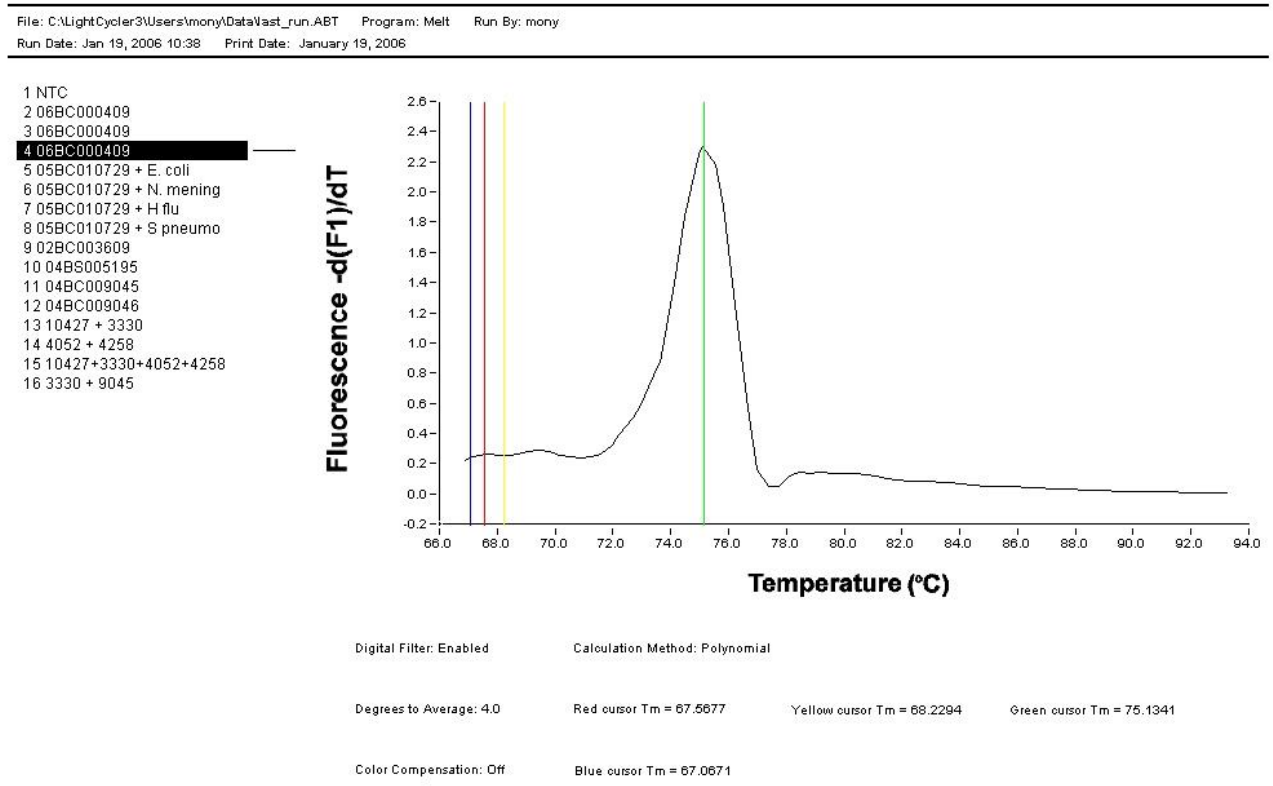
Molecular Detection- Malaria

- Roche LightCycler®-based real time PCR assay implemented at WSLH
- Specimen type: EDTA blood
- Validated for speciation of *Plasmodium* found in the microscopic exam of stained blood smears
- Ability to detect mixed infections
- Aid in the rule out of *Babesia* species infection

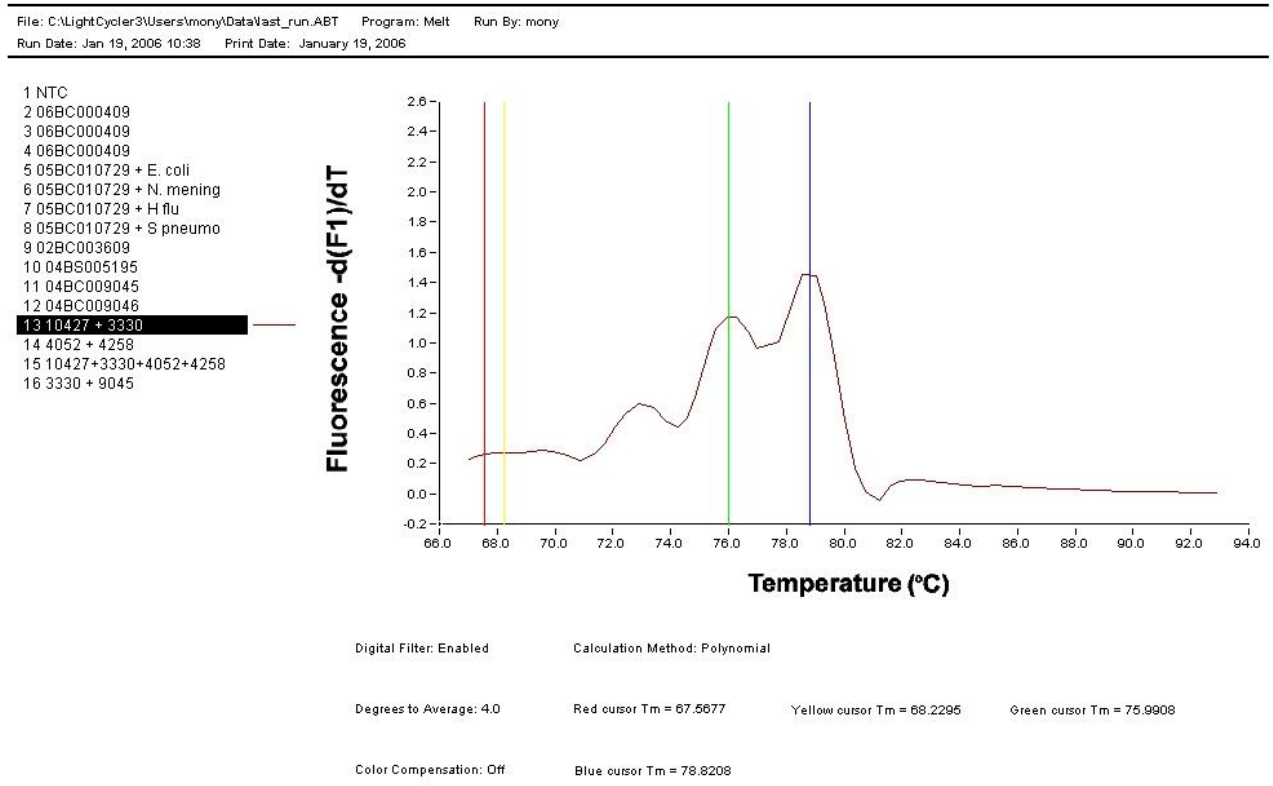
Malaria PCR- Melt Curve Analysis



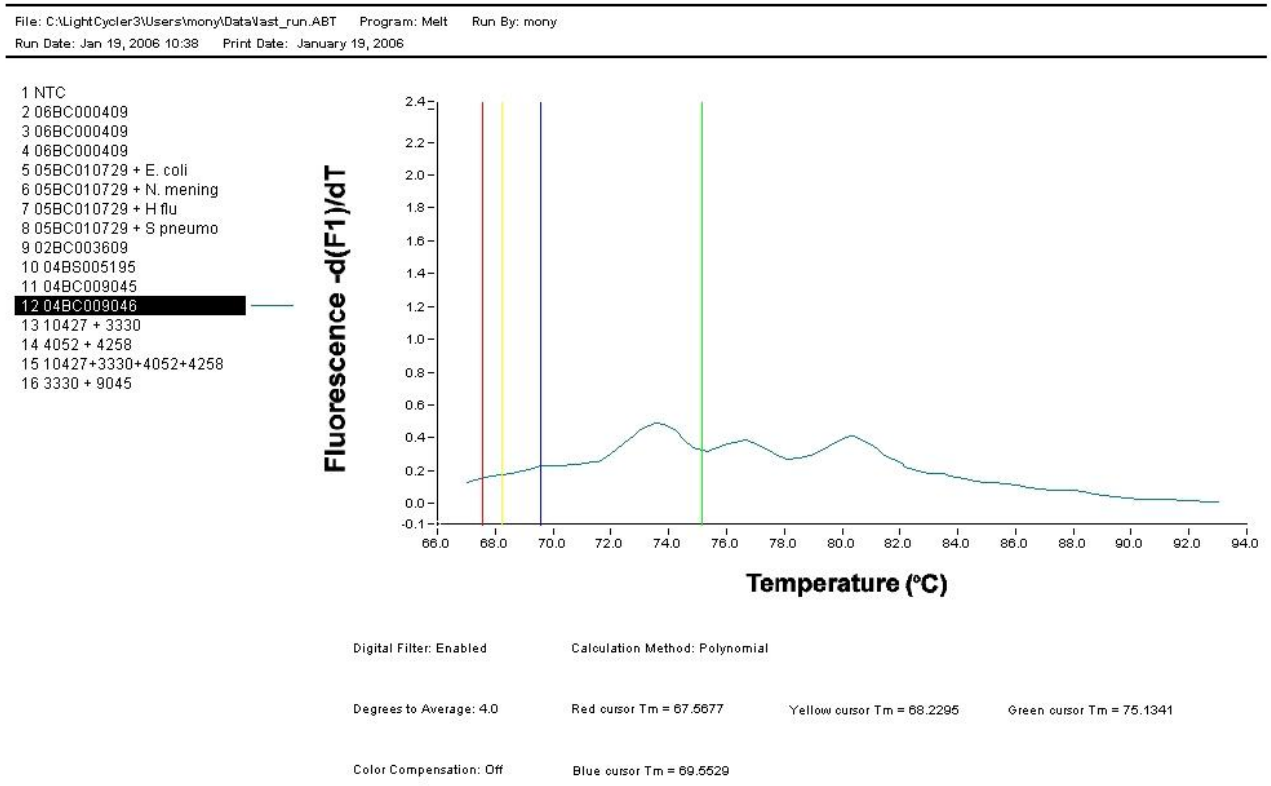
Malaria PCR- Positive Result



Malaria PCR- Mixed Infection



Malaria PCR- *Babesia* Positive



Malaria PCR- New Developments

- New test in development at WSLH; looking at assays developed at CDC and the Wadsworth Center in New York
- Intention is to implement an ABI 7500 Fast-based assay with specific targets for the four common human *Plasmodium* species
- Will keep ability to detect mixed infections while gaining sensitivity
- Will announce change…stay tuned!

Babesia PCR- Implementation

JCM
Journals.ASM.org

A New Real-Time PCR Assay for Improved Detection of the Parasite *Babesia microti*

Allen E. Teal, Andrea Habura, Jill Ennis, Janet S. Keithly, and Susan Madison-Antenucci
Division of Infectious Diseases, Wadsworth Center, New York State Department of Health, Albany New York, USA

Babesiosis is an emerging zoonosis with important public health implications, as the incidence of the disease has risen dramatically over the past decade. Because the current gold standard for detection of *Babesia* is microscopic examination of blood smears, accurate identification requires trained personnel. Species in the genus cannot be distinguished microscopically, and *Babesia* can also be confused with the early trophozoite stage (ring forms) of *Plasmodium* parasites. To allow more accurate diagnosis in a format that is accessible to a wider variety of laboratories, we developed a real-time PCR assay targeting the 18S rRNA gene of *Babesia microti*, the dominant babesiosis pathogen in the United States. The real-time PCR is performed on DNA extracted from whole-blood specimens and detects *Babesia microti* with a limit of detection of ~100 gene copies in 5 μl of blood. The real-time PCR assay was shown to be 100% specific when tested against a panel of 24 organisms consisting of *Babesia microti*, other *Babesia* species, *Plasmodium* species, tick-borne and other pathogenic bacteria, and other blood-borne parasites. The results using clinical specimens show that the assay can detect infections of lower parasitemia than can be detected by microscopic examination. This method is therefore a rapid, sensitive, and accurate method for detection of *Babesia microti* in patient specimens.

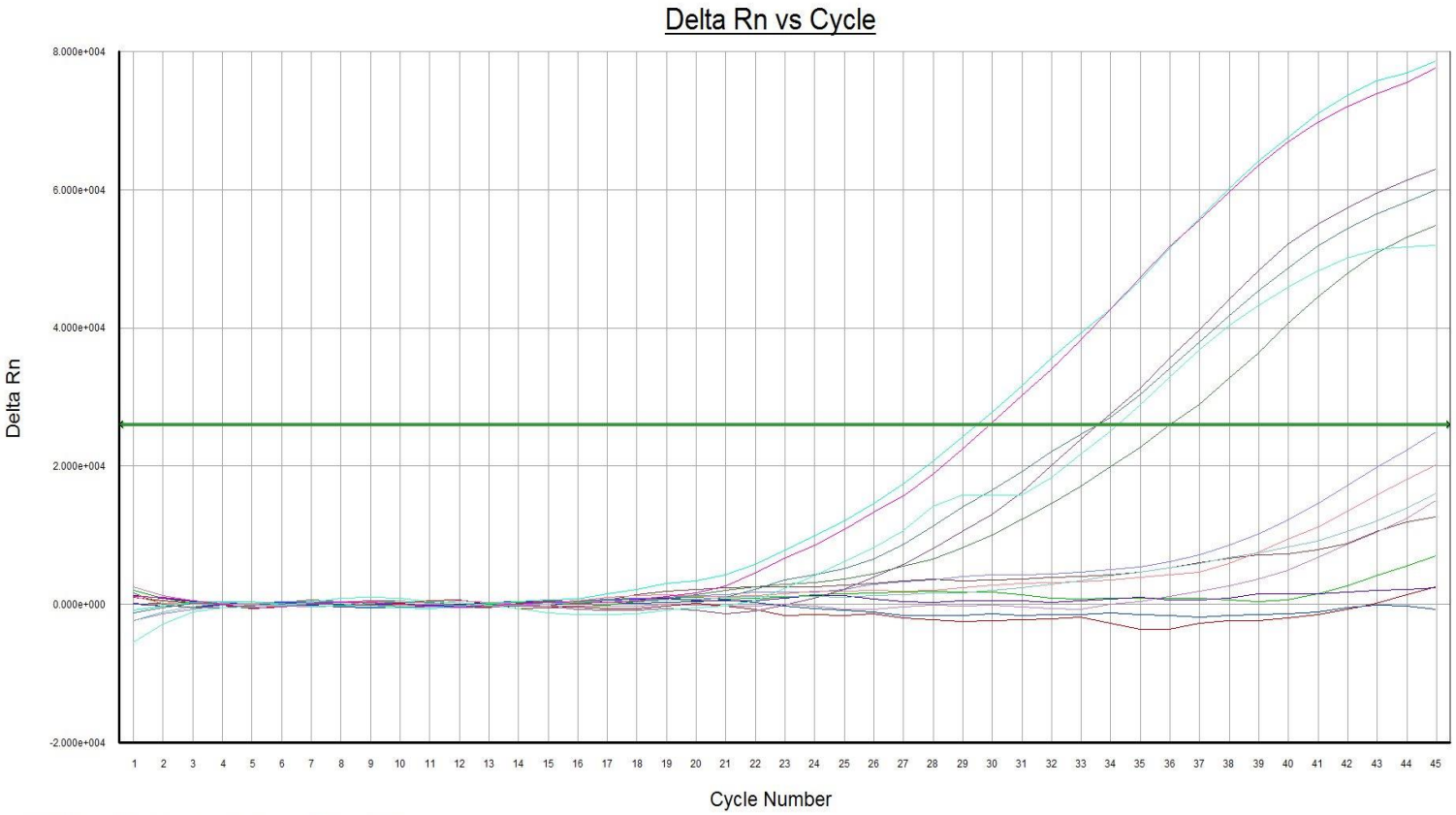
Received 19 September 2011 Returned for modification 12 October 2011
Accepted 5 December 2011
Published ahead of print 14 December 2011
Address correspondence to Susan Madison-Antenucci, sxm31@health.state.ny.us.

doi:10.1128/JCM.05848-11

Babesia PCR- WSLH

- New test offered for 2015
- ABI 7500 Fast-based real time PCR assay
- Ability to confirm the presence of *Babesia microti* in cases with positive smear results
- Ask that all positive *Babesia* blood specimens be submitted to WSLH for confirmation of *B. microti* by PCR and submission to CDC for further studies; Can use Dunham Express courier for fee exempt overnight submission

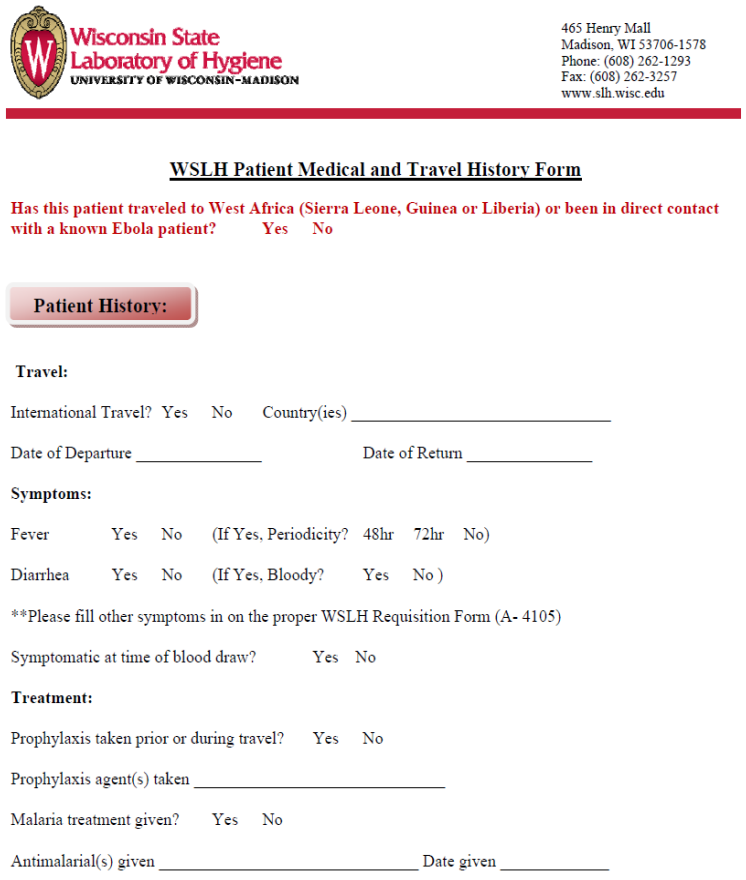
Babesia Real Time PCR



Blood Specimen Submission

- All *Plasmodium*- and *Babesia*-positive blood specimens are asked to be sent to WSLH for molecular speciation and submission to CDC for surveillance testing
  - Fee exempt confirmation of positive specimens
  - Susceptibility testing of *Plasmodium* species at CDC if the parasite is viable
  - Please submit thick and thin blood smear slides (stained or unstained) with EDTA blood

WSLH Patient Medical and Travel History Form



Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN-MADISON

465 Henry Mall
Madison, WI 53706-1578
Phone: (608) 262-1293
Fax: (608) 262-3257
www.slh.wisc.edu

WSLH Patient Medical and Travel History Form

Has this patient traveled to West Africa (Sierra Leone, Guinea or Liberia) or been in direct contact with a known Ebola patient? Yes No

Patient History:

Travel:

International Travel? Yes No Country(ies) _____

Date of Departure _____ Date of Return _____

Symptoms:

Fever Yes No (If Yes, Periodicity? 48hr 72hr No)

Diarrhea Yes No (If Yes, Bloody? Yes No)

**Please fill other symptoms in on the proper WSLH Requisition Form (A- 4105)

Symptomatic at time of blood draw? Yes No

Treatment:

Prophylaxis taken prior or during travel? Yes No

Prophylaxis agent(s) taken _____

Malaria treatment given? Yes No

Antimalarial(s) given _____ Date given _____

- Available on WSLH external web site
- All blood parasite diagnostic test requests should have this completed form submitted with the WSLH req form (A)
- Required for Ebola-endemic countries

Resources

Wisconsin State Laboratory of Hygiene

- Contact WSLH CDD Customer Service

Dunham Express Courier

- (800)236 – 7127
- Account 7271
- Next day delivery except on Sat/Sun
- Call WSLH customer service **(800)862-1013** during normal work hours or the WSLH pager service **(800)263-3280** after hours or weekends to discuss STAT testing

Resources

Centers for Disease Control and Prevention- Division of Parasitic Disease

- DPDx- Site maintained by the Division of Parasitic Diseases and Malaria (DPDM)
  - http://www.cdc.gov/dpdx/
- Can submit digital images to DPDx via email for telediagnosis; generally they ask that you fill out specimen submission form 50.34 (available from their web site or from WSLH)

Resources

- Information regarding which tests CDC offers can be acquired from the CDC web site:
  - http://www.cdc.gov/laboratory/specimen-submission/list.html
- If submitting directly to CDC, we ask that you fill out a WSLH req form (A) and fax it along with the CDC form 50.34 (if filled out) to WSLH (608-890-2548); All reports come back through WSLH; if already in the WSLH system, reporting will be expedited

CDC Test Directory

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People.™

Submitting Specimens to CDC

Test Directory

CDC's Infectious Diseases Laboratories provides an online Test Directory that allows you to identify the right test for your needs. The searchable Test Directory features an up-to-date list of orderable tests and provides information on specimen requirements, contact information, test turnaround times, and other supplemental information. Access the directory here or while completing a Specimen Submission Form.

You may also download a copy [379 pgs, 2.60 MB] (/laboratory/specimen-submission/cdc-lab-tests.pdf) of the entire Test Directory.

Effective December 5th 2014, an updated test directory is available. View the major list of changes here [PDF - 32 KB] (/laboratory/specimen-submission/pdf/TestOrderUpdates-current.pdf).

Search

Narrow the results with a keyword, test title, test synonym, or point of contact:

Go

A (#A) B (#B) C (#C) D (#D) E (#E) F (#F) G (#G) H (#H) I (#I) J (#J) K (#K) L (#L) M (#M) N (#N) O (#O) P (#P) R (#R) S (#S) T (#T) V (#V) Y (#Y)

Showing 354 of 354 tests.

Test Name	Test Code
Acanthamoeba Molecular Detection (/laboratory/specimen-submission/detail.html?CDCTestCode=CDC-10471)	CDC-10471
Actinomyces – Anaerobic ID (/laboratory/specimen-submission/detail.html?CDCTestCode=CDC-10483)	CDC-10483
Actinomycetes-Aerobic -ID (/laboratory/specimen-submission/detail.html?CDCTestCode=CDC-10148)	CDC-10148

Summary

- *Plasmodium* species, *Babesia microti* and *T. cruzi* (and *Ehrlichia/ Anaplasma*?) might be coming to a microscope slide near you soon
- Microscopy is still an effective way to diagnose bloodborne parasites but new methodologies are available and in use
- Useful diagnostic resources are an email away; telediagnosis can be an effective and efficient means to a quick and accurate diagnosis

Summary

- Patient history remains a critical piece of the diagnostic identification process for blood parasites; patient health and travel history should accompany all test requests
  - Can use the WSLH patient health and travel history form on the WSLH web site
  - Testing will not begin until history is obtained
  - Special precautions will be taken for those patients that have traveled to Ebola outbreak countries in Africa

Contact Information

WSLH

Ann Valley- ann.valley@slh.wisc.edu

Tim Monson- timothy.monson@slh.wisc.edu

WSLH Customer Service- (800)862-1088

WDPH

Diep Hoang-Johnson- (608)267-0249

Jim Kazmierczak- (608)266-2154

CDC Division of Parasitic Diseases

(404) 718-4100/ dpd@cdc.gov

Thank You!

Google images

Questions?

Google images