Blood Parasite Identification

BP-06 Introduction

Thick and thin Giemsa-stained smears were obtained from a 9-month-old adoptee from Nigeria with fever and night sweats. The specimen contained *Plasmodium falciparum*. A response of *"Plasmodium falciparum", "Plasmodium* sp., NOS would refer or request another specimen, or perform additional molecular testing" and "*Plasmodium* sp./*Babesia* sp. seen, referred for identification" were considered satisfactory.

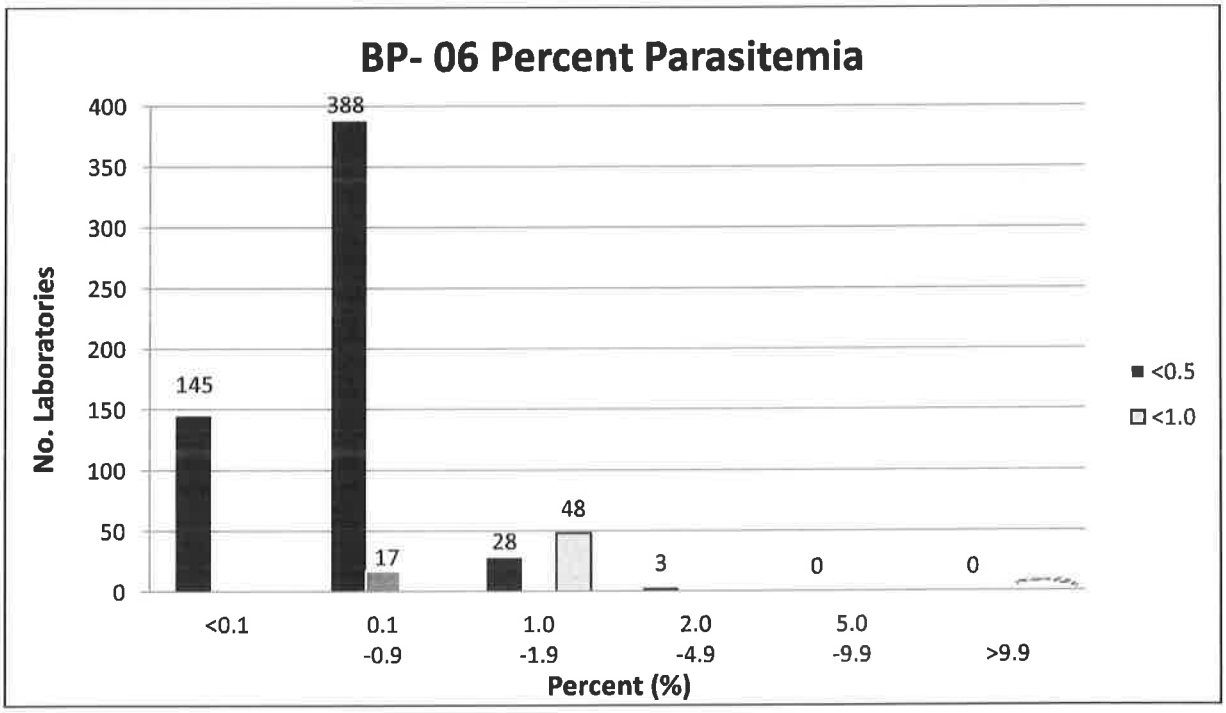
BP-06

Parasite Identification	Referees No.	Referees (49) %	Participants No.	Participants (471) %
Plasmodium falciparum	44	89.8	412	87.5
Plasmodium sp., NOS would refer or request another specimen, or perform additional molecular testing	4	8.2	58	12.3
Babesia sp.	1	2.0	1	0.2
Plasmodium vivax/ovale, NOS	1	2.0	1	0.2

Parasite Screen	Referees No.	Referees (60) %	Participants No.	Participants (799) %
Plasmodium sp./*Babesia* sp. seen, referred for identification	57	95.0	767	96.0

BP-06

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)



Key morphologic features on thin blood film that suggests a diagnosis of *P. falciparum* may include:

- Normal size and shape of infected erythrocytes
- Smaller, more delicate ring-form trophozoites (approximately 1/5 the diameter of the erythrocyte) frequently with two chromatin dots (so-called "head phone" forms)
- Erythrocytes infected with multiple parasites
- Presence of ring-form trophozoites at the edge of the erythrocyte (appliqué forms)
- Usually an absence of mature trophozoites and schizonts in the peripheral blood film (may be seen if there is a delay in processing the blood specimen, or in patients with a very high parasitemia or underlying conditions)
- Presence of crescent-shaped gametocytes (not always seen)
- Absence of Schüffner's stippling. Larger, comma-shaped dots (Maurer's clefts) may be seen, especially when the stain buffer is at a pH of 7.0-7.2

Distinguishing *P. falciparum* from *Babesia* spp. can be challenging, given that both have a predominance of small ring forms, infect RBCs of all ages, and there may be multiple parasites per erythrocyte. The presence of Maurer's clefts, malarial pigment, and cresent-shaped gametocytes eliminate *Babesia* infection from consideration. Furthermore, *Babesia* parasites are usually more pleomorphic with spindled, elliptical and ovoid forms. Extracellular forms are also more common in babesiosis. Finally, identification of the classic "Maltese cross" or tetrad form of *Babesia* sp. is diagnostic for babesiosis although it may rarely be observed. Molecular or antigen-detection methods, in addition to clinical/travel history may be useful adjuncts for distinguishing between these two similar appearing parasites.

Discussion

Causal Agents

There are four species of *Plasmodium* that cause human malaria: *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*. *Plasmodium ovale* is sometimes divided into two subspecies which may actually represent valid species, *P. o. walkeri* and *P. o. curtisi*. 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*, human infections of which appear to be increasing on the Malaysian peninsula (see also Table 1).

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 patchier 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 Central Asia, the Indian subcontinent, southeast Asia, and the Americas.

Table 1.
Plasmodium species recorded infecting humans, their geographic distributions, and natural intermediate (mammalian) hosts.

Species	Geographic Distribution	Natural Intermediate Hosts
*Plasmodium brasilianum**	South America	Howler monkeys, spider monkeys, tits, capuchins, bearded saki, woolly mokeys, squirrel monkeys
Plasmodium cynomolgi	Southeast Asia	Macaques, surilis
Plasmodium falciparum	Circumtropical	Humans
Plasmodium inui	Southeast Asia	Macaques
Plasmodium knowlesi	Southeast Asia	Macaques
Plasmodium malariae	Africa (primarily tropical sub-Saharan), southeast Asia, South America, southern Central America, Caribbean	Humans
Plasmodium ovale	Africa (primarily western and tropical sub-Saharan), southern and southeastern Asia	Humans
Plasmodium schwetzi	Tropical western Africa	Chimpanzees, gorillas
*Plasmodium simium**	Brazil	Howler monkeys
Plasmodium vivax	Africa (East, Horn of Africa and Madagascar), Central and South America, Central Asia, Indian Subcontinent, Southeast Asia, Korean Peninsula	Humans

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

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. With *P. vivax* and *P. ovale*, some parasites will remain in the liver as hypnozoites, resulting in relapses months or years later. 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 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.

Diagnosis

General Considerations

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 an

identification cannot be made morphologically or there is morphologic evidence of a mixed infection. 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 (e.g. 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 $\geq$ 2% in immunologically naïve patients and $\geq$ 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).

Table 2.
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 in relation to uninfected RBCs. Between 1,000-10,000 RBCs should be counted (ideally at least 1000).
2. Apply the formula:

$$\frac{\text{\# of parasitized RBCs}}{1000 \text{ (or } 200)} \times 100 = \% \text{ parasitemia}$$

Procedural notes:

- an infected RBC containing multiple parasites is calculated only once
- fields devoid of parasites should be included, if encountered
- gametocytes should not be included in the count. The justification is because (1) some 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.

Important parasitemia thresholds

Clinicians take several components into consideration, including the percent parasitemia, when making treatment decisions. In general, levels of parasitemia ≥ 5% are indicative of severe disease and should be treated aggressively with parenteral antimalarial therapy in all patients (see also Treatment, below). A lower threshold parasitemia of ≥ 2% may indicate severity in the non-immune traveler. Other clinical criteria that are indicative of severe disease include impaired consciousness, renal failure, severe anemia (Hb <7g/dL), acute respiratory distress syndrome, hemoglobinuria, jaundice, hypotension, disseminated intravascular coagulation, and spontaneous bleeding. In patients with *P. falciparum* or *P. knowlesi* hyperparasitemia (parasitemia >10%), more aggressive interventions may be considered. The role of exchange transfusion is controversial but may be useful for removing parasites from the blood stream, improving oxygen carrying capacity and improving blood viscosity.

Molecular Detection

Molecular detection and identification of *Plasmodium* species is becoming increasingly popular in the diagnostic laboratories, although it can often be cost prohibitive for some labs, especially smaller labs with a lower specimen volume. There are currently no FDA-approved commercial assays for routine clinical use in the United States, and to date all available assays are laboratory-derived tests (LDTs). However, several molecular assays are approved for use in Europe and Canada. Multiple assays have been described, including DNA/RNA hybridization, loop-mediated isothermal amplification (LAMP), conventional and real-time PCR, and nucleic acid sequence-based amplification (NASBA). The preferred specimen type for molecular detection is whole blood collected in EDTA, although several assays have been validated for finger-stick blood collected on dried blood spots such as filter papers.

Table 3.
The following table highlights the advantages and disadvantages for the molecular detection of *Plasmodium* species (adapted from Mathison and Pritt 2017)

Advantages	Disadvantages
• More sensitive than microscopy and RDTs • Less subjective than microscopy • Improved diagnosis of mixed infections • Requires less training time of personnel than microscopy • Allows for detection of polymorphisms associated with drug resistance.	• Still cost-prohibitive in many places, especially for routine diagnosis • Often not performed on a STAT basis • High-complexity method that requires special training of personnel • Should not be used to evaluate treatment success

Antigen Detection

There are over 40 rapid detection tests (RDTs) commercially available on a worldwide basis for the detection of *Plasmodium*. However, in the United States, there is only one that is approved by the FDA for human use, the BinaxNOW® Malaria Test (Abbott, Lake Forest, IL). This test targets *P. falciparum*-specific Hrp2 and aldolase common to the four human species of *Plasmodium*. According to the package insert, the BinaxNOW® test has sensitivities for the detection of *P. falciparum* and *P. vivax* of 100% and 81.6%, respectively, using blood obtained by venous draw, however the sensitivity drops to 30% for other species. Regardless of the results when performing the BinaxNOW® Malaria Test, the results should be confirmed my microscopy. Also, the BinaxNOW® Malaria Test should not be used to monitor treatment success as residual antigen can result in false-positive results for as long as 28 days in the case of Hrp2.

Antibody Detection

Antibody detection is not typically recommended for routine clinical diagnosis of malaria, except for a few clinical scenarios, including but not limited to: (1) febrile patients with recent travel to endemic areas that are repeatedly smear negative, (2) diagnosis of suspected tropical splenomegaly syndrome, and (3) trace-back investigations of donors in transfusion-associated cases.

Clinical Significance

Nearly half of the world's population is at risk of malaria. In 2017, there were an estimated 219 million cases of malaria in 90 countries, with 435,000 deaths. Most malaria cases (92%) and deaths (93%) occur sub-Saharan Africa. There are approximately 1,000 cases of malaria diagnosed in the United States each year, almost all in recent travelers and immigrants. The visiting friends and relatives (VFR) population contribute to the vast majority of cases in travelers returning to non-endemic areas. Although the *Anopheles* mosquito is endemic in parts of North America, malaria transmission was largely eliminated 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* and *P. knowlesi* 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 or amodiaquine or atovaquone-proguanil. In recent years, resistance to artemisinins has been detected in Cambodia, Laos, Myanmar, Thailand and Viet Nam. Chemoprophylaxis can be achieved with atovaquone-proguanil, doxycycline, and meflqouine as examples. For confirmed *P. vivax* and *P. ovale* infections, radical cure can be achieved with treatment using primaquine or tafenoquine, in order to prevent relapse due to the hypnozoite form, except in high-transmission settings where re-infection is likely. Mixed-species malarial infections are not common but may be underestimated by routine microscopy.

References

1. Ash LR, Orihel TC. Atlas of human parasitology, ed 5, Chicago:ASCP Press, 2007.
2. Centers for Disease Control and Prevention (CDC)a: Malaria. Atlanta, GA. https://www.cdc.gov/parasites/malaria/index.html Accessed online June 14, 2019.
3. Foundation for Innovative New Diagnostics (FIND), Special Programme for Research and Training in Tropical Diseases (TDR) and World Health Organization (WHO): Malaria rapid diagnostic test performance. Results of WHO product testing of malaria RDTs: Round 4 (2012). TDR/World Health Organization, Geneva, Switzerland: 2012.
4. Mace KE, Arguin PM, Tan KR. Malaria surveillance – United States, 2015, Morbidity and Mortality Weekly Report, 2018:67.
5. Mathison BA, Pritt BS. Update on malaria diagnostics and test utilization. *J Clin Microbiol.* 2017;55:2009.
6. National Committee for Clinical Laboratory Standards (NCCLS): Laboratory diagnosis of blood borne parasitic diseases; approved guideline. NCCLS Document M15-A, Wayne, Pa.:NCCLS; 2000.
7. Pritt BS. Chapter 139: Plasmodium and Babesia. In: Carroll JC, Pfaller MA, Landry ML, et al (eds). Manual of Clinical Microbiology, ed 12. Washington, D.C.:ASM Press;2019.

BP-07 Introduction

Thick and thin Giemsa-stained smears were obtained from a college female returning from studying 3 months in Israel. A response of "No parasite(s) seen" or "Specimen screened for blood parasites, no organisms seen" was considered satisfactory.

BP-07

Parasite Identification	Referees (50) No.	%	Participants (440) No.	%
No parasite(s) seen	50	100.0	429	97.5

Parasite Screen	Referees (60) No.	%	Participants (832) No.	%
Specimen screened for blood parasites, no organisms seen	59	98.3	797	95.8

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. 2016. *Diagnostic Medical Parasitology*, 6th ed., Washington, DC. ASM Press.

BP-08 Introduction

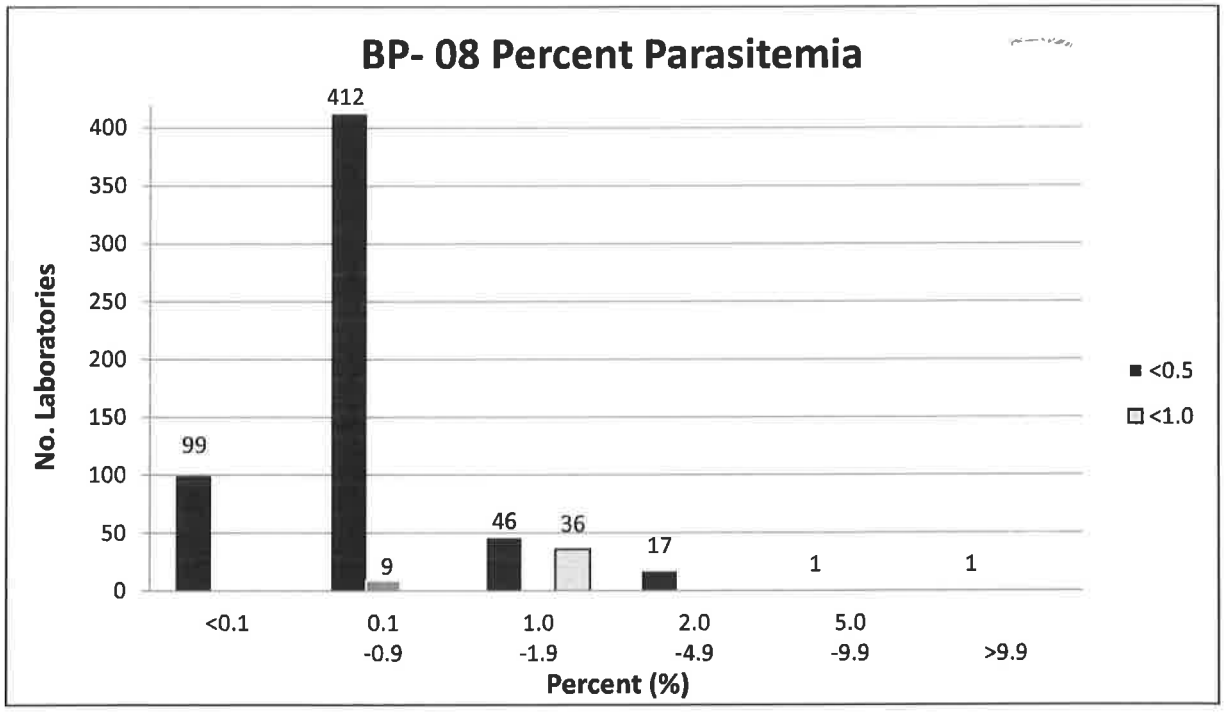
Thick and thin Giemsa-stained smears were obtained from a 25-year-old returning from an eco-expedition in Malaysia. The specimen contained *Plasmodium ovale*. A response of "*Plasmodium ovale*", "*Plasmodium vivax/ovale* NOS", "*Plasmodium* sp., not *P. falciparum* referred for identification", "*Plasmodium* sp., NOS would refer or request another specimen, or perform additional molecular testing", and "*Plasmodium* sp./*Babesia* sp. seen referred for identification" would have been considered satisfactory; however, consensus was not reached for parasite identification.

BP-08

Parasite Identification*	Referees (50) No.	%	Participants (475) No.	%
Plasmodium ovale	1	2.0	49	10.3
Plasmodium vivax/ovale, NOS	12	24.0	127	26.7
Plasmodium sp., not *P. falciparum,* referred for identification	18	36.0	149	31.4
Plasmodium sp., NOS would refer or request another specimen, or perform additional molecular testing	5	10.0	43	9.1
Plasmodium malariae	10	20.0	48	10.1
Plasmodium vivax	4	8.0	69	14.5
Parasite Screen	**Referees (59) No.**	**%**	**Participants (795) No.**	**%**
Plasmodium sp./*Babesia* sp. seen, referred for identification	58	98.3	789	99.3

* Parasite identification was not graded due to lack of participant and referee consensus.

BP-08 **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)**



Both thick and thin blood films should be prepared when the diagnosis of malaria is suspected. Examination of the thick blood film is considered the gold standard for identification since a larger blood volume can be examined than with the thin film, thus enabling the detection of low levels of parasitemia. In contrast, thin blood films provide the best morphology for species differentiation. In this Survey, the challenge was to recognize the presence of *Plasmodium ovale*. Consensus was not reached by participants or referees.

Key morphologic features on thin and thick blood films that characterize *P. ovale* may include:

1. Enlarged size of the infected red blood cells (seen with both *P. ovale and P. vivax)*.
2. Infected red blood cells with fimbriations and an ovoid shape (seen in up to 1/3 of infected cells)
3. Presence of Schüffner's stippling (seen in both *P. ovale and P. vivax*). Note: These may not be seen in early ring-form trophozoites in *P. ovale*
4. Mature schizonts with 8-12 merozoites
5. Compact ring compared to the more amoeboid trophozoite of *P. vivax.*
6. Large, round gametocytes usually with coarser pigment than as usually seen with *P. vivax*.

Distinguishing *P. ovale* from *P. vivax* morphologically may be challenging when the trophozoites do not have characteristic features and fimbriations are not abundant. The absence of schizonts also makes the differentiation between the two species more difficult. In these cases a diagnosis of *Plasmodium vivax/ovale* may be acceptable. If available, molecular testing can be used confirm the species when morphology is not definitive.

Note: *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.

References

1. Ash LR, Orihel TC. Atlas of human parasitology, ed 5, Chicago:ASCP Press, 2007.
2. Centers for Disease Control and Prevention (CDC)a: Malaria. Atlanta, GA. https://www.cdc.gov/parasites/malaria/index.html Accessed online June 14, 2019.
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7. Pritt BS. Chapter 139: Plasmodium and Babesia. In: Carroll JC, Pfaller MA, Landry ML, et al (eds). Manual of Clinical Microbiology, ed 12. Washington, D.C.:ASM Press;2019.

Discussion

Please refer to discussion starting on page 3.

BP-09 Introduction

Thick and thin Giemsa-stained smears were obtained from a 35-year-old returning from the Democratic Peoples Republic of Congo (DRC) with fever and chills. The specimen contained *Trypanosoma brucei.* A response of "*Trypanosoma brucei* (*gambiense* or rhodesiense)", "Blood flagellate, NOS, referred for identification" and "Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification" was considered satisfactory.

BP-09

Parasite Identification	Referees (50) No.	%	Participants (450) No.	%
Trypanosoma brucei (*gambiense* or *rhodesiense*)	49	98.0	442	98.2
Trypanosoma cruzi	1	2.0	9	2.0
Parasite Screen	**Referees (60) No.**	**%**	**Participants (818) No.**	**%**
Blood flagellate, NOS, referred for identification	58	96.7	715	87.4
Blood or tissue parasite, not *Plasmodium* sp. or *Babesia* sp., referred for identification	2	3.3	89	10.9

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 spp. 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* 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 diving 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

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 combination therapy with nifurtimox and eflornithine is recommended for second-stage disease. 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.* https://www.cdc.gov/dpdx Accessed June 13, 2019.
3. Garcia LS. 2007. *Diagnostic Medical Parasitology*, 5th ed., Washington, DC. ASM Press.

BP-10 Introduction

Thick and thin Giemsa-stained smears were obtained from a routine screening of a 17-year-old female refugee from South Sudan. A response of "No parasite(s) seen" or "Specimen screened for blood parasites, no organisms seen" was considered satisfactory.

BP-10

Parasite Identification	Referees (50) No.	%	Participants (440) No.	%
No parasite(s) seen	50	100.0	434	98.6

Parasite Screen	Referees (60) No.	%	Participants (827) No.	%
Specimen screened for blood parasites, no organisms seen	59	98.3	799	96.6

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. 2016. *Diagnostic Medical Parasitology*, 6th ed., Washington, DC. ASM Press.

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 analytes with an exception reason code, review and document the acceptability of performance as outlined below and retain documentation of review for at least 2 years. The actions 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.	Applies to a response that is not formally evaluated when a peer group is not established due to fewer than 10 laboratories reporting. Document that the laboratory performed a self-evaluation using the data presented in the Participant Summary and compared its results to a similar method, or all method, or all participant statistics if provided. Perform and document the corrective action of any unacceptable results. 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. Perform and document the corrective action of any unacceptable results.
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 results 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.	Review participant summary report for comparative results and document performance accordingly. Evaluation criteria are not established for educational challenges. Laboratories should determine their own evaluation criteria approved by their laboratory director for self-evaluation. Response to the CAP is not required.
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.	Applies to a response that is not formally evaluated when a less than or greater than sign is reported. Document that the laboratory performed a self-evaluation and compared its results to the proper statistics supplied in the Participant Summary. Verify detection limits. Perform and document the corrective action of any unacceptable results.
30	Scientific Committee decision.	Applies to a response that is not penalized based on Scientific Committee Decision. Document that the laboratory has reviewed the proper statistics supplied in the Participant Summary.

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 analytes with an exception reason code, review and document the acceptability of performance as outlined below and retain documentation of review for at least 2 years. The actions laboratories should take include but are not limited to:

Code	***Exception Reason Code Description***	***Action Required***
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 41	Results for this kit were not received. Results for this kit were received past the evaluation cut-off date.	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.
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, 46	Various codes.	No action required.

COLLEGE of AMERICAN PATHOLOGISTS

Attestation of Participation of Self-Reported Training*

We the participants ~~below~~ have completed the review of the CAP BP-B 2019 (Product Mailing, Year) Participant Summary/Final Critique report and can self-report the recommended 0.5 (Education Hours) hours towards fulfilling education and certification of maintenance requirements.

Participant	**Date**	**Participant**	**Date**

Director (or Designee) Signature - I have verified that the individuals listed above have successfully participated in this activity. Date

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**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.*