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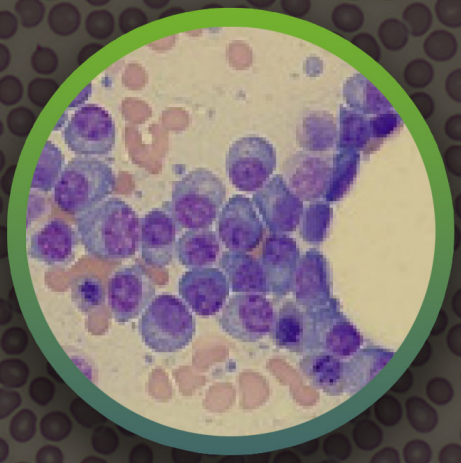
BIOMEDICAL SYSTEMS

AEROSPRAY® HEMATOLOGY PRO SLIDE STAINER / CYTOCENTRIFUGE
APPLICATION NOTE

"A good picture is worth a thousand words."

Margreet Schoorl, MD



Keywords:
Morphology, Peripheral Blood Smears, Bone Marrow Aspirates, Microscopy, Staining Protocol, Validation

Morphological Examination

Peripheral blood contains red blood cells (erythrocytes), platelets (thrombocytes), and different types of white blood cells (leukocytes).

In a standard morphological examination of the blood, one drop of blood is spread out on a slide. Freshly made smears should be stained with May-Grünwald Giemsa or Wright-Giemsa stain and examined for white blood cells, red blood cells, and platelet abnormalities. The aspect (shape, color, and size) of the cell types is assessed and the different types of leukocytes are classified.[1]

Peripheral Blood

A peripheral blood smear is usually evaluated in relation to the results of the cell counts obtained with a hematology analyzer. The software of this equipment gives a message ('suspect flag') if the leukocyte subpopulations cannot be properly distinguished from each other and in the presence of abnormal cell populations in the blood sample. This includes the presence of abnormal lymphocytes, immature granulocytes, and/or blasts. In that case, additional microscopic examination is needed. Microscopic evaluation of blood and bone marrow is still considered the gold standard.[1,2]

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Morphological Examination of Bone Marrow

Sometimes a blood test is not enough to determine the cause of, for example, anemia. There are conditions in which a bone marrow examination is needed, such as in the case of (suspected) leukemia.

Leukemia

Leukemia is a disease in which one of the different cell types that are produced in the bone marrow grows unrestrained. The result is an accumulation of the same type of cells, which impedes the production of the other cells. This creates an abnormal distribution of the cells in the blood.

Bone marrow material can be obtained in two ways: via an aspirate or a biopsy. A combination of both techniques enables an optimal assessment of the nature and extent of the disease process in the case of hematological abnormalities.[2-5]

Aspirate

An aspirate is obtained by inserting a needle into the bone marrow and aspirating a small amount of bone marrow using a syringe. Bone marrow chunks of this material are smeared on a slide to form the so-called PLET preparations, which are then stained with May-Grünwald Giemsa or Wright-Giemsa stain for optimal microscopic assessment of cell structures, cytoplasmic granules, and nuclear chromatin.[2, 4, 5]

Biopsy

During a bone marrow biopsy, a small piece of bone is removed for examination using a thick needle.

A biopsy provides additional important information with regard to the aspirate, such as the structure of the hematopoiesis, the cellularity, the location and extent of lesions, and the degree of fibrosis.[2, 4, 5]

May-Grünwald Giemsa Staining and Wright-Giemsa Staining

The May-Grünwald Giemsa stain and Wright-Giemsa stain are standard staining techniques used in every laboratory. These stains have been developed for the microscopic evaluation of cells in, among others, blood and bone marrow.

Both stains are derived from the original Romanowsky staining technique, which uses eosin and methylene blue. The red eosin is an acidic dye that binds preferentially to basic substances in the cytoplasm, while the basic methylene blue mainly binds to the nucleic acids in the nucleus of the cell. The main difference between the two stains is the addition of methylene acetic acid in the May-Grünwald Giemsa technique, which makes the staining of the cell more intense and richer in contrast. [2, 6, 7]

Several *pre-analytical aspects* are important for a reliable microscopic evaluation, such as the quality of the sample and the slide. For the *quality of the sample*, you can think of the freshness and purity of the sample. Quality aspects for a good slide are a proper distribution of the cells where the cells have kept their original shape and size without artifacts.

In addition, *strong and reliable staining* of the cells is essential for the assessment of nucleus- and cytoplasm details and the clear visibility of granules. The quality of the staining reagents and a good staining procedure are therefore important aspects.

Laboratories prefer to use standardized and validated procedures to confirm that the examination procedures are suitable for the intended use.[8]

Aerospray® Hematology Pro Stainer / Cytocentrifuge

The Aerospray Hematology Pro Slide Stainer / Cytocentrifuge is a dual purpose instrument, slide stainer and cytocentrifuge, that allows for the preparation and staining of various cell suspensions and body fluids. The slides are mounted in a rotating carousel for processing. The rotor capacity for staining preparations is 30 slides. Fresh reagents are applied by spray nozzles atomizing stain onto microscope slides prepared with blood, bone marrow, or other body fluid specimens.

The cells on the slide are stained with the staining reagents thiazin and eosin.

Staining options include a rapid stain mode, Wright-Giemsa, and May-Grunwald Giemsa (MGG) staining procedures. These modes allow for variation in the eosin/thiazin ratio and the spin time. In addition, it is also possible to program custom-made staining procedures/protocols.

A few benefits of the Aerospray staining technology are the standardized and clean performance of the staining procedure, the low reagent consumption, and the homogeneous staining of the slide.

Figure 1. *Aerospray Hematology Pro Slide Stainer / Cytocentrifuge - Model 7152*

Reagents

- Hematology Reagent A, buffer pH 6.8
  ref. SS-171A-EU
- Hematology Reagent A, buffer pH 7.2
  ref. SS-172A-EU
- Reagent B, thiazin stain (blue)
  ref. SS-071B-EU
- Reagent C, eosin stain (red)
  ref. SS-071C-EU
- Aerospray Nozzle clean
  ref. SS-029C-EU

Staining Protocols

Each staining protocol uses various combinations of the six process steps. The rapid mode and the Wright-Giemsa stain are similar and produce stained slides virtually identical to the classic Wright-Giemsa stained slides. With the May-Grünwald Giemsa staining protocol, the cells are stained similarly to the classical May-Grünwald Giemsa stain.

Table 1 (page 4) shows the standard program settings of the May-Grünwald Giemsa staining protocol.

The steps of *fixation*, *concentrate red/blue ratio, spin, diluted red/blue ratio, and end rinse* can be modified using sliders to adjust the staining result so that all cell details are clearly visible.

1) Front Panel with Touch Screen Display
2) Bowl
3) Carousel
4) Lid with Safety Lock
5) Right Side Panel with Label Indicating Reagent Positions:
- **A - Rinse**
- **B - Thiazin**
- **C - Eosin**
- **D - Methanol or Aerofix®**

6) Reagent Tray

		Intensity Settings									
		1	2	3	4	5	6	7	8	9	
Steps	1. Fixation (Setting)	4	4	4	4	4	4	7	7	7	Modifiable
	2. Concentrate Intensity (Setting)	2	2	2	2	2	2	2	2	2	
	a. Red/Blue Ratio	60/40	60/40	60/40	60/40	60/40	60/40	60/40	60/40	60/40	Modifiable
	b. Spin (seconds)	35	35	35	35	35	35	35	35	35	Modifiable
	3. Mid Rinse (Setting)	5	5	5	5	5	5	5	5	5	
	4. Dilute Stain Intensity (Setting)	1	2	3	4	5	6	7	8	9	
	c. Red/Blue Ratio	30/70	30/70	30/70	30/70	30/70	30/70	30/70	30/70	30/70	Modifiable
	d. Stain/Buffer Ratio	30/70	30/70	30/70	30/70	30/70	30/70	30/70	30/70	30/70	
	e. Spin (seconds)	1	1	1	1	1	1	1	1	1	
	5. End Rinse (Setting)	4	4	4	4	4	4	4	4	4	Modifiable
	6. Dry Time (Setting)	4	4	4	4	4	4	4	4	4	

Table 1. *Standard program settings of the May-Grünwald Giemsa staining protocol.*
NOTE: The three default programs provided in the May-Grünwald Giemsa stain mode are highlighted in green in the table above.

Since subjective criteria and laboratory preferences often play a role when evaluating a new staining method, the settings can be adjusted with minor changes as required.

Appendix D of the application manual describes the effects of adjustments in these steps. [9, 10]

Validation and Verification of Staining Protocols

For the *validation* of the above May-Grünwald Giemsa staining protocol, on peripheral blood smears and PLET preparations from bone marrow aspirates, the standard settings were used.

The staining protocols used hematology reagent buffer pH 6.8.

Incrementally, minor adjustments were made to the settings, evaluating the effect of each change on slides from different patient samples.

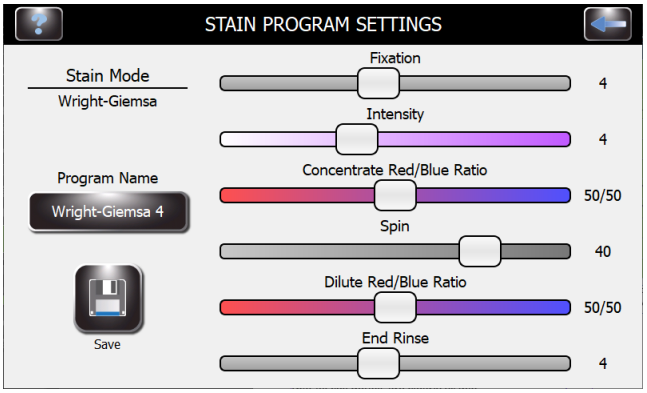

Figure 2. *Use the sliders to adjust the settings: Move right to increase, and left to decrease the value. Use the numeric values shown in the right column to confirm each setting. Settings appear as time (in seconds) or percent (X/Y).*

It was assumed in the assessment that:

- All cell types, in blood smears and bone marrow PLET preparations, can be reliably identified with the microscope
- The staining of the cells is of good quality in the parts of the slides that are optimal for assessment
- The staining result does not depend on the cell richness
- No artifacts are visible
- Blood and bone marrow preparations can be stained with 1 setting
- The staining result meets the ergonomic needs of the lab technician who looks into the microscope for a long period

A large number of samples, including benign and reactive abnormalities, malignant abnormalities, and samples without abnormalities, were evaluated for the following aspects:

- Color of the cells
- Structure of the nucleus
- Color intensity of nucleus, cytoplasm, and (hypo- and hyper-) granulation
- Distinction between blasts and myeloid cells
- Inclusions (Döhle bodies, Auer rods)
- Megakaryopoiesis and erythropoiesis

The reproducibility *(intra-assay)* of the staining was tested by staining and evaluating slides from the same patient for 7 days.

This resulted in the following settings shown in Table 2 for reproducibly staining cells in blood smears and bone marrow PLET preparations.

Blood Smears Bone Marrow PLET Preparations	
Fix	4
Concentrate Intensity	1
Concentrate Red/Blue Ratio	60-40
Concentrate Spin	45
Mid Rinse	5
Dilution Intensity	5
Dilution Red/Blue Ratio	60-40
Dilution Stain Buffer	30-70
Dilution Spin	1
End Rinse	5
Dry Time	4

Table 2. *Blood smears and bone marrow PLET preparations*

For *verification*, approximately 300 bone marrow samples were then stained and assessed with these settings and compared with the May-Grünwald Giemsa staining that had been used in the laboratory for many years.

No significant discrepancies were observed. The same settings were verified again in another hematology laboratory, with a limited number of blood smears and bone marrow preparations.

Conclusion

- With the settings mentioned, blood smears and bone marrow preparations can be stained
- The staining result does not depend on the cell richness
- All cell lines in the bone marrow, erythropoiesis, granulopoiesis, megakaryopoiesis, lymphocytes, and plasma cells are well stained
- Macrophages, mast cells, and blasts are also clearly recognizable

These settings for the May-Grünwald Giemsa staining using the Aerospray Hematology Pro Slide Stainer / Cytocentrifuge are a good starting point for other laboratories to verify a new staining protocol, for blood smears, and for bone marrow PLET preparations.

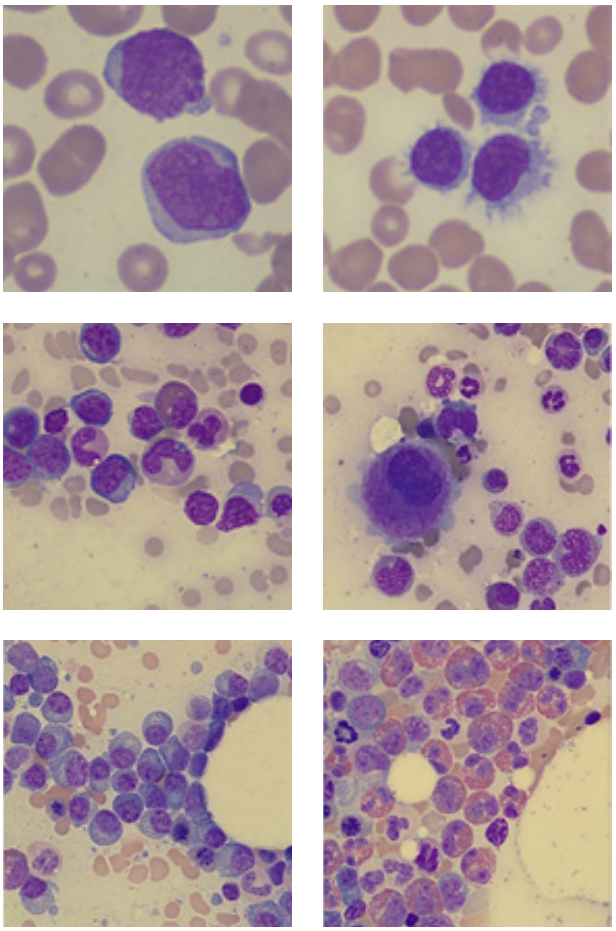

Figure 3. *Examples from the laboratory for special hematology of the LUMC (Leiden, The Netherlands), prepared with the Aerospray Hematology Pro Slide Stainer / Cytocentrifuge and the validated staining protocol.*

Acknowledgment

The adjusted settings for the May-Grünwald Giemsa stain were validated in the laboratory for special hematology at the Leiden University Medical Center (Leiden, The Netherlands) and then verified with approximately 300 randomly collected bone marrow samples.

The same settings were verified again in the laboratory for Clinical Chemistry, Hematology, and Immunology of the Northwest Clinics (Alkmaar, The Netherlands), with a limited number of blood smears and bone marrow preparations.

The programming and validation work for this study was conducted in 2019 and this documentation was completed 2021-2022.

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About the Author

Margreet Schoorl, MD

Margreet Schoorl is trained in the field of clinical and medical biochemistry and marketing and innovation in healthcare.

She completed her thesis, “Innovative hematological parameters in clinical practice,” in June 2015.

Since the beginning of 2018, she has been a member of the editorial board of the *International Journal of Laboratory Hematology*.

She worked as manager of the department for hematology, coagulation, and flow cytometry in the Department for Clinical Chemistry, Hematology, and Immunology at the Northwest Clinics, a top clinical teaching hospital in Alkmaar, the Netherlands.

In 2020, with her team of lab technicians, she completed the total laboratory automation (TLA) project of a fully automated track system for both 24-hour laboratories.

She retired in October 2021.

With various co-authors, Margreet has published approximately 55 peer-reviewed articles in internationally recognized journals.

Margreet’s focus in the field of marketing and innovation in healthcare is sharing knowledge with medical specialists regarding the use of new hematology equipment and methods in daily laboratory practice and the added value of new diagnostic hematological parameters.

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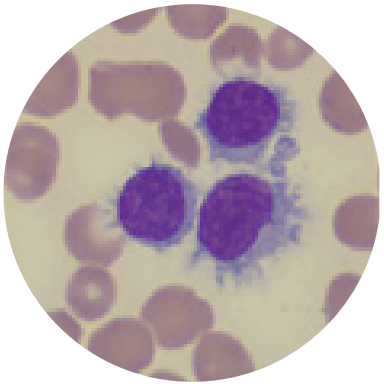

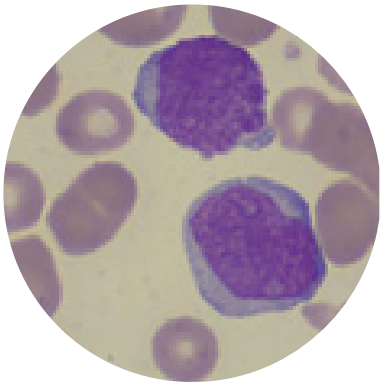

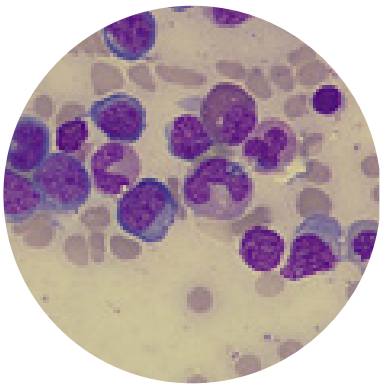

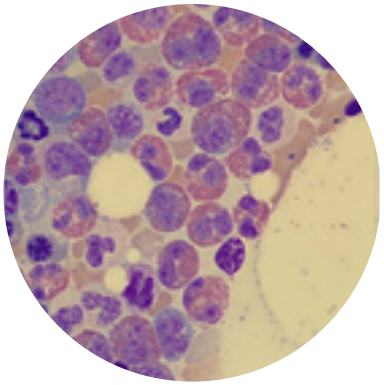

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