What to consider when performing method comparisons on blood gas analyzers

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Gitte Wennecke
Radiometer Medical ApS
Åkandevej 21
DK-2700 Brønshøj
Denmark

When performing method comparisons, it is important to address a number of preparatory and preanalytical issues to ensure that comparisons solely reflect the analytical differences between the two methods in question.

This article provides preparatory and preanalytical checklists that can be used when comparing two or more blood gas analyzers.

Introduction

The purpose of method comparison is to determine the agreement between two or more methods or analyzers measuring the same analyte.

The experiment is preferably performed on split samples measured on both methods, and any difference found between the two methods should be interpreted as analytical difference.

A method comparison is recommended whenever a new method or analyzer is introduced into a healthcare institution as part of a method validation. If two or more methods/analyzers are used simultaneously for the same analyte, these can, as a way to ensure equality, be compared regularly.

In the US, for instance, there is a legal requirement to perform method comparisons twice a year to validate this.

As the purpose is to determine if there is an analytical difference between the two methods, it is extremely important to eliminate inconsistent contributions from the preanalytical phase, which by experience is a major source of error during method validation of blood gas analyzers.

The objective of this article is, therefore, to provide checklists with the preanalytical and preparatory considerations when performing method comparisons on blood gas analyzers.

The typical parameters found on a blood gas analyzer are:

Blood gases and pH: pH, pO_2, and pCO_2
Electrolytes: cNa^+, cK^+, cCa^{2+}, and cCl^-
Metabolites: *c*tBil, *c*Lac, and *c*Glu
Oximetry: *c*tHb, FCOHb, FO_2Hb, FMetHb, and sO_2

Preparatory considerations

When conducting a method comparison there are some preparatory issues that are important to address to ensure that the method comparison will reflect only the analytical differences between the two methods.

The following table is a checklist with general issues to consider in the preparatory phase of the method comparison. The next table is a more detailed description of preanalytical considerations for each of the parameter groups typically measured on blood gas analyzers.

Checklist with general preparatory considerations when performing method comparisons:		
1.	Expected outcome	Prior to a method comparison it is necessary to determine the allowable difference (or bias if the comparative method is a reference method) on the analytes which is acceptable to the laboratory.
	Performance specifications	In order to determine the allowable difference, it is necessary to take the known performance specifications of each of the two methods into account. Performance specifications such as inaccuracy and imprecision are often determined by the manufacturer and should include questionable results from calibration solutions, electrodes, etc.
	Matrix effect	Analytical differences caused by a matrix effect [1] should be identified. An example is the measurement of electrolytes on direct or indirect ISE.
	Interfering substances	It is also important to investigate whether the manufacturer has identified any interfering substances [2], which could influence the method performance.
2.	Test protocol	The aim and the requirements of the study should be defined prior to starting the test. A detailed description of the test is needed for all participants.
3.	Thorough training	All personnel participating in the test should be familiar with methodologies, maintenance, etc., on both methods prior to starting the test.
4.	Patient population	The sample material should reflect the patient population from which the routine samples will come from.
5.	Range of results	Within the given patient population it is important to have a sufficient range of results, representing also the extremes. If the samples are only used for method comparison, extended storage time is one way to gain values in the outer range.
6.	Sample material	The sample material used for the test should reflect the material used for routine testing. It is preferable to analyze on split samples, if possible. If capillaries are used for neonatal testing, it is important that the samples are drawn at the same time and from the same site.
7.	Adequate sample volume	Prior to the test, it is necessary to determine the volume of blood needed.
8.	Adequate number of samples	It is debatable what the adequate number of samples is, and it may vary due to the nature of the analyte. The general recommendation by the NCCLS [3] is a minimum of 40 samples.
9.	Analyzer preparations	Prior to the test, it is important to ensure that the analyzers are in control by doing QC and calibrations according to the manufacturers' recommendations.

10.	Storage of samples before measurements	If the samples used for the method comparison are also used for patient evaluation, storage time before measurement should be according to the manufacturers' or the laboratory's procedures!
11.	Storage of samples between measurements	As a blood sample is living material, storage between measuring on the two methods will change the values of most parameters and should, therefore, be minimized.

TABLE I

Preanalytical considerations

The preanalytical issues listed in the table below are the most important considerations when performing blood gas analysis.

If the samples used for the method comparison are also used for patient evaluation, all of the listed preanalytical considerations are important and even more variables may be considered. However, if the results are only used for method comparison, some of the issues are less important, e.g. storage time before analysis.

By far the most important preanalytical consideration in connection with a method comparison is to ensure uniform quality of the sample that is introduced into the two methods.

For instance, minimizing storage time between the two measurements is critical. In order to avoid non-analytical errors, the best approach is to perform measurements on routine patient samples with the analyzers side by side.

Detailed description of preanalytical considerations when performing method comparisons:		
Blood gas and pH (pH, pO_2, pCO_2)		
1.	Air bubbles	*Air bubbles* will have an effect on the pO_2 results. Air bubbles must be removed prior to each measurement.
2.	Storage time	*Storage time*[1] between the measurements will affect pO_2 and eventually pCO_2 and pH. The maximum time between measurements on two methods should be 1-2 min.
3.	Storage temperature	*Storage temperature*[1] is less important when the sample is only used for a method comparison.
Electrolytes (cNa^+, cK^+, cCa^{2+}, and cCl^-)		
4.	Hemolysis	*Hemolysis* will affect cK^+ and cCa^{2+}. Avoid cooling a sample directly on ice or mixing it vigorously between measuring on the two methods.
5.	Storage time	*Storage time*[1] between the measurements can affect cK^+ if the sample is stored cold for more than 30 min. The maximum time between measurements on the two methods should be 1-2 min.
6.	Evaporation	*Evaporation* is another consequence of storage and can occur from open tubes or microcups.
7.	Anticoagulant	It is important to ensure an even distribution of *anticoagulant* prior to the first analysis.
8.	Storage temperature	*Storage temperature*[1] is less important when the sample is only used for a method comparison.

Metabolites (*c*Glu, *c*Lac, and *c*tBil)		
9.	Storage time	Avoiding *storage time*[1] between measurements is one of the most essential considerations, especially for cGlu and cLac. The maximum time between measurements on the two methods should be 1-2 min.
10.	Evaporation	Evaporation is another consequence of storage and can occur from open tubes or microcups.
11.	Hemolysis (Glu + Lac)	*Hemolysis* affects cGlu and cLac on some enzymatic measuring methods and should be avoided [4]. Avoid cooling a sample directly on ice or mixing it vigorously between measuring on the two methods.
12.	Light	*Light* degradation of bilirubin may affect the result [5].
Oximetry		
13.	Mixing	Settled samples are the most common cause of errors on especially ctHb. Mixing the sample very thoroughly prior to the first measurement and between measurements is essential.
14.	Air bubbles	As *air bubbles* affect the pO_2 result, the sO_2 will also be affected. Air bubbles must be removed prior to each measurement
15.	Storage time	The maximum *storage time*[1] between measurements on the two methods should be 1-2 min.

TABLE II

[1] The recommended storage time for samples used for patient evaluation is max. 10 min. at room temperature and max. 30 min. at 0-4 oC (32-39 oF) [6]. **The NCCLS recommedation is max. 3 min. at room temperature.**

Other considerations

Below is a list of other considerations when performing method comparison.
According to NCCLS [3] it is recommended that you…

1. Use at least 40 patient samples.
2. Split the sample between the two analyzers.
3. Alternate the sample sequence between the two methods.
4. Perform the test over five operating days.
5. Analyze each patient sample in duplicate on both methods.
6. Whenever possible, 50 % of all samples should be outside the laboratory's reference interval. This will help validate patient samples outside normal reference values.
7. After the comparison, NCCLS recommends that you analyze the data for outliers and plot the data in a scatter plot and a bias plot. The data analysis can be performed by using a statistical tool, such as the EP evaluator. All documentation from the method comparison needs to be saved for the regulatory inspection and presented during the site inspection.

   Other recommendations are to…

8. Remember always to expel a few drops of blood from the blood gas syringe prior to the measurement and to wipe off the inlet to avoid contamination of the successive sample.
9. When mixing, small air bubbles may develop. Be careful not to inject or aspirate them into the analyzer.
10. If you are comparing an injection analyzer to an aspirating analyzer, use the injection analyzer first to avoid air bubbles contaminating the sample.

Conclusion

The objective of this article was to provide checklists with preparatory and preanalytical issues to be considered to ensure that a method comparison only reflects the analytical difference between two or more methods.

Misinterpretation of the results caused by preanalytical errors may lead to a lot of extra work, or, in the worst case, erroneous acceptance of a failing analyzer.

The entire process, if handled correctly, should give the laboratory confidence that their new system is operating correctly and is substantially equivalent to the old/other method.

References

1. Lasky FD, Boone DJ, Eckfeldt JH *et al*. Evaluation of matrix effects. NCCLS Publication EP14-A. Wayne, Pa: NCCLS, 2001; 21,3.

2. Powers DM, Boyd JC, Glick MR *et al*. Interference testing in clinical chemistry. NCCLS Publication EP7-P. Villanova, Pa.: NCCLS, 1986, 6,13.

3. Krouwer JS, Tholen DW, Garber CC *et al*. Method comparison and bias estimation using patient samples. 2nd ed. NCCLS Publication EP9-A2. Wayne, Pa: NCCLS, 2002; 22,19.

4. Young DS. Effects of preanalytical variables on clinical laboratory tests. 2nd edition. Washington: The American Association of Clinical Chemistry, Inc., 1997.

5. Skurup A. Guide to evaluating bilirubin measurements - designing the optimal test. Radiometer Publication Bulletin no. 24-2001. Copenhagen: Radiometer Medical A/S, 2001.

6. Lock R, Francke K, Nötzli B. The whole blood sampling handbook. Ed. 0005A. Copenhagen: Radiometer Medical A/S, 2000.