Letter to the Editor

To follow or not to follow the recommendations regarding microscopic analysis of the Clinical and Laboratory Standards Institute H20-A2 to validate the criteria for blood smear review?

Dear Editor,

We read with great interest the Letter to the Editor by Grotto on the need to follow the Clinical and Laboratory Standards Institute 2007 H20-A2 guidelines during microscopic analysis in reply to the study by Comar et al. We appreciate the comments that ensured extensive discussion on this subject.

The work of Barnes et al., which represents the core of the criteria for blood smear review (BSR) recommended by the International Society for Laboratory Hematology (ISLH), emanates from an international consensus among 20 experts in 2002 during a conference in Indian Wells, CA, USA. In consensus, Barnes et al. proposed a nine-step protocol for validating the BSR criteria in routine laboratory practices. Step 4 of this protocol is as follows: “Perform a slide review of all samples. Limit the reviews to only one or two senior technologists for consistency. Manual differentials should only be performed if there is a specific need to do so (e.g., Vote out, abnormal cell-type flags, etc.”

The step-wise protocol by Barnes et al. does not mention the NCCLS H20-A as a sine qua non condition for the microscopic review of blood smears. In their work, Barnes et al. did not mandate the application of the NCCLS H20-A guidelines, as interpreted by Grotto. Thus, Barnes et al. did not exclude the possibility of one observer counting 100 cells to validate the BSR criteria. We therefore understand that counting performed by either one or two observers is equally acceptable.

The CLSI H20-A2 (formerly NCCLS H20-A) is a reference document to evaluate hematology analyzers that perform automated leukocyte differential counts and consider the visual leukocyte differential count as the gold standard. Most studies that rigorously followed this guideline specifically evaluated the automated leukocyte differential count and the suspect flags of the hematology analyzers. On the other hand, studies evaluating sets of criteria for BSR did not necessarily follow the recommendations of the NCCLS H20-A or CLSI H20-A2 regarding the microscopic analysis. Thus, we emphasize that, in the study of Comar et al. the step-wise rules of Barnes et al. that exclusively deal with the validation of the BSR criteria were followed.

We believe that Barnes et al. recommended slide review by either one or two observers, without specifying a set number of slides per sample nor the number of cells to be counted per slide, to enable application of the same protocols of sample collection and processing as in routine protocols for validation purpose, thus simulating the real-time conditions of most hematology laboratories.

We evaluated the criteria for BSR by using the hematology analyzers provided by Sysmex Corporation. The application of the criteria for BSR adapted from ISLH resulted in high false negative (FN) (>5%) and microscopic review rates (MRR). Similar results were reported by Xing et al. in an analysis of 2400 samples using the ADVIA 120/2120 hematology analyzer, according to the screening criteria proposed by ISLH and their own positive smear findings [FN = 5.5%, false positives (FP) = 28.1%, and MRR = 50.2%]. It is important to emphasize that we did not conclude “the inadequate performance of both pieces of equipment” in any instance of the proposals by Comar et al. We explained that 30% of the FP results (i.e., 6.98% of the total samples or 138 samples in 1977) occurred due to the presence of suspect flags in the samples. This percentage represents the sum of all suspect flags generated in all samples and whose microscopic counterpart did not provide any positive smear finding. We believe that the FP rates observed by Comar et al. can be partially attributed to the profile of the samples analyzed and not to the brand or type of hematology analyzer used. As evidence, in another laboratory where one of the authors works and which generally attends outpatients, the application of the same criteria for BSR using similar hematology analyzers resulted in a daily MRR of 5–20%, an FP rate of 3–10%, and an FN rate of <5% (unpublished data).
In our experience, in individual analysis, the main suspect flags delivered the following results using the XE-2100D hematology analyzer for samples similar to those used by Comar et al. The FN rate and efficiency for immature granulocytes were 1.15% and 94.71%, the FN rate for blasts was 0.17% \((n = 3)\) samples; and the efficiency, sensitivity, and specificity for Left Shift were 82.4%, 44%, and 92.08%, respectively. Therefore, unlike Grotto’s (1) interpretation, the performances of these suspect flags were almost similar to those reported by Stamminger et al. and Ruzicka et al.

In summary, each laboratory should establish its own criteria for BSR of blood counts according to their peculiarities, possibilities, and limitations, and it should follow the appropriate guidelines and tools to validate such criteria in routine laboratory practices. After a careful analysis of the results discussed above, we conclude that the use of the rules proposed by Barnes et al. was adequate in the study of Comar et al.

**Conflicts of interest**

The authors declare no conflicts of interest.

**REFERENCES**


Samuel Ricardo Comar*, Mariester Malvezzi, Ricardo Pasquini

Universidade Federal do Paraná (UFPFR), Curitiba, PR, Brazil

*Corresponding author at: Laboratório de Hematologia, Unidade de Apoio Diagnóstico, Hospital de Clínicas, Universidade Federal do Paraná (UFPFR), Rua Padre Camargo, 280, Alto da Glória, 80060-240 Curitiba, PR, Brazil.

E-mail address: srcomar@ufpr.br (S.R. Comar).

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