Random access gram stain automation: a review of current approaches

By Bruce Bartholomew

Random access processing of gram stain slides provides high-volume laboratories an ideal mix of standardization, labor-efficiency, and throughput. Batch-design automated staining systems have provided some advantages over manual processing, such as increased throughput and reduced waste, but critical drawbacks prevent universal appeal. Cuvette-design stainers can deliver random access capabilities to microbiology labs. Combined with electro-optical decolorizing technology now available, laboratories can realize high-quality slide staining, increased standardization, labor-efficiency, throughput, and reduced waste in their gram stain operations.

Random access: definition and discussion

Random access instruments have become the norm in most parts of the laboratory. The benefits associated with random access analyzers are well-established and include improved turnaround times, reduced error rates, and reduced labor costs. Random access instruments allow individual specimens to be tested as they are received by the lab. Moreover, they permit unique testing profiles or conditions to be applied to each specimen. Other features, such as reagent and waste monitoring, automated calibration, and automated maintenance routines are also commonly associated with random access systems. At a minimum, "random access," as it applies to gram stain automation, must be defined by at least three features:

- Single slides. A single slide may be loaded and analyzed one at a time.
- Continuous load. Slide(s) may be loaded and testing initiated at any time.
- Individual profile. Unique staining conditions may be applied to each slide.

Past gram stain automation options do not meet these three criteria. Systems are not designed to efficiently process one sample. Second, once a run has begun, current systems do not permit additional samples to be loaded. Third, for each batch setup, all samples are subjected to identical staining conditions. The recent introduction of multi-slide, cuvette-design automated stainers represents a leap forward in delivering random access features for gram staining.

Automation challenges

Slide preparation: The variability of specimen sources requiring the gram stain has made standardized automation of sample preparation (the application of sample material to a microscopic slide) unfeasible thus far. Swabs, sputum, CSF, and cultures are delivered to the lab in different containers, volumes, and consistency. They do not lend themselves to standardized sample prep automation. While new automation offers the potential to automate slide preparation for some specimen types, most labs continue to prepare slides manually. For this reason, gram stain automation approaches have focused on processing slides after samples are applied and fixed to the slide.

Decolorization: Decolorization is the step most likely to cause problems in the gram stain procedure. The rate of decolorization depends upon many factors including sample type, slide fixation, wash steps, and reagent quality.[1] All automated systems using time-based decolorization suffer from the inherent problem of sample variability. While a skilled laboratorian can start, monitor, and stop decolorization for an individual slide using visual cues, batch processors by design apply the same decolorization timing to every slide in the batch, regardless of variability. As a result, some slides will be over-decolorized and some will be under-decolorized in batch-design systems.

Labs have attempted to work around this weakness by 1) batching certain types of samples together, 2) running some samples manually, or 3) "reading around" non-optimally decolorized slides. These options necessarily create compromises. Holding certain specimen types for batching delays turnaround time and risks slide misplacement. Running samples manually results in decreased labor efficiency, increased waste generation, and reduced standardization. "Reading around" poor-quality slides consumes more time and is a skill-dependent solution, raising the risk of errors.

Single-slide processing: In a random access gram stain system, each slide should receive its own unique reagents and washes. Individual slide processing avoids two problems inherent in bath stainers: over/under-decolorization and carryover. Without single-slide processing, it is impossible to adjust decolorization to individual slide requirements, as all slides are subjected to the same decolorizing conditions. In addition, bath stainers hold greater potential of carryover or precipitate effects. Incompletely fixed sample material may wash off in the bath and create anomalies that potentially affect other slides in the bath.

With single-slide processing in mind, automation options would likely mandate parallel processing in order to meet throughput requirements. Given that individual gram stains take two to four minutes each, a lab running more than 50 gram stains per day cannot wait for the sequential analysis of so many slides. A system must parallel-process stain incubations for turnaround times to be acceptable.

Review of current automation options

With these definitions and challenges in mind, let us review the advantages and disadvantages of current gram stain options. We limit this discussion to six desired features, summarized in the priority shown from the input of more than 50 high-volume gram staining labs:

- *Reduced maintenance.* Non-burdensome maintenance
- *Accuracy/quality.* Distinct, easy-to-read staining results

- *Standardization/consistency.* The same results across all staff and shifts
- *Ease of use/simplicity.* Reduced complexity and intuitive operation
- *Labor efficiency.* Conserved time and reduced opportunity costs
- *Reduced waste generation.* Reduced hazards and lower costs of disposal

Manual staining. Manual staining by a skilled technologist offers the best quality slides to the laboratory. For this reason, many high-volume labs continue to run manually, or supplement their automation with manual staining of certain slides or sample types. The disadvantage of manual staining is that laboratorians are not equally skilled, and therefore standardization is impossible. Manual staining is not labor-efficient and generates large amounts of waste for which the disposal can be a significant cost.[2]

Bath stainers. Bath stainers incorporate a series of reagent baths and wash stations into which multi-slide carriers are immersed. The primary advantage of bath stainers is their high throughput. Standardization is also an advantage over manual staining, especially within-run. Accuracy/Quality is considered a disadvantage for two reasons: some bath-stainer users express carryover concerns and report precipitate formation; and bath stainers are subject to non-optimal decolorization.

Spray strainers. Spray-design stainers use nozzles to spray reagents onto slides loaded inside a carousel/centrifuge carrier. In this way, each slide receives unique reagents, and the carryover risk of bath stainers is avoided. These stainers consume the smallest volumes of reagents, making waste generation a primary advantage. Spray stainers offer the advantage of standardization, as each slide in a batch receives identical staining parameters, configured for each run. The primary disadvantage is the high maintenance required to keep the systems operating well. Laboratories using these spray-stainers often refer to their extensive maintenance burden. Spray stainers also may suffer from non-optimal decolorization, negatively impacting accuracy/quality, which frequently induces labs to run some samples manually.

Cuvette stainers. Cuvette-designed stainers have an inherent advantage in delivering "custom" decolorization for each slide. In this design, reagents are pumped into and immerse slides in a narrow, slide-shaped cuvette. In one manufacturer's design, an "electro-optical eye" continuously monitors the effluent draining from an actively-decolorizing slide and identifies the optimal "stopping point," just as a skilled technologist stops decolorizing based on visual cues. In this way, cuvette-designed stainers offer a means of adjusting decolorization automatically for each slide. This is a major advantage in generating consistently high-quality slides, independent of sample type and thickness variability.

Multi-slide systems available today incorporate automated maintenance and calibration routines, making operation simple and vastly reducing the maintenance burden associated with spray and bath stainers. They offer standardization across all slides and consume minimal reagents, generating large reductions in waste disposal over manual staining.

Multi-slide cuvette stainers with electro-optical decolorization now offer the benefits of random access gram staining to high-volume microbiology laboratories. Cuvette stainers process each slide separately. They adjust decolorization for every slide, offering labs the ability to deliver unique staining conditions to every gram stain slide. Finally, multi-slide cuvette stainers, which parallel process slides in multiple cuvettes, deliver high-volume throughput, permit labs to load one or many slides at a time, and offer continuous load capability.

Editor's note: *The author analyzes manual staining and the currently available automation options of bath stainers, spray stainers, and cuvette stainers, reaching the conclusion that the last of these is the best choice. MLO would be pleased to print concurring or opposing views, both from clinical laboratory professionals as well as industry leaders, in an upcoming issue.* □

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References

1. Sutton S. The gram stain. *Pharmaceutical Microbiology Forum Newsletter.* 2006;12(2):4.
2. Mayne DJ, McVay JD. Comparison of PREVI Color Gram automated gram stain to manual gram stain. Poster accessible at *http://www.biomerieux-usa.com/upload/previ-color-gram-vs-manual-gram-stain-1.pdf.* Accessed May 17, 2014.