COLLEGE of AMERICAN PATHOLOGISTS

Surveys and Anatomic Pathology Education Programs

Blood Parasite
BP-C 2016

Participant Summary

1.0 Credit of Continuing Education Available

This document is available on e-LAB Solutions.

TABLE OF CONTENTS

2016 BP-C
PARTICIPANT SUMMARY/FINAL CRITIQUE

Program Update

Don't Miss Out on this Educational Opportunity!
With your participation in CAP's Surveys programs, *every member of your team* can take part in education activities: earn Continuing Education (CE) credits or receive Self-Reported Training* at no additional charge.

Survey mailing includes an online education activity to earn 1.0 CE credit. To access the activity, see page 18.

**CAP Self-Reported Training activities do not offer CE credit, but can be used towards fulfilling requirements for certification of maintenance by agencies such as the American Society of Clinical Pathology (ASCP). Please verify with your certifying agency to determine your education requirements.*

Evaluation Criteria

Analytes regulated for proficiency testing appear in **bold** type.

Analyte	Evaluation Criteria
Parasite Identification	80% Participant or Referee Consensus
Parasite Screen	80% Participant or Referee Consensus

The CAP is required to submit PT results to the Centers for Medicare and Medicaid Services (CMS) for all labs that have provided a CLIA identification number. If you do not notify the CAP that your lab has discontinued testing of a regulated analyte, **a score of zero will be given**. Your reporting preferences are outlined on the CMS Analyte Reporting Selections document. If new products are ordered and/or canceled, this may affect your reporting selections, so it is recommended that you periodically check this report on e-*LAB* Solutions™, which will always reflect the most up-to-date information. This information can also be obtained by contacting the Customer Contact Center at 800-323-4040, Option 1, or 001-847-832-7000, Option 1 (international).

In the event a result is not graded, a numeric code will appear next to your result. A definition of the code will appear on the first page of your evaluation. Please see "Actions Laboratories Should Take when a PT Result is Not Graded" on page 17.

To provide a timely evaluation of your results, statistics presented in this participant summary report reflect participant data received by the due date.

Blood Parasite Identification

BP-11 Introduction

Thick and thin Giemsa-stained smears were obtained from a 31-year-old immigrant from Sudan. The specimen contained *Trypanosoma brucei*. A response of "*Trypanosoma brucei* (*gambiense* or *rhodesiense*)", "Blood flagellate, NOS, referred for identification" or "Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification" was considered satisfactory.

BP-11

Parasite Identification	Referees (30) No.	Referees (30) %	Participants (440) No.	Participants (440) %
Trypanosoma brucei (*gambiense* or *rhodesiense*)	30	100.0	438	99.5
Parasite Screen	**Referees (19) No.**	**Referees (19) %**	**Participants (781) No.**	**Participants (781) %**
Blood flagellate, NOS, referred for identification	18	94.7	672	86.0
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	-	-	90	11.5

Discussion

Causal Agents

Human African trypanosomiasis (HAT) is caused by two subspecies of *Trypanosoma brucei, T. b. gambiense* (West and Central Africa) and *T. b. rhodesiense* (Eastern and Southeastern Africa). The type subspecies, *T. b. brucei*, does not cause human infection.

Biology and Life Cycle

Trypanosoma brucei ssp. are transmitted by tsetse flies in the genus *Glossina*. When an infected tsetse fly takes a blood meal, metacyclic trypomastigotes are injected into the bloodstream where they transform into bloodstream trypomastigotes. There are two forms of bloodstream trypomastigotes, slender and stumpy. The slender trypomastigotes multiply by binary fission and perpetuate the blood cycle. Stumpy forms are adapted to be picked up by the tsetse fly vector. Within the midgut of the vector, stumpy forms develop into procyclic forms and multiply by binary fission. After a while, some procyclic forms leave the midgut and migrate via the hemocoel to the salivary glands, where they develop into epimastigote and eventually metacyclic forms. Metacyclic forms are non-dividing and are the infectious stage for the vertebrate host. Unlike with *T.cruzi*, there is no amastigote formation in the human host tissue, although *T. brucei* spp. can cross the blood-brain barrier and cause central nervous system involvement.

Diagnosis

Diagnosis of *T. brucei* is made by the finding of trypomastigotes in blood, chancre fluid, lymph node aspirates, bone marrow, and CSF. A wet preparation may be examined for motility. Concentration techniques may increase the chances for a morphologic diagnosis, including centrifugation and examination of the buffy coat.
Trypomastigotes (the only stage seen in the human host) are 14-33 µm long, have a large central nucleus, a small, terminal kinetoplast at the posterior end and a free flagellum leaving the body anteriorly. In stained blood films, it is possible to find divding forms, something not seen in cases with *T cruzi*. Currently, serologic, molecular, and rapid diagnostic (RDT) tests are not routinely available in the United States.

Clinical Significance

According to the World Health Organization in 2014, 3796 cases were reported with fewer than 15,000 estimated cases of HAT.Human infection with *Trypanosoma brucei* presents with two clinical manifestations. In the first, the parasite is found in the peripheral blood and symptoms include fevers, headaches, malaise, and muscle and joint aches. In the second, parasites cross the blood-brain barrier to involve the central nervous system and can be found in the cerebrospinal fluid. During second stage disease, neurologic symptoms develop and mental status declines, eventually leading to coma and death. Disease progresses at different rates depending on which subspecies is involved, with *T. b. gambiense* having a more chronic, indolent course spanning years while *T. b. rhodesiense* progresses more rapidly over a period of months. If left untreated, both forms of African trypanosomiasis are fatal.

Treatment

All people with trypanosomal infection should be treated. First-line therapy depends on stage of disease and subspecies involved. For *T. b. gambiense* infections, pentamidine isethionate is the drug of choice for first-stage disease while nifurtimox-eflornithine combination therapy (NECT) is now standard of care for second-stage Gambiense (HAT). For T*. b. rhodesiense* infections, suramin is the first-line treatment for first-stage disease while melarsoprol is recommended for second-stage disease. These therapies are generally effective yet have varying toxicity profiles. Of note, adverse reactions to melarsoprol can be severe and life-threatening with 5-18% of patients developing an encephalopathic reaction which is fatal in 10-70% of these patients.

References

1. Ash LP, Orihel TC. *Atlas of Human Parasitology*, 5th ed. Chicago, IL: ASCP Press: 2007.
2. Centers for Disease Control and Prevention, Division of Parasitic Diseases (DPD). *Laboratory Identification of Parasites of Public Concern.* http://www.cdc.gov/dpdx
3. Garcia LS. 2007. *Diagnostic Medical Parasitology*, 5th ed., Washington, DC. ASM Press.

BP-12 Introduction

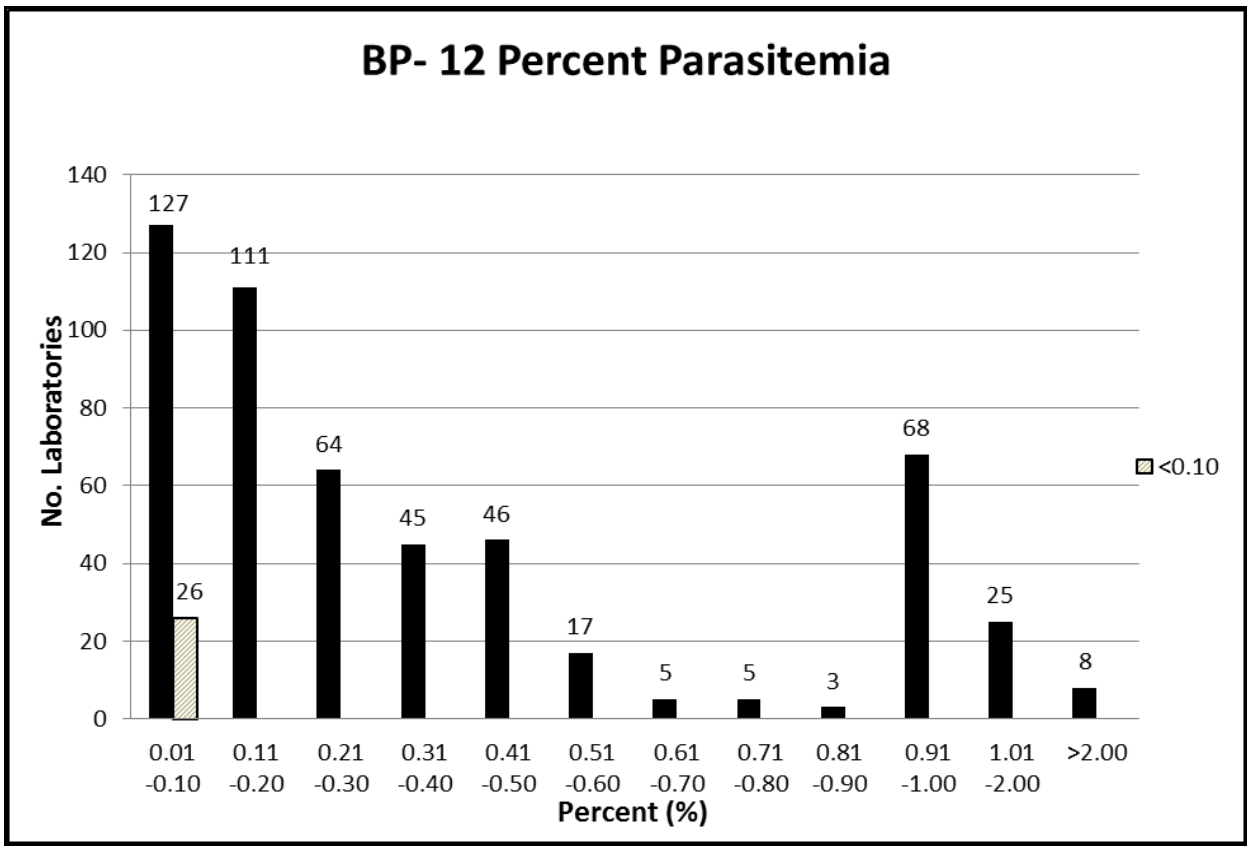
Thick and thin Giemsa-stained smears were obtained from a 19-year-old missionary returning from a month in Honduras. The specimen contained *Plasmodium vivax.* A response of "*Plasmodium vivax", "Plasmodium vivax/ovale,* NOS*", "Plasmodium* sp., NOS would refer or request another specimen, or perform additional molecular testing*","Plasmodium* sp*.,* not *P. falciparum,* referred for identification*"* and *"Plasmodium* sp*./Babesia* sp.seen, referred for identification" was considered satisfactory.

BP-12

Parasite Identification	Referees (30) No.	%	Participants (467) No.	%
Plasmodium vivax	16	53.3	265	56.8
Plasmodium vivax/ovale, NOS	9	30.0	87	18.6
Plasmodium sp., not *P. falciparum*, referred for identification	5	16.7	82	17.6
Plasmodium sp., NOS would refer or request another specimen, or perform additional molecular testing	-	-	11	2.4
Parasite Screen	**Referees (19) No.**	**%**	**Participants (755) No.**	**%**
Plasmodium sp*./Babesia* sp. seen, referred for identification	19	100.0	744	98.5

BP-12

If you have identified a *Plasmodium* sp. or *Babesia* sp.: Report percent of infected RBCs seen (number of infected RBCs per 100 red blood cells). (Ungraded)



Discussion

In the case of an actual patient specimen, both thick and thin blood films should be prepared for evaluation of malaria. Examination of the thick blood film is considered the gold standard for diagnosis because a larger blood volume can be examined enabling the detection of low levels of parasitemia. Thin blood films are helpful with species identification. In this specimen the primary challenge was the identification of *P. vivax*. In this particular challenge, the travel history to Honduras was also important, as transmission of *P. ovale* is not known from the New World.

Key morphologic features on a thin blood film that would suggest the diagnosis of *P. vivax* are:

1. Enlarged size of the infected RBCs compared to the uninfected cells.
2. The ring-form trophozoites usually with sturdy cytoplasm and one or two (often more commonly one) chromatin dots.
3. Developing trophozoites ameboid.
4. Gametocytes round to pleomorphic (in the latter, may appear to 'hug' surrounding RBCs).
5. Schüffner's dots are typically present in all cells except early ring forms, when stained with Giemsa at a pH of.8-7.2.
6. Mature schizonts with 12-24 merozoites.
7. All stages are usually present.

Causal Agents:
There are four species of *Plasmodium* that cause human malaria: *P. falciparum*, *P. malariae*, *P. ovale* (comprised of two subspecies, *P. o. wallikrei* and *P. o. curtisi*), and *P. vivax*. In addition, there are at least six species of simian *Plasmodium* that have been documented to cause zoonotic infections in humans, the most notable being *P. knowlesi*, infections of which appear to be increasing on the Malaysian peninsula.

Plasmodium falciparum occurs nearly worldwide in the tropics and subtropics, particularly in Africa and Southeast Asia. *Plasmodium malariae* also occurs nearly worldwide in the tropics and subtropics, but has a more patchy distribution than *P. falciparum*; most common in tropical Africa, Indian subcontinent, and Southeast Asia. *Plasmodium ovale* occurs primarily in tropical western Africa, but also New Guinea and Southeast Asia; *P. ovale* has not yet been documented from the New World. *Plasmodium vivax* occurs nearly worldwide in the tropics, subtropics, and some regions of northern and eastern Africa, the Middle East, the Indian subcontinent, Southeast Asia, and the Americas.

Biology and Life Cycle:
Plasmodium spp. are transmitted by mosquitoes in the genus *Anopheles*. Infected female mosquitoes inject sporozoites when taking a blood meal. Sporozoites are carried via blood to the liver where they invade hepatocytes and form schizonts. The liver schizonts rupture, releasing large numbers of merozoites that then invade erythrocytes starting the erythrocytic cycle. Early ring forms develop into mature trophozoites and take one of two pathways: 1) they develop into schizonts (which rupture and continue the erythrocytic cycle) or 2) develop into gametocytes. Gametocytes are a dead-end stage in the human host but are required for sexual reproduction in the mosquito. In the mosquito host, microgametocytes (=males) exflagellate and fertilize macrogametocytes (=females), resulting in an ookinete. Ookinetes further develop into oocysts, which when mature rupture and release the infective sporozoites. In *P. falciparum*, late trophozoites and schizonts express a protein on the surface of the erythrocytic membrane causing the infected erythrocyte to sequester to the endothelial lining of capillaries in internal organs. Thus, only ring forms and gametocytes are usually seen in well-prepared peripheral blood smears.

Diagnosis:

The ideal specimen for laboratory identification of malaria is fresh capillary blood from a finger or heel stick, with immediate preparation of thick and thin blood films. Since this is not feasible in most settings, however, venous blood collected in EDTA anticoagulant is also acceptable. It is important to transport the blood as quickly as possible to the laboratory for examination since prolonged exposure to EDTA may result in distortion of the malaria parasites and compromised morphology.

Malaria is primarily diagnosed by the identification of *Plasmodium* parasites on thin and thick blood films stained with Giemsa, Wright, or Wright-Giemsa stain. Molecular methods such as PCR may be employed when a species-level identification cannot be made morphologically or there is morphologic evidence of a mixed infection. Rapid diagnostic tests (RDTs) for antigens such as histidine-rich protein (hrp) and parasite lactate dehydrogenase (LDH) are also commonly used to screen for *P. falciparum* from other malaria infections. Due to lack of analytical sensitivity, RDTs may miss low level malaria infections. Serology is not used for routine diagnosis but may be helpful during transfusion investigations.

Typically, thick films are used for the recognition of *Plasmodium*, with a species-level identification performed on the thin film. Thin films should be read at 1000x magnification with oil for at least 100 microscopic fields. Immunologically naïve patients (eg, returning travelers born in non-endemic areas) may present with stronger clinical manifestations at a lower parasitemia. Severe malaria is currently defined as a parasitemia ≥ 2% in immunologically naïve patients and ≥ 5% in non-naïve patients.

Morphologic Identification:

Two important questions regarding morphologic identification of malaria are:

1) Is it malaria?
2) Is it *Plasmodium falciparum*?

Recognition of *Plasmodium* is based on observing stages of the parasite inside infected red blood cells. In a well-prepared specimen, the cytoplasm will stain blue and the chromatin red. Pigment (which is absent in *Babesia* infections) will present as golden-brown to black flecks. In some species, structures such as Schüffner's stippling or Maurer's clefts may be present when stained at an appropriate pH. Identification of *Plasmodium* to the species level is extremely important for patient management, as different species may be treated differently (for example, it is important to target the liver stages of *P. ovale* and *P. vivax* to prevent relapse of the disease).

The following table compares the morphologic features of the four stages of human *Plasmodium* spp.

Morphologic Criteria	*Plasmodium falciparum*	*Plasmodium malariae*	*Plasmodium ovale*	*Plasmodium vivax*
Size of infected RBC	Normal	Normal to smaller	Enlarged	Enlarged
Rings (early trophozoites)	Common; usually with thin, delicate cytoplasm and double chromatin dots; often multiple rings per infected RBC; appliqué forms common	Cytoplasm sturdy, usually with single, large chromatin dot; occasional 'birds-eye' forms	Cytoplasm sturdy, with 1-2 large chromatin dots	Large, sturdy cytoplasm, usually with large, single chromatin dot
Developing trophozoites	Rare, but may be seen if a delay in processing; form compact, pigment usually evident	Variable; may be compact to elongate (band-form) or pleomorphic and vacuolated (basket-form); pigment coarse	Compact to slightly amoeboid with dark pigment; elongation and fimbriation may be observed	Pleomorphic to grossly amoeboid; pigment diffuse and golden-brown to nearly black
Schizonts	Rare in peripheral blood; 8-24 small merozoites when mature; pigment dark, compact	6-12 merozoites when mature, often in a rosette pattern around central mass of pigment	6-14 merozoites when mature; pigment dark brown to black and discrete when mature; elongation and fimbriation may be present	12-24 merozoites when mature; may fill entire RBC; often noticeable enlarged
Gametocytes	Crescent-shaped; chromatin discrete (macrogametocyte) or diffuse (microgametocyte); Laveran's bib may be present	Small, round, compact; pigment coarse and diffuse	Round to oval, compact; if elongated and fimbriated may not fill entire infected RBC; pigment coarse, dark	Large and round to pleomorphic (may 'hug' surrounding RBCs); may fill most of infected RBC; pigment golden-brown to nearly black
Other Features	Maurer's clefts may be present; ring-form trophozoites usually predominate	Generally smaller; pigment coarse; Ziemann's stippling may be present; all stages seen	Schüffner's stippling may be present at appropriate pH; elongation and fimbriation may be observed; all stages seen	Schüffner's stippling may be present at appropriate pH; enlargement of infected RBCs usually pronounced; all stages seen

Calculating Percent Parasitemia:
The percent parasitemia is very important to calculate for prognostic purposes and also to evaluate response to antimalarial therapy.

Parasitemia can be calculated on a thin blood film as follows:

1. Count the number of infected RBCs per 100 RBCs in different oil immersion fields.
2. Apply the formula:

$$\frac{\text{\# of infected RBCs}}{\text{total \# of RBCs counted}} \times 100 = \% \text{ parasitemia}$$

Notes:

1) At least 500 RBC's should be counted, with counting 2000 or more RBCs providing the most accurate estimation of parasitemia
2) An infected RBC containing multiple parasites is calculated only once
3) Fields devoid of parasites should be included, if encountered
4) Gametocytes should not be included in the count. Justification is because: 1) many antimalarial drugs are not gametocidal and the presence of gametocytes post-treatment is not indicative of the effectiveness of the treatment and 2) gametocytes are a dead-end stage in the human host.

Clinical Significance:
In 2014, ninety seven countries and territories had ongoing malaria transmission. Over half a million people die from malaria each year. Most malaria cases and deaths occur in children in sub-Saharan Africa. In 2011, a 40-year high of 1,925 cases of malaria were reported to the CDC in the United States, almost all in recent travelers and immigrants. Although the *Anopheles* mosquito is endemic in parts of North America, malaria transmission was largely eradicated in the 1940s through public health efforts.

Malaria infection can be classified as either uncomplicated or severe (complicated). In uncomplicated infections, patients present with nonspecific symptoms including fever, chills, sweats, headaches, nausea/vomiting, body aches and malaise. Symptoms classically (but infrequently observed) recur either in a two-day cycle (*P. falciparum*, *P. vivax* and *P. ovale*) or in three-day cycle (*P. malariae*). In severe infections, organ failure and/or metabolic abnormalities occur including severe anemia, acute respiratory distress syndrome, acute kidney failure, metabolic acidosis, cerebral infection, and coagulation abnormalities. Severe infection is considered a medical emergency requiring urgent treatment. *Plasmodium falciparum* can cause severe illness and death whereas *P. vivax*, *P. malariae* and *P. ovale* tend to cause less severe illness. The hypnozoite form of *P. vivax* and *P. ovale* can remain dormant in a patient's liver and cause relapsing infection.

Treatment:
Treatment of malaria should ideally wait until a laboratory diagnosis has been made. Treating "presumptively" should occur only when no other option exists. Therapy is guided by the infecting species of *Plasmodium*, the clinical status of the patient and the drug susceptibility of the infecting parasites (dependent on geographic area and previous anti-malarial treatment). Because of the rapid progression of *P. falciparum* infections and a high risk of fatality, urgent treatment is essential.

If the infection is uncomplicated, oral anti-malarial medication can provide effective treatment. However, severe infections necessitate parenteral therapy. *Plasmodium falciparum* and *P. vivax* have different drug resistance patterns in different geographic regions. Although not readily available in North America, the WHO recommends artemisinin-based combination therapy as first-line treatment in uncomplicated *P. falciparum* malaria (oral administration), severe malarial infections (intravenous administration) and *P. vivax* infections in areas of known chloroquine resistance. Other, non-artemisinin based combination treatments include sulfadoxine-pyrimethamine

plus either chloroquine, amodiaquine, or atovaquone-proguanil. In recent years, resistance to artemisinins has been detected in Cambodia, Laos, Myanmar, Thailand and Vietnam. For confirmed *P. vivax* and *P. ovale* infections, radical cure can be achieved with treatment using primaquine and in order to prevent relapse due to the hypnozoite form. In high-transmission settings re-infection with P. vivax is likely. Mixed-species malarial infections are not common but may be underestimated by routine microscopy.

References

1. Centers for Disease Control and Prevention. Treatment of Malaria: Guideline for Clinicians. Available at: http://www.cdc.gov/malaria/diagnosis_treatment/clinicians3.html. Accessed June 23, 2015.
2. World Health Organization. Guidelines for the Treatment of Malaria. 3rd ed. Geneva, 2015.
3. World Health Organization. Malaria: Fact Sheet #94. Updated December 2014. Available at: http://www.who.int/mediacentre/factsheets/fs094/en/ Accessed online October 26, 2015.
4. Garcia LS. *Diagnostic Medical Parasitology.* 5th ed. Washington, DC. ASM Press; 2007.
5. CDC: Malaria surveillance – United States 2005. MMWR 2007;56(SS06);23-38.
6. Greenwood BM, Bojang K, Whitty CJ, Targett GA. Malaria. *Lancet*.2005;365:1487-1498.

BP-13 Introduction

A photograph challenge was submitted from an international traveler with a painful ulcer on his left hand. Participants were asked to identify the presence or absence of parasites. The image contained *Leishmania* amastigotes. A response of "*Leishmania* sp.", "Blood flagellate, NOS, referred for identification" or " Blood or tissue parasite, not *Plasmodium* spp. or *Babesia* sp., referred for identification" was considered satisfactory.

BP-13

Parasite Identification	Referees (24) No.	Referees (24) %	Participants (372) No.	Participants (372) %
Leishmania sp.	24	100.0	356	95.7

Parasite Screen	Referees (13) No.	Referees (13) %	Participants (370) No.	Participants (370) %
Blood flagellate, NOS, referred for identification	2	15.4	65	17.6
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	7	53.8	255	68.9

Discussion

Causal Agents

Leishmaniasis is caused by flagellated protozoans in the genus *Leishmania*. Depending on the taxonomy employed, at least twenty species belonging to four subgenera (*Leishmania*, *Viannia*, *Paraleishmania*, and *Sauroleishmania*) and species complexes have been recorded from humans (see Table on the next page).

Biology and Life Cycle

Leishmania spp. have a complex life cycle involving both vertebrate and insect vector hosts. Infection initiates when sand flies (primarily the genera *Phlebotomus* in the Old World and *Lutzomyia* in the New World) inject infectious promastigotes while taking a blood meal. Promastigotes are phagocytized by macrophages and other mononuclear phagocytic cells and transform into amastigotes. Amastigotes multiply by binary fission and infect other mononuclear phagocytic cells. The sand fly becomes infected when it ingests amastigotes while taking a blood meal. In the gut of the fly, amastigotes transform into promastigotes. Promastigotes multiply in the gut and then migrate to the proboscis to continue the cycle. Various factors, many unknown, will determine how the disease will present clinically in the human host, (i.e. visceral or cutaneous). Visceral leishmaniasis can also be acquired congenitally, by blood transfusion, and organ transplantation. Some species in the subgenus *Viannia* can also cause mucocutaneous leishmaniasis. This form of infection occurs when parasites causing cutaneous leishmaniasis disseminate to the mucosae of the head and neck. It has been hypothesized that the mucocutaneous form of leishmaniasis is mediated by a virus infecting the *Leishmania* organisms.

Species	Geographic Distribution	Primary Clinical Presentation
Leishmania (*Viannia*) *braziliensis*	Central and South America	Cutaneous, Mucocutaneous
Leishmania (*V.*) *guyanensis*	Central and South America	Cutaneous, Mucocutaneous
Leishmania (*V.*) *lainsoni*	Brazil, Bolivia, Peru, Colombia	Cutaneous
Leishmania (*V.*) *lindbergi*	Brazil	Cutaneous
Leishmania (*V.*) *naiffi*	Brazil, French Guyana	Cutaneous
Leishmania (*V.*) *panamensis*	Central and northern South America	Cutaneous, Mucocutaneous
Leishmania (*V.*) *peruviana*	Andean Peru and Bolivia, Panama, Costa Rica	Cutaneous
Leishmania (*V.*) *shawi*	Brazil	Cutaneous
Leishmania (*Leishmania*) *aethiopica*	Africa (Ethiopia, Kenya, Uganda), Yemen	Cutaneous
Leishmania (*L.*) *donovani*	Indian subregion, China, Africa, South America	Visceral, Cutaneous
Leishmania (*L.*) *infantum*	Mediterranean region, northern Africa, Middle East	Visceral, Cutaneous
Leishmania (*L.*) *chagasi*[1]	Central and South America	Visceral, Cutaneous
Leishmania (*L.*) *tropica*	Mediterranean region, Middle East, India, Africa (Gambia, Mauritania)	Cutaneous
Leishmania (*L.*) *major*	Northern Africa, Middle East, India, China	Cutaneous
Leishmania (*L.*) *mexicana*	Texas, Mexico, Belize, Guatemala	Cutaneous
Leishmania (*L.*) *amazonensis*	Amazonian South America	Cutaneous, Mucocutaneous
Leishmania (*L.*) *pifanoi*[2]	Brazil, Venezuela	Cutaneous
Leishmania (*L.*) *venezuelensis*	Venezuela	Cutaneous
Leishmania (*Paraleishmania*) *equatoriensis*	Colombia	Cutaneous
Leishmania martiniquensis[3]	Martinique, Thailand	Cutaneous, Visceral
Leishmania siamensis[3]	Central Europe, Thailand, USA	Cutaneous, Visceral

[1]many specialists consider a synonym of *L. infantum*.
[2]many specialists consider a synonym of *L. mexicana*.
[3]not currently assigned to subgenus.

Diagnosis

Initial diagnosis of leishmaniasis is made by the finding of amastigotes in clinical specimens, including bone marrow, touch preparations of skin lesions, and biopsy specimens. When collecting specimens of skin lesions, it is best to collect dermal material from the outer edges of the ulcer, where amastigote activity is highest. Species-level identification cannot be made based on amastigote morphology, and amastigotes of *Leishmania* spp. are morphologically indistinguishable from those of *Trypanosoma cruzi*. Therefore, clinical correlation and additional testing should be considered for differentiation of the two parasites. Amastigotes are round to oval and measure 1-5 µm long by 1-2 µm wide and possess a large nucleus and a prominent rod-shaped kinetoplast. Both the nucleus and kinetoplast should be visible in order to make a definitive identification. Promastigotes do not occur naturally in human tissues but may occasionally be seen in clinical specimens if there is a delay in processing. When detected, they are pyriform to slender and measure approximately 10-12 µm in length. They possess both a nucleus and a kinetoplast as well as a long, anteriorly-directed flagellum.

Species-level identification should be performed to identify or rule-out those species that can cause the mucocutaneous form of the disease (see Clinical Presentation, below). Traditionally, species-level identification was made by performing isoenzyme analysis on isolates derived from culture, a process that can take a few weeks. Recently, molecular analysis, usually PCR followed by sequencing analysis, has greatly improved turn-around times

for species-level identification, as the initial PCR can be performed directly on the clinical specimen rather than waiting for promastigotes to grow in culture.

Serology can be used for diagnosis of visceral leishmaniasis, but is less reliable for the cutaneous form of the disease as some patients of the latter do not develop a significant antibody response. Also, cross-reactivity can occur with *T. cruzi*.

Clinical Significance

In 2014, over 90% of new cases of human leishmaniasis reported to the World Health Organization occurred in six countries: Brazil, Ethiopia, India, Somalia, South Sudan, and Sudan. Human leishmaniasis encompasses several clinical syndromes depending upon the species involved, geographic location and the host immune response: cutaneous, mucocutaneous, and visceral leishmaniasis (a.k.a. kala-azar).

Cutaneous leishmaniasis typically begins as a firm, painless papule which develops over weeks to months to years at the site of a sand fly bite. This papule evolves into a volcano-like ulcer and then eventually heals with scarring. In South America, cutaneous leishmaniasis due to some species in the subgenus *Viannia* carries the additional risk of progression to mucocutaneous leishmaniasis (a.k.a. espundia), a highly destructive infection of the oral, nasopharyngeal, and laryngeal mucosa. If untreated, up to 15% of cutaneous infections with *L*. (*V*.) *braziliensis* can progress to mucocutaneous leishmaniasis. Unlike cutaneous leishmaniasis, mucocutaneous leishmaniasis does not spontaneously resolve and results in progressively disfiguring and debilitating disease.

Visceral leishmaniasis is the most severe form of the disease and is characterized by fever, weight loss, abdominal enlargement, malaise, hepatosplenomegaly and anemia. In endemic areas, including regions of southern Europe, visceral leishmaniasis is an important opportunistic and sometimes deadly infection in patients co-infected with human immunodeficiency virus (HIV). Post-kala-azar dermal leishmaniasis manifests as skin sequelae in unsuccessfully treated cases of visceral leishmaniasis, consisting of a macular, papular or nodular rash occurring on the face, upper arms, trunk or other parts of the body months to years after apparent treatment of visceral disease.

Treatment

Lesions of simple cutaneous leishmaniasis often resolve spontaneously without treatment. However, to reduce scarring and to prevent recurrence and/or dissemination, treatment is advocated. The optimal course of therapy remains ill-defined. Options range from cryotherapy, local heat therapy, surgical removal, to chemotherapy via intralesional injection, topical application or systemic administration. Medications used for treatment include the azoles, amphotericin B, miltefosine, paromomycin, pentavalent antimonials and pentamidine. A large variation in response to therapy has been observed depending on the species of *Leishmania* involved and the form of the disease. Identifying the infecting species of *Leishmania* can therefore aid in clinical decision-making. Due to the risk of development into mucocutaneous leishmaniasis, systemic treatment is generally recommended for cutaneous leishmaniasis acquired in South America. Visceral leishmaniasis must be treated with systemic medication such as amphotericin B, miltefosine, paromomycin and pentavalent antimonials. Post-therapy follow-up smear and cultures are recommended to ensure effectiveness of therapy. The CDC DPDx provides more detailed treatment information (http://www.cdc.gov/dpdx/leishmaniasis/tx.html).

References

1. Akhoundi M, Kuhls K, Cannet A, *et al*. A historical overview of the classificatrion, evolution, and dispersion of *Leishmania* parasites and sandflies. *PLoS Negl Trop Dis*. 10: e0043490. doi:10.137 1/journal.pntd.0004349.
2. Ash LR, Orihel, TC. *Atlas of Human Parasitology*. 5th edition. Chicago, IL: American Society of Clinical Pathologists; 2007.
3. DPDx: Laboratory Identification of Parasites of Public Health Concern. Atlanta, GA: Centers for Disease Control and Prevention. Accessed October 16, 2016.
4. Jorgensen JH, American Society for Microbiology. *Manual of Clinical Microbiology*, 11th ed. Washington, DC: ASM Press; 2015.
5. Magill AJ. Leishmaniasis, pp. 739-760. *In*: Magill AJ, Ryan ET, Hill DR, Solomon T (eds.) *Hunter's Tropical Medicine and Emerging Infectious Diseases*. 9th ed. New York, NY: Elsevier; 2012.
6. World Health Organization. Leishmaniasis Eradication Programme Website. http://www.who.int/leishmaniasis. Accessed October 17, 2016.

BP-14 Introduction

Thick and thin Giemsa-stained smears were obtained from 40-year-old male from Guyana with scrotal swelling. A response of “No parasite(s) seen” or “Specimen screened for blood parasites, no organisms seen” was considered satisfactory.

BP-14

Parasite Identification	Referees (31) No.	%	Participants (424) No.	%
No parasite(s) seen	31	100.0	418	98.6
Parasite Screen	**Referees (18) No.**	**%**	**Participants (797) No.**	**%**
Specimen screened for blood parasites, no organisms seen	18	100.0	768	96.4

Discussion

Identification

Careful examination of multiple thin and thick blood films is imperative to exclude the diagnosis of blood parasites, particularly for patients living in endemic areas. For thin films: (1) all blood components (erythrocytes, white blood cells, and platelets) should be intact, (2) the background should be clean and free from debris, (3) erythrocytes should stain a pale grayish-pink, and neutrophilic leukocytes should have deep purple nuclei and well defined granules, and (4) erythrocytes at the terminal, feathered end of the film should be adjacent, but not overlap (one layer thick). For thick films: (1) the background should be clean, free from debris, with a pale mottled-gray color derived from lysed erythrocytes, (2) leukocytes should stain deep purple with pale purple cytoplasm, and (3) eosinophilic granules should stain a bright purple-red and neutrophilic granules should stain deep pink-purple.

Thick films are most useful for screening since they provide a larger quantity of blood for examination. Thin films, on the other hand, are most useful for speciation since they provide the best red blood cell (RBC) and parasite morphology. All requests for peripheral blood smear examination to detect *Plasmodium* spp. should be performed without delay. Both thick and thin films should first be fully screened at low power (ie,using the 10x objective) to detect microfilaria which may be present in low numbers anywhere on the slides and which may not be detected in the standard 300 field slide review at higher magnification.

Due to the severe implications of a misdiagnosis, laboratory personnel should then examine at least 300 oil immersion fields (using the 100X oil immersion objective) for each thick and thin blood film. In addition, one set of blood films is not sufficient to exclude the diagnosis of malaria and the laboratory should recommend collection of multiple blood specimens approximately at 6-8 hour intervals to definitively exclude the presence of blood parasitemia. This comment should accompany the final report “No blood parasites seen.”

References

1. Garcia LS. 2007. *Diagnostic Medical Parasitology*, 5th ed., Washington, DC. ASM Press.
2. CDC: Malaria surveillance - United States 2008. *MMWR* 2010;59(SS07);1-15.
3. Greenwood BM, Bojang K, Whitty CJ, Targett GA. Malaria. *Lancet.* 2005;365:1487-1498.

BP-15 Introduction

Thick and thin Giemsa-stained smears were obtained from a 25-year-old refugee from Argentina. The specimen contained *Trypanosoma cruzi*. A response of "*Trypanosoma cruzi*", "Blood flagellate, NOS, referred for identification" or "Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification" were considered satisfactory.

BP-15

Parasite Identification	Referees (30) No.	%	Participants (441) No.	%
Trypanosoma cruzi	30	100.0	433	98.2

Parasite Screen	Referees (19) No.	%	Participants (777) No.	%
Blood flagellate, NOS, referred for identification	18	94.7	651	83.4
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	1	5.3	95	12.2

Discussion

Causal Agent

American Trypanosomiasis (also called Chagas disease) is caused by *Trypanosoma cruzi*, a flagellated protozoan endemic to the American tropics. Although *T. cruzi* is enzootic in the United States, the feeding/defecation patterns of the Nearctic triatomine bugs, in combination with improved living conditions, do not allow for efficient vector-borne transmission.

Biology and Life Cycle

Trypanosoma cruzi is transmitted by triatomine ('kissing') bugs as the bug releases infective trypomastigotes in the feces while taking a blood meal. Trypomastigotes enter the bite site when scratched into the wound, or other mucus membranes such as the conjunctiva. *Trypanosoma cruzi* has also been transmitted in fruit juices and other foods, when infected bugs contaminate fruits and other food sources. At the infection site, parasites differentiate into intracellular amastigotes. Amastigotes multiply by binary fission and differentiate into trypomastigotes and are released into the bloodstream. Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in the new infection sites. Only amastigotes replicate; trypomastigotes do not divide (unlike with the African trypanosome, *T. brucei*). Triatomine bugs become infected when they take a blood meal from an infected human or animal with circulating trypomastigotes. Ingested trypomastigotes transform into epimastigotes in the midgut and multiply there. Epimastigotes migrate to the hindgut where they become infective metacyclic trypomastigotes.

Diagnosis

Trypanosoma cruzi can be challenging to diagnose. During the acute stage of the disease, trypomastigotes may be observed in peripheral blood or CSF. Trypomastigotes are approximately 20 µm long, have a central nucleus, and a large subterminal kinetoplast at the pointed posterior end. The single flagellum is anteriorly directed. Dividing forms are not seen.

During the chronic stage of the disease, amastigotes may be found in tissue biopsy specimens, although serologic testing is recommended. Molecular diagnosis (PCR) is often employed in cases of transplant or transfusion transmission or when congenital cases are suspected. PCR can also be useful for early detection of *T. cruzi* in transplant-transmitted recipients of organs from donors with chronic disease. The diagnosis of chronic Chagas in patients without immunosuppression should be performed with serology.

Clinical Significance

Between six and seven million people are thought to be infected with *T. cruzi* in the Americas. The clinical presentation of Chagas is biphasic. Acutely, over a period of two months, individuals can be asymptomatic or present with skin changes such as swelling of eyelids accompanied with fever, myalgia, and lymphadenopathy. Disease with this pathogen can be cured if treated early. Complications of chronic disease include heart (30%), gastrointestinal (10%), neurological (5%), and mixed disease. If untreated, cardiomyopathy and neurological deficits can lead to sudden death. Blood donor and organ screening is critical to prevent transfusion or organ related transmission. Other forms of transmission include consumption of food contaminated with triatomine excrement, congenital infection, and laboratory accidents usually with infected human specimens.

Treatment

Specific anti-Chagas drug therapy can be achieved with benznidazole and nifurtimox. Both agents are effective in the acute phase, but efficacy is proportionally lower as the disease progresses into the chronic phase. Treatment in the acute phase can be protracted (up to 2 months) and complicated by adverse drug reactions such as kidney and liver injury. Cardiac and gastrointestinal disease may require targeted therapy to correct the anatomical dysfunction caused by chronic disease. Immunosuppressive regimens associated with autoimmune or neoplastic disease can lead to reactivation of Chagas which also requires anti-parasitic therapy.

References

1. Ash LP, Orihel TC. *Atlas of Human Parasitology*, 5th ed. Chicago, IL: ASCP Press: 2007.
2. Centers for Disease Control and Prevention, Division of Parasitic Diseases (DPD). *Laboratory Identification of Parasites of Public Concern.* http://www.cdc.gov/dpdx
3. Mandell GL, Bennett JE, Dolin R. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 7th ed. Philadelphia, PA: Churchill Livingstone Elsevier, 2009.

Actions Laboratories Should Take when a PT Result is Not Graded

The College uses Exception Reason Codes that signify the proficiency testing (PT) for an analyte has not been graded. The Exception Reason Code is located on the evaluation report in brackets to the right of the result. Your laboratory must identify all of the analytes with an Exception Reason Code and investigate the acceptability of performance with the same rigor as if it were an unacceptable performance. The actions accredited laboratories should take include but are not limited to:

Code	***Exception Reason Code Description***	***Action Required***
11	Unable to analyze.	Document why the specimens were not analyzed (eg, instrument not functioning or reagents not available). Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
20	No appropriate target/response; cannot be graded.	Document that the laboratory performed a self-evaluation using the data presented in the Participant Summary and compared its results to a similar method, all method, or all participant statistics if provided. If comparison is not available, perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
21	Specimen problem.	Document that the laboratory has reviewed the proper statistics supplied in the Participant Summary. Perform and document alternative assessment for the period that commercial PT was not tested to the same level and extent that would have been tested. Credit is not awarded in these cases.
22	Result is outside the method/ instrument reportable range.	Document the comparison of results to the proper statistics supplied in the Participant Summary. Verify detection limits.
24	Incorrect response due to failure to provide a valid response code.	Document the laboratory's self-evaluation against the proper statistics and evaluation criteria supplied in the Participant Summary. Perform and document the corrective action of any unacceptable results. Document corrective action to prevent future failures.
25	Inappropriate use of antimicrobial.	Document the investigation of the result as if they were unacceptable and review the proper reference documents to gain knowledge of the reason your response is not appropriate.
26	Educational challenge.	Response to the CAP is not required. Laboratory should document its review.
27,31	Lack of participant or referee consensus.	Document that the laboratory performed a self-evaluation and compared its results to the intended response when provided in the Participant Summary. If comparison is not available, perform and document alternative assessment (ie, split samples) for the period that commercial PT reached non-consensus to the same level and extent that would have been tested.
28	Response qualified with a greater than or less than sign; unable to quantitate.	Document that the laboratory performed a self-evaluation and compared its results to the proper statistics supplied in the Participant Summary. Verify detection limits.
30	Scientific Committee decision.	Document that the laboratory has reviewed the proper statistics supplied in the Participant Summary.
33	Specimen determined to be unsatisfactory after contacting the CAP.	Document that the laboratory has contacted the CAP and no replacements specimens were available. Perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
40	Results for this kit were not received.	Document why results were not received, corrective action to prevent recurrence and the laboratory's self-evaluation of the results by comparing results to the proper statistics and evaluation criteria supplied in the Participant Summary. If PT specimens were not analyzed, perform and document alternative assessment (ie, split samples) for the period that commercial PT was not tested to the same level and extent that would have been tested.
41	Results for this kit were received past the evaluation cut-off date.	
42	No credit assigned due to absence of response.	The Participant Summary indicates which tests are graded (see evaluation criteria) and which tests are Not Evaluated/Educational. Updates to grading will also be noted. If a test is educational, the laboratory is not penalized for leaving a result(s) blank. The code 42 that appears on the evaluation is **not** a penalty. However, if a test is graded (regulated and non-regulated analytes) and your laboratory performs that test, results cannot be left blank. The laboratory is required to submit results for **all** challenges within that test or use an appropriate exception code or indicate test not performed/not applicable/not indicated. Exceptions may be noted in the Kit Instructions and/or the Result Form. Document corrective actions to prevent future failures.
44	This drug is not included in our test menu. Use of this code counts as a correct response.	Verify that the drug is not tested on patient samples and document to ensure proper future reporting.
45	Antimicrobial agent is likely ineffective for this organism or site of infection.	Document that the laboratory performed a self-evaluation of written protocols and practices for routine reporting of antimicrobial susceptibility reports to patient medical records. Document that routine reporting of this result to clinicians for patient care is compliant with specific recommendations of relevant Medical Staff and Committees (eg, infectious Diseases, Pharmacy and Therapeutics, Infection Control). Response to the CAP is not required.
77	Improper use of the exception code for this mailing.	Document the identification of the correct code to use for future mailings.
91	There was an insufficient number of contributing challenges to establish a composite grade.	Document the investigation of the result as if it were an unacceptable result. Perform and document the corrective action if required.
35, 43, 88, 92	Various codes.	No action required.

COLLEGE of AMERICAN PATHOLOGISTS

Don't Miss Out On This Opportunity to Earn Continuing Education Credit

***** Enter the information below and distribute to your laboratory staff. *****

Program Mailing and Year:	BP-C 2016
Activity Start Date:	October 31, 2016
Activity Expiration Date:	October 30, 2017

How to Access Your Online Education Activities

1. **Access Your Account**
   a. Go to cap.org.
   b. Under the MY CAP menu, click **Log In**.
      - If you are unsure whether you have an *individual* web account with the CAP, or do not remember your user ID and password, click on the **Reset password** or **Email temporary password.**
      - If you do not have an individual web account, click on **Register with the CAP.** Complete and submit the account request form. You will be notified within one business day that your individual account has been activated.

   c. **If you are associated to one lab** that purchased kits for this activity, the system will register you into the activity. A kit will be associated to your registration.
   d. **If you are associated to more than one lab** that purchased kits for this activity, you will need to select the lab by clicking the **Select or Change Laboratory** button. The system will register you into the activity. A kit will be associated to your registration.
   e. **If you are not associated to a lab**, you need to add lab affiliation.
      - Under the MY CAP menu, click **Update My Profile**.
      - Click the **Business/Professional** tab, then (in the upper-right corner) click **+ Add Affiliation** to enter the appropriate information.

2. **Access Your Online Education Activities**
   a. Go to cap.org.
   b. Under the MY CAP menu, click **Log In**.
   c. Click on the **Learning** tab.
   d. Enter the Program code in the Search box (eg, BMD, CGL), then click the arrow icon  .
   e. In the list of results, click the **Register** button of your activity.
   f. After reviewing the Activity Details page, click the **Register** button.
   g. Click **Resume** to access the Activity.
   h. Click the confirmation checkbox at the bottom of the Activity Overview page, and then click the **Continue** button.
   i. If you choose to return to the activity later, it can be found on the In-Progress Learning tab. Click the activity title to return to the activity.

View courses with one of the following browsers: Internet Explorer 7.x or newer, Firefox, Google Chrome, or Safari. Pop-up blockers must be turned off to complete the activity.

Important: Refer to the System Requirements document located on cap.org.

For assistance, please call a Customer Contact Center representative at 800-323-4040 or 847-832-7000 option 1.

Continuing Education Information

CE (Continuing Education for non-physicians)
The CAP designates this educational activity for a maximum of 1 credit of continuing education. Participants should only claim credit commensurate with the extent of their participation in the activity.

The American Society for Clinical Pathology (ASCP) Board of Certification (BOC) Certification Maintenance Program (CMP) accepts this activity to meet the continuing education requirements.

This activity is approved for continuing education credit in the states of California and Florida.

Disclosure Statement

The following authors/planners have financial relationships to disclose:
None

The following authors/planners have no financial relationships to disclose:
D. Jane Hata, PhD; Blaine A. Mathison, M(ASCP);Robin Rolf, MT(ASCP)

The following In-Kind Support has been received for this activity:
None

The following Commercial Support has been received for this activity:
None

All authors/planners of a CAP educational activity must disclose to the program audience any financial interest or relationship with the manufacturer(s) of any commercial product(s) that may be discussed in the educational activity or with the manufacturer of a product that is directly competitive with a product discussed in the presentation. Relevant financial relationships are considered to be any financial relationships in any amount occurring within the past 12 months that create a conflict of interest.

The College of American Pathologists does not view the existence of these interests or uses as implying bias or decreasing the value to participants. The CAP feels that this disclosure is important for the participants to form their own judgment about each presentation.

All College educational activities hold to the highest standards of educational quality and are dedicated to promoting improvement in health care quality and not a specific proprietary business interest of a commercial interest. All recommendations and/or planning criteria involving clinical medicine are based on evidence that is accepted within the profession of medicine as adequate justification for this indication and contraindication in the care of patients.

Learning Objectives

Upon completing the reading and answering the learning assessment questions, you should be able to:

1. Understand the biology and life cycle of *Plasmodium* species and how they pertain to human disease.
2. State the diagnostic morphologic features of the four species of *Plasmodium* that cause human malaria.
3. Differentiate *Plasmodium* species from *Babesia* species.
4. Discuss diagnostic laboratory techniques for malaria, the appropriate specimen types, and reason for their application.
5. Understand the clinical manifestations of malaria and proper treatment algorithms.

CAUSAL AGENTS

Malaria is caused by apicomplexan protozoan parasites in the genus *Plasmodium*. There are four species that use humans as part of their natural life cycle: *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*. It should be noted that molecular data suggest that *P. ovale* is actually a complex consisting of 2 different species or subspecies (*P. o. wallikeri* and *P. o. curtisi*); however such separation is not believed to be clinically relevant. In addition, there are 2 simian *Plasmodium* species—*P. cynomolgi* and *P. knowlesi*—which have been documented as causing zoonotic malaria naturally acquired in humans, and four other simian species that have been experimentally shown to infect humans (Table 1).

Table 1. Non-human primate *Plasmodium* species that have been known to, or have the potential to, cause zoonotic malaria in humans.

Species	Human Species Most-Closely Resembles	Geographic Distribution	Natural Hosts
*Plasmodium brasilianum**	*Plasmodium malariae*	South America	Howler monkeys, spider monkeys, titis, capuchins, bearded saki, woolly monkeys, squirrel monkeys
Plasmodium cynomolgi	*Plasmodium vivax*	Asia	Macaques and surilis
Plasmodium inui	*Plasmodium malariae*	Asia	Macaques
Plasmodium knowlesi	*Plasmodium falciparum* (early stages); *P. malariae* (later stages)	Asia	Macaques
Plasmodium schwetzi	*Plasmodium ovale*	Africa	Chimpanzees and gorillas
*Plasmodium simium**	*Plasmodium vivax*	South America	Howler monkeys

*Molecular data suggest *P. simium* and *P. brasilianum* may just be *P. vivax* and *P. malariae*, respectively, which adapted to non-human primates after introduction to South America.

GEOGRAPHIC DISTRIBUTION

With an increase in global travel, and better surveillance through molecular characterization, the knowledge of the distribution of human *Plasmodium* species is ever-changing.

- *Plasmodium falciparum* is distributed throughout the tropics and subtropics wherever malaria cases occur. This species is most prevalent in tropical and western Africa, parts of Southeast Asia, and Haiti.
- *Plasmodium malariae* also has a broad geographic distribution, although more focally located than as seen with *P. falciparum*, with most cases coming from western Africa and parts of Southeast Asia.
- *Plasmodium ovale* is most common in western and tropical Africa, with cases also reported from Southeast Asia. *Plasmodium ovale* transmission has not yet been documented in the New World.
- *Plasmodium vivax* probably has the widest geographic distribution and is most common in eastern and northern Africa, parts of the Middle East, the Indian subcontinent into Southeast Asia, and Central and South America.

Historically, the rule of thumb is that in Africa, *P. ovale* is western and southern, and *P. vivax* is eastern and northern. As more data become available, it appears that both *P. ovale* and *P. vivax* are more widely distributed on the continent than they are historically known for. Morphologic separation of these 2 species in general can be quite challenging and any isolate resembling *P. vivax* from tropical Africa can be confirmed by molecular methods such as polymerase chain reaction (PCR) for epidemiologic purposes.

BIOLOGY AND LIFE CYCLE

The following Centers for Disease Control and Prevention (CDC) chart illustrates *Plasmodium* species having a two-host life cycle involving a mosquito in the genus *Anopheles* that serves as a vector and definitive host, and a vertebrate intermediate host. Infected female mosquitoes inject sporozoites when taking a blood meal (Figure 1.1). Sporozoites are carried via blood to the liver where they invade hepatocytes (Figure 1.2) and form schizonts (Figure 1.3). The liver schizonts rupture (Figure 1.4), releasing large numbers of merozoites that then invade RBCs starting (Figure 1.5) the erythrocytic cycle. Early ring forms develop into mature trophozoites and take one of 2 pathways: (1) they develop into schizonts (which produce merozoites, eventually rupturing and continuing the erythrocytic cycle (Figure 1. 6) or (2) develop into gametocytes (Figure 1.7). Gametocytes are a dead-end stage in the human host but are required for sexual reproduction in the mosquito. When taken up by an *Anopheles* mosquito during a blood meal (Figure 1.8), microgametocytes (=males) exflagellate and fertilize macrogametocytes (=females) (Figure 1.9), resulting in an ookinete (Figure 1.10). Ookinetes further develop into oocysts (Figure 1.11), which when mature rupture (Figure 1.12) and release the infective sporozoites. Sporozoites migrate to the salivary glands of the mosquito hosts and can be transmitted to the next human host during feeding.

During human infection, late trophozoites and schizonts of *P. falciparum* express a protein on the surface of the erythrocytic membrane causing the infected erythrocyte to adhere to the endothelial lining of capillaries in internal organs. Thus, only ring forms and gametocytes are usually seen in well-prepared peripheral blood smears. In *P. vivax* and *P. ovale*, the parasites may remain in the liver in the form of hypnozoites. Activation of these hypnozoites may cause relapses months or years later if the patient is not treated properly.

Figure 1. Life cycle of *Plasmodium* species

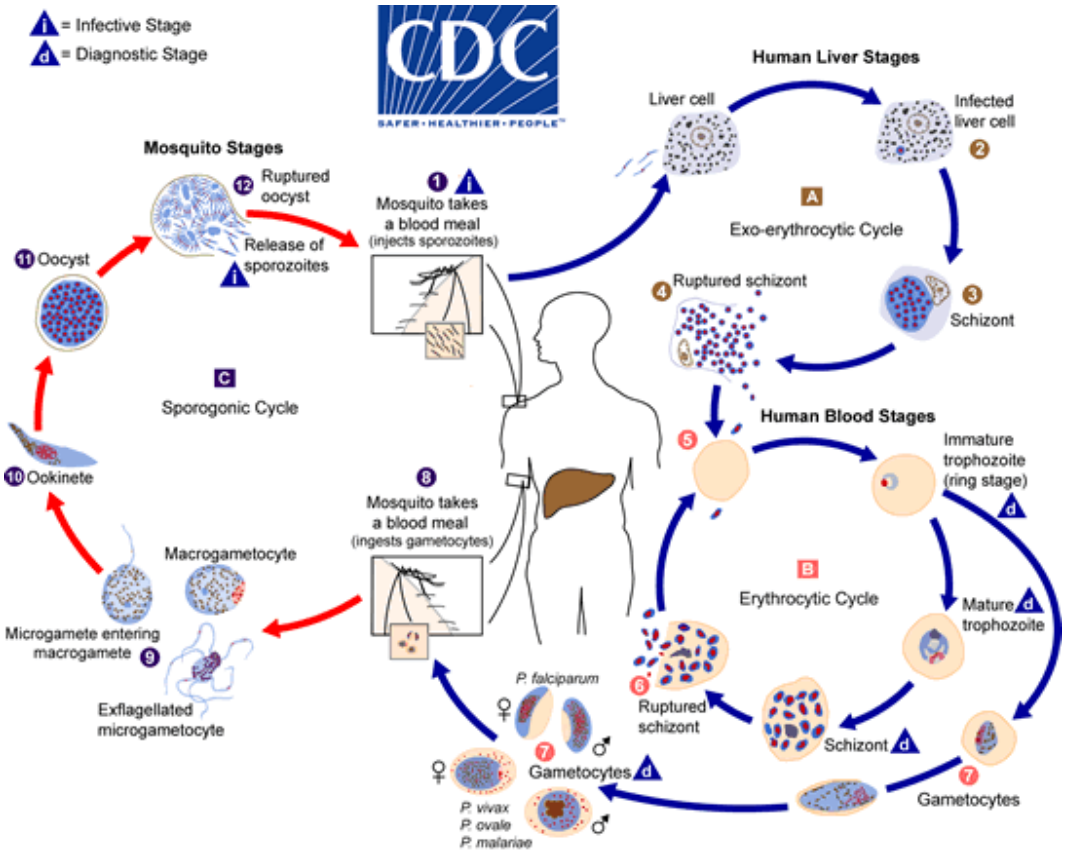


Image courtesy of the CDC-DPDx.

DIAGNOSIS

Morphology

The gold standard for malaria diagnosis and species-level determination continues to be morphologic analysis of thick and thin smears. Proper collection, processing, and staining are critical to achieve an accurate diagnosis.

- Blood Collection
  The ideal specimen for morphological analysis is the fingerstick with slides made at bedside. This is not always practical, and venous blood specimens preserved in ethylenediaminetetraacetic acid (EDTA) are also suitable for morphology. It is important that smears be made as soon as possible after collection as delay can result in changes to parasite morphology and staining characteristics that can hinder the chances of making an accurate diagnosis.

- Smear Preparation
  Typically both thick and thin blood films are examined for *Plasmodium* parasites. The thick film consists of a layer of dehemoglobinized (lysed) red blood cells (RBCs). The blood elements (including parasites, if any) are more concentrated and this method is thus used primarily for detection of parasites. The thin film is used for species-level identification and consists of blood spread in a layer such that the thickness decreases progressively toward the feathered edge. In the feathered edge, the cells should be in a

monolayer, not touching one another. Thick and thin films can be made on the same slide or separate slides.

It is important to let both the thick and thin smears dry completely before fixing and/or staining. Thick films take longer to dry than thin films, and this could result in a delay of the diagnosis. The scratch method for thick films is an alternate method for making thick films that allows for improved adherence and faster turnaround times. The process is similar to making a normal thick film, but instead of using a stick to spread the blood, the edge of a glass microscope slide is used, while applying firm pressure to create small scratches in the underlying slide. The scratches allow for improved adherence of the blood film to the slide without affecting the smear morphology. The smear can then be stained as soon as it is dry, generally within 20 - 30 minutes of smear preparation.

- Fixation
  The thin films (only) should be fixed in absolute methanol and allowed to dry completely prior to staining. If thin and thick films are made on the same slide, it is very important not to contaminate the thick film with methanol or vapors derived therefrom. Otherwise, the thick film may become inadvertently fixed and possible rendered inadequate for reading.

- Staining
  Thick and thin films for malaria diagnosis are typically stained with Wright, Giemsa, or a combined Wright-Giemsa stain. Wright stain is more commonly used in the United States, as it can be used in automated systems. However, with Wright stain you may not detect certain morphologic features (eg, Schüffner's stippling and Maurer's clefts) that are best visualized using Giemsa stain at a pH of 7.2. Field stains are useful for rapid diagnosis but are not recommended for routine malaria diagnosis.

- Microscopic Examination
  Examine the thick film first for parasites, using the thin film to identify organisms to the species level. Both the thick and thin films should be examined at 100x magnification with oil for a minimum of 100 fields for malaria parasites. Immunologically naïve patients (ie, those without previous *Plasmodium* exposure) may be symptomatic at lower parasitemias; therefore, screening of at least 300 fields may be recommended.

- Morphologic Features
  A species-level identification is made by examining the morphologic features of the ring-form trophozoites, mature trophozoites, gametocytes, and schizonts (see Table 2). It is important when attempting to make a species-level identification, to look at the whole picture and not just ascribe textbook definitions to every individual parasite seen. Some features, especially those seen in the early ring-form trophozoites, may be seen in more than one species, and are not always diagnostically helpful. Characteristics to look for in each of the stages can include:

  - Ring-form trophozoites. Not always diagnostically helpful. Note the form of the cytoplasm and the size (and number) of chromatin dots. How many parasites are present in infected RBCs? Are other stages present?
  - Developing trophozoites. Note whether developing trophozoites are becoming compact or amoeboid. Is the pigment coarse or fine? Features such as Schüffner's stippling, fimbriation, and enlargement of infected RBCs may become present at this stage.
  - Gametocytes. Note the shape: round or crescent-shaped. Does the parasite fill the entire RBC? Is the infected RBC enlarged? Schüffner's stippling (dots), fimbriation, and other features may be evident at this stage.

- Schizonts. Count the number of merozoites in mature schizonts only; mature schizonts can be recognized as having the pigment coalesced and not scattered throughout the RBC. Are the merozoites large? Are they arranged in a rosette pattern? Schüffner's stippling (dots), enlargement, fimbriation, and other features may be evident at this stage.

Table 2. Comparison the morphologic features of the 4 stages of human *Plasmodium* spp.

Morphologic Criteria	*Plasmodium falciparum*	*Plasmodium malariae*	*Plasmodium ovale*	*Plasmodium vivax*
Size of infected RBC	Normal	Normal to smaller	Enlarged	Enlarged
Rings (early trophozoites)	Common; usually with thin, delicate cytoplasm and double chromatin dots; often multiple rings per infected RBC; appliqué forms common	Cytoplasm sturdy, usually with single, large chromatin dot; occasional "birds-eye" forms	Cytoplasm sturdy, with 1 - 2 large chromatin dots	Large, sturdy cytoplasm, usually with large, single chromatin dot
Developing trophozoites	Rare, but may be seen if a delay in processing; form compact, pigment usually evident	Variable; may be compact to elongate (band-form) or pleomorphic and vacuolated (basket-form); pigment coarse	Compact to slightly amoeboid with dark pigment; elongation and fimbriation may be observed	Pleomorphic to grossly amoeboid; pigment diffuse and golden-brown to nearly black
Schizonts	Rare in peripheral blood; 8 - 24 small merozoites when mature; pigment dark, compact	6 - 12 merozoites when mature, often in a rosette pattern around central mass of pigment	6 - 14 merozoites when mature; pigment dark brown to black and discrete when mature; elongation and fimbriation may be present	12 - 24 merozoites when mature; may fill entire RBC; often noticeable enlarged
Gametocytes	Crescent-shaped; chromatin discrete (macrogametocyte) or diffuse (microgametocyte); Laveran's bib may be present	Small, round, compact; pigment coarse and diffuse	Round to oval, compact; if elongated and fimbriated may not fill entire infected RBC; pigment coarse, dark	Large and round to pleomorphic (may "hug" surrounding RBCs); may fill most of infected RBC; pigment golden-brown to nearly black
Other Features	Maurer's clefts may be present; ring-form trophozoites usually predominate	Generally smaller; pigment coarse; Ziemann's stippling may be present; all stages seen	Schüffner's stippling may be present at appropriate pH; elongation and fimbriation may be observed; all stages seen	Schüffner's stippling may be present at appropriate pH; enlargement of infected RBCs usually pronounced; all stages seen

Plasmodium falciparum

It is most important to identify or rule-out *P. falciparum*, given the severity of disease this species can cause. Classic features for this species, such as thin, delicate ring-form trophozoites with double chromatin dots, multiply-infected RBCs, and appliqué (accolé) forms, are not specific to this species. However, these features combined, especially with a moderate to heavy parasitemia and the absence of other stages, can be diagnostic (Figure 2A). Developing trophozoites are not typically found in circulating blood, but may be seen in clinical specimens if there is a prolonged delay in processing. Developing trophozoites are typically small, compact, and will usually have discernible pigment. In both the ring-form and developing trophozoite stages, Maurer's clefts (Figure 2B) may be seen when slides are stained with Giemsa at an appropriate pH. Schizonts are also not commonly seen; when they are present they are not often diagnostically helpful due to the wide range in number of merozoites (6 - 24) in a mature schizont. The *P. falciparum* gametocyte is the most diagnostically helpful stage, given its unique crescent-shaped appearance (Figure 2C). Occasionally, remnants of the host RBCs can be observed in the form of a Laveran's bin (inset, Figure 2C). In all stages, infected RBCs are not enlarged. *Plasmodium falciparum* also needs to be distinguished from *Babesia* species (see below).

Plasmodium malariae

In cases of *P. malariae*, all developmental stages may be seen in peripheral blood. Early ring-form trophozoites of *P. malariae* are very similar to those of other species. "Birds-eye" forms (Figure 2E) may be seen whereby the chromatin is enclosed by cytoplasm; however, these can also be seen with *P. falciparum*. Developing trophozoites become amoeboid and may take on band forms that appear to stretch across the RBC (Figure 2F, lower left) or become vacuolated and appear basket-shaped (Figure 2F, upper right). Pigment is coarse and dark. Mature schizonts have 6 - 12 merozoites that are often arranged in a rosette pattern around the coalesced pigment (Figure 2H). Gametocytes (Figure 2G) are round, typically fill the entire host RBC, and have dark, coarse pigment. In all stages, infected RBCs may appear smaller than non-infected RBCs.

Plasmodium ovale

Plasmodium ovale can be difficult to separate from *P. vivax* (below), especially in the absence of good travel history. Both species are clinically managed in a similar manner so even an identification of *Plasmodium vivax*/*ovale* can be beneficial.

With *P. ovale*, all stages may be seen in peripheral blood. Early ring-form trophozoites often have sturdier cytoplasm and a larger chromatin dot than as seen with *P. falciparum* and *P. malariae*. Single chromatin dot forms predominate (Figure 2I), but double-dot forms are not uncommon (often more common than with *P. vivax*). As trophozoites develop, they may become amoeboid, but not to the extent of *P. vivax*. As the parasites develop, infected RBCs may appear enlarged and elongate and become fimbriated (inset, Figure 2I; Figure 2J). Fimbriation should not be confused with crenation (Figure 3B). Pigment is typically coarser and darker than as seen with *P. vivax*. Schüffner's stippling (dots) will be evident in specimens stained with Giemsa at an appropriate pH (Figure 2J). Schizonts are typically enlarged and contain 6 - 14 merozoites, which are sometimes arranged in a rosette pattern similar to as seen with *P. malariae*. Gametocytes are large, round and often completely fill the host RBCs.

Plasmodium vivax

With *P. ovale*, all stages may be seen in peripheral blood. Early ring-form trophozoites often have sturdier cytoplasm and a larger chromatin dot than as seen with *P. falciparum* and *P. malariae*. Single chromatin dot forms predominate, but double-dot forms are not rare. Developing trophozoites become amoeboid, often grossly so (Figure 2N). As the trophozoites develop, infected RBCs may appear enlarged and pigment might be golden to dark brown. Schüffner's stippling (dots) will be evident in specimens stained with Giemsa at an appropriate pH (Figure 2N). Schizonts are large and typically fill the host RBC; merozoites are large and usually number 12 - 24.

Gametocytes are large, round, and often completely fill the host RBCs. In later stages, infected RBCs may become pleomorphic, and appear to contour around the surrounding RBCs (Figure 2N). This phenomenon may be seen with *P. ovale* as well, but rarely to the extent seen with *P. vivax*.

Figure 2. Developmental stages of *Plasmodium* species.

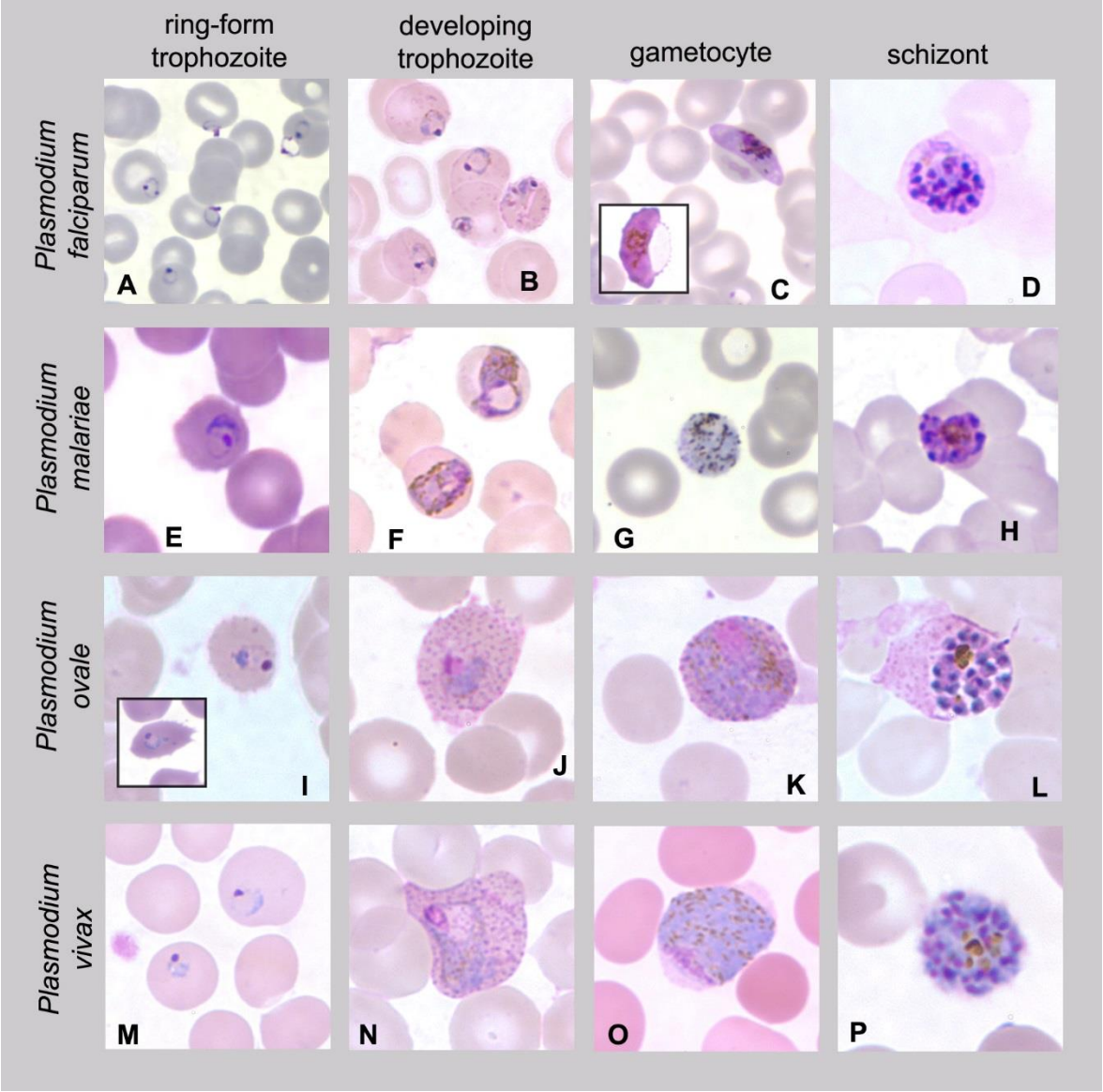
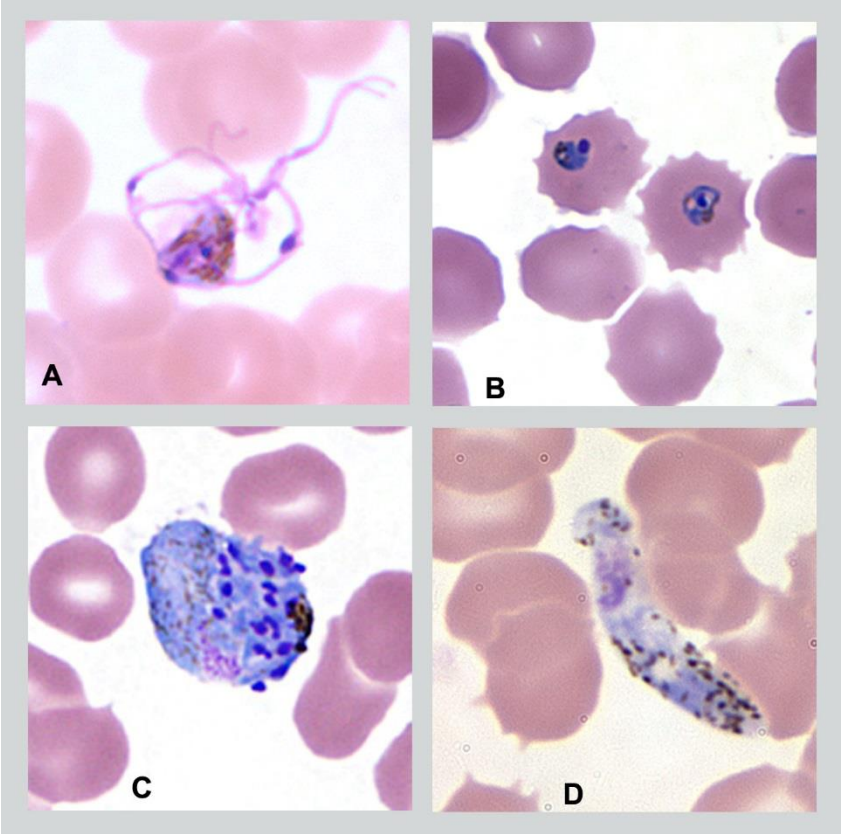


Figure 3. Atypical forms of *Plasmodium* that may be seen in clinical specimens.



(A) Exflagellating microgametocyte of *P. falciparum*; exflagellation may occur when is there is a noticeable delay in processing. (B) trophozoites of *P. falciparum* in crenated RBCs; crenation of the host RBC occurs when there is a noticeable delay in processing and should not be confused with fimbriation seen with *P. ovale*. (C) multiply-infected RBC containing a gametocyte (left) and schizont (right) of *P. vivax*. (D) ookinete of *P. vivax*; ookinetes are typically only seen in the mosquito host, but may form in EDTA blood if left to sit for a prolonged time under conditions similar to those of the mosquito (eg, temperature). In this example, the ookinetes may be confused for a gametocyte of *P. falciparum*; however, it is too large and the pigment is too widely dispersed.

Differential Diagnosis from *Babesia* species

Ring-form trophozoites of *Plasmodium* spp., especially *P. falciparum*, need to be distinguished from *Babesia* species. *Babesia* is a genus of apicomplexan protozoan parasites transmitted by ticks in the genus *Ixodes*. There are several species of *Babesia* that can infect humans, including at least 3 in the US: *B. microti* (Northeastern states), *B. duncani* (California and the Pacific Northwest), and *Babesia* MO-1 (Midwestern US). Trophozoite stages of *Babesia* are similar to the early ring forms of *Plasmodium*, but may differ in being more pleomorphic (including pyriform, spindle-shaped, or occurring in small chains), vacuolated, and always lack pigment. Merozoites may present in a characteristic tetrad formation (Figure 4) and extracellular forms may occur. Gamonts and other sexual stages are not known to occur in the human host. The parasitemia for *Babesia* species may be very high, even in asymptomatic or low symptomatic cases.

Figure 4. *Babesia* sp. in a thin blood film.

Notice the wide variety of shapes among the trophozoites and a tetrad-forming merozoites (arrow) can also be seen.

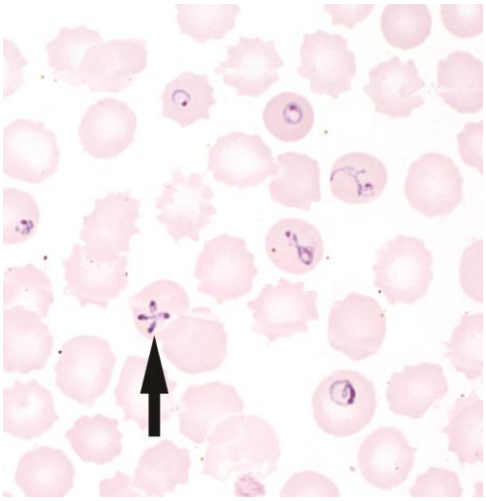


Image courtesy of Bobbi S. Pritt, MD. Parasitology Benchtop Reference Guide, College of American Pathologists. 2014.

Figure 5. *Plasmodium falciparum* in a thin blood film.

Notice there is more uniformity among the size and shape of the parasites.

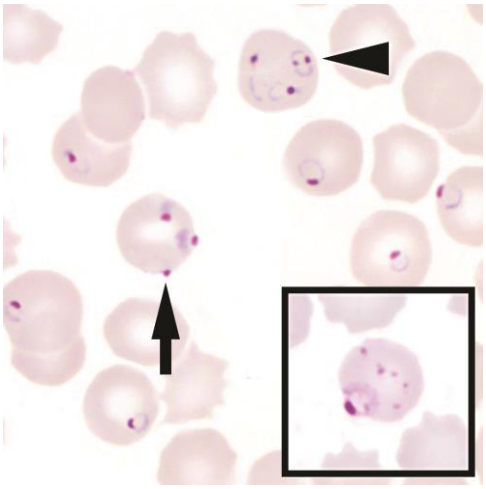


Image courtesy of Bobbi S. Pritt, MD. Parasitology Benchtop Reference Guide, College of American Pathologists. 2014.

Calculating Percent Parasitemia

Calculating the percent parasitemia (or, more accurately, the percent infected RBCs) may be used to guide therapy, predict prognosis, and access response to treatment in cases of malaria. Percentages may be calculated using thick or thin films, although the thin film gives the most accurate results.

The formula for the thin film is as follows: (# of infected RBCs/total # of RBCs counted) x 100

Procedural notes:

- perform on an area of the thin film in which there is minimal overlap of RBCs and approximately 250 cells per 100x field
- at least 500 RBCs should be counted, with 2000 or more RBCs providing the most accurate estimation of parasitemia; count the RBCs in a given field even if no parasites are present in the given field
- do not include gametocytes in counts
- an RBC containing multiple parasites should be counted only once
- extracellular forms (eg, liberated merozoites) should not be counted

Molecular Methods

While parasitology diagnosis is still predominately performed by morphological analysis, molecular methods such as PCR are becoming more common. PCR is typically not used as a screening tool for *Plasmodium* species, and is more commonly used in larger reference and public health labs. Whole blood preserved in EDTA is the preferred specimen type. Possible scenarios that may warrant PCR testing include, but are not limited to:

- when a species-level identification cannot be made by morphology (eg,, low parasitemia, poor specimen quality)
- when the morphologic features suggest a mixed infection
- testing seropositive donor specimens during transfusion-associated investigations
- patients manifesting with symptoms suggestive of cerebral malaria, even in the absence of parasites on blood films
- when there are discordant results by other methodologies (eg, morphology vs. rapid diagnostic tests [RDTs])

It should be noted that PCR results may be negative when performed on post-treatment blood; rarely does PCR continue to be positive after morphologic detection ceases following treatment.

RDTs

There are hundreds of lateral flow immune-chromatographic tests, or (RDTs), available for malaria diagnosis. However, only one is available for clinical use in the US, Binax NOW. The Binax NOW test is best used for initial screening of patients presenting with cyclic fever with recent travel to a malaria endemic region. The test uses the HRP-II antigen for *P. falciparum* and a pan-malarial aldolase for all 4 species. The test offers roughly 95% sensitivity and 94% specificity for *Plasmodium falciparum*, but had a much lower sensitivity and specificity for non-*falciparum* species. The Binax NOW test should always be performed in conjunction with microscopy, as the limits of detection for both *P. falciparum* and non-*falciparum* species may not allow for detection with low levels of parasitemia. The test should not be used to evaluate treatment, as circulating antigen in the patient's blood may result in positive results up to 2 weeks post treatment. Molecular methods may be used when there are discordant results between the RDT and microscopy.

Serology

Serologic testing is not practical for diagnosing acute cases of malaria, as it can take a week or longer for a patient to develop a detectable antibody response. Serology is most commonly performed on donor specimens during transfusion-associated cases or on patients who have recently been treated for malaria but the diagnosis is in question. Antibodies to *Plasmodium* persist, and as such a positive result cannot differentiate a recent infection from a past infection.

CLINICAL MANIFESTATIONS

In 2014, 97 countries and territories reported ongoing malaria transmission. It is estimated over half a million people die from malaria each year. Most malaria cases and deaths occur in children in sub-Saharan Africa. In 2011, a 40-year high of 1,925 cases of malaria were reported to the CDC in the US, almost all in recent travelers and immigrants. Although the Anopheles mosquito is endemic in parts of North America, malaria transmission was largely eradicated in the 1940s through public health efforts. Significant progress has been made in decreasing incidence and mortality due to malaria, however, substantial challenges remain.

Infections due to *P. falciparum* may cause severe disease and death, whereas *P. vivax*, *P. malariae,* and *P. ovale* tend to result in less severe illness. Depending upon the infective species of *Plasmodium*, the typical incubation period of malaria may vary from 9 - 18 days from exposure. Development of symptoms may be as short as 7 days for *P. falciparum* to as long as 40 days for *P. malariae*. Some strains of *P. vivax* may exhibit a delayed initial presentation of up to 12 - 18 months after a bite from an infected mosquito. Hypnozoite (dormant) liver forms of *P. vivax* or ovale may result in disease relapse months to years after initial infection. Variations in length of incubation period may also occur in cases of inadequate prophylaxis, inadequate immune response, or relapse of disease.

The primary symptoms of acute uncomplicated malaria are nonspecific, and most often consist of fever, chills, nausea, vomiting, myalgias, back and abdominal pains, and diarrhea. Periodicity of fever may or may not be observed. Splenomegaly, anemia, and mild jaundice may be noted on physical examination. Anemia is common in children and pregnant women. Additional manifestations of malaria in pregnant women include spontaneous abortion, premature delivery, low birth weight, congenital malaria infection, and stillbirth. Differential diagnosis of malaria may include dengue fever, viral hemorrhagic fevers (including Ebola viral disease), typhoid fever, leptospirosis, pneumonia, and sepsis due to bacteremia.

Severe/complicated malaria will manifest itself with impaired consciousness or seizures, respiratory distress progressing to acute respiratory distress syndrome, pulmonary edema, circulatory shock, hepatic failure, disseminated intravascular coagulation (DIC), or renal failure. Physical findings may include jaundice, petechiae, splenomegaly, and/or hepatomegaly. Rarely, “blackwater fever” may be noted by the presence of dark urine secondary to large amounts of excreted malarial pigments and hemoglobin due to intravascular hemolysis. This is often associated with secondary or multiple infections with *P. falciparium*. Parasitemia often exceeds 5% in cases of severe malaria, and is most often associated with *P. falciparum* (rarely *P. vivax*). This emphasizes the importance of accurate species-level identification. Severe malaria may be most often noted in non-immune individuals, pregnant women, children from 6 months - 3 years of age, and immunocompromised hosts.

Cerebral malaria is the most severe manifestation of *P. falciparum* malaria. This syndrome is thought to be due to sequestration of parasitized red blood cells in the cerebral microcirculation, or high levels of inflammatory mediators and metabolic factors resulting in brain swelling, intracranial hypertension, retinal hemorrhage, or impaired consciousness. Cerebral malaria is universally fatal if untreated; with treatment, mortality is 15% - 20%. Significant neurological sequelae are more common in children than adults. Young or old age, HIV infection, pregnancy, poor nutrition, and asplenia may increase the risk of cerebral malaria.

TREATMENT RECOMMENDATIONS

Therapy is guided by the infecting species of *Plasmodium*, the clinical status of the patient, and the drug susceptibility of the infecting parasites (dependent on geographic area and previous anti-malarial treatment).

A laboratory diagnosis of malaria is considered a critical (panic) value, and warrants rapid initiation of treatment. Accurate species-level identification by smear or RDT and determination of the circulating percentage of parasite density when possible are keys to successful management. Due to geographic patterns of anti-malarial drug resistance, an accurate travel history can provide important information to guide therapy. Patients with proven *P. falciparum* or *Plasmodium* not identified to the species level should be admitted to a hospital upon treatment initiation to monitor for treatment compliance, and reduction of parasitemia. Patients with severe malaria may not be able to take oral medications reliably, and require parenteral treatment. Treatment should continue until the patient improves clinically, and blood films are negative.

Chloroquine phosphate is recommended as first-line treatment of uncomplicated infections due to *P. malariae* and *P. ovale*. *Plasmodium falciparum* infections acquired in Hispaniola, Central America west of the Panama Canal, parts of China and the Middle East are considered susceptible to chloroquine. *P. vivax* infections acquired outside of Papua New Guinea and Indonesia are also susceptible. Hydroxychloroquine may be used as a second-line agent. In areas where chloroquine resistance occurs (Papua New Guinea and Indonesia), atovaquone-proguanil, artemether-lumefantrine, quinine plus tetracycline, doxycycline or mefloquine may be used for primary treatment of non-*P. falciparum* species. These options may also be used in areas where infections with chloroquine-resistant *P. falciparum* may occur.

Alternative treatment regimens including mefloquine and primaquine may be used in specific clinical situations. Mefloquine may be used for treatment of *P. falciparum* infections; however mefloquine-resistant strains have been reported in Southeast Asia. Mefloquine can be associated with neuropsychiatric reactions, including auditory hallucinations, and is contraindicated in individuals with a history of depression, anxiety disorders, or psychosis. US-FDA labeling requires mefloquine carry a black box warning due to these adverse effects.
Primaquine is an effective agent in the treatment of malaria due to *P. vivax* or *P. ovale* due to its ability to eradicate the hypnozoite forms of these parasites. Patients must be screened for glucose-6-phosphate dehydrogenase (G6PD) deficiency prior to use of primaquine due to the risk of intravascular hemolysis in affected individuals. In patients who cannot tolerate primaquine, long-term prophylaxis (years) with chloroquine or mefloquine may be required to eradicate hypnozoite forms.

Artemisinin derivatives are key components of malaria treatment worldwide, and are available on a limited basis in the United States. Artemisinin and its derivatives should not be used as oral monotherapy, due to the potential for development of resistance to these agents. Artemisinin resistance has been reported in Cambodia, Laos, Myanmar, Thailand and Vietnam. The World Health Organization recommends use of combination agents consisting of artemisinin derivatives with a long acting partner drug such as lumefantrine, amodiaquine, or mefloquine.

In pregnant women, chloroquine phosphate has been proven safe for treatment of *P. falciparum*. In uncomplicated infections due to *P. falciparum* acquired in geographic regions of chloroquine resistance, a regimen of quinine plus clindamycin may be used. Although there is a possibility of fetal hypoglycemia secondary to quinine treatment, this is outweighed by the potential risk of untreated falciparum malaria. Primaquine is not recommended for use, as hemolytic disease can result if the fetus is G6PD-deficient. Tetracycline and doxycycline are also contraindicated due to effects on the developing fetus. Atovoquone-proguanil is not recommended due to limited efficacy data in pregnancy, but may be considered in certain cases where quinine/clindamycin or quinine monotherapy is not tolerated. Mefloquine is generally not recommended for use during pregnancy.

In children, chloroquine phosphate is well-tolerated for treatment of *P. falciparum*, if infection is acquired in an area where resistance is not an issue. Treatment regimens consisting of tetracycline and doxycycline are

contraindicated due to permanent staining of tooth enamel and decreased linear skeletal growth rate. A combination of clindamycin and quinine is an acceptable alternative. Mefloquine may be used in children > 5 kg. Primaquine may be considered in children who do not have a G6PD deficiency.

In cases of severe malaria, intravenous quinidine gluconate plus doxycycline, tetracycline or clindamycin is approved for use in the US quinidine is cardiotoxic and must be administered in an ICU setting with cardiac monitoring. Outside the US, parenteral artenunate, artemether, or quinine may be used for severe malaria. Parasite density (based on blood smear) should decrease by 90% over the first 48 hours of quinidine therapy. If parasitemia exceeds 10%, or cerebral malaria exists, exchange transfusion should be considered to reduce parasite load until pharmacological control can be achieved.

An updated list of treatment regimens may be found at:
http://www.cdc.gov/malaria/resources/pdf/treatmenttable.pdf

Consultation on patient treatment and assistance with drug acquisition is available through the CDC Malaria Hotline (770-488-7788 or 855-856-4713 toll free) or http://www.cdc.gov/malaria.

REFERENCES

1. Ash LR, Orihel, TC. 2007. Atlas of Human Parasitology. 5th ed. American Society of Clinical Pathologists. Chicago, IL.
2. Breman JG. Clinical Manifestations of Malaria. UpToDate [serial on the Internet]. 2015: Available from: http://www.uptodate.com/contents/clinical-manifestations-of-malaria?source=machineLearning&search=malaria&selectedTitle=1%7E150&sectionRank=1&anchor=H3#H3 . Accessed September 15, 2016.
3. Coatney GR, Collins WE, Warren M, Contacos PG. 1971. The Primate Malarias. US Department of Health, Education, and Welfare. Bethesda, MD.
4. DPDx – Laboratory Identification of Parasitic Diseases of Public Health Concern. Centers for Disease Control and Prevention website. http://www.cdc.gov/dpdx/.
5. Griffith KS, Lewis LS, Mali S, Parise ME. 2007. Treatment of malaria in the United States: a systematic review. *JAMA.* 297:2264-2277.
6. Guimarães LO, Bajay MM, Wunderlich G, Bueno MG, Röhe F, Catão-Dias J, et al. 2012. The genetic diversity of *Plasmodium malariae* and *Plasmodium brasilianum* from human, simian, and mosquito hosts in Brazil. *Acta Tropica*. 124:27-32.
7. Idro R, Marsh K, John CC, Newton CR. 2010. Cerebral malaria: mechanisms of brain injury and strategies for improved neurocognitive outcome. *Pediatr Res.* 68:267-274.
8. Lim CS, Tazi L, Ayala FJ. 2005. *Plasmodium vivax*: recent world expansion and genetic identity to *Plasmodium simium*. *Proc Natl Acad Sci USA*. 102:15523-15528.
9. Norgan AP, Arguello HE, Sloan LM, Fernholz EC, Pritt BS. 2013. A method for reducing the sloughing of thick blood films for malaria diagnosis. *Malar J.* 12:231-235.
10. Pritt BS. 2014. Parasitology Benchtop Reference Guide. College of American Pathologists. Chicago, IL.
11. Sulzer AJ, Wilson M. 1971. The fluorescent antibody test for malaria. *Crit Rev Clin Lan Sci*. 2:601-609.
12. World Health Organization. Guidelines for the Treatment of Malaria. Second edition. Geneva, April 2015
13. World Health Organization. Malaria: Fact Sheet #94. Updated December 2014. http://www.who.int/mediacentre/factsheets/fs094/en/ Accessed online February 22, 2015.

AUTHORS

Blaine A. Mathison M(ASCP)
D. Jane Hata, PhD
Microbiology Resource Committee

NOTES

COLLEGE of AMERICAN PATHOLOGISTS

325 Waukegan Road
Northfield, IL 60093-2750
800-323-4040
847-832-7000 (Country code: 001)