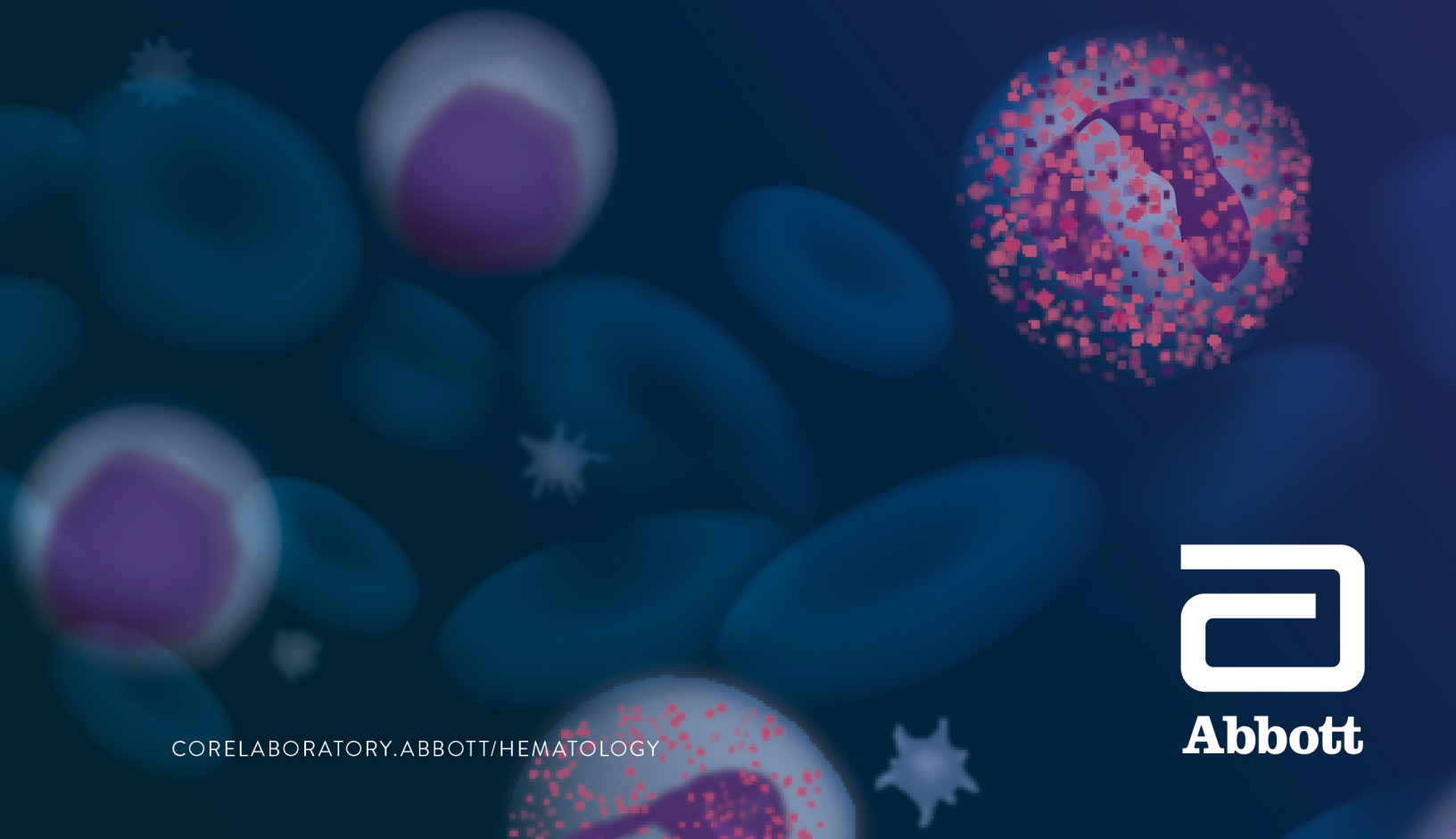
HEMATOLOGY ACADEMY

LEARNING GUIDE

THE ALINITY H-SERIES STAINING GUIDE



CORELABORATORY.ABBOTT/HEMATOLOGY

Abbott

CONTENTS

ALINITY H-SERIES STAINING GUIDE

The Alinity hs is the slide maker stainer module for the Alinity h-series of hematology systems. Utilizing blank glass slides, smear fix, phosphate buffer and stains, the system creates smeared and/or stained slides using whole blood samples or externally prepared whole blood smears.

This guide provides details of whole blood smear staining on the Alinity hs, including descriptions of the stains used, the methodology and the different pre-configured and customizable staining protocols available.

INTRODUCTION TO STAINING

Stained blood smears are used in hematology primarily to confirm or provide additional clinical information to supplement the CBC+Diff results from an automated analyzer. The staining of the smears distinguishes cellular components and morphological abnormalities. In hematology, smears are typically stained using a combination of Romanowsky dyes to produce a consistent coloration. Table 1 shows an example ideal coloration for hematology stains in a typical laboratory.1 Preferences in staining intensity and exact coloration vary by laboratory and morphologist. There are no specific international standards that dictate the exact stain or method that should be used.

Table 1: Ideal coloration of stained hematology smears[1]

Cell/ Cellular Components	Color
Red Blood Cells	Light to moderate pink, not gray or blue
Neutrophils - Nuclei	Blue to dark blue to purple
Neutrophils - Granules	Reddish purple lilac
Neutrophils - Cytoplasm	Pale pink
Eosinophils - Nuclei	Blue to dark blue to purple
Eosinophils - Granules	Red to red orange
Basophils - Nuclei	Purple to dark blue to black
Basophils - Granules	Purple
Lymphocytes - Nuclei	Dark purple
Lymphocytes - Cytoplasm	Sky blue
Monocytes - Nuclei	Purple
Monocytes - Cytoplasm	Sky blue/blue-gray
Platelets - Granules	Violet to purple

There are several publications that offer best practices for staining smears, as summarized below. Details are provided in the "References" section of this guide.

- **CLSI H20-A2** - Provides guidelines for the collection and preparation of stained smears.
- **ICSH and ISLH** - Guidelines published in 1984 provide a complete method for manually staining smears using May Grünwald Giemsa (MGG) stain.[3] More recent guidelines reference other publications, such as "Blood Cells: A Practical Guide" or "Practical Haematology" for guidance on staining.[4,5,6]
- **French Society of Clinical Cytology (SFCC)** - Provides information on previous publications regarding smear and staining guidelines as well as recommendations for MGG staining.[7]
- **UK-NEQAS** - Provides a handbook for the smearing and staining of whole blood samples. It covers 4 major categories: pre-staining or smearing, nuclear staining, cytoplasmic staining and post staining.[8]
- **Blood Cells: A Practical Guide** - Reviews smearing and staining procedures including some of the common causes for staining variation.[5]

The goal for smear staining is to achieve consistent coloration and intensity, with adequate differentiation between the blood components, including white blood cells (WBCs), red blood cells (RBCs), RBC inclusions and platelets (PLTs). A change in technology, staining procedure or stain should be evaluated with morphological reviewers to ensure the changes meet the laboratory needs.

Older publications typically provide detailed protocols and focus on manual methods for smearing and staining.[3,4] More recent publications and studies have demonstrated that automated slide maker stainer systems can consistently produce good quality smears and provide flexibility for laboratories to define their ideal coloration.[9,10] However, with expansion of digital morphology, additional standardization of stain quality may be needed in the future to ensure consistent results globally.[11]

MAY GRÜNWALD GIEMSA AND WRIGHT GIEMSA STAINS

While there are a number of different stains used in laboratories worldwide, two of the most commonly used stains are May Grünwald Giemsa (MGG) and Wright Giemsa (WG). Many laboratories have preferences for one stain based on a historical preference and geography. MGG stains tend to be more common in Europe, while WG stains tend to be more predominately used in the USA.[8]

Both MGG and WG stains utilize Romanowsky dyes dissolved in methanol and mixed with co-solvents and stabilizers. The exact dyes and stabilizers used vary by the manufacturer of the stain and the stain itself.[12,13,14] However, all these dyes consist of a blue basic dye and a red acidic dye that contribute to the coloration of the cells. A description of the dyes and the effect they have on the cells is summarized in Table 2.

Table 2: Effect of Romanowsky dyes on stained cells[12]

Stains	Blue Basic Dye	Red Acid Dye
Example dyes	Methylene blue, Azure B	Eosin Y, Eosin B
Sites stained	Basophilic sites	Acidophilic sites
Appearances impacted	Lymphocyte cytoplasm and nucleoli Dual staining causes the purple color of the chromatin and nuclear material, neutrophil granules, and platelets	Eosinophil granules, RBCs

In addition to the dyes used in the stain, variation in the coloration can be caused by other factors including the stabilizers, the pH of any buffer solutions and the timing of the staining procedures.[12] For example, dimethyl sulfoxide in the stains reduces the precipitate and contributes to the shelf life of pre-prepared staining solutions, and a high pH buffer solution can cause RBCs to appear blue or green.[8,15]

Given the geographical differences in stain preferences the impact of multiple variables in staining coloration and the flexibility of existing guidelines, most manufacturers of automated slide maker stainers provide multiple stain options and procedures to support laboratories to achieve their desired staining performance.

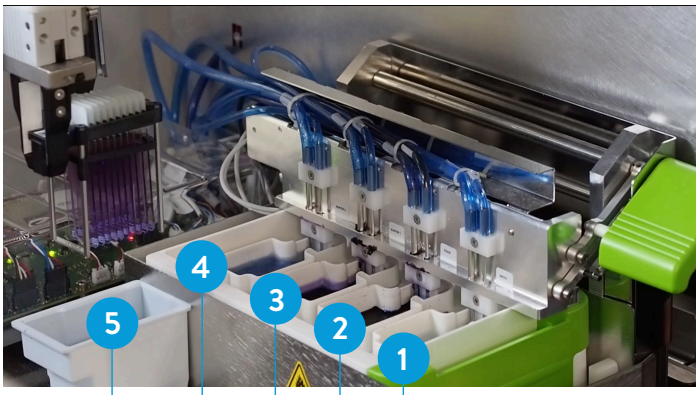
STAINING ON THE ALINITY HS

The Alinity hs is designed to stain smears prepared internally on the module, or externally prepared with either the Alinity h-series MGG or the Alinity h-series WG stains, which have been developed specifically for use with the Alinity h-series. The MGG stain contains methylene blue, eosin and Giemsa dyes in over 70% methanol solution along with approximately 8% dimethyl sulfoxide.[16,17] The WG stain uses Wright and Giemsa dyes in 57% methanol and 25% dimethyl sulfoxide.[18,19] They are available in bottles with keyed caps to prevent loading in the incorrect location on the system.

In addition to the choice of stain, the Alinity hs offers multiple pre-configured and customizable staining protocols to provide flexibility in adjusting the appearance of the stained smears to a laboratory's desired coloration and intensity. These protocols will be reviewed later in this guide.

THE STAINING PROCEDURE

The Alinity hs uses a "dip and dunk" method for staining smears. Slides are placed into slide carriers and are stained in batches of up to 10 slides. The volume of liquid in the baths is automatically adjusted to compensate for the number of slides. The Alinity hs contains 4 baths and a drying station. Slide carriers are moved through the baths sequentially and then dried in the drying station before being returned to the loading area for ejection. The baths, their contents and function and the drying station are described in Figure 1.



1. **Smear Fix Bath**
   Contains a methanol-based smear fix reagent that fixes and preserves the cells.
2. **Stain Bath**
   Contains either the Alinity h-series MGG or the Alinity h-series WG stain which introduce dyes to the cells and begins coloration development.
3. **Buffer Bath**
   Contains pH 6.8 phosphate buffer that further develops the smear color.[20] It may also contain a spike with additional stain to help darken cellular components.
4. **Buffer Wash Bath**
   Contains only phosphate buffer to rinse the slide and remove excess stain.
5. **Drying Station**
   Air dries the stained smears with warm and ambient air before they are ejected.

Figure 1: Locations in the staining process on the Alinity hs

ADJUSTING THE STAINING PROCEDURE

Each step in the staining process influences the appearance of the completed stained smear. Staining procedures can be impacted by different factors, including the pH of the stain and buffer, the temperature of the laboratory, the cleanliness of the system and the age of the stains.[5] The Alinity hs attempts to keep these variables constant with production controls on the stains and buffer, an automated cleaning procedure, and periodic refreshing of the baths. The different staining protocols on the Alinity hs offer the capabilities for setting different times in each bath as well the amount of buffer spike added to the buffer bath. These steps and their associated impact on the final stained smear are discussed below:

SMEAR FIX BATH

Depending on the stain used, the time in the smear fix bath can impact the darkness of cells and background stain. When using MGG stain, reducing time in the smear fix bath may darken the appearance of nuclei and granules and increase the background (Figure 2).

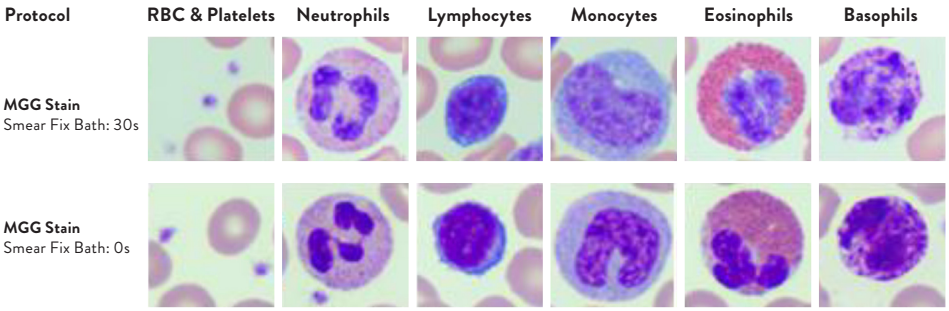


Figure 2: Decreasing time in the smear fix bath

STAIN BATH

Time in the stain bath impacts the darkness of cells. If time in the baths is insufficient, then the cells will be incompletely stained and appear too light. However, once the time in the bath exceeds a certain threshold there are diminishing returns or even a slight lightening in the intensity of nuclear and platelet staining. This lightening can be seen in the example in Figure 3 below.

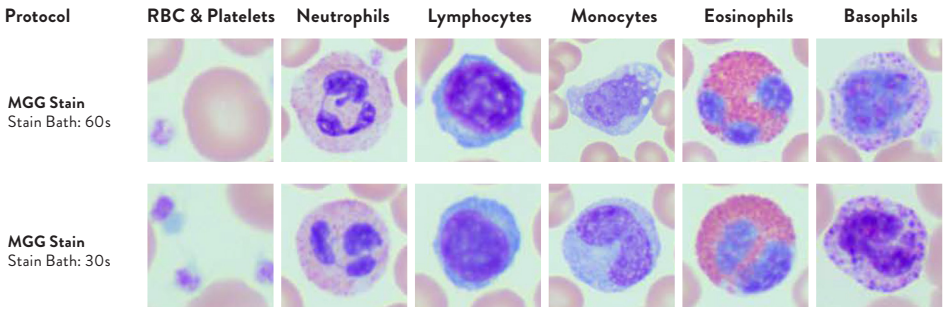


Figure 3: Decreasing time in the stain bath

BUFFER BATH

The buffer bath intensifies the stain color. A darker stain can be achieved by increasing the time in the bath and by adding stain to the buffer solution (called a buffer spike). However, increased precipitate and background coloration may be seen if a large buffer spike is implemented or if the time in the bath with a buffer spike is too long. Additionally, increasing the buffer spike beyond a certain amount may have diminishing returns. The examples in Figures 4-6 below show the impact of these variables. Figure 4 shows the effect of increasing the time in the bath with a minimal buffer spike, Figure 5 shows the effect of increasing the buffer spike concentration and Figure 6 shows the effect of increasing the time in the bath with a significant buffer spike.

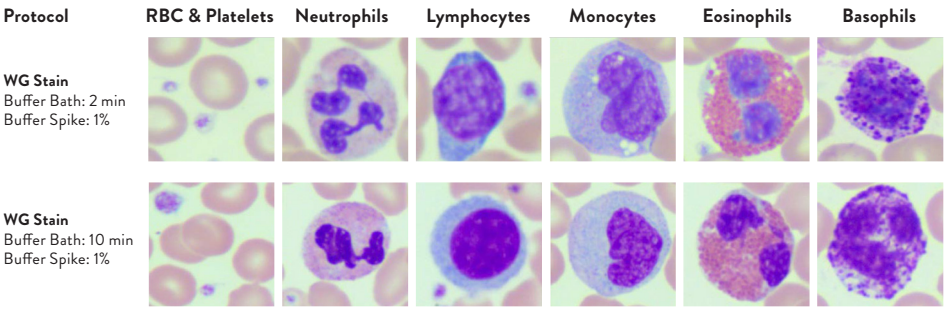


Figure 4: Increasing time in the buffer bath with a minimal buffer spike

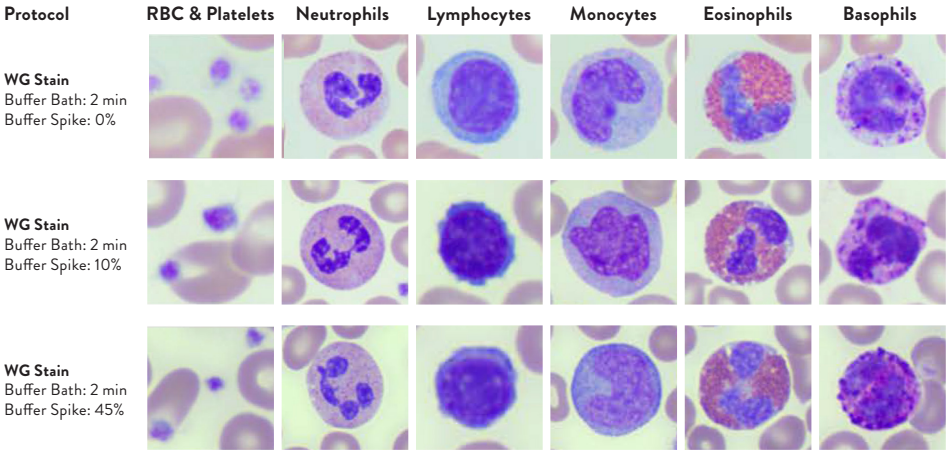


Figure 5: Increasing stain concentration in the buffer bath (buffer spike)

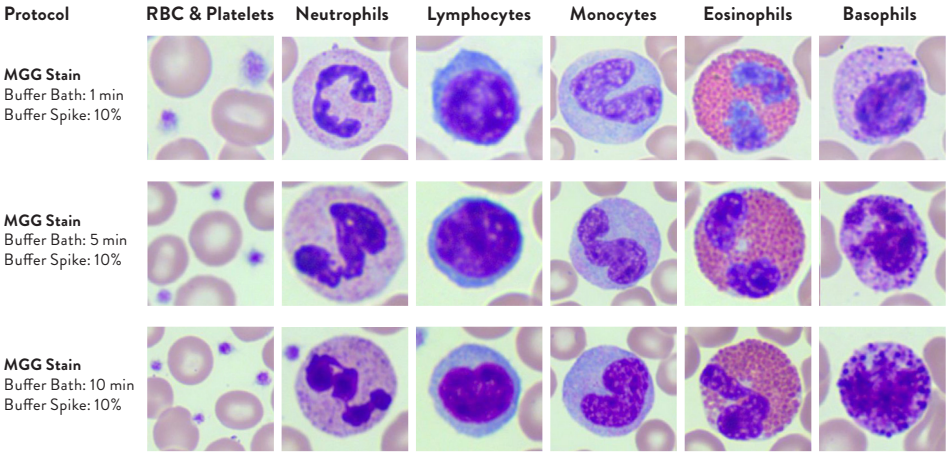


Figure 6: Increasing time in a spiked buffer bath

BUFFER WASH BATH

Time in the buffer wash bath can affect the precipitate and background on the slides. Increasing the time in the buffer wash bath can reduce the precipitate and background stain and may counter-balance additional time in the stain bath or a buffer bath with a high stain spike concentration. No spike can be added to this bath.

PRE-CONFIGURED PROTOCOLS

The Alinity hs provides pre-configured and customizable stain protocols to allow flexibility to meet a laboratory's staining preferences. The pre-configured protocols on the system have a range of different staining options and can simplify the process of selecting or creating a staining protocol. These protocols differ with the timings in the stain and buffer baths as well as the amount of buffer spike. A protocol can be selected by an "admin level" user from the sample processing configuration screen (Figure 7).

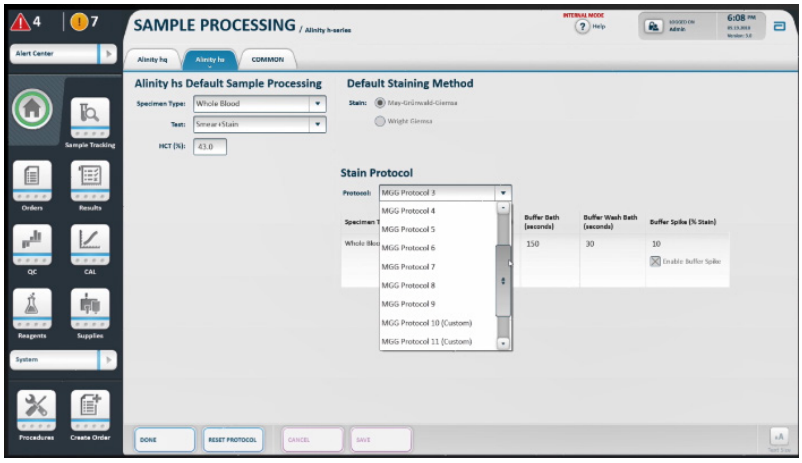


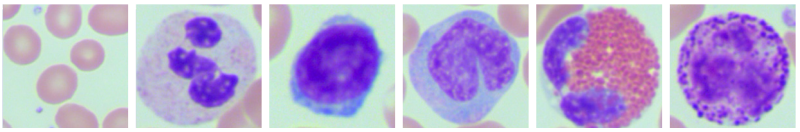
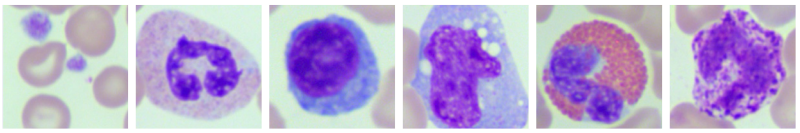
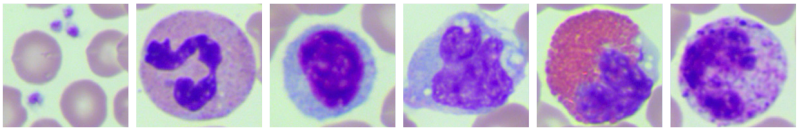
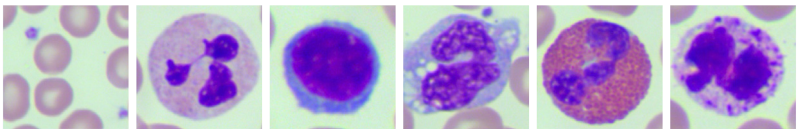
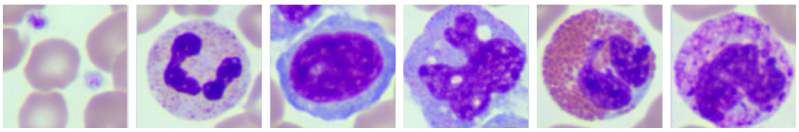
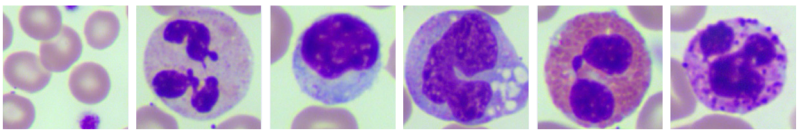
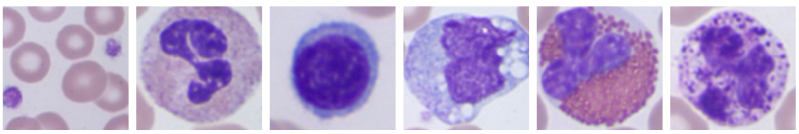
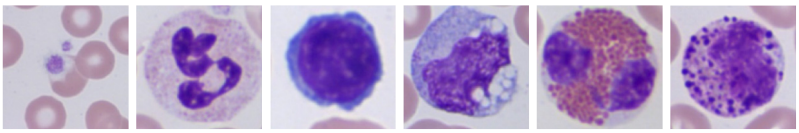
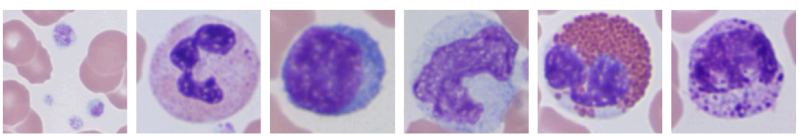
Figure 7: Protocol selection on the Alinity hs

There are default protocols for use with both the MGG and WG stains. When selecting the most appropriate protocol, a lab may choose to try the default protocol initially and then use Tables 3 and 4 to select an alternative based on their assessment of the default. When transitioning to the Alinity hs from a different staining technology, comparing smears stained with the pre-configured protocols with the previous technology or a preferred method may facilitate protocol selection. Once the most appropriate protocol has been determined, further refinements can be made using the customizable protocols should they be required.

MGG PROTOCOLS

There are 9 different MGG pre-configured protocols on the system. Protocols 1-6 are arranged from the lightest to the darkest and protocols 7-9 are alternative colorations with different timings based on feedback from customers. Table 3 provides information on the different protocols, example images of the different cell types and the reasons for the differences in appearance.

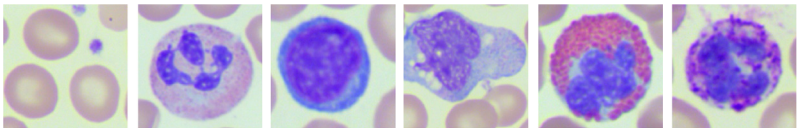
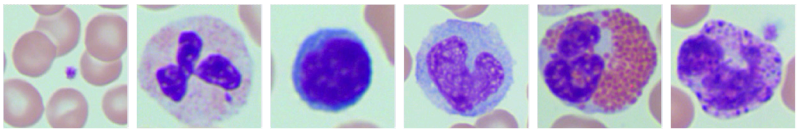
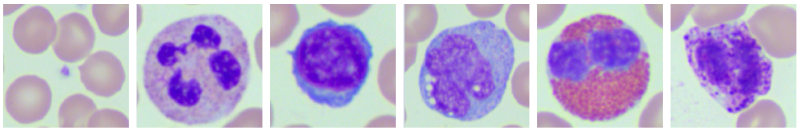
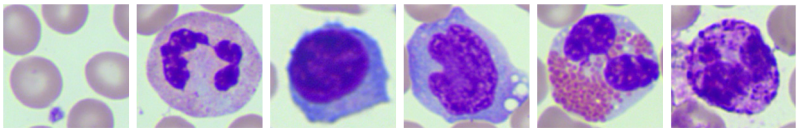
Table 3: MGG Pre-configured staining protocols

Protocol	RBC & Platelets	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
MGG 1 Buffer Spike: 0% Smear Fix: 30s Stain: 440s Buffer: 200s Buffer Wash: 30s						
MGG 1 is lighter than MGG 2 and the MGG 3 (default) due to the removal of the buffer spike						
MGG 2 Buffer Spike: 5% Smear Fix: 30s Stain: 60s Buffer: 60s Buffer Wash: 30s						
MGG 2 is lighter than the MGG 3 (default) due to the reduction buffer spike, the time in the stain bath and time in the buffer bath. It is darker than MGG 1 despite the decrease in stain and buffer bath time due to the addition of a 5% buffer spike						
MGG 3 (default) Buffer Spike: 10% Smear Fix: 30s Stain: 180s Buffer: 150s Buffer Wash 30s						
MGG 3 is the default protocol						
MGG 4 Buffer Spike: 10% Smear Fix: 30s Stain: 595s; Buffer: 250s Buffer Wash: 30s						
MGG 4 is darker than MGG 3 (default) protocol due to increased time in the stain and buffer baths						
MGG 5 Buffer Spike: 10% Smear Fix: 30s Stain: 80s Buffer: 250s Buffer Wash: 30s						
MGG 5 is slightly darker than MGG 4 due to the reducing time in the stain bath, demonstrating the lightening effect of extending time in the stain bath beyond a certain threshold						
MGG 6 Buffer Spike: 10% Smear Fix: 30s Stain: 390s Buffer: 570s Buffer Wash: 30s						
MGG 6 is darker than MGG 5 and MGG 3 (default) protocol due to increasing the time in both the stain and buffer baths						
MGG 7 Buffer Spike: 5% Smear Fix: 30s Stain: 15s Buffer: 90s Buffer Wash: 30s						
MGG 7 shows the effect of reducing the time in the stain bath to the minimum of 15s						
MGG 8 Buffer Spike: 4% Smear Fix: 30s Stain: 80s Buffer: 275s Buffer Wash: 30s						
MGG 8 shows the effect of a slightly reduced buffer spike of 4%. The increased time in the stain and buffer baths offsets the difference in the time in the smear fix bath and results in similar coloration to MGG 7						
MGG 9 Buffer Spike: 0% Smear Fix: 20s Stain: 80s Buffer: 250s Buffer Wash: 30s						
MGG 9 shows the effect of reducing the time in the smear fix bath. This offsets some of the impact of the lack of buffer spike compared to MGG 8						

WG PROTOCOLS

There are 4 different WG pre-configured protocols on the system. Like the MGG protocols, are numbered from lightest to darkest. Example images of the cell types and parameters for each of protocols are described in Table 4.

Table 4: WG Pre-configured staining protocols

Protocol	RBC & Platelets	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
WG 1 Buffer Spike: 0% Smear Fix: 30s Stain: 100s Buffer: 40s Buffer Wash: 15s						
WG 1 is lighter than WG 2 and the WG 3 (default) due to the reduced time in the buffer bath						
WG 2 Buffer Spike: 0% Smear Fix: 30s Stain: 55s Buffer: 320s Buffer Wash: 15s						
WG 2 is comparable or slightly lighter than WG 3 (default) demonstrating the lightening effect of extending the time in the stain and buffer baths						
WG 3 (default) Buffer Spike: 0% Smear Fix: 30s Stain: 30s Buffer: 120s Buffer Wash: 15s						
WG 3 is the default protocol. It may be used as a starting point when selecting a staining protocol						
WG 4 Buffer Spike: 5% Smear Fix: 30s Stain: 55s Buffer: 415s Buffer Wash: 15s						
WG 4 is darker than WG 3 (default) intensity due to the inclusion of a 5% buffer spike						

CUSTOMIZABLE PROTOCOLS

The Alinity hs also allows the use of customizable protocols which support optimization of the appearance of stained smears to meet laboratory preferences.

When creating a custom staining protocol, it is recommended to start with the pre-configured protocol that stains closest to preference and adjusting as needed. Creating a protocol requires “admin level” user access on the Alinity hs, using the sample processing screen on the System Control Center (Figure 8). Up to three protocols can be customized, for both MGG or WG stains; protocols 5, 6 or 7 for WG stain and 9, 10 or 11 for MGG stain.

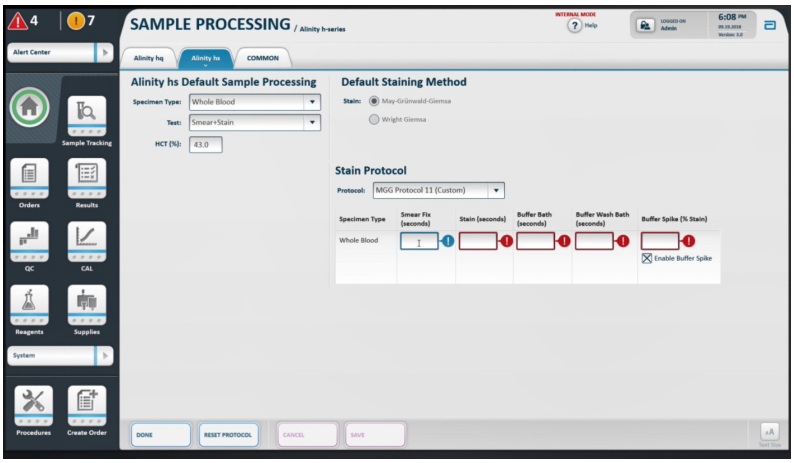


Figure 8: Configuring custom protocols

The customizable protocols on the Alinity hs offer maximum flexibility through adjustment of the following variables:

- Timings in the smear fix, stain, buffer, and buffer wash baths
- Inclusion of a spike in the buffer bath
- Concentration of the spike

Table 5 summarizes the impact of configurable changes on the appearance of the stained smear. Example images showing some of these changes can be found in the detailed "Adjusting the Staining Procedure" section of this guide.

Table 5: The impact of configuration changes on stained smear appearance

Change	Effect
Adjust time in the smear fix bath	Increase: Reduce blueness in the background due to inadequate fixation[5] Decrease: May increase the uptake of stain and darken cellular components
Adjust time in the stain bath	Increase: Reduce the redness seen in all cell types, darken the coloration[21, 22] Decrease: Lighten cellular components, decrease background color
Adjust time in the buffer bath	Increase: Darken all cellular components Decrease: Lighten cellular components
Adjust or add a buffer spike	Increase: Increase the intensity of staining Decrease: Lighten all cellular component types; reduce the precipitate seen on slides, decrease background coloration[21]
Adjust time in the buffer wash bath	Increase: Decrease the color of the background, reduce precipitate seen on slides Decrease: Smears may appear bluer if the time causes inadequate washing[5]

The lower and upper limits that can be configured are listed in Table 6. Inclusion of a long timing setting, or large spike concentration may impact system throughput and stain consumption respectively. The time in the drying station is fixed and cannot be adjusted.

Table 6: Configurable staining protocol setting limits

Custom Stain Protocol Limits	Buffer Spike	Smear Fix Bath	Stain Bath	Buffer Bath	Buffer Wash Bath
Lower limit	4% stain*	15 seconds	15 seconds	15 seconds	15 seconds
Upper limit	50% stain	900 seconds	1800 seconds	1800 seconds	600 seconds

*Can be set to 0% stain by disabling the spike.

Overall, the Alinity hs is a flexible system designed to meet the different staining preferences of laboratories worldwide. It achieves this through the choice of two different stain types and both pre-configured and customizable protocols. The information in this guide provides a foundation of knowledge on the staining procedures of the Alinity hs and how laboratories can utilize them to optimize the quality of stained smears.

REVIEW QUESTIONS

1. True or false, laboratories can vary in their preferences for the coloration and intensity of the stained smears.

   A. True

   B. False

2. Which of the following items can impact the final appearance of the stained smear?

   A. The staining protocol used

   B. The temperature of the laboratory

   C. The pH of buffer solutions

   D. All of the above

3. On the Alinity hs, the buffer spike is added to which bath?

   A. The smear fix bath

   B. The stain bath

   C. The buffer bath

   D. The buffer wash bath

4. What is the default protocol for WG stain?

   A. Protocol 1

   B. Protocol 2

   C. Protocol 3

   D. Protocol 4

REFERENCES

1. O'Connor BH. A Color Atlas and Instruction Manual of Peripheral Blood Cell Morphology. Williams & Wilkins; 1984.
2. Koepke JA, Clinical and Laboratory Standards Institute. CLSI H20-A2 Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods : Approved Standard. Clinical and Laboratory Standards Institute; 2007.
3. International Committee for Standardization in Haematology. ICSH reference method for staining of blood and bone marrow films by azure B and eosin Y (Romanowsky stain). British Journal of Haematology. 1984;57(4):707-710. doi:10.1111/j.1365-2141.1984.tb02949.x
4. Palmer L, Briggs C, McFadden S, et al. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. International Journal of Laboratory Hematology. 2015;37(3):287-303. doi:10.1111/ijlh.12327
5. Bain BJ. Blood Cells: A Practical Guide. Oxford, Uk Barbara J. Bain; 2006.
6. Bain BJ, V J, Lewis SM. Dacie and Lewis Practical Haematology. Churchill Livingstone; 2012.
7. UK-NEQAS Staining Criteria Handbook - Non Gynae Diagnostic Cytology (Edition 3, February 2015). Available at: http://www.ukneqascpt.org.uk/Documents/NEQMANMA003-%20special%20stain%20criteria%20handbook%20non%20gynae%20edition%203%20jan%202015.pdf
8. Piaton E, Fabre M, Goubin-Versini I, et al. Guidelines for May-Grünwald-Giemsa staining in haematology and non-gynaecological cytopathology: recommendations of the French Society of Clinical Cytology (SFCC) and of the French Association for Quality Assurance in Anatomic and Cytologic Pathology (AFA. Cytopathology. 2016;27(5):359-368. doi:10.1111/cyt.12323
9. SIMSON E, GASCON-LEMA MG, BROWN DL. Performance of automated slidemakers and stainers in a working laboratory environment - routine operation and quality control. International Journal of Laboratory Hematology. 2010;32(1p1):e64-e76. doi:10.1111/j.1751-553x.2009.01141.x
10. Briggs C, Culp N, Davis B, d'Onofrio G, Zini G, Machin SJ. ICSH guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting. International Journal of Laboratory Hematology. 2014;36(6):613-627. doi:10.1111/ijlh.12201
11. Kratz A, Lee S, Zini G, Riedl JA, Hur M, Machin S. Digital morphology analyzers in hematology: ICSH review and recommendations. International Journal of Laboratory Hematology. 2019;41(4). doi:10.1111/ijlh.13042
12. Horobin RW. How Romanowsky stains work and why they remain valuable - including a proposed universal Romanowsky staining mechanism and a rational troubleshooting scheme. Biotechnic & histochemistry : official publication of the Biological Stain Commission. 2011;86(1):36-51. doi:10.3109/10520295.2010.515491
13. Wright Giemsa Stains. Sigma-Aldrich. www.sigmaaldrich.com/industries/tissue-diagnostics/wright-stain.html. Accessed 13 Nov. 2020.
14. May-Grünwald | Sigma-Aldrich. Sigmaaldrich.com. Published 2013. https://www.sigmaaldrich.com/catalog/search?term=May-Gr%C3%BCnwald&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product. Accessed November 13, 2020
15. Dunning K, Safo A. The ultimate Wright-Giemsa stain: 60 years in the making. Biotechnic & Histochemistry. 2011;86(2):69-75. doi:10.3109/10520295.2010.515496
16. Alinity h-series May-Grunwald-Giemsa Stain Package Insert G72243R03 - July 2020
17. Alinity h-series May-Grunwald-Giemsa Stain SDS - February 2020
18. Alinity h-series Wright-Giemsa Stain Package Insert G72240R03 - July 2020

19. Alinity h-series Wright-Giemsa Stain SDS - December 2018

20. Alinity h-series Phosphate Buffer SDS - February 2020

21. ALQEP. BLOOD FILM STAINING EFFECTS. College of Physicians and Surgeons of Alberta; 2004. Accessed November 13, 2020. http://www.cpsa.ca/wp-content/uploads/2016/08/Blood-Film-Staining-Effects-Educational-Document.pdf

22. ALQEP. THE PERIPHERAL BLOOD FILM Preparing the Peripheral Blood Film. College of Physicians and Surgeons of Alberta; 2003. Accessed November 14, 2020. http://www.cpsa.ca/wp-content/uploads/2016/08/The-Peripheral-Blood-Film.pdf

23. Alinity h-series Operations Manual 80000023-107 - September 3 2019

24. Abbott internal studies on staining

CORRECT RESPONSES

1. **A** True
2. **D** All of the above
3. **C** The buffer bath
4. **C** Protocol 3

CORELABORATORY.ABBOTT/HEMATOLOGY

Rx Only

The Alinity h-series is available in select countries. Alinity hq is a Class I laser product. For *in vitro* diagnostic use only. Refer to the Operations Manual for operational precautions, limitations, and hazards.

ADD-128970-GBL-EN 11/23.

Abbott