Mechanisms to Assess Gram Stain Proficiency of
Technologists at Satellite Laboratories

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Running title: Note

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ABSTRACT

To address Gram stain proficiency in a satellite/centralized microbiology laboratory paradigm, non-microbiology technologists were required to interpret standardized Gram-stained preparations of clinical material (quality assurance program 1). Clinical Gram stains prepared and read by satellite laboratorians were reviewed by microbiologists (quality assurance program 2). Satisfactory performance was achieved (94%) in quality assurance program 1. In contrast, quality assurance program 2 had a significantly lower overall performance (89%; \( P < 0.0001 \)) due to poorer identification of host cells (93%) and bacteria (84%). A variety of intervention mechanisms, including continuous monitoring, resulted in overall performance improvement (\( P \leq 0.006 \)). While technologist challenge has educational merit, review of previously-read slides is a better indicator of technologist Gram stain proficiency.
Gram staining of primary clinical specimens has tremendous clinical utility. Studies have shown that Gram staining sputum can be predictive for several etiologies of lower respiratory tract disease [1,10,11,15,16,17,19,23]. Gram stain data also influence the treatment of clinically-significant bloodstream infection [8,13]. In addition, proper performance of the primary Gram stain can guide the processing of expectorated sputum specimens [14] and aid in interpretation of cultured skin and soft tissue specimens [3,26].

Bartlett [2] cited virtual elimination of house staff laboratories (as an indirect result of the Clinical Laboratory Improvement Amendments of 1988) and outsourcing of primary specimens as two factors contributing to the decline in clinical microbiology studies for patients with lower respiratory tract disease. Others have raised concerns over Gram stain clinical diagnostic value in terms of protocol standardization [5,7,18]. As a result, many have discouraged utilization of Gram-stained smears as predictors of certain clinical infections, particularly those involving the lower respiratory tract [10,20,21].

Technologist competency has become of even greater importance with the centralization of microbiology testing and creation of satellite processing (rapid response) laboratories staffed by non-traditional microbiologists [6]. Moreover, accrediting agencies mandate proficiency documentation of all technologists who execute clinical Gram staining protocols. In this report, we describe a mechanism for objectively assessing the Gram stain interpretation competency of non-microbiology laboratorians and present a quality assurance program for monitoring and increasing proficiency of Gram staining.
Full-spectrum clinical laboratories were present at three Milwaukee, Wisconsin inpatient facilities until 1988 when two hospitals (B and C) consolidated their clinical microbiology laboratories to a free-standing building (central laboratory). Subsequently, the central laboratory assimilated the clinical microbiology services of a third hospital (A) in 1999. Algorithms were devised in which primary clinical specimens for routine bacteriology were processed to appropriate media [22] and smeared for Gram staining and interpretation [25] by trained non-microbiology technologists at the satellite laboratories. Within 24 hours, Gram-stained smears were forwarded to the central laboratory for confirmation of results.

Three primary clinical specimens from a variety of sources were smeared and Gram stained quarterly by an experienced microbiologist at the central laboratory (quality assurance program 1). The number of cells (viewed with 100x magnification for eukaryotic cells and 1000x magnification for prokaryotic cells) was identified as rare (<1 per field), few (1-5 per field), moderate (5-20 per field), and abundant (>20 per field). Included within these primary clinical specimens were sputa to be evaluated for culture processing acceptability, based on the criteria of Murray and Washington [14]. Seventy satellite laboratory technologists were made aware of the specimen source and asked to provide Gram stain interpretation and semi-quantitation of eukaryotic and prokaryotic cells from the challenge slides. Results were forwarded to the central laboratory microbiologist. Furthermore, central laboratory microbiologists (on a rotating basis) daily selected a number of the satellite laboratory-prepared smears that was commensurate with relative microbiology volume generated by that hospital (quality assurance program 2). A descriptive Gram stain report was documented by the microbiologist and forwarded to the microbiology supervisor.
Review of the Gram-stained preparations in quality assurance program 1 and 2 was documented using the following system: one point was given for each correct Gram stain reaction; one point for each bacterial morphology present; one point for each host cell (squamous epithelial or inflammatory) morphology present; and, one point for the quantity of each host or bacterial cell type present. The acceptable range for quantitation was ± one gradation of the expected value. Credit for quantity, Gram reaction, or cell type involved was not granted if a sputum specimen was rejected; the technologist received a slide value of 0. In addition, points were subtracted from the score when additional cell types or bacteria were reported. Finally, point values for host cells (quantity plus presence/absence) and bacteria (quantity plus Gram and morphology) were summed per preparation and expressed as percentage of expected value. Standard error of the mean was calculated for all measures of Gram stain proficiency testing. Comparisons of inter-laboratory performance, cell type, and the two measures of Gram stain proficiency testing were facilitated by the t-test for independent samples. Assessment of temporal change (improvement) in laboratory performance was facilitated by one-way analysis of variance [24]. The alpha level was set at 0.05 before investigations commenced and all P values are two-tailed.

The overall Gram stain proficiency rate of 70 satellite laboratory technologists, as determined by quality assurance program 1, was 94% (Fig. 1). However, host cells were interpreted with a higher success rate than bacteria (98% versus 89%; $P < 0.0001$). When data were examined by hospital (Fig. 1), personnel at hospital C showed the lowest proficiency for host cell recognition and presence of bacteria. Upon quality assurance program 2 review of 2517 random Gram-stained preparations, no significant differences in proficiency for detection of host cells or
bacteria were detected among the laboratorians of the three satellite laboratories. Similar to quality assurance program 1, greater proficiency in the interpretation of host cells was demonstrated by satellite laboratorians when compared to bacterial observations \( (P < 0.001) \).

When results from the two quality assurance programs were compared, quality assurance program 2 was 5.4% lower than that determined by quality assurance program 1 \( (P < 0.0001; \text{Fig. 2}) \). Analogous differences were detected between the programs for identifying bacteria and host cells \( (P \leq 0.03) \). Upon delineation of individual hospital data by cell type, higher rates of proficiency were generally noted in quality assurance program 1 when compared to those of quality assurance program 2 \( (P \leq 0.04) \).

Implementation of quality assurance program 1 and 2 caused a significant increase in Gram stain proficiency in the fourth quarter in 2006 \( (P \leq 0.006; \text{Fig. 3}) \). Improvements occurred in host cell and bacterial identification among participants of quality assurance program 1 \( (P \leq 0.01) \) and host cell indicators with participants of quality assurance program 2 \( (P = 0.03) \). Among the hospitals, the greatest improvement occurred with hospital C \( (P \leq 0.005; \text{Fig. 4 A and B}) \). Non-microbiology laboratorians at hospital C had consistently performed less skillfully than laboratorians at hospital A and B prior to implementation of the quality assurance programs.

Gram stain diagnostic value has been questioned from a variety of perspectives. In a meta-analysis, Reed et al. [21] reported Gram stain sensitivity of 15-100% for diagnosis of pneumococcal pneumonia, with specificity ranging between 11% and 100%. In a regional proficiency testing program, Church et al. [5] reported a significant lack of standardization in
reporting quantities of host cells and bacteria and raised concern how this could impact patients over a continuum of care. Cooper et al. [7] documented low inter-technologist agreement rates for cell quantitation from Gram-stained lower respiratory tract specimens. In a broad sense, following a regional microbiology laboratory restructuring, a number of subsequent proficiency challenges revealed that error rates increased in a number of laboratories, such as rural facilities (noted to neither possess a technologist devoted to microbiology duties nor have on-site pathologist or medical microbiologist oversight) that took on additional microbiology responsibilities [6]. Taken together with the clinical and bench-level utility of the Gram stain procedure, the preceding factors provide an impetus for evaluating and maintaining the proficiency of microbiologists and technologists who perform this method.

Central laboratory microbiologists who determined expected values of slides throughout these quality assurance programs were subjected to in-house Gram stain proficiency testing. On a quarterly basis, all microbiologists reported findings on a randomly-selected slide set. Data were pooled and the expected value of each slide was derived from the majority of responses for each parameter. Unlike quality assurance program 1 that granted credit for ± one gradation of an expected value for quantitation, in-house proficiency testing used higher stringency (i.e., only one acceptable value for quantitation). The microbiologist who prepared proficiency material and graded responses for quality assurance program 1 was one of two microbiologists who performed at 100% of expected value for in-house proficiency testing during the timeframe of this investigation. Central laboratory microbiologists demonstrated a mean in-house proficiency rate of 95%. No significant differences existed between average microbiologist proficiency
score and the mean demonstrated by the microbiology laboratory as a whole ($P \geq 0.06$). These
data confirmed the validity of the expected values.

Data from both quality assurance programs demonstrated competency of satellite laboratorians in
the interpretation of Gram-stained material. However, greater proficiency was found in host cell
analysis ($\geq 93\%$) than in bacteria analysis ($\geq 84\%$). The rate of successful interpretation of host
cells was markedly higher than that observed in an intra-laboratory study [7]. Cooper et al. [7]
limited their study to lower respiratory tract smears and exercised more stringency in their
grading of quantitative measures. However, satellite technologists in our study may have had
more breadth of knowledge in hematology and sterile body fluid analysis.

Quality assurance program 1 yielded higher rates of proficiency than did quality assurance
program 2. This was true in each component of the monitor and was realized at all three satellite
laboratories. While intra-laboratory technologist collaboration may or may not have played a
role in the increased frequency of correct responses in quality assurance program 1, one aspect
that could have played a role was the nature of the Gram-stained smear itself. To ensure
consistency in the survey, specimens were pre-selected and smeared onto slides, with contents
stained, coverslipped, and previewed by the microbiologist at the central laboratory before
presentation to the three satellite laboratories. In contrast, Gram-stained smears encountered in
quality assurance program 2 were prepared during routine operations of the satellite laboratory.
As such, there may have been greater opportunity for microbiologists to review overdecolorized
Gram positive cocci and degenerated leukocytes--cell types that may have been incorrectly
reported by satellite technologists.
A key component of any continuous quality assurance monitor is *ad libitum* intervention [9]. Throughout this study, four major means of correction were implemented. First, Gram-stained smears from either quality assurance program 1 or 2 that yielded an egregiously-incorrect response from a satellite laboratorian were returned to a designated point person from that laboratory. This individual personally reviewed the slide with the technologist and discussed any discrepancies. Secondly, one-on-one interactions at a dual-headed microscope took place between a central laboratory microbiologist and the satellite laboratorian. Third, personnel from the satellite laboratories and the central laboratory formed a systemwide microbiology subcommittee which provided a conduit for exchange of data, policy, and concerns. Finally, digital images of interesting findings from quality assurance program 2 were disseminated via inter-laboratory electronic mail in the format of a case presentation. Comprising this document were chart review, organism epidemiology, and discussion of the relevance of the Gram-stained smear in the specific case. These mailings also provide a forum for discussion of aberrant findings in Gram-stained preparations, such as organisms partially treated with antimicrobial agents [4,12].

Analysis of variance data for four quarters of quality assurance program 1 (Fig. 3) revealed an increase in Gram stain proficiency. Analogous improvement was seen via quality assurance program 2. However, neither hospital A nor hospital B demonstrated any improvement in quality assurance program 1 or 2 throughout the course of this monitor. It is likely that these two laboratories had the most experience with satellite microbiology processing. In further support of this statement, hospital A and B possessed the two highest proficiency rates (Fig. 1). In
contrast, hospital C showed significant improvement in total Gram stain proficiency (Fig. 4) and interpretation of bacteria in both quality assurance programs.

In conclusion, we describe a quantitative measure of Gram stain proficiency that can be applied to challenges of satellite laboratorians and an off-site review of technologist performance. These monitors can assess proficiency to the level of the laboratory, technologist, cell type, and quantitative measure. Technologist challenge is best utilized as an educational tool. However, off-site review is likely a better indicator of overall day-to-day Gram stain proficiency. When combined, these measures enable a multi-site microbiology service to generate valid data both for patient care and for practices of the clinical microbiology laboratory.
REFERENCES


Figure 1. Gram stain proficiency of three satellite laboratories, determined by quality assurance program 1 and delineated by interpretation of bacteria (solid bars), host cells (open bars), and sum of the two categories (shaded bars).
Figure 2. Comparison of quality assurance program 1 and 2 for measure of overall Gram stain proficiency.
Quality Assurance Program 1

Percentage of Expected Value

Quality Assurance Program 2
Figure 3. Non-microbiology laboratorian Gram stain proficiency, determined by quality assurance program 1 (boxes) and 2 (diamonds), for four quarters of 2006.
Figure 4. Gram stain proficiency of hospital C non-microbiology laboratorians, measured at the onset and later stages of quality assurance program 1 (A) and 2 (B).