ESTIMATION OF BIAS USING REFERENCE MATERIAL.

17-10-30. Anders Kallner. Dept Clin Chem Karolinska university hospital, Stockholm Sweden. anders.kallner@ki.se.

Overview

By definition, bias can only be expressed in relation to a known value. Previously this 'known value' was assumed to be the true value, but modern thinking defines bias as systematic error. However, bias cannot be estimated without comparison to a value that has a degree of legitimacy. Certified reference materials fulfill these criteria and other materials with an assigned or conventional value that are traceable to a 'higher metrological order' can also be used. All these materials are hereafter referred to as 'reference materials'.

To be useful for bias estimates, the reference material needs to have a value with a known uncertainty. This can usually be found on the insert or obtained from the manufacturer. Materials with an assigned value may have an uncertainty of zero. It is also important, and desirable, that the material shall be commutable, i.e. the relation between the signal and the concentration shall be the same in the reference materials and patient samples. (cf. VIM) "the closeness of agreement between the relation among the measurement results for a stated quantity in this material, obtained according to two given measurement procedures, and the relation obtained among the measurement results for other specified materials"

Special precautions are necessary if the measurand – the quantity (component concentration) intended to be measured – is poorly defined. Poorly defined measurands are common, particularly in the measurement of proteins and protein hormones, often assayed using immunochemical procedures.

This program addresses the difference between an observed concentration and a nominal value with a given uncertainty.

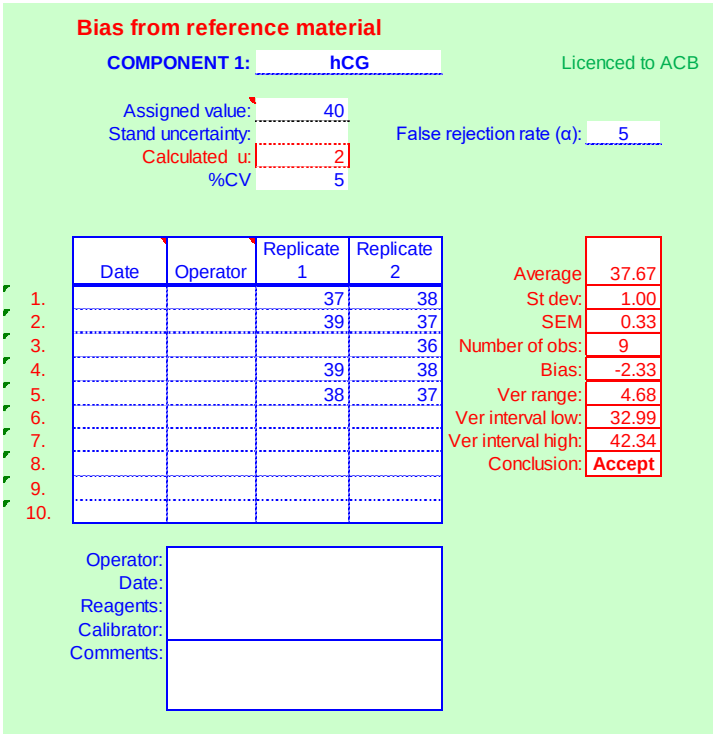
Short instruction

- Copy the example data (*2018-07 ACB Trueness (bias) from reference materials - example data.xlsx*) into the spreadsheet using "Paste special", "Values (123)".
- Enter the target values (D4 and O4) and their uncertainty (SEM), D5 or D7 and O5 or O7, as appropriate.
- The false rejection rate is set to 5 % by default but can be changed.
- Adjust the Y-axis of the graph to fit purpose.

Experimental design and software

- A sample of a suitable reference material with a known concentration and uncertainty is measured in duplicate at least five times. Both the assigned value and its uncertainty must be known. The latter can be entered as a standard error of the mean (standard uncertainty) or corresponding coefficient of variation (relative standard uncertainty). If both are entered, the standard uncertainty takes precedence.
- It is assumed that the uncertainty of the assigned value represents the standard error of the mean.
- The measurements should be spaced in time to emulate a possible variation between measurement series. The evaluation is based on the average and standard deviation of all the observations.

- It may be feasible to prepare aliquots of the material and store safely under appropriate conditions until assayed.
- Insert the assigned value in cell D4 or O4. The uncertainty may be entered as an absolute value (D5 or O5) or a relative (D7 or O7). If an absolute value is entered it takes precedence over the relative.
- The spreadsheet program allows input of up to ten duplicate measurements (Figure 1) at two concentrations. Missing values will be handled correctly; cells may thus be left blank.



Bias from reference material

COMPONENT 1: hCG — Licenced to ACB

Assigned value: 40
Stand uncertainty:
Calculated u: 2
%CV 5

False rejection rate (α): 5

	Date	Operator	Replicate 1	Replicate 2
1.			37	38
2.			39	37
3.				36
4.			39	38
5.			38	37
6.				
7.				
8.				
9.				
10.				

Average	37.67
St dev:	1.00
SEM	0.33
Number of obs:	9
Bias:	-2.33
Ver range:	4.68
Ver interval low:	32.99
Ver interval high:	42.34
Conclusion:	**Accept**

Operator:
Date:
Reagents:
Calibrator:
Comments:

Figure 1. Input and report tables

In the report table, the results are continuously updated. The verification range is the sum of the variances of the reference material and the squared estimated SEM of the measurements. The coverage factor is taken from the *t*-distribution considering the false rejection rate and the number of observations. The evaluation is reported in plain text, if the target value of the reference material is outside the verification interval it is reported as 'Reject', otherwise as 'Accept', where 'Reject' signifies that the deviation from the assigned value is not statistically significant. For a detailed account of the calculations, the reader is referred to the CLSI EP 15 A2 [1].

The evaluation resembles the calculation of a minimal value for a statistically significant difference (MD), which is also based on the propagation rules.

$$MD = k \times \sqrt{sd_1^2 + sd_2^2}$$

The coverage factor is then usually set to 2. In this case the measurements are supposed to be performed by the same procedure at different times and thus the variance is assumed to be constant. The MD then approaches 2 × sd × $\sqrt{2}$ = 2.8 × sd. It should be noted that if the measured result is compared to a reference value with an assigned value (e.g. in diagnostic guidance

documents) without an uncertainty, the MD is reduced to 2 × sd, i.e. diagnostic deviations from a reference value become significant at smaller values than in a trend analysis of repeated values.

References

1. Clinical and Laboratory Standards Institute (CLSI). User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition. CLSI document EP15-A2 (ISBN 1-56238-574-7). Wayne, Pennsylvania 19087-1898 USA, 2005.