remel

GIEMSA PLUS STAIN KIT

INTENDED USE
Remel Giemsa Plus Stain Kit is a rapid, differential stain intended for use in the detection and identification of blood and tissue parasites. The Giemsa Plus Stain is also suitable for use as a routine differential hematology stain.

SUMMARY AND EXPLANATION
The diagnosis of infections caused by blood and tissue parasites is based on detection and identification of the parasites in stained films of peripheral blood or tissues.[1] In 1970, Witlin introduced a water-soluble Wright stain, consisting of two separate aqueous solutions. Giemsa Plus Stain Kit is a modification of this formula which produces staining characteristics that parallel those of the conventional Wright stain. The staining time is reduced to 15 seconds, allowing for rapid identification of blood and tissue parasites.

PRINCIPLE
Smears are fixed using the Giemsa Plus Fixative. Slides are immersed in Giemsa Plus Reagent A and in Giemsa Plus Reagent B, individually, to differentially stain specific cellular components. Reagents A and B are anionic and cationic dyes, respectively. These charged dye molecules form ionic bonds with oppositely charged sites on proteins. The cellular components stain either basophilic (blue) or eosinophilic (red). The color intensity can be varied by adjusting the staining time in each reagent.

REAGENTS (CLASSICAL FORMULA)*

Giemsa Plus Fixative:	2.0 mg/ml Malachite Green in Methanol
Giemsa Plus Reagent A:	0.1% Eosin in buffer and 0.1% Formaldehyde, pH 6.8-7.2
Giemsa Plus Reagent B:	0.91 g/l Thiazine dye mixture in buffer, pH 6.8-7.2

*Adjusted as required to meet performance standards.

PRECAUTIONS
Giemsa Plus Fixative – **CAUTION: POISON!** May be fatal or cause blindness if swallowed. **FLAMMABLE!** Keep away from heat, sparks or flame. **VAPOR HARMFUL!** Causes eye irritation.

Giemsa Plus Reagent A – **CAUTION: Contains Formaldehyde.** Suspected human carcinogen.

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully. Refer to Material Data Safety Sheet for additional information on reagent chemicals.

STORAGE
Store product in its original container at room temperature until used. Keep container tightly closed during storage.

PRODUCT DETERIORATION
This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Coplin jars or staining jars with covers, (2) Microscope slides, (3) Microscope with oil immersion lens, (4) Immersion oil, (5) Fresh blood smears or other quality control slides.

SPECIMEN COLLECTION AND PREPARATION[1-4]
Blood Specimens:
For optimal stain results make smears from blood without anticoagulant, such as that obtained from a finger stick or ear lobe puncture. Blood obtained by venipuncture can be used; however, it is preferable to use the blood remaining in the needle because it is anticoagulant-free. If smears cannot be made immediately, EDTA is the anticoagulant of choice. Fresh blood smears provide optimum results.

Thin Smears: The thin smear is prepared in the same manner as for a differential leukocyte count. Place one drop of blood near one end of a glass microscope slide. Hold a second spreader slide at a 40-45° angle, draw into the drop of blood, and allow it to spread to the width of the slide. Rapidly and smoothly push the spreader slide to the other end of the slide, pulling the blood behind it. A well-prepared smear is thick at one end and thin at the other.[2,3]

Thick Smears: The thick smear is prepared by spreading 1 drop of blood into an area approximately 1.5 cm in diameter. A properly prepared thick smear should be thin enough so that newsprint can barely be read through it. Allow the smear to dry overnight. Stain within 3 days. **Do not heat fix.**

Tissue Specimens:
Tissue or fluid specimens obtained from biopsy or necropsy may be used to make impression smears or histologic sections.

REAGENT PREPARATION
Giemsa Plus reagents are ready for use. No additional preparation is required. Pour reagents into Coplin jars or staining jars with covers.

PROCEDURE
A. Staining Procedure for Thin Smears:

1. Dip slides in Giemsa Plus Fixative 5 times, 1 second per dip. Drain excess.
2. Dip in Giemsa Plus Reagent A 5 times, 1 second per dip. Drain excess.
3. Dip in Giemsa Plus Reagent B 5 times, 1 second per dip.
4. Rinse slide with distilled water.
5. Allow the smear to air dry.
6. Examine under oil immersion.

B. Staining Procedure for Thick Smears:

Note: The same staining procedure is used for thick smears. Before staining, remove the hemoglobin from the erythrocytes by elution, as described below.

1. Prepare a thick smear and allow it to dry overnight.
2. Place the slide flat on a staining rack.
3. Carefully overlay the entire smear with demineralized water
4. Allow the water to evaporate and the slide to air dry completely. Do not heat or disturb the slide during this phase.
5. Stain the dried smear following the procedure for staining thin smears.

RESULTS AND INTERPRETATION
Refer to appropriate references for appearance of blood and tissue parasites and/or differential characteristics of Giemsa stained blood and tissue smears.[1,4]

QUALITY CONTROL
All lot numbers of Giemsa Plus Stain Kit have been tested and found to be acceptable. Testing of controls should be performed in accordance with established laboratory quality control procedures. The patient smear can serve as quality control to verify the efficacy of the staining reagents.[3] If the leukocytes and erythrocytes exhibit typical colors, parasites can be expected to stain correctly. In addition, a smear made from a patient blood specimen (previously identified as positive) with at least one parasite per oil immersion field may also be included to verify differential staining characteristics and compare with specimen stain results.[2] If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS

Giemsa Plus is an aqueous stain. The water-soluble portion of the cellular components may take up the stain differently than the traditional alcohol-based Wright stain, resulting in basophils which appear "washed out". If basophils are suspected in the specimen, it should be stained using a Wright stain.

TROUBLESHOOTING

1. If an overall lighter stain is desired, decrease the number of dips in Reagent A and B to no less than 3 dips. Each dip should be 1 full second.
2. The intensity of the stain may be altered by varying the number of dips in Reagents A and B.
   a. To increase eosinophil staining, increase the number of dips in Reagent A.
   b. To increase basophilic staining, increase the number of dips in Reagent B.
   c. To increase the overall intensity of the stain, increase the number of dips in both Reagent A and Reagent B.

BIBLIOGRAPHY

1. Garcia, L.S. and D.A. Bruckner. 1997. Diagnostic Medical Parasitology. 3rd ed. ASM Press, Washington, D.C.
2. Clinical and Laboratory Standards Institute (CLSI). 2000. Laboratory Diagnosis of Blood-Borne Parasitic Diseases; Approved Guideline. M15-A. CLSI. Wayne, PA.
3. Isenberg, H.D. 2004. Clinical Microbiology Procedures Handbook. 2nd ed., Vol. 2. ASM Press, Washington, D.C.
4. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.

PACKAGING

REF R246403, Giemsa Plus Stain Kit ..... 3 X 250 ml

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

Manufactured for Remel Inc.

IFU 246403, Revised December 16, 2009 Printed in U.S.A.

12076 Santa Fe Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Website: www.remel.com Email: remel@remel.com
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128