Efficient Utilization of the Sysmex SP-100™ in the United States

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In the United States, changes surrounding the management of the delivery of health care have been occurring very rapidly. The medical centers in the United States have needed to respond to this change by ensuring high volume throughput of patient samples, while maintaining quality in result reporting. Implementation of systems for diagnostic testing are crucial to the effective management of the process of delivery of health care. Workflow efficiency and quality processing of hematological specimens has been enhanced by systems that incorporate cell counting, smear preparation and smear staining.

This paper presents a summary of how efficient utilization of the Sysmex SP-100 slide preparation unit, enhances the laboratory’s capability to achieve quality patient reporting. The SP-100, as utilized on an automated hematology system, is manufactured by TOA Medical Electronics Co., Ltd., Japan. The SP-100 is designed to be utilized in conjunction with Sysmex hematology analyzers in an automated system that is customized to meet the needs of a particular medical center.

This paper will focus on:
1) The automation features of the SP-100.
2) A review the Wright staining process as utilized in the United States.
3) Discussion of the inherent subjectivity of evaluation of quality stained smears.
4) Comparison of the staining features of the SP-100 with conventional methods.
5) Outlining the key steps to successful implementation of the SP-100.

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**Key Words** Automation, Wright Staining, SP-100

INTRODUCTION

Processing of patient samples for hematology studies has been streamlined dramatically in the past 15 years. This has been achieved primarily by the introduction of hematology analyzers that perform a complete blood count (CBC) including a WBC differential. Until the introduction of the Sysmex SP Series, however, the laboratorian did not have a safe and uniform method for preparation of a blood film for review of cell populations.

Responding to this need, TOA Medical Electronics, Co., Ltd., Japan, introduced the SP slide preparation unit in 1990. This product enhanced the productivity and safety of laboratory specimen testing by eliminating the need to make a smear manually. However, in order to complete the process of making a hematology system completely

Fig. 1 SP-100 slide preparation unit
automated and as a result more efficient, the hematology laboratorians requested introduction of a product that offered the capability of smear preparation and staining of the blood film. In 1994, the Sysmex SP-100™ was introduced to address this need (Fig. 1).

**SP-100 AUTOMATION**

The SP-100 is designed for use on a fully automated hematology system that incorporates a single CBC analyzer (Sysmex Alpha system), or as part of a multi-instrument automated system (Sysmex HST™). A laboratory may incorporate multiple CBC and reticulocyte analyzers along with the slide making and staining capabilities of the SP-100. The SP-100 allows the laboratory to customize the staining process as desired for their diagnostic needs. The SP-100 can be programmed to stain a blood film by the standard Romanowsky (Wright stain) method, or by staining a blood film using a combination of stains (double stain methods) of their choice.

This combination of technologies, automated cell counting and staining, is key to a successful response to the demand for quality care of the patient while minimizing costs. The hematology system as described, when used by a well trained staff, can provide consistent quality reporting of a complete hematology profile for a patient. Cost savings are achieved with the automation, consistency and reliability that is incorporated in the SP-100.

The SP-100 allows the laboratory to select staining of one or two smears for each patient. The process for operational flow of a blood sample on the SP-100 is described (Fig. 2). The SP-100 aspirates 100μL of blood and dispenses a uniform amount of blood onto a glass smear. The volume of sample dispensed is automatically adjusted by the analyzer, based on the hematocrit of the sample. A normal sample would have a volume of 10μL used in the preparation of the smear by the SP-100. The angle and speed of the spreader glass that is applied to the sample while making the smear, is adjusted for patient hematocrit as well.

The patient identification and sample identification information is read from the bar coded sample and once this information is printed on the smear, the smear is fan dried and the process of staining begins.

**STAINING OF SMEARS ON THE SP-100**

The staining process utilized on the SP-100 is achieved by dispensing stain into a specially designed cassette which holds the dried blood film. The customer selects the stain process desired (Wright...
The sample is then stained by a “dispense” method to deliver and discharge the stain, buffers and rinse solutions. At the conclusion of the staining process, the smear is then dried and is held in the single cassette for the laboratorian to remove and examine the smear at the microscope.

The SP-100 staining process most often utilized in United States medical centers is the modified Romanowsky (Wright single stain) process. This is utilized because of its' practicality as a stain of desired quality for the review of samples for routine studies. A single stain is also utilized because of the high volume throughput that can be achieved on the SP-100. Using a single stain method, 120 samples per hour can be processed, with processing including smear preparation, staining and drying1).

QUALITIES OF A WELL STAINED SMEAR

Before beginning a discussion of individual laboratory preferences for staining, it is necessary to review established textbook criteria for definition of a quality stain. The modified Romanowsky or Wright stain is a combination stain. It combines methylene blue and eosin. After several steps in the manufacturing process, methanol is added to the stain combination. The methods for combination of stains must be controlled so that the combined stain is a neutral compound dye. In its final for this reason, most laboratories purchase commercially prepared stains.

When the stain is used in combination with buffers, ionization of the stains occur. This is how staining of the cells occur. Eosin ions carry a negative charge and stain the basic components of the cells a pink color. Components of the cells that are acidic have an affinity toward the positively charged basic methylene blue ions. The neutral components of the cells are stained by both components of the dye. Wright stain, as manufactured, has a slight variability from lot to lot2).

Buffers that are used in the staining process as available in the marketplace, can be mono or dibasic or combination mono and dibasic phosphate buffers. Their pH can range from 6.8 to 7.2. Individual laboratories usually select the combination of stain and buffer that provides them with what they consider to be the desired color for identification of each cell population.

Once the stain and buffer are selected, the laboratory then determines the timing for the stain process that produces staining of the platelets and the red and white cells that they determine are the most desirable. Commercial manufacturers often market their product offerings by suggesting that specific stains are best utilized in conjunction with a specific pH buffer.

It is generally agreed that a smear that is well stained will show RBCs that are an orange-pink color. The morphologist will note that there are dark purple stained nuclei in the lymphocytes and neutrophils, and lighter purple stained nuclei in the monocyte. Platelets will stain an intense purple color. The cytoplasm of the WBC are to stain as follows: the neutrophil will have light pink cytoplasm with lilac granules, the monocytes will have a gray-blue cytoplasm with fine red granules and the lymphocyte will have a cytoplasm with varying shades of blue1).

Characteristic inclusions to the RBCs and WBCs as seen on a Wright’s stained blood film are related to specific disease states. It is imperative that the routine stained smear provide appropriate staining of these inclusions to alert the laboratorian to perform a more in depth review of the patient history. These samples with abnormal inclusions are often stained with a second or double stain to ensure accurate diagnosis. For a description of these inclusions and their relation to disease states, the reader is best referred to a Hematology/Oncology Pathology text reference, available in a medical library.
SUBJECTIVITY OF IDENTIFICATION OF A QUALITY STAIN

The staining process, which includes the fixation, staining, buffering and drying of a blood film varies from laboratory to laboratory, as previously noted. What staining characteristics constitutes a “quality” stain is also quite variable. In the United States, each laboratory identifies quality of staining that is desirable for their purposes of cell identification.

Therefore, the process for implementing a new staining unit such as the SP-100, needs to be examined thoroughly. The process requires much experimentation and cooperation from all of the hematology laboratory staff. This includes the pathologist who is responsible for final diagnostic decisions and for all members of the hematology staff who review patient blood smears and evaluate individual characteristics of all cell populations.

This process of agreement as to the desired quality of the smear as stained, should be approached as a very important task. Once the staff within the department agree on a quality staining process, their level of satisfaction with their ability to provide best patient care is enhanced. The staining process then can be considered to be “optimized” for their laboratory. Along with their optimal use of their CBC and Reticulocyte analyzer, once the SP-100 staining process is optimized, the laboratory can function at their highest efficiency.

There are now over fifty SP-100 units in use on Sysmex automated hematology systems in North America. Each customer has a desired standard of staining that is to be achieved. The process of achieving that desired stain quality is no easy task. The process must be considered to be as vital as all of the steps involved in implementing a new hematology analyzer. Before a new hematology analyzer is utilized for patient reporting, the laboratory performs an intensive correlation study. Similar studies are to be performed for the staining process.

Over the past two years, the Sysmex hematology applications specialists, have worked in conjunction with our customers in the process of implementing the SP-100. Medical centers may be converting from a manual stain method to the SP-100 process, or may be converting from use of either an automated “dip” or “platen” stainer. Less than 10% of our customers are utilizing a double stain process. Most are using a Romanowsky (Wright) stain manually or on their automated stainer. The single stain process is preferred because of the need to process specimens quickly and also because the double stain increases the cost of specimen processing. Romanowsky staining is recommended by the ICSH as well.

In the United States, it is recognized that there are many different stain and buffer manufacturers. The United States customers want to be able to choose the stain and buffer combination that they feel provides an “optimized” stained blood film. The SP-100 was designed as an “open” system. Therefore, the customer can choose the stain and buffer combination that best meets their needs as related to their staining criteria.

In the process of implementing the SP-100 we find that our customers need to re-evaluate the stain and buffers that they have been using, to see if they are amenable to a quality stain as produced by the SP-100. Several commercially prepared stains are less than acceptable for use on the SP-100. The majority are acceptable or perform very well for our customers.

Buffers produced in the United States all prove to perform to our customers’ expectations when in the range of pH 6.8-7.2. What is most critical to the implementation of the SP-100 is finding the combination of stain and buffer that meet each laboratories’ needs, as the SP-100 uses a dispense method for delivery of stain and buffer. At a minimum, a laboratory first utilizing the SP-100 needs to re-evaluate the time of either staining or buffering, or consider a slight change to both stain and buffer timing. Occasionally, a laboratory needs to investigate use of a totally different Wright stain.
compared to what they are currently using.

The process of implementing the SP-100 is similar to an instrument correlation process. The evaluation can take several days in order to experiment with the stain, buffer and timing options that the customer can select. The most difficult aspects of performance to address completely are those related to individual criteria. Repeatedly we recognize that optimum performance of the SP-100 is dependent more on subjective criteria of individual users than mechanical performance capabilities of the SP-100.

SUCCESSFUL IMPLEMENTATION

The process of implementation may take several days to fine tune. Key variables that have an effect on the implementation include:

- Stain
- Buffer
- Timing of stain and buffer
- pH of rinse
- Cleaning of cassettes
- Achievement of agreement (consensus) on “quality” staining

The Sysmex SP-100 has the capability of offering the customers the flexibility to change and evaluate each of these variables. To streamline the process, we give the customer general guidelines to follow. These guidelines include:

Stain
- Methanol content is to be 96% or greater. This is not an issue for Wright stain, as this is a U. S. Manufacturing requirement for the Wright single stain.
- Fresh stain is the most desirable (purchase with longest outdate).

Buffer
- To be in the range of pH 6.8-7.2.
- Buffers must be purchased commercially if the laboratory is unable to determine the pH of their water supply or if the pH of the water supply varies.

Timing
- Longer times intensified the stain and/or buffering of the cells, thus the laboratory need to determine the stain and buffer time that meet their “optimized” criteria.
- Longer timing for staining may or may not be acceptable for a laboratory that must meet very demanding timing for reporting (throughput is one slide every 30 seconds, but a delay will be seen if a long timing is set for the initial sample being run).

Rinse
- Buffer or water used for the rinse must be near a neutral pH.

Cassettes
- Cassettes must be cleaned daily to avoid seeing precipitate on the smear.
- Cassettes must be dry in order not to dilute the methanol in the stain.

Consensus as to “optimized” stain
- Gain pathologist support for use of the automated stainer.
- Gain pathologist support for determination of “optimized” staining.
- Reach consensus on “optimized” stain with all staff members.
Additional challenges are seen in the implementation process. These challenges are related to variables such as poor quality of glass slides, poor storage of reagents, contamination of buffers and samples that are not stored at refrigerator temperature that are more than 24 hours old. Managing these variables are a challenge for every laboratory and not reflective of performance capabilities of the SP-100.

**CONCLUSION**

With over fifty SP-100 units in use in the United States, we feel confident that the SP-100 can meet the need for “optimized” staining as part of the processing of patient samples for hematological studies. As the health care marketplace changes and the need for efficiency and standardization in testing increases, systemization will be key for success of hematology laboratories. In fact, the shift from a full CBC with manual differential to limited morphological review, has been noted as a mandate for care in the United States. Hematology laboratories need to correlate the numerical and flagging data supplied by automated instrumentation to limit smear review to those samples that require further testing. This is another indication of the need for more efficient and effective analysis of patient blood samples.

Automated systems help to assure high quality of reporting while realizing standardization, efficiency, safety and consistency. The Sysmex SP-100, with the capability of preparation and staining of a blood film, completes the automated hematology laboratory and meets the need of the United States marketplace for quality and efficiency.

**References**