IFU

Instructions for Use

ONE STEP WRIGHTS STAIN (BUFFERED BLOOD STAINING SOLUTION)

Cat. no. SS016	One Step Wrights Stain, 16 fl. oz.	One bottle/package
Cat. no. SS032	One Step Wrights Stain, 32 fl. oz.	One bottle/package

INTENDED USE

One Step Wrights Stain is a hematology stain that is used for differential staining of blood smears, bone marrow and blood parasites.

SUMMARY AND PRINCIPLES

The traditional Wrights Stain dates from the early 1890's. The original Wrights Stain was an alcoholic solution of methylene blue and eosin Y. Since then there have been many modificiations, most involving partial oxidative demethylation of the methylene blue to improve polychroming. Modern day samples of the dye usually contain mixtures of methylene blue, azure A, thionin and eosin Y. They also contain some amount of giemsa stain.

The traditional stain is diluted 1:1 with giordano buffer before use. One Step Wrights Stain contains the buffer already dissolved in the stain. The slides are stained in the undiluted stain and differentiated by decolorizing in purified water.

FORMULA

Reagent	**CAS No.**
Wright Stain	68988-92-1
Giemsa Stain	51811-82-6
Methanol	67-56-1
Buffer Salts	
Intensifier	

STORAGE AND SHELF LIFE

Storage: Upon receipt store One Step Wrights Stain at 15-30ºC. The staining solution will absorb water from the air, therefore, store the stain in a tightly stoppered container at all times.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Methanol is a poison. Ingestion may be harmful or fatal. It cannot be made non-poisonous. Avoid contact with skin. Wear protective gloves during use and/or wash thoroughly after use. Avoid breathing the fumes. Use only in rooms with adequate ventilation.

Warning: This product is flammable; keep away from heat, sparks, or flames.

PROCEDURE

SPECIMEN COLLECTION:

Either capillary or venous blood is acceptable. EDTA should be used as anticoagulant. The sample should not be excessive heat. Hemolysis will render the sample unsatisfactory. Slides should be stained within 4 hours of preparation or fixed. Blood smears may be fixed with methanol if required.

STAINING PROCEDURE FOR BLOOD SMEARS AND BONE MARROW:

A. Dip Procedure

1. Prepare a film of blood or bone marrow on a microscope slide and allow to air dry.

2. Prepare three containers (e.g. coplin jars, or staining dishes). Fill one container with One Step Wrights Stain and the second and third containers with distilled or deionized water.

3. When stain volume in container 1 becomes insufficient, replace the stain. Do not replenish by adding new stain to the old.

4. To prevent evaporation, keep stain tightly covered when not in use.

5. Change the water in container 2 or 3 when an iridescent scum forms on the surface or when a dark blue discoloration occurs. It is very important to keep the rinse water clean.

6. Dip air dried slides in One Step Wrights Stain 15-30 seconds.

7. Dip slide in distilled or deionized water in container 2 for 15-45 seconds.

8. Dip slide in distilled or deionized water in container 3 for 25 seconds, using quick dips. As an alternative, the slide

may be "swished" in distilled or deionized water for 25 seconds to remove the stain.

9. Wipe the back of slide.

10. Dry slides in vertical position, on absorbent surface. Do not blot the smear.

11. Apply oil and examine microscopically.

* For bone marrow smears, double all the above times.

B. Rack Procedure

1. Prepare a film of blood or bone marrow on a microscope slide and allow to air dry.

2. Place slide on staining rack.

3. Apply sufficient One Step Wrights Stain may be applied to the slide using dropper bottles or pipettes.

4. Wait 15-30 seconds and add an equal volume of distilled or deionized water.

5. Mix the stain and water with a fine air stream for 15-45 seconds (gentle blowing).

6. Pour stain and water mixture off the slide.

7. Dip the slide in distilled or deionized water 25 seconds using quick dips. As an alternative, the slide may be "swished" in distilled or deionized water 25 seconds to remove the stain, or it may be washed using a wash bottle.

8. Wipe the back of slide.

9. Dry slide in vertical position, on an absorbent surface. Do not blot smear.

10. Apply oil and examine microscopically.

* For bone marrow smears, double all the above times.

Staining times are approximate and should be varied as needed.

INTERPRETATION OF RESULTS

Erythrocytes:	Pink-tan with degrees of chromasia.
Lymphocytes:	Clear blue cytoplasm, red purple granules may be present.
Monocytes:	Mosaic of pink and blue cytoplasm, azure granules usually present.
Neutrophils:	Light purplish-pink or lavender granules in cytoplasm.
Eosinophils:	Bright red or reddish-orange granules in cytoplasm.
Basophils:	Deep purple and violet black granules in cytoplasm.
Platelets:	Clearly demarcated red-purple granules in light blue cytoplasm.

LIMITATIONS

Smears should not be too thick. All glassware should be scrupulously clean. Smears should be fixed and stained as soon as possible, preferably within one hour of collection. The rinsing should be deionized or distilled water. Tap water is not suitable because the chlorine will bleach the stain.

Precipitate formulation on the slide may be due to inadequate or incorrect washing. Rinse water must be kept clean.

Dust or a dirty slide will also cause precipitate.

Excessive blue stain may be due to over staining, excessive alkalinity of the water or inadequate washing. Excessive red stain may be due to under staining or the water being too acid. If deionized water is too acid, the use of a phosphate buffer may be required to improve staining quality.

If bleach is used to clean the stain jars, they must be thoroughly rinsed to remove all traces of the bleach. If any bleach residue exists, the slides will be to light in color.

MATERIALS REQUIRED BUT NOT PROVIDED

Additional materials needed include microscope slides, cover slips, microscope with oil immersion lens, immersion oil, blood collecting supplies, jars, racks, distilled or deionized water.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

One Step Wrights Stain should appear opaque, and deep violet in color.

PACKAGING

One Step Wrights Stain:
- SS016 16 fluid oz. One bottle/package
- SS032 32 fluid oz. One bottle/package

ALSO AVAILABLE FROM HARDY DIAGNOSTICS:

Coplin Staining Jars:
- VCJ-001 Holds 5 slides One jar/box
- Slides, Immersion Oil, Containers, etc., See catalog.

REFERENCES

1. Wright, J.H. 1902. *Med. Res.*; 7:138.

2. Davidosh, I. and Henry, J.B. Saunders. 1974. *Clinical Diagnosis by Laboratory Methods*, 15th ed.

3. Wintrobe, M.M. *Clinical Hematology*, 7th ed. Lea & Febiger, Philadelphia, PA.

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]