California Association for Medical Laboratory Technology

Distance Learning Program

POTENTIAL PROBLEMS WITH THE DIAGNOSIS OF MALARIA IN THE UNITED STATES;
LABORATORY IDENTIFICATION OF MALARIA

Course # DL-002

by
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Approved for 2.0 CE/Contact Hours
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Level of Difficulty: Intermediate

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DISTANCE LEARNING ANSWER SHEET

Please circle the one best answer for each question.

COURSE NAME: POTENTIAL PROBLEMS WITH THE DIAGNOSIS OF MALARIA IN THE UNITED STATES  COURSE # DL-002

NAME__________________________________________________ LIC. # ________________ DATE_________

SIGNATURE (REQUIRED) _______________________________________________________________________

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7. a b c d  17. a b c d
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1. Overall, I was satisfied with the quality of this Distance Learning course.
   5  4  3  2  1
2. The objectives of this Distance Learning course were met.
   5  4  3  2  1
3. The difficulty of this Distance Learning course was consistent with the number of CE hours.
   5  4  3  2  1
4. I will use what I learned from this Distance Learning course.
   5  4  3  2  1
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POTENTIAL PROBLEMS WITH THE DIAGNOSIS OF MALARIA IN THE UNITED STATES; Laboratory Identification of Malaria

OBJECTIVES
Upon completion of this course, the participant will be able to:

● Discuss the symptomatic differences seen in immunologically naïve patients compared with those from endemic areas with past exposure to malaria.
● Outline the patient history information required for the Clinical Laboratory Scientist to perform diagnostic testing with the best and most relevant diagnostic results outcome.
● Describe the steps required for STAT testing recommended for the diagnosis of malaria infections.
● Outline appropriate procedures for the laboratory identification of malaria.
● Discuss the pros and cons for the FDA-approved rapid test for malaria.
● Differentiate among the five species of human malaria, including potential difficulties associated with morphologic identification to the species level.
● Describe appropriate report comments designed to provide the physician with the most relevant patient information.

INTRODUCTION
Malaria is an ancient human disease, with fatal periodic fevers discussed as early as 2700 BC in Egyptian and Chinese writings. Infections with Plasmodium spp were documented in Rome by 200 BC, spread throughout Europe during the twelfth century, and arrived in England by the fourteenth century. European explorers, conquistadors, and colonists probably imported Plasmodium malariae and Plasmodium vivax to the Americas. The arrival of Plasmodium falciparum coincided with the importation of African slaves, and by the early 1800s malaria was found worldwide.

Malaria has had a greater impact on world history than any other infectious disease including the outcome of wars, population movements, and the rise and fall of various nations. Before the American Civil War, malaria was found as far north as southern Canada; however, by the early 1950s it was no longer an endemic disease within the United States. More than 300 to 500 million individuals worldwide are infected with Plasmodium spp, and 1.5 to 2.7 million people a year, most of whom are children, die from the infection. Malaria is endemic in over 90 countries with an approximate population of 2.4 billion people; this represents nearly 40% of the world’s population. About 90% of the malaria deaths occur in Africa. Despite continuing efforts in vaccine development, malaria prevention is difficult, and no drug is universally effective.

Of the four most common species that infect humans, P. vivax and P. falciparum cause 95% of the infections. P. vivax is responsible for about 80%, since this species has the widest distribution, throughout the tropics, subtropics, and temperate zones. P. falciparum is generally confined to the tropics, P. malariae is sporadically distributed, and Plasmodium ovale is confined mainly to central West Africa and some South Pacific islands.

A fifth malaria has been implicated in human disease. Plasmodium knowlesi, a malaria parasite of long tailed macaque monkeys, has been confirmed in a number of human cases from Malaysian Borneo, Thailand, Myanmar, and the Philippines (9-11). Although it is well known
that under laboratory conditions some monkey malarias can be transmitted to humans, it is now well established that \textit{P. knowlesi} is emerging as an important zoonotic human pathogen (11).

Although malaria is often associated with travelers to endemic areas, other situations resulting in infection include blood transfusions, use of hypodermic needles contaminated by prior use, bone marrow transplantation, congenital infection, and transmission within the United States by indigenous mosquitoes that acquired the parasites from imported infections (9).

In nonendemic areas, it is important for health care personnel to understand the potential problems associated with malarial diagnosis, specifically the fact that symptoms are often nonspecific and may mimic other conditions. When traveling to endemic areas, individuals are susceptible to malarial infection, and prophylaxis may be recommended. Also, due to an increase in the number of people traveling from the tropics to malaria-free areas, the number of imported malaria cases is also increasing.

It is possible that mosquitoes can transmit the infection among people who live or work near international airports; these mosquitoes can also reach areas far removed from the airports (10). This situation has been termed “airport malaria,” and diagnostic testing for malaria should be considered in patients who work or live near an international airport and who present with an acute febrile illness. In addition to asking a patient, “where have you been, and when were you there?” one should also ask, “where do you live?”

Contributing factors to malaria-related fatalities in United States travelers include:

1. failure to seek pre-travel advice
2. failure to prescribe correct chemoprophylaxis
3. failure to obtain prescribed chemoprophylaxis
4. failure to adhere to chemoprophylaxis regimen
5. failure to seek medical care promptly for illness
6. failure to take adequate patient history
7. delay in diagnosis of malaria
8. delay in initiating treatment of malaria

One or more of these potential problems can lead to the death of the patient.

**LIFE CYCLE**

The female anopheline mosquito is the vector for malaria. When the mosquito takes a blood meal, infective sporozoites contained in the salivary glands are discharged into the puncture wound (10). Within about an hour, the sporozoites are carried via the blood to the liver, where they penetrate hepatocytes and begin to grow, thus initiating the pre-erythrocytic or primary exoerythrocytic cycle. Detailed study of sporozoite entry into the hepatocytes indicates that the process involves parasite encoded surface proteins and host molecules. The sporozoites become round or oval and begin dividing repeatedly. This schizogony results in large numbers of exoerythrocytic merozoites. Once these merozoites leave the liver, they invade the red blood cells (RBCs), thus initiating the erythrocytic cycle. It has been reported that a secondary or dormant schizogony may occur in \textit{P. vivax} and \textit{P. ovale} organisms, which remain quiescent in the liver until a true relapse at a later time. These resting forms are called hypnozoites. Delayed schizogony does not occur in \textit{P. falciparum}, \textit{P. knowlesi}, or \textit{P. malariae}.

Recrudescence refers to a situation in which the RBC infection is not eliminated by the immune system or by therapy, and the parasite numbers in the RBCs begin to increase again with subsequent clinical symptoms. All species may cause a recrudescence. A recurrence or true relapse refers to a situation in which the erythrocytic infection is eliminated, and a relapse occurs
later because of a new invasion of the RBCs from liver merozoites. As indicated above, this occurs only in *P. vivax* and *P. ovale* infection.

When the RBCs and reticulocytes have been invaded, the parasites grow and feed on hemoglobin. Within the RBC, the merozoite (or young trophozoite) is vacuolated, ring shaped, more or less ameboid, and uninucleate. The excess protein, an iron porphyrin, and hematin left over from the metabolism of hemoglobin combine to form malarial pigment.

Once the nucleus begins to divide, the trophozoite is called a developing schizont. The mature schizont contains merozoites (whose number depends on the species), which are released into the bloodstream. Many of the merozoites are destroyed by the immune system, but others invade RBCs, in which a new cycle of erythrocytic schizogony begins.

After several erythrocytic generations, some of the merozoites begin to undergo development into the male and female gametocytes (8). Sexual commitment appears to be highest in merozoites derived directly from the liver schizont, and there is a progressive loss of commitment with increased asexual cycles. Environmental stimuli may support an increase in parasite density. Also, there may also be some links between sublethal doses of chloroquine and an increase in gametocyte formation in chloroquine resistant parasites.

The asexual and sexual forms circulate in the bloodstream during infections by four of the *Plasmodium* species. However, in *P. falciparum* infections, as the parasite continues to grow, the RBC membrane becomes sticky and the cells tend to adhere to the endothelial lining of the capillaries of the internal organs. Thus, only the ring forms and the gametocytes (occasionally mature schizonts) normally appear in the peripheral blood in *P. falciparum*.

When the mosquito takes a blood meal, gametocytes are ingested, which mature into gametes while in the mosquito gut. The male microgametocytes undergo nuclear division by exflagellation, in which the microgametes break out of the RBC, become motile, and penetrate the female macrogamete, with the fertilized stage being called the zygote. This zygote then elongates, becomes motile, and is called the ookinete. This stage migrates to the mosquito midgut, secretes a thin wall, and grows into the oocyst, which extends into the insect's hemocele. Within days, the oocyst matures, with the formation of hundreds of sporozoites, some of which migrate to the salivary glands. When the mosquito next takes a blood meal, the sporozoites are injected with saliva into the host. Unfortunately, when blood tubes are held at room temperature, especially with the cap removed, the parasites assume they are now within the mosquito (reduced temperature and oxygenation of the blood). Thus, the process of exflagellation can occur within the tube of blood. Subsequently, when the blood films are prepared, these microgametocytes can be confused with *Borrelia* spp (10).

**CLINICAL DISEASE**

For a week or so after the original mosquito bite, the patient remains asymptomatic. However, during this time, the organisms are undergoing multiplication in the liver (preerythrocytic cycle). Although several broods begin to develop when the liver merozoites invade the RBCs; one will eventually dominate and suppress the others, thus beginning the process of periodicity. Once the cycle is synchronized, the simultaneous rupture of a large number of RBCs and liberation of metabolic waste by-products into the bloodstream precipitate the paroxysms of malaria. In an early infection, particularly in an immunologically naive patient who has never been exposed to malaria, the patient may have nonspecific symptoms with no periodicity seen.

Symptoms of malaria include anemia, splenomegaly, and the classic paroxysm, with its cold stage, fever, and sweat (3-7). Although the periodic febrile episodes strongly suggest infection,
many patients who are seen in the early stages of the infection do not exhibit any typical fever pattern. They may have a fever or several small, random peaks of fever each day. Therefore, all patients should have their blood drawn IMMEDIATELY when they have a fever, since the fever may not be periodic. Since the symptoms associated with malaria are so nonspecific, the diagnosis should be considered in any symptomatic patient with a history of travel to an area where malaria is endemic. In a patient with an established periodicity, the typical paroxysm begins with the cold stage and rigors lasting 1 to 2 hours. During the next few hours, the patient spikes a high fever and feels very hot, and the skin is warm and dry. The last several hours are characterized by marked sweating and a subsequent drop in body temperature to normal or subnormal.

Anemia can be caused by a number of mechanisms, including:
1. direct RBC lysis as a function of the life cycle of the parasite
2. splenic removal of both infected and uninfected RBCs (coated with immune complexes)
3. autoimmune lysis of coated infected and uninfected RBCs
4. decreased incorporation of iron into heme
5. increased fragility of RBCs
6. decreased RBC production from bone marrow suppression

Malaria mimics many other diseases, such as gastroenteritis, pneumonia, meningitis, encephalitis, or hepatitis. Other possible symptoms include lethargy, anorexia, nausea, vomiting, diarrhea, and headache. Leukopenia can also be seen in malaria, as can an occasional elevated white blood cell count with a left shift. Eosinophilia and thrombocytopenia may also be seen but are much less frequent.

DIAGNOSIS

Infections with Plasmodium spp can be life-threatening, and laboratory orders, processing, specimen examination, and reporting for blood smear examination and organism identification should all be treated as “STAT” requests (10). In terms of health care personnel training, it is important to recognize that parasite recovery and identification often tend to be more difficult than expected, particularly in patients with a low parasitemia. Patient history details should be available to the laboratorian as seen below.

Patient Information

When requests for malarial smears are received in the laboratory, some patient history information should be made available to the laboratorian. This information should include the following:
<table>
<thead>
<tr>
<th>QUESTION</th>
<th>COMMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Where has the patient been, and what was the date of return to the United States?</td>
<td>May be helpful in suggesting particular species and/or resistance information.</td>
</tr>
<tr>
<td>Where do you live? This is particularly relevant if the patient lives by an airport.</td>
<td>Well documented that mosquitoes can be transmitted to nonendemic areas through airplanes and/or baggage.</td>
</tr>
<tr>
<td>Has malaria ever been diagnosed in the patient before? If so, what species was identified?</td>
<td>If the response is “yes” – often a relapse case may exhibit a lower parasitemia; species will dictate therapy if P. vivax or P. ovale are involved (potential relapse).</td>
</tr>
<tr>
<td>What medication (prophylaxis or otherwise) has the patient received, and how often? When was the last dose taken?</td>
<td>Prophylaxis tends to reduce the parasitemia; thus microscopic examination of blood films may need to be extended; dose information will influence microscopic examination, as well.</td>
</tr>
<tr>
<td>Has the patient ever received a blood transfusion? Is there a possibility of other needle transmission (drug use)?</td>
<td>Both situations represent malaria transmission possibilities – may have nothing to do with being in an endemic area.</td>
</tr>
<tr>
<td>When was the blood specimen drawn, and was the patient symptomatic at the time? Is there any evidence of a fever periodicity?</td>
<td>If periodicity present, will influence interpretation of blood film findings; if no periodicity, may represent an early infection with a very low parasitemia.</td>
</tr>
</tbody>
</table>

**Conventional Light Microscopy**

Single-draw blood films or specimens are not sufficient to exclude the diagnosis of malaria, especially when the patient has received partial prophylaxis or therapy. Partial use of antimalarials reduces the parasitemia, which complicates making the correct diagnosis, even when serious disease is present. Patients with a relapse case or an early primary case may also have few organisms in the blood smear.

Regardless of the presence or absence of any fever periodicity, both thick and thin blood films should be prepared immediately, and at least 200 to 300 oil immersion fields should be examined on both films before a negative report is issued. One set of negative films will not rule out malaria; additional blood specimens should be examined over a 36-hour time frame (10).

The characteristics of the thin film include:

- RBC morphology is preserved
- less blood is examined when compared with the thick blood film
- the overall sensitivity is less than the thick blood film
- the ability to identify the organisms to the species level is greater than on the thick film

The characteristics of the thick blood film include:

- the RBCs are laked (ruptured prior to or during staining)
- more blood is examined when compared with the thin blood film
- the overall sensitivity is greater than the thin blood film
• the ability to identify the organisms to the species level is less than on the thin blood film

Although Giemsa stain has been recommended for all parasitic blood work, the organisms can also be seen if other blood stains, such as Wright stain, Wright-Giemsa, Field’s, or the rapid stains are used. Regarding staining quality control, the patient’s thick and thin blood films being stained serve as their own quality control; if the WBCs look good, any parasites present will exhibit acceptable staining characteristics. Thus, your QC slide is the actual slide you are staining. Any parasites present will stain like WBCs, so your QC is built into the system.

Blood collected using EDTA anticoagulant is preferred over heparin; however, if the blood remains in the tube for any length of time before smears are made, after they are stained, Schüffner’s dots may not be visible (P. vivax, as an example) and other morphologic changes in the parasites will be seen. Also, the proper ratio between blood and anticoagulant is required for good organism morphology, so each collection tube should be filled to the top. Finger-stick blood is recommended, particularly when the volume of blood required is minimal (i.e., when no other hematologic procedures have been ordered). The blood should be free-flowing when taken for smear preparation, and should not be contaminated with alcohol used to clean the finger prior to the stick. However, the use of finger-stick blood is much less common than in the past, and venipuncture blood is the normal specimen obtained. Usually the amount of blood taken during a finger stick is quite small, while venipuncture blood is often approximately 7 ml. Also, when finger stick blood is spread onto the glass slide, the Plasmodium spp life cycle is stopped at that point, while the life cycle continues in venipuncture blood that remains in the tube prior to blood film preparation.

If a tube of blood containing EDTA cools to room temperature and the cap has been removed, several parasite morphologic changes can occur. The parasites within the RBCs will respond as if they were now in the mosquito after being taken in with a blood meal. The morphology of these changes in the life cycle and within the RBCs can cause confusion when examining blood films prepared from this blood. Also, after 4-6 hours, parasites begin to disappear from the blood specimen. Also, the following changes may be possible:

1. stippling (Schüffner’s dots) may not be visible
2. the male gametocyte (if present) may exflagellate and mimic spirochetes
3. the ookinetes of Plasmodium species other than P. falciparum may develop as if they were in the mosquito and may mimic the crescent-shaped gametocytes of P. falciparum

Identification to the species level determines which drug(s) is recommended. In early infections, patients with P. falciparum infections may not have the crescent-shaped gametocytes in the blood. Low parasitemias with the delicate ring forms may be missed; consequently, oil immersion examination at 1000x is mandatory. Some microscopists may use the 50x or 60x oil immersion objectives to screen the blood films; however, oil immersion examination using the 100x objective is required before reporting the final results.

Patients with malaria may appear for diagnostic blood work when least expected. Laboratory personnel should be aware of the "STAT" nature of such requests and the importance of obtaining some specific patient history information. The typical textbook presentation of the blood smears may not be seen by the technologist. It becomes very important that the smears be examined at length and under oil immersion. The most important thing to remember is that even though a low parasitemia may be present on the blood smears, the patient may still be faced with a serious,
Malaria is one of the few parasitic infections considered to be immediately life-threatening, and a patient with the diagnosis of P. falciparum or P. knowlesi malaria should be considered a medical emergency because the disease can be rapidly fatal. Any laboratory providing the expertise to identify malarial parasites should do so on a 24-h basis, 7 days/week.

Parasite Morphology

Although specific morphologic characteristics of the various Plasmodium spp are provided for the reader, (see below), with a very low parasitemia it can be very difficult to identify the parasites to the species level. It is always important to do so, if possible; however, in many cases it may not be possible. Remember that the thick film is designed to allow you to detect the presence of the organism(s), while the thin film is designed to allow identification to the species level (providing the parasitemia is sufficient). It is mandatory that both be examined.

*Plasmodium vivax* (benign tertian malaria) (2, 5, 9, 10)
1. 48-hour cycle
2. Tends to infect young cells
3. Enlarged RBCs
4. Schüffner's dots (true stippling) after 8-10 hours (may not be visible from EDTA)
5. Delicate ring; occasional multiple rings per cell
6. Very ameboid trophozoite
7. Mature schizont contains 12-24 merozoites
8. All stages can be found in the peripheral blood
9. Hypnozoites in the liver can initiate true relapse at a later time

*Plasmodium malariae* (quartan malaria) (6, 9, 10)
1. 72-hour cycle (long incubation period)
2. Tends to infect old cells
3. Normal size RBCs
4. No stippling
5. Thick ring, large nucleus
6. Trophozoite tends to form "bands" across the cell
7. Mature schizont contains 6-12 merozoites
8. All stages can be found in the peripheral blood
9. No true relapse from the liver; recrudescence possible

*Plasmodium ovale* (7, 9, 10)
1. 48-hour cycle
2. Tends to infect young cells
3. Enlarged RBCs with fimbriated edges (oval)
4. Schüffner's dots appear in the beginning (in RBCs with very young ring forms in contrast to *P. vivax*)
5. Smaller ring than *P. vivax*
6. Trophozoite less ameboid than that of *P. vivax*
7. Mature schizont contains average 8 merozoites
8. All stages can be found in the peripheral blood
9. Hypnozoites in the liver can initiate true relapse at a later time

**Plasmodium falciparum** (malignant tertian malaria) (6, 9, 10)
1. 36-48-hour cycle
2. Tends to infect any cell regardless of age, thus very heavy infection may result
3. All sizes of RBCs
4. No Schüffner's dots (Maurer's dots: may be larger, single dots, bluish)
5. Multiple rings/cell (only young rings, gametocytes, and occasional mature schizonts are seen in peripheral blood)
6. Delicate rings, may have two dots of chromatin/ring, appliqué or accolé forms
7. Crescent-shaped gametocytes
8. Usually only the rings, gametocytes and an occasional mature schizont are seen in the peripheral blood
9. No true relapse from the liver

**Plasmodium knowlesi** (simian malaria)* (9, 10, 11)
1. 24-hour cycle
2. Tends to infect any cell regardless of age, thus very heavy infection may result
3. All sizes of RBCs, but most tend to be normal size
4. No Schüffner's dots (faint, clumpy dots later in cycle)
5. Multiple rings/cell (may have 2-3)
6. Delicate rings, may have two or three dots of chromatin/ring, appliqué forms
7. Band form trophozoites commonly seen
8. Mature schizont contains 16 merozoites, no rosettes
9. Gametocytes round, tend to fill the cell
10. All stages can be found in the peripheral blood
11. No true relapse from the liver

*Early stages mimic *P. falciparum*; later stages mimic *P. malariae*

**Alternative Methods**

A number of alternative methods have been developed, including different approaches to microscopy, flow cytometry, biochemical methods, immunoassay, and molecular methods. The aim of these procedures has been to reduce cost, reduce requirements for expensive equipment, increase sensitivity, and provide simple, rapid methods that do not require conventional microscopy.

Several rapid malaria tests (RMTs) are now commercially available, some of which use monoclonal antibodies against the histidine-rich protein 2 (HRP2) whereas others detect species-specific parasite lactate dehydrogenase (pLDH) (1, 10, 12). These procedures are based on an antigen capture approach in dipstick or cartridge formats. Only one of these RMTs is Food and Drug Administration approved for use within the United States. The BinaxNOW Malaria Test is a rapid immuno-diagnostic assay for differentiation and detection of circulating *Plasmodium falciparum* (P.f.) antigen and the panspecific antigen common to all to malarial species: *Plasmodium vivax* (P.v.), *Plasmodium ovale* (P.o.), *Plasmodium malariae* (P.m.), and *Plasmodium knowlesi* (P.k.) in whole blood.

Although these tests are rapid, the maximum sensitivity is for a parasitemia >5,000
parasites/µl (0.1%). As one can see from the chart below, many patients will have a parasitemia less than 0.1%; thus any negative result must revert immediately back to the STAT thick and thin blood films. Many travelers who have never been exposed to malaria before (immunologically naïve) may present with an extremely low parasitemia that can be missed using rapid methods, automation, and even routine thick/thin blood films.

<table>
<thead>
<tr>
<th>Parasitemia (%)</th>
<th>No. of Parasites/µl</th>
<th>Clinical Correlation †</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001-0.0004</td>
<td>5-20</td>
<td>Number of organisms required for positive thick film (sensitivity)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Examination of 100 thick-blood-film (TBF) fields (0.25 µl) may miss up to 20% of infections (sensitivity 80-90%); at least 300 fields should be examined before reporting a negative result</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Examination of 100 thin-blood-film fields (THBF) (0.005 µl); at least 300 fields should be examined before reporting a negative result; <strong>both</strong> thick and thin blood films should be examined for every specimen submitted for a suspect malaria case (report final results using 100 x oil immersion objective)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>BinaxNOW® rapid lateral flow method (dipstick) (0-100 = 53.9% sensitivity for <em>P. falciparum</em>)</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>BinaxNOW® rapid lateral flow method (dipstick) (0-100 = 6.2% sensitivity for <em>P. vivax</em>)</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>One set (TBF + THBF) of negative blood films does not rule out a malaria infection</strong></td>
</tr>
<tr>
<td>0.002</td>
<td>100</td>
<td><strong>Patients may be symptomatic below this level, particularly if they are immunologically naïve</strong> (no prior exposure to malaria)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Example: travelers)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>BinaxNOW® rapid lateral flow method (dipstick) (100-500 = 89.2% sensitivity for <em>P. falciparum</em>)</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>BinaxNOW® rapid lateral flow method (dipstick) (100-500 = 23.6% sensitivity for <em>P. vivax</em>)</strong></td>
</tr>
<tr>
<td>0.02</td>
<td>1,000</td>
<td><strong>Level often seen in travelers (immunologically naïve) – results may also be lower than this</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>BinaxNOW® rapid lateral flow method (dipstick) (1000-5000 = 99.2% sensitivity for <em>P. falciparum</em>) (500-1000 = 92.6% sensitivity for <em>P. falciparum</em>)</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>BinaxNOW® rapid lateral flow method (dipstick) (1000-5000 = 81.0% sensitivity for <em>P. vivax</em>) (500-1000 = 47.4% sensitivity for <em>P. vivax</em>)</strong></td>
</tr>
<tr>
<td>0.1</td>
<td>5,000</td>
<td><strong>BinaxNOW® rapid lateral flow method (dipstick) (&gt;5000 = 99.7% sensitivity for <em>P. falciparum</em>)</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>BinaxNOW® rapid lateral flow method (dipstick) (&gt;5000 = 93.5% sensitivity for <em>P. vivax</em>)</strong></td>
</tr>
<tr>
<td>0.2</td>
<td>10,000</td>
<td><strong>Level above which immune patients will exhibit symptoms</strong></td>
</tr>
<tr>
<td>2</td>
<td>100,000</td>
<td><strong>Maximum parasitemia of <em>P. vivax</em> and <em>P. ovale</em> (which infect young RBCs only)</strong></td>
</tr>
</tbody>
</table>
### Table

<table>
<thead>
<tr>
<th>Antigen Load (µl)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-5</td>
<td>Hyperparasitemia, severe malaria(^a); increased mortality</td>
</tr>
<tr>
<td>10</td>
<td>Exchange transfusion may be considered; high mortality</td>
</tr>
</tbody>
</table>

\(^a\)The BinaxNOW® malaria test (Inverness Medical, Scarborough, ME) is FDA approved. The BinaxNOW® Malaria Test detects antigen from both viable and non-viable malaria organisms, including gametocytes and sequestered *P. falciparum* parasites. Test performance depends on antigen load in the specimen and may not directly correlate with microscopy performed on the same specimen. Samples with positive rheumatoid factor titers may produce false positive results. Analytical reactivity testing demonstrates that the pan malarial test line on the BinaxNOW® test is capable of detecting all five malaria species. However, during clinical trials, insufficient data was generated to support clinical performance claims for the detection of *P. malariae*, *P. ovale*, or *P. knowlesi*. Clinical performance claims for this test are made for *P. falciparum* and *P. vivax* detection only. The test is not intended for use in screening asymptomatic populations. (BinaxNOW® malaria test package insert (Inverness Medical, Scarborough, ME). The BinaxNOW Positive Malaria Control, as well as the BinaxNOW® Malaria test, is available commercially (Inverness Medical, Scarborough, ME).

\(^b\)World Health Organization criteria for severe malaria are parasitemia of >10,000/µl and severe anemia (hemoglobin, <5 g/liter). Prognosis is poor if >20% of parasites are pigment-containing trophozoites and schizonts and/or if >5% of neutrophils contain visible pigment.

Potential diagnostic problems with the use of automated differential instruments have been reported (10). Some cases of malaria, as well as *Babesia* infections, have been completely missed using these methods. The number of fields scanned by a technologist on instrument-read smears is quite small; thus, failure to detect a light parasitemia is almost guaranteed. In both cases, after diagnosis had been made on smears submitted to the parasitology division of the laboratory, all previous smears examined by the automated system were reviewed and found to be positive for parasites. Failure to make the diagnosis resulted in delayed therapy. Although these instruments are not designed to detect intracellular blood parasites, the inability of the automated systems to discriminate between uninfected RBCs and those infected with parasites may pose serious diagnostic problems in situations where the parasitemia is >0.5%.

**Reporting Results.** It is extremely important that result reports convey as much information as possible. There are particular circumstances where report comments are very valuable, and specific examples are provided below:
RESULT*  REPORT COMMENT(S)  INTERPRETATION DISCUSSION

No Parasites Seen  The submission of a single blood specimen will not rule out malaria; submit additional bloods every 4-6 hours for 3 days if malaria remains a consideration.  It is important to make sure the physician knows that examination of a single blood specimen will not rule out malaria.

Plasmodium spp Seen  Unable to rule out *Plasmodium falciparum* or *P. knowlesi*  Since *P. falciparum* and *P. knowlesi* cause the most severe symptoms, it is important to let the physician know these species have NOT been ruled out.

Plasmodium spp, possible mixed infection  Unable to rule out *Plasmodium falciparum* or *P. knowlesi*  Since *P. falciparum* and *P. knowlesi* cause the most severe symptoms, it is important to let the physician know these species have NOT been ruled out.

Negative for parasites using automated hematology instruments  Automated hematology instruments will not detect low malaria parasitemias seen in immunologically naïve patients (travelers with no prior exposure to malaria)  In patients who have never been exposed to malaria (immunologically naïve), they will become symptomatic with very low parasitemias that will not be detected using automation (0.001 to 0.0001%)

* NOTE: Use of the BinaxNOW (Inverness Medical) malaria rapid test may help detection of mixed infections (STAT test). This test is FDA approved and the external malaria control is now also available (BinaxNOW® Malaria Product Fact Sheet Test Kit & Positive Control). However, if test is negative, thick and thin blood film examination is mandatory (STAT).

REFERENCES

REVIEW QUESTIONS
Course #DL-002
Choose the one best answer

1. When malaria smears are requested, what patient information should be obtained?
   a. diet, age, sex
   b. age, antimalarial medication, sex
   c. travel history, antimalarial medication, date of return to U.S.
   d. fever patterns, travel history, diet

2. *Plasmodium vivax* and *Plasmodium ovale* are similar because they:
   a. exhibit Schüffner’s dots and have a true relapse in the life cycle
   b. have no malarial pigment but do have multiple rings per RBC
   c. commonly have appliqué forms in the red cells
   d. have true stippling, do not have a relapse stage, and infect old red cells

3. The main differences between finger stick and venipuncture blood are:
   a. oxygen content and number of RBCs
   b. volume of blood and *Plasmodium* spp parasites do not continue developing in finger stick blood
   c. parasitemia visible and number of ring forms
   d. presence of gametocytes and mature schizonts

4. A traveler who has acquired a *Plasmodium* spp infection, but who has never been exposed to malaria before is classified as being:
   a. immunologically deficient
   b. immunologically immunosuppressed
   c. immunologically mature
   d. immunologically naïve

5. The main benefit of the thick blood film is:
   a. the volume of blood is larger than the thin blood film
   b. the thick blood film is less sensitive in terms of organism detection
   c. parasite morphology is superior to that on the thin blood film
   d. WBCs are no longer visible

6. What is the best anticoagulant to use for blood specimens for parasitology (venipuncture)?
   a. heparin
   b. EDTA
   c. no anticoagulant is required
   d. type of anticoagulant not relevant

7. What type of QC slides should be used for blood parasite work?
   a. positive slides containing malarial parasites
   b. positive slides containing any blood parasite
   c. slides stained with Giemsa stain only
   d. patient slide that you are currently staining
8. The infective stage from which the patient acquires malaria from the mosquito vector is:
   a. the sporozoite
   b. the ookinete
   c. the gametocyte
   d. the exflagellating male gametocyte

9. A mature schizont is described as having 8 merozoites arranged around the excess malarial pigment and is seen in an infected RBC that is relatively small in size. The *Plasmodium* species is most probably which of the following:
   a. *Plasmodium falciparum*
   b. *Plasmodium vivax*
   c. *Plasmodium ovale*
   d. *Plasmodium malariae*

10. When is the most appropriate time to draw blood for thick and thin blood film preparation for the diagnosis of malaria?
    a. when the fever peaks
    b. after the fever peaks
    c. every 2 hours
    d. immediately on request

11. Acceptable stain options for blood parasite work include which of the following:
    a. Giemsa stain
    b. Giemsa, Wright, Wright-Giemsa, Field’s stains
    c. Delafield’s hematoxylin stain, rapid blood stains
    d. all of the above

12. How should malaria blood films (both thick and thin films) be examined?
    a. 10 min each using 100x oil immersion objective
    b. 300 oil immersion fields (using 100x oil objective)
    c. 10 min thin, 20 min thick films using 60x oil immersion objective
    d. screen using 60x oil immersion objective, 5 min using 100x oil immersion objective

13. RBCs containing multiple rings/cell are usually seen in infections with:
    a. *Plasmodium vivax*
    b. *Plasmodium ovale*
    c. *Plasmodium malariae*
    d. *Plasmodium falciparum*

14. Finding only ring forms on two sets of blood films drawn 6 h apart may suggest:
    a. the possibility of a mixed infection
    b. the possibility of an infection with *P. vivax*
    c. the probability of an infection with *P. falciparum*
    d. the probability of an infection with *P. ovale*
15. Why aren’t gametocytes of *P. falciparum* seen in many patients presenting to the Emergency Room (ER)?
   a. no gametocytes are formed in most infections
   b. it is too early in the cycle to see gametocytes
   c. treatment has destroyed the gametocytes
   d. the parasitemia is too low

16. A developing trophozoite has been described as follows: enlarged RBC, very ameboid troph, presence of Schüffner’s dots. The species is most likely:
   a. *Plasmodium vivax*
   b. *Plasmodium ovale*
   c. *Plasmodium knowlesi*
   d. *Plasmodium falciparum*

17. Which of the following is **not** important to include in a report from blood film examination for malaria?
   a. genus, species
   b. parasitemia, possible mixed infection
   c. ability to “rule out” *P. falciparum* and *P. knowlesi*
   d. sex of a patient

18. Crescent-shaped gametocytes are most often associated with:
   a. *Plasmodium vivax*
   b. *Plasmodium ovale*
   c. *Plasmodium malariae*
   d. *Plasmodium falciparum*

19. Early ring stages of the fifth human malaria, *Plasmodium knowlesi*, resemble those of:
   a. *Plasmodium malariae*
   b. *Plasmodium ovale*
   c. *Plasmodium falciparum*
   d. *Plasmodium vivax*

20. The infected RBCs containing developing trophozoites tend to be small in which of the following species:
   a. *Plasmodium ovale* and *P. falciparum*
   b. *Plasmodium malariae* and *P. knowlesi*
   c. *Plasmodium vivax* and *P. ovale*
   d. *Plasmodium falciparum* and *P. vivax*