**Laboratory error rate**

**Q** We have been trying to determine an acceptable quality threshold for laboratory error rate. We formerly had our threshold set at 1%. When that seemed too lenient, we moved it to ≤0.25%. Does that seem appropriate? Do you have any resources or references I can check?

**A** It is necessary to start with the realization that a small percentage of a big number is itself a big number. A 1% error rate for a laboratory that reports 1,000,000 tests per year is experiencing 10,000 errors per year, or 830 errors per month, or about 30 errors per day, or about 1.25 errors per hour! How many patient lives would a laboratory be adversely affected with this “99% quality” level?

The measurement standard most in use in business and industry is the concept of Six Sigma — which translates to a laboratory error rate of 3.4 errors per million test results — or 99.999997% accuracy. Higher Sigma levels equate to higher quality because they represent a lower error rate. A laboratory with an error rate of 0.25% on a base of 1 million tests per year experiences 2,500 errors per year, or 208 errors per month, or about seven errors per day. This “99.75% quality” level represents Three Sigma. While the percentage looks low, the actual error rate is still quite high.

Healthcare processes, with their inherently manually directed nature, have been shown to function at the Two to Three Sigma level, leaving much room for improvement toward industry goals. Perhaps laboratories should measure error rates and set thresholds in terms of number of errors per 10,000 tests, a figure that can be reported in whole numbers but can also easily be translated into Sigma levels.

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**nRBC and giant-platelet interference on a cell counter**

**Q** Our lab recently purchased a cell counter that will enumerate nucleated red blood cells (nRBCs) and report a corrected white blood cell count (WBC) if the size of the nRBCs is at the threshold for triggering a cellular interference flag. It is our protocol to first estimate the WBC under hi-dry to verify the counter’s corrected WBC. We then go to 100x to see if nRBCs or other interferences are present, such as clumped platelets, giant platelets, and so forth.

If just nRBCs are seen, we perform a diff and enumerate the number of nRBCs. We report the counter’s nRBC result if it agrees with our manual count; we report the counter’s WBC if it agrees with our estimate.

The scenario gets tricky if both nRBCs and giant platelets are seen on smear. How would the panel verify/determine the corrected WBC if both interferences are seen?

This is what we do:
1. Perform separate counts to enumerate giant platelets and nRBCs/100 cells counted as WBCs on the counter by a) enumerating nRBCs by counting the number of nRBCs/100 cells (when counting 100 cells include both giant platelets and WBCs), and b) enumerating giant platelets by counting the number of giant platelets/100 cells (when counting 100 cells, include both nRBCs and WBCs);
2. Add the number of giant platelets and nRBCs from the first step together; and
3. Calculate WBC correction as follows:  
   - Corrected WBC = Uncorrected WBC minus Total Cellular Interference  
   If this corrected WBC result is close to the counter’s corrected WBC, we report out the counter results. If this does not agree with the counter result but agrees with our estimate from the smear, we report out our calculated result. Does that panel agree with this? If not, can you recommend a detailed procedure?

**A** You are correct in recognizing that the counter will enumerate nRBCs and can generate an nRBC and cellular interference flag. It is our laboratory’s experience that there are situations where a cellular interference flag is generated and no nRBCs are enumerated, as well as times where the nRBC flag will be present and no cellular interference flag is present. Our laboratory has taken the approach that if either of these flags are present, we review the smear, and if there are nRBCs present we will report the number of nRBCs seen on the smear and the corrected WBC.

The issue arose regarding what to do if neither the cellular interference flag nor the nRBC flags are present; but on manual review of the blood smear, nRBCs are noted. We initially wanted to utilize the uncorrected WBC count as part of our correction formula. When we reviewed this approach with the manufacturer, its representatives indicated that the uncorrected WBC count is simply a number to indicate how many particles greater than 35 fL are present and that it could not be utilized as an accurate WBC count or used in any calculation. We decided that we would run such a sample on a different complete blood count instrument that did not provide/calculate a “corrected” WBC count and then perform the manual correction utilizing the formula of: WBC x 100/100 + nRBCs noted on smear review.

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While our laboratory has never corrected for giant platelets, we do utilize a similar correction for the presence of circulating megakaryocyte nuclei. We take the number of nRBCs plus the number of megakaryocyte nuclei and apply the same formula.

Utilizing the same approach for giant platelets, you would recalculate the WBC for giant platelets in those situations where you did not have an nRBC flag or a cellular interference flag, and on smear review where you saw giant platelets and your platelet smear estimate did not match the platelet count. I do have some concerns related to which platelets (i.e., how giant) will end up being counted by the instrument and how you would be able to ensure that the platelets you are counting on smear review are or are not being incorporated into the corrected WBC count. A giant platelet is defined as a platelet larger than a red cell (typically >100 fL), but we know that particles >35 fL may impact the WBC count. How would you be able to reliably ensure that the platelets that are interfering with the white cell count (those between 36 fL and 100 fL) are appropriately identified by the technologist when they review the smear? Unless the giant platelets were numerous concurrent with a very low white count, the impact to the white count and then subsequent clinically significant change in WBC and associated values, such as the absolute neutrophil count, are minor. While the issue you raise has technical merit, the inability to reliably compensate for a technical issue with minimal clinical impact may end up having a negative impact on the overall quality and consistency of your result.

Finally, I would add that WBC counts can be estimated by peripheral smears, and this will aid in the verification of the corrected WBC count. The use of a disposable diluting pipette system and a hemacytometer for a manual white blood cell count, however, can be useful in the event that the corrected count disagrees with your peripheral estimate due to interference of any sort.

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Colony age for susceptibility testing

Q The Clinical and Laboratory Standards Institute (CLSI) says that susceptibility testing in particular (and probably ID panels as well) should be done using 18 to 24 hours’ growth. In real life, this is not altogether practical. We are really good at not doing ID/susceptibility panels on growth that is more than 24 hours old. Instead, we subculture and do it on fresh growth — although this does delay results by 18 hours (Gram negative bacilli) to 24 hours (staph and enterococcus). When we err, it is by setting up the panels on growth that is only 10 to 14 hours old.

Is it better to subculture the organism and delay results by a day, or do an ID/susceptibility panel on growth that is between 24 and 48 hours old? My understanding is that the problem with “old” organisms is that some may be dead but still give turbidity when preparing the inoculum. Therefore, the panel would be under-inoculated and results might be falsely susceptible. That is why we are strict about only using 18- to 24-hour growth in this scenario.

What is the downside of using growth that is “too young”? Can we get erroneous results by using growth that is less than 18 hours old? We use conventional panels (not rapid). Since some rapid panels rely on enzymatic reactions, I can see how too young a growth could affect those results. I am not as clear on what erroneous results we could get in an overnight (conventional) panel.

A CLSI (formerly NCCLS) states that a suspension of an 18- to 24-hour-old culture can be used for direct broth or saline preparation for testing of antimicrobial susceptibility of bacterial isolates.1,2

This is the recommended method for testing fastidious organisms, Haemophilus spp., Neisseria spp., and Streptococcus spp., and for staphylococci when testing for potential oxacillin resistance.

Alternatively to this, the CLSI standards also indicate that at least three to five well-isolated colonies of the same morphological type can be selected from an agar plate culture (age not specified). The top of each colony is touched with a wire loop, and the growth is transferred to a tube containing four mL to five mL of a suitable broth medium, such as tryptic soy both. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually two to six hours). This suspension can then be used to prepare your 0.5 McFarland standard for use in antimicrobial-susceptibility testing.

Thus, either the direct colony suspension method or the growth method can be used with confidence to prepare bacterial isolates for susceptibility testing. There is an exception to this, however. The manufacturer of your panels says in its package insert that for Staphylococcus aureus only the direct colony suspension method should be used if looking for methicillin-resistant S aureus, or MRSA. Always follow the manufacturer’s directions, and discuss any concerns you have with appropriate representatives from the company concerning their instructions.

I would not recommend extensive subculturing of organisms in order to prepare susceptibility testing, which would delay patient-result reporting but, instead, incorporate one or both of the two methods outlined above and standardized by the CLSI into your routine workflow. I would look at your workflow and try to redesign it so that you are not delaying your patient reports, especially on critical specimens (e.g., positive blood cultures, body fluids), but working on them first thing in the morning so that you can get them on your susceptibility-testing instrument the same day. Work towards this with all of your clinically significant isolates.

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References

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