KETTERING Health Network

Detection of Blood Parasites

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Retired: Date: _____

I. PRINCIPLE

During certain stages in their life cycle, species of *Plasmodium* (malaria), *Babesia*, *Trypanosoma*, *Leishmania donovani*, and filariae are detectable in human blood. *Plasmodium* and *Babesia* species are found within the RBCs; trypanosomes and microfilariae, the larval stage of filariae , are found outside the RBCs; *Leishmania* amastigotes are occasionally found within monocytes.

Microscopic examination of stained blood films is best accomplished by beginning with a thorough search of both the thin and thick films with low power magnification for microfilariae. If larvae are found, magnification at a higher power will reveal the finer morphological details necessary to make a definitive identification. Other blood parasites require examination with oil immersion magnification of both thin and thick films. Trypanosomes, even those detected in the thick film, are more frequently identified in the thicker portion of the thin film. *Plasmodium* and *Babesia* spp., being intracellular parasites, are detected in the thick film, but are more readily identifiable in the thinner portion of the thin film.

Satisfactory examination requires approximately 5 to 10 minutes for the thick film (about 100 fields) and 15 to 20 minutes for the thin film (about 300 fields).

II. SPECIMEN COLLECTION, TRANSPORT, AND HANDLING

A. Whole EDTA (purple) tube
B. Slides should be made within 2 hours of blood collection

III. MATERIALS

A. Coplin Jars or Slide holder
B. Deionized water
C. Wright Giemsa Stain
D. Glass microscope slides – Frosted
E. Pencil to label slides
F. Applicator sticks
G. Paper with newsprint size print
H. Microscope
I. Ocular micrometer
J. Color atlas of hematology

IV. QUALITY CONTROL

Macroscopically, blood films appear pinkish-purple microscopically, RBCs appear tan to pinkish red, and WBCs have bright blue nuclei and lighter cytoplasm. Slight variations may appear in the colors described, depending on the batch of stain used and the character of the blood itself, but if the various morphological structures are distinct, the stain is satisfactory.

V. PROCEDURE

A. **Thick smear preparation – Hematology department will make 3 thick smears.**
   1. Place a clean glass microscope slide on a horizontal surface.

2. Label the slide with a pencil on the frosted part of the slide. Label slides with patient's name and accession number.
3. Place a drop (30-40 uL) of blood onto the center of the slide about ½ inch from the end.
4. Immediately place the thick film over small print and be sure that the print can just barely be read through it. If not, expand the size of the blood film until the print can be read
5. Allow the film to dry in a horizontal position protected from dust for at least 1 hour. Do not use any type of heat, because heat will fix the RBCs, and they will not lyse in the staining procedure.
6. Place smears in deionized water for 30 seconds in Coplin Jar to lyse the red cells. Let slides air dry thoroughly after removal from deionized water, keep slides at an angle to dry
7. Stain slides using **Wright-Giemsa Stain**. (Hematology stainer)

B. **Thin smear preparation – Hematology department will make 3 thin smears.**
1. Make slides using the same procedure for differentials.
2. Allow the film to dry in a horizontal position protected from dust.
3. Label the slides with a pencil on the frosted part of the slide. Label slides with patient's name and accession number.
4. Stain slides using **Wright-Giemsa Stain**. (Hematology stainer).

Advantages of thick and thin blood films

Thin blood film advantages	Thick blood film advantages
RBC morphology (size, shape, stippling) can be seen after fixation with methanol prior to staining (Giemsa) or as a part of the staining process (Wright). RBCs are laked in the thick film. Other blood stains are also acceptable (including the rapid stains).	Higher number of parasites per field than with the thin blood film. RBCs are laked, so WBCs, platelets, and parasites will be visible after staining.
Identification to the *Plasmodium* species level is easier, since the parasite can be seen within the RBC. The size of the parasites within the RBCs can provide information necessary for identification to the species level.	Phagocytized malaria pigment can be seen within the WBCs, even with a low parasitemia.
Parasitemia (%) can be calculated from the thin film; determination of parasitemia is mandatory for all species of *Plasmodium* and is particularly important to monitor therapy for *P. falciparum*. Parasitemia should be reported with every set of positive blood films for malaria or *Babesia*.	Stippling may be seen in a well-stained thick film. However, this will depend on how long the blood has been in contact with anticoagulant if not collected as a fresh specimen (finger stick).

C. Microscopic examination
1. Both thin and thick slides are to be screened by Hematology department first. These results are recorded on a Pathologist Review Form BS1.
2. The slides are then taken to Microbiology for screening.

3. Examine the entire area of both thin and thick preparations under low power (10X) for the presence of microfilariae or trypanosomes.
4. Examine a minimum of 300 oil immersion fields (100X) before reporting results.
5. All smears for the detection of blood parasites must be reviewed by a Pathologist before a final report can be issued.

VI. REPORTING RESULTS

A. Reporting Results

1. The Hematology tech will record their results on a Pathologist Review Form in the Other Abnormalities section.
2. Microbiology department results one of the following as Preliminary Results. ***Refer to Appendix A for images***. ***Refer to Appendix B for instructions on resulting in LIS.*** Pathologist will give the Final Results after review:
   a. Blood flagellate, not otherwise specified, referred for identification
   b. Microfilaria, not otherwise specified, referred for identification
   c. *Plasmodium sp./Babesia sp.* Seen, referred for identification
   d. Specimen screened for blood parasites, no organisms seen
   e. Blood or tissue parasite, not *Plasmodium sp.* or *Babesia sp.*, referred for identification
3. A lab comment "To be reviewed by a pathologist" will automatically be applied to the result.
4. Take all slides and Pathologist Review Form to pathologist covering blood smears for that day. If specimen is received after hours leave slides for pathologist confirmation the next day.
5. After the pathologist has reviewed the smears. Finalize report with a lab comment "Reviewed by: ___ (name of pathologist) ____ (.ptr)
6. Organisms present should be quantified by Microbiology tech as to the number (percent) of infected RBCs.
   a. Parasitemia can be calculated on a thin blood film as follows:
      1) Count the number of infected RBCs per 100 RBC in different oil immersion fields
      2) Apply the formula:
         $\frac{\text{\# of infected RBCs}}{\text{Total\# of RBCs counted}} \times 100 = \%\ \text{Parasitemia}$
7. The appropriate county health department must be notified for any positive specimen.
8. Positive blood parasites will be sent to ODH for identification. Send out requirements:
   a. Thick and thin slides (used at KMC for testing)
   b. EDTA blood
   c. ODH Microbiology Specimen Submission Form
   d. CDC Form 50.34
   e. Send all items to:
      ATTN: Parasitology
      Ohio Department of Health
      Bureau of Public Health Laboratory
      8995 East Main Street, Bldg 22
      Reynoldsburg, OH 43068
9. Report any parasite found to the Infection Control and the physician or nursing unit as soon as possible

B. Expected Results

1. Specimen screened for blood parasites, no organisms seen.

VII. CRITICAL DETERMINANTS

A. All smears for the detection of blood parasites must be reviewed by a Pathologist
B. If the smears are prepared from the anti-coagulated blood that is more than one hour old, morphology of parasites may not be typical, and the film may wash off the slide during the staining procedure.
C. At least 300 oil immersion fields must be examined before reporting the test as negative
D. Giemsa stain should be stored at room temperature

VIII. DECISION CRITERIA

A. Visually, the thick smear should be round to oval and approximately 2.0cm across.
B. Newsprint should barely be readable through the wet or dry thick film.
C. The film should not have any clear areas or smudges.
D. Make certain the films are protected from dust and not exposed to heat while drying.
E. The microscope objectives should be calibrated for an ocular micrometer.
F. Finding no parasites in one set of blood films does not rule out a parasitic infection.
G. Reference materials (Atlas) should be used to aid in identification and are available in the department.

IX. FORMS ASSOCIATED WITH PROCEDURE:

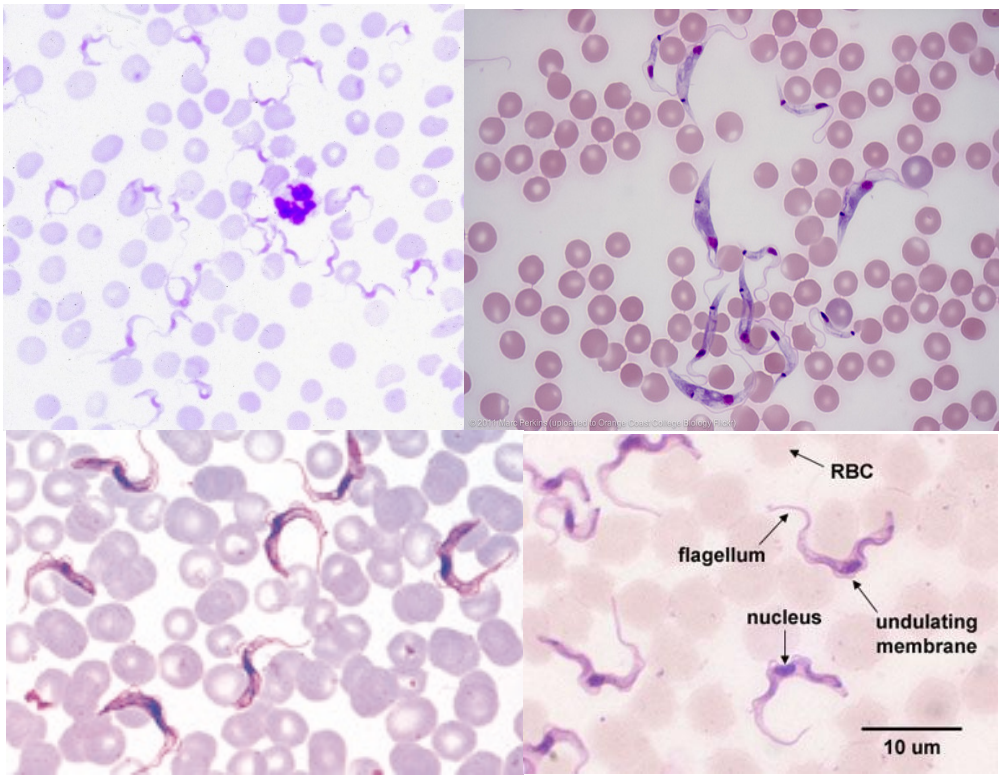
A. BS1 Pathologist Review Form

X. REFERENCES

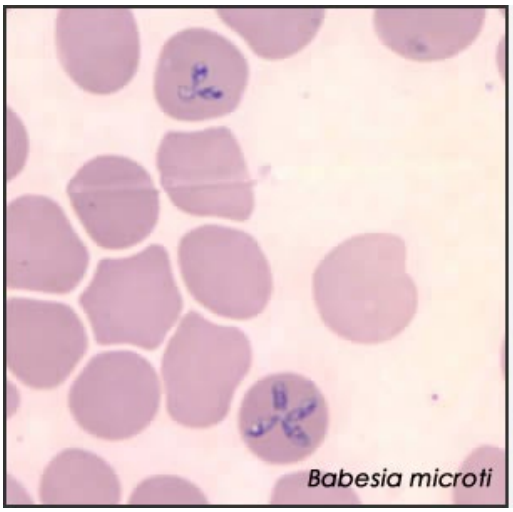
A. Manual of Clinical Microbiology**,** 9th ed. American Society for Microbiology, Washington, D.C., 2007
B. Thermo Scientific Richard-Allan Scientific Giemsa Stain Solution Instructions for Use 2015
C. *Manual of Clinical Microbiology 11th Edition American Society for Microbiology.* Washington: ASM Press. Jorgensen, J. H., Pfaller, M. A., Carroll, K. C., Landry, M. L., Funke, G., Richter, S. S., et al. (2015)
D. Color Atlas of Hematology CAP 1998

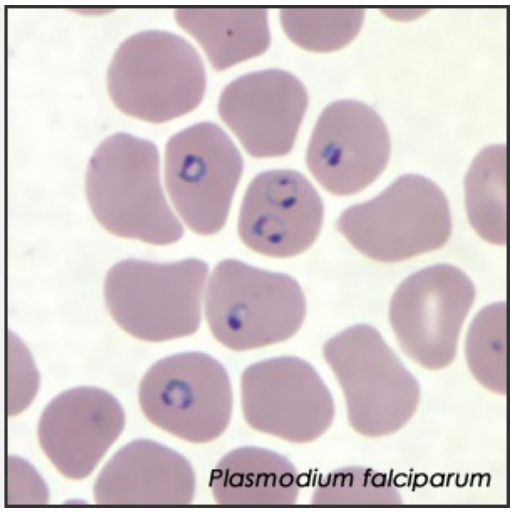
Appendix A

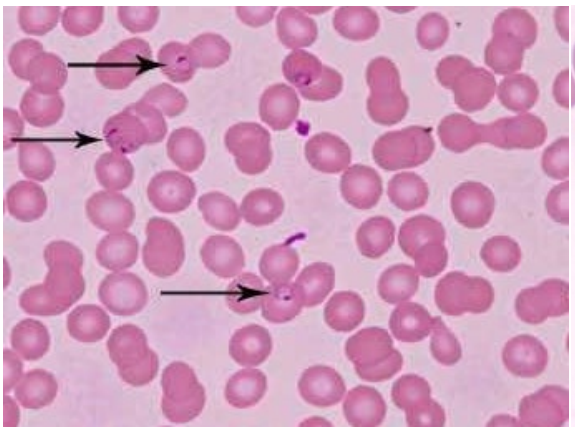
Blood flagellate: An organism with 1or more whip-like appendages called flagella.

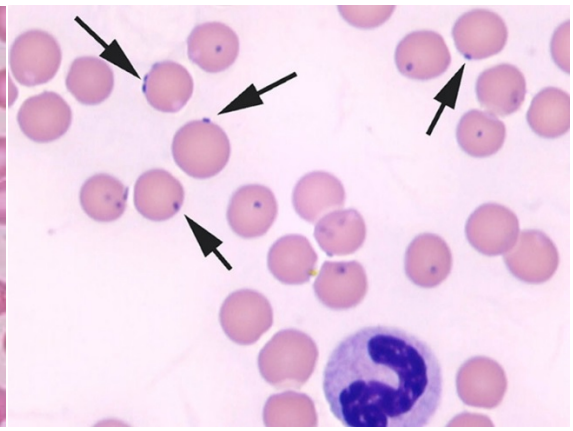


***Plasmodium sp and Babesia sp*: An organism with different shapes and appearances at various stages of development.**

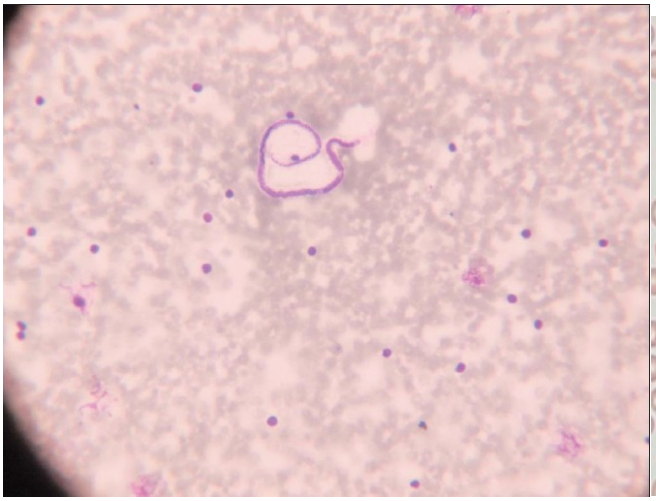


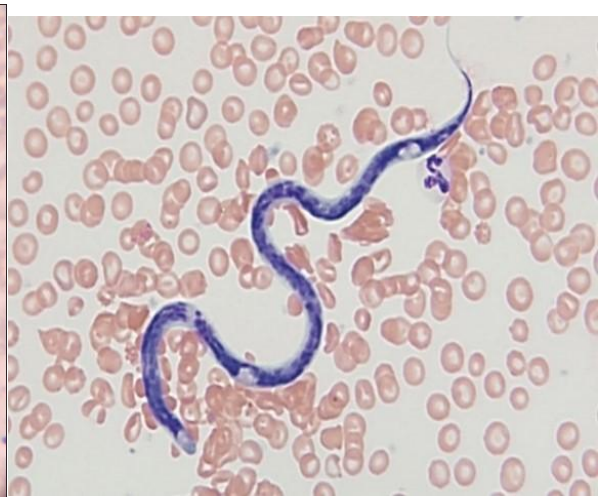


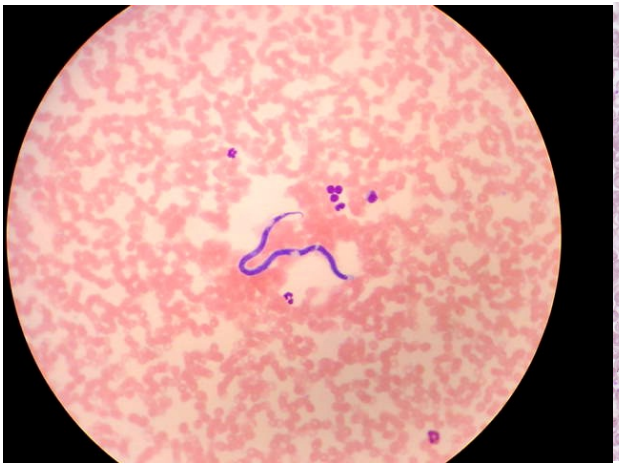


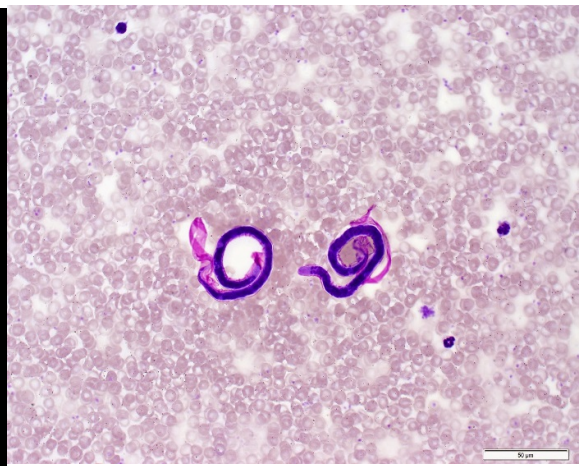


Microfilaria: An organism 160-315um in length, depending on species. They have elongated cylindrical bodies with one tapered end, one rounded end and smooth contours. Nuclei are arranged in a chain, filling most of the body. Some species have a thin covering transparent sheath.



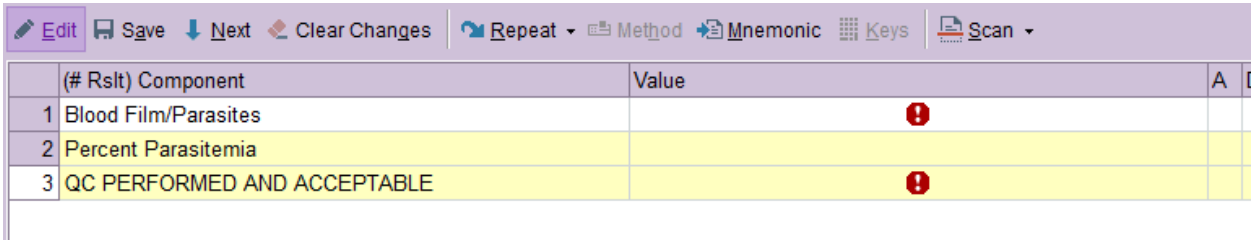




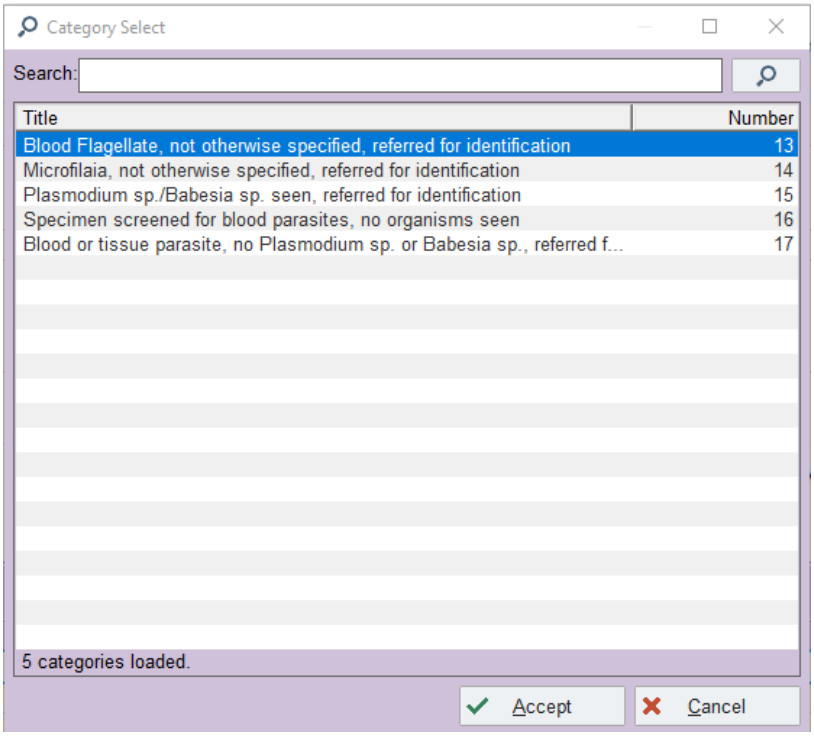


Appendix B

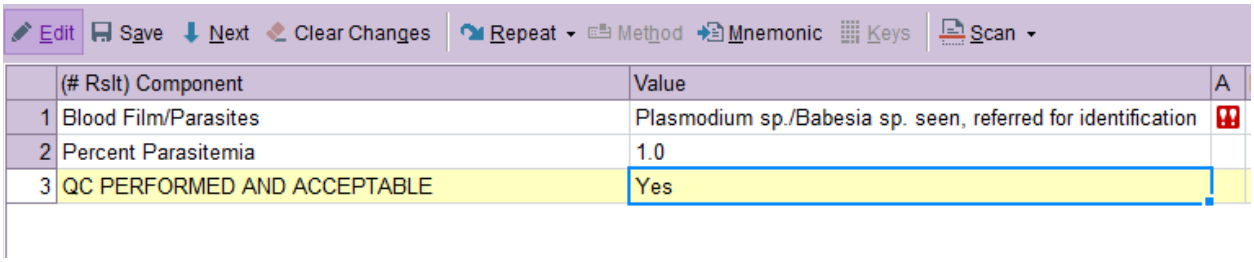
- Select Result Entry in Beaker and enter specimen number
  - Verify patient information matches patient information of the organism being tested.
- Click on “Edit”



- Click on the magnifying glass under value and select the appropriate result from the category list.

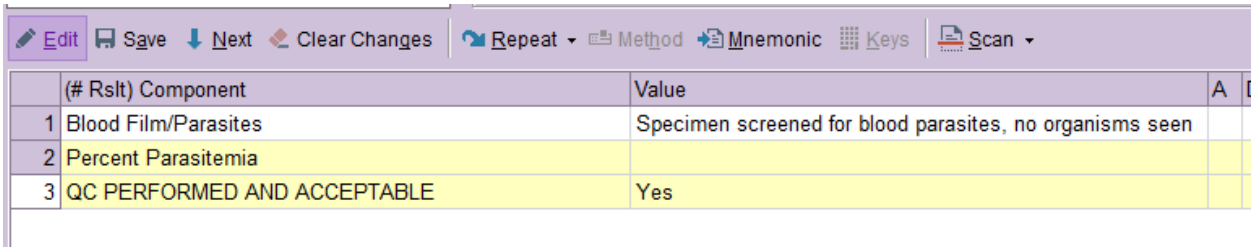


- If Plasmodium sp./Babesia sp. is seen, calculate and enter Percent Parasitemia



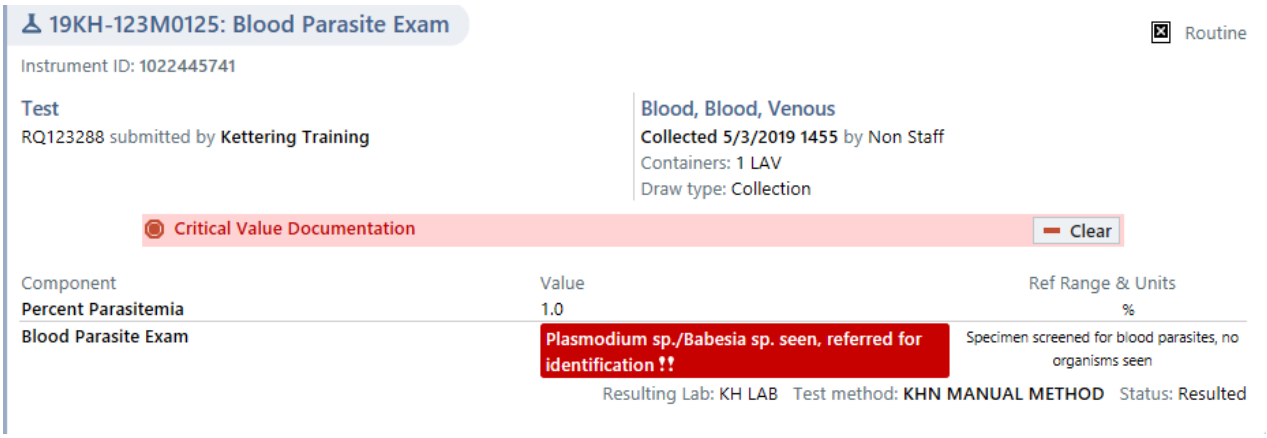
	(# Rslt) Component	Value	A
1	Blood Film/Parasites	Plasmodium sp./Babesia sp. seen, referred for identification	!!
2	Percent Parasitemia	1.0	
3	QC PERFORMED AND ACCEPTABLE	Yes	

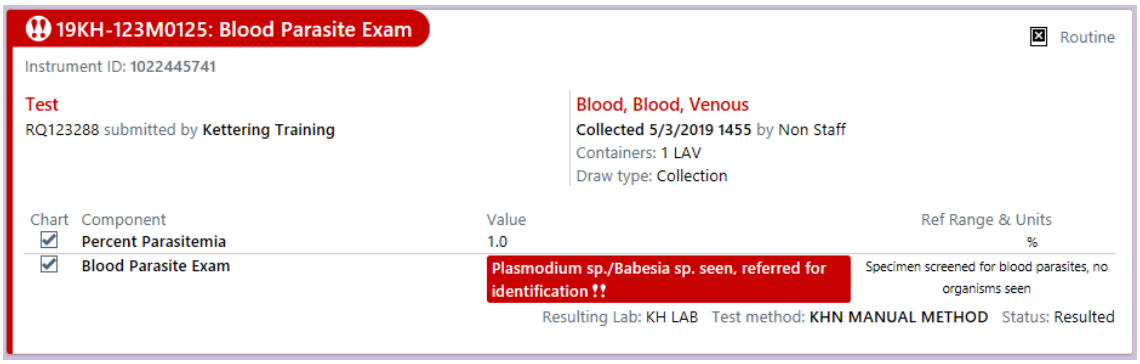
- If no parasites are seen do not put a result in the Percent Parasitemia field



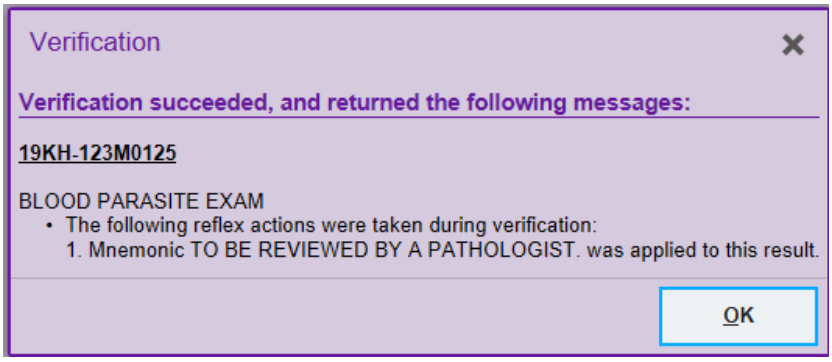
	(# Rslt) Component	Value	A
1	Blood Film/Parasites	Specimen screened for blood parasites, no organisms seen	
2	Percent Parasitemia		
3	QC PERFORMED AND ACCEPTABLE	Yes	

- QC PERFORMED AND ACCECTABLE is to document the acceptability of the stain.
- Prelim Verify.
    - The critical value must be documented in order to prelim verify the result

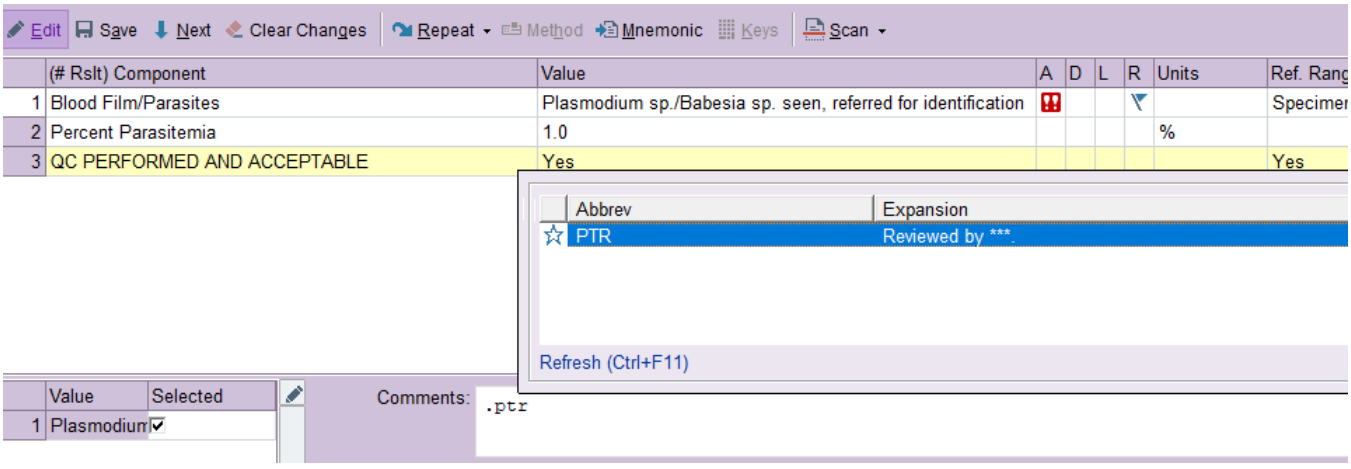




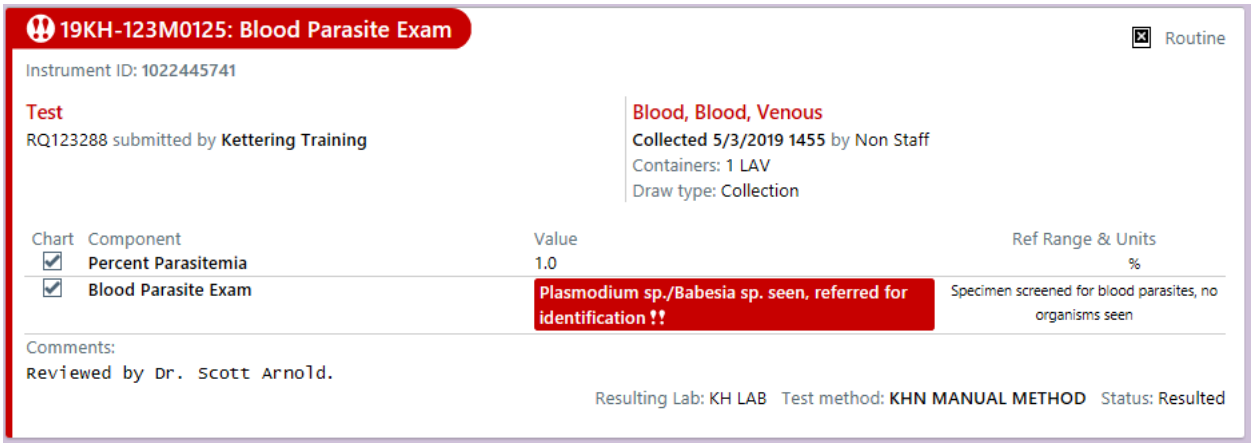
- A comment will be automatically added that states “TO BE REVIEWED BY A PATHOLOGIST”



- After pathologist reviews the slides return to Result Entry and Edit. Take out the To be reviewed by a Pathologist comment and replace it with .ptr this will add Reviewed by *** put in name of pathologist that reviewed the slides and any comments from the review.



- Final Verify



19KH-123M0125: Blood Parasite Exam

Routine

Instrument ID: 1022445741

Test
RQ123288 submitted by **Kettering Training**

Blood, Blood, Venous
Collected 5/3/2019 1455 by Non Staff
Containers: 1 LAV
Draw type: Collection

Chart	Component	Value	Ref Range & Units
☑	**Percent Parasitemia**	1.0	%
☑	**Blood Parasite Exam**	**Plasmodium sp./Babesia sp. seen, referred for identification !!**	Specimen screened for blood parasites, no organisms seen

Comments:
Reviewed by Dr. Scott Arnold.

Resulting Lab: KH LAB Test method: **KHN MANUAL METHOD** Status: Resulted