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PROPOSALS FOR THE DESCRIPTION AND MEASUREMENT OF CARRY-OVER EFFECTS IN CLINICAL CHEMISTRY

(Recommendations 1991)

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Proposals for the description and measurement of carry-over effects in clinical chemistry (Recommendations 1991)

Abstract - Carry-over phenomena occur both in manual and mechanised procedures and are often neglected in practice. They are observed even with the latest clinical chemistry analysers. This paper makes recommendations concerning the terminology of carry-over and its classification. The conceptual aspects of specimen-dependent and specimen-independent carry-over are outlined, and both the experimental determination and calculation of carry-over effects are described in detail.

INTRODUCTION

The phenomenon of carry-over has been discussed by the members of the Commission on Automation and Clinical Chemical Techniques (VII.1) and by the Commission on Analytical Nomenclature (V.3). The following suggestions are made.

DEFINITIONS

The term *carry-over* is commonly used to describe a process by which materials are carried into a reaction mixture to which they do not belong. These materials can be either parts of a specimen, or reagents including the diluent or wash solution. In such cases, carry-over means the transfer of material (specimen or reagents) from one container, or from one reaction mixture, to another one. It can be either unidirectional or bidirectional in a series of specimens or assays.

The term *hysteresis* is recommended in the International Vocabulary of Basic and General Terms in Metrology (ISO-IEC-OIML-BIPM) (ref. 1) for the carry-over from specimen to specimen. However, due to its established usage, the term *carry-over effect* is used throughout this document.

In a recommendation of the International Federation of Clinical Chemistry, carry-over has been defined as the influence of one sample upon a following one (ref. 2). This definition theoretically could include instances such as influences or interactions between samples which may occur during the counting process in radiochemical measurements. In fact, the term *interaction* has also been proposed (ref. 3) instead of carry-over. Since interaction is widely used for cross reactions between drugs *in vivo* the term is not recommended here.

The influence of viscous samples on the result from a preceding sample, which has been termed a *carryback effect* (ref. 4) or *downstream specimen interaction* (ref. 5), should not be considered as a carry-over phenomenon.

CLASSIFICATION

Carry-over can be classified either according to the material which is carried over, or according to the site where the carry-over occurs:

(i) carry-over of specimen, diluent, reagent, reaction mixture, or wash solution;

(ii) carry-over in a specimen cup, sample probe, reagent probe, reaction system, signal detection system, or wash station.

In practice, the following combinations have been observed (ref. 6-8):

Carry-over from specimen to specimen in a sample probe.
(specimen-to-specimen carry-over)

Carry-over from diluent to specimen in a specimen cup.
(diluent-to-specimen carry-over)

Carry-over from reaction mixture to reaction mixture in a processing system or signal detection system (reagent-to-reaction mixture carry-over). Carry-over by splash-over effects (ref. 6) or by insufficient washing and drying belongs to this group.

Carry-over from reagent(s) to reaction mixture by a reagent probe.

Carry-over from a preceding sample probe into a following specimen cup is a special case. This effect has been called *contamination*. It will influence not just one result, but all assays on that specimen, or with that reagent. Contamination is easy to imagine, but has not been reported so far (ref. 9). Similarly, contamination of the bulk of one reagent by another is possible.

CONCEPTUAL ASPECTS OF THE DETERMINATION OF CARRY-OVER

The concept of carry-over implies the transfer of part of one reaction mixture to another one. It is measured in terms of volume, mass or concentration fraction and may be determined either independently of the analytical procedure using dye solutions or radionuclides, or by one of the analytical procedures being applied.

In analytical chemistry, the error produced by carry-over within a particular procedure is of interest. It is dependent on the method including all the reagents involved. Therefore, carry-over effects, rather than carry-over itself, are to be determined. For this purpose, a sequence of specimens, usually with high (a) and low (b) concentrations of the analyte to be measured, are processed: e.g. $\underline{a}_1$, $\underline{a}_2$, $\underline{b}_1$, $\underline{b}_2$, $\underline{b}_3$.

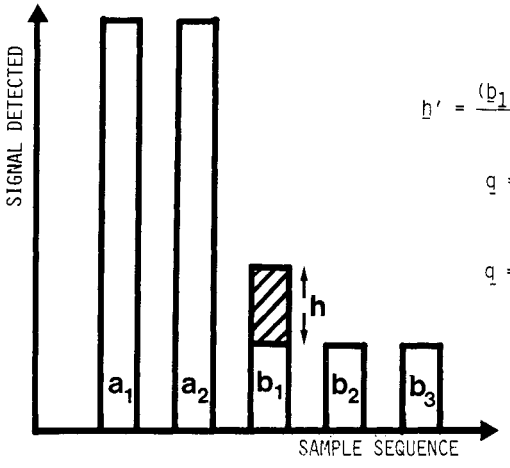
The *carry-over effect* ($\underline{q}$) can be calculated (see figure 1) using either equation I (ref. 3):

$$\underline{q} = \frac{(\underline{b}_1 - \underline{b}_3)}{(\underline{a}_2 - \underline{b}_3)} \qquad (I)$$

or equation II (ref. 5):

$$\underline{q} = \frac{(\underline{b}_1 - \underline{b}_3)}{(\underline{a}_2 - \underline{b}_1)} \qquad (II)$$

q may also be called the *carry-over ratio* and is sometimes expressed as "percentage value" ($\underline{Q}$ %=100q). This implies that the influence of carry-over effects on the analytical results is inversely proportional to the concentration difference of the component to be determined by the analytical procedure in the two specimens.



$$\underline{h} = (\underline{b}_1 - \underline{b}_3)$$

$$\underline{h}' = \frac{(\underline{b}_1 - \underline{b}_3)}{\underline{b}_3} \qquad \underline{h}'(\%) = \underline{h}' \cdot 100$$

$$\underline{q} = \frac{\underline{h}}{(\underline{a}_2 - \underline{b}_3)} \quad \text{(Eq. I)}$$

$$\underline{q} = \frac{\underline{h}}{(\underline{a}_2 - \underline{b}_1)} \quad \text{(Eq. II)}$$

Fig. 1: CALCULATION OF THE CARRY-OVER EFFECT

The last assumption is probably valid in many cases (ref. 7), however, it does not apply for pH/blood gas analysers nor for some selective analysers, where complicated carry-over phenomena may occur from reagents to reaction mixtures. Another disadvantage of calculating the carry-over effect ($\underline{q}$) is that it does not indicate directly to what extent the results may be erroneously influenced. For example, a carry-over ratio of $\underline{q}$=0.01 ($\underline{Q}$=1%) can lead to clinically misleading results in one case, whereas a value of $\underline{q}$=0.1 ($\underline{Q}$=10%) may be irrelevant in another case (Note a).

Note a: First example, q=0.01: the apparent ALT value following a specimen with 2000 $U.L^{-1}$ will be 40 $U.L^{-1}$ instead of 20 $U.L^{-1}$. Second example, q=0.1: the apparent chloride concentration following a specimen with 140 $mmol.L^{-1}$ will be 86 $mmol.L^{-1}$ instead of 80 $mmol.L^{-1}$. Both examples occur in clinical practice. In the first situation, the carry-over effect erroneously leads to a pathological result with clinical relevance, whereas in the second the carry-over is clinically unimportant, although it is ten times higher.

PROPOSALS FOR THE DETERMINATION OF CARRY-OVER EFFECTS

Since the carry-over in an analytical system depends partly on the reagents, carry-over effects in a spectrometer (chosen as an example) should be determined preferably with the reagents used in the cuvette or flow cell of a particular spectrometer, rather than with dye solutions.

These effects should be expressed in terms of the units in which the results are to be presented, e.g. $mol.L^{-1}$ or $U.L^{-1}$ and not as quantity fractions. Carry-over effects can be either specimen-dependent or specimen-independent.

1. Specimen-dependent carry-over

1.1 With constant sample volume

Where the determination of a specimen with a high concentration of the analyte to be measured interferes with that of a specimen with a low concentration (or *vice versa*), a sequence of at least two successive aliquots of a specimen with a high value (a) followed by at least three successive aliquots of one with a low value (b) should be used to determine the carry-over effect.

The concentration of (b) should be chosen to be close to the most relevant decision level, which in many cases is the upper limit of the reference interval (e.g. 20-30 $U.L^{-1}$ in the case of aminotransferase catalytic activity).

The concentration of (a) should represent the extreme values which may occur (e.g. 1000-2000 $U.L^{-1}$ for the aminotransferase activity concentration, or 50 $mmol.L^{-1}$ for the glucose concentration). The volume in the container from which the sample is taken must be defined (usually it should be filled to two-thirds of its maximum capacity).

This experiment should be repeated ten times (ref. 10). For the comparison of the paired values $\underline{b}_1$ ($\overline{\underline{b}}_1$) and $\underline{b}_3$ ($\overline{\underline{b}}_3$), the Wilcoxon signed rank test is recommended. If a highly significant ($\alpha \leqslant 1\%$) difference is detected, then the mean carry-over effect ($\underline{h}$) is calculated (fig. 1):

$$\underline{h} = \overline{\underline{b}}_1 - \overline{\underline{b}}_3 \qquad \text{(III)}$$

The carry-over effect may be expressed as a percentage of the value b_3 which is less influenced by carry-over:

$$\underline{h}' \ (\%) = \frac{(\overline{\underline{b}}_1 - \overline{\underline{b}}_3)}{\overline{\underline{b}}_3} \cdot 100 \qquad \text{(IV)}$$

When the carry-over effect is not highly significant, the procedure can be classified as *carry-over safe* in an interval between the quantity measured in specimen (b) [lower limit] and the quantity measured in specimen (a) [upper limit]. Once a carry-over effect has been detected, it is common practice to repeat a test when the difference between its result and the previous one suggests that a significant error has occurred due to carry-over effects. The difference that can just be tolerated (the *carry-over safe interval*) is usually estimated by intuition. However, it can also be determined experimentally. For this purpose, the experiment reported above (leading to equation III) must be repeated with lower values of (a) which are close to the upper limit of the linear range. If a significant carry-over effect cannot now be detected, the upper limit of the carry-over safe interval is assumed to be identical with the upper limit of the linearity interval. If, on the other hand, the carry-over effect is still significant, then the concentration at the upper limit of the carry-over safe interval, $\underline{a}_S$, can be estimated from equation V, for details of which ref. 6 should be consulted. Assuming that:

$$\overline{\underline{b}}_1 - \overline{\underline{b}}_3 \leqslant \overline{\underline{b}}_3 + 2\underline{s}$$

and that the carry-over effect increases linearily with the difference of quantities measured in specimens (a) and $\underline{b}_1$:

$$\underline{a}_S = \frac{2\underline{s}\ (1+q)}{\underline{q}} + \overline{\underline{b}}_3 \qquad \text{(V)}$$

where $\underline{s}$ is the standard deviation calculated from the ten $\underline{b}_3$-values determined in the previous carry-over experiment. The value of q is

calculated by equation I from the results of the previous carry-over experiment (the a-value is close to the upper limit of the linear range):

$$\underline{q} = \frac{(\overline{\underline{b}}_1 - \overline{\underline{b}}_3)}{(\underline{a}_2 - \overline{\underline{b}}_3)} \qquad \text{(VI)}$$

After the carry-over safe interval has been determined, all results close to the reference range of the particular analytical procedure should be regarded as erroneously increased if they follow a result above the carry-over safe interval. This range is not valid for results which are far below the value of (b) chosen for the calculation of the carry-over effect. In some cases, the carry-over safe interval may be relatively small and too many analyses have to be repeated. Although each result can then be corrected by using a factor (q) calculated according to either equation I or II, such a situation should be avoided.

Carry-over effects can be observed and determined either from high to low, or from low to high values. Usually both effects have the same value (ref. 8), but different values have also been reported. If carry-over from low to high values is suspected and may be medically relevant, it has to be determined with the following specimen sequence (the high value should be at the upper end of the linearity range): $\underline{b}_1$, $\underline{b}_2$, $\underline{a}_1$, $\underline{a}_2$, $\underline{a}_3$. This experiment should be repeated ten times and the results treated analogously to the carry-over effects from high to low values:

$$\underline{h} = \overline{\underline{a}}_3 - \overline{\underline{a}}_1 \qquad \text{(VII)}$$

1.2 With varying sample volume

In some multi-test analysers the sample volume may vary from one method to another, so it is necessary to repeat the experiments for specimen-dependent carry-over with the specimen sequence $\underline{a}_1, \underline{a}_2, \underline{b}_1, \underline{b}_2, \underline{b}_3$. With some instruments it is not possible to use various volumes for one procedure. Then for (b) a procedure with the lowest sample volume is chosen, and (a) should be pipetted with the highest possible sample volume that is used in the particular instrument. The concentrations of specimens (a) and (b) should be chosen as indicated above (1.1), although specimen (a) may be processed by a method different from that used for specimen (b). The carry-over effect is calculated using equations III and IV.

2. Specimen-independent carry-over

Specimen-independent carry-over represents another type of carry-over which can be caused by diluent (*diluent carry-over*) or by reagents (*reagent carry-over*). Reagent probe carry-over may, for example, be encountered in analysers where the same reagent probe is used to dispense reagents on a random basis from two or more reagent containers.

The reagent probe carry-over can be determined from the following sequence using the same specimen and different methods (identified by letters):

$\underline{a}_1$, $\underline{a}_2$, $\underline{a}_3$, $\underline{a}_4$, $\underline{a}_5$, $\underline{b}$, $\underline{a}_6$, $\underline{c}$, $\underline{a}_7$, $\underline{d}$, $\underline{a}_8$, $\underline{e}$, $\underline{a}_9$, $\underline{a}_{10}$, $\underline{a}_{11}$, $\underline{a}_{12}$, $\underline{a}_{13}$, $\underline{a}_{14}$.

The reagent probe carry-over from reagents used in method b is calculated according to equation VIII:

$$\underline{h}_b = \underline{a}_6 - \overline{\underline{a}} \qquad \text{(VIII)}$$

$\overline{\underline{a}}$ is the mean calculated from $\underline{a}_1$ to $\underline{a}_5$ and $\underline{a}_{10}$ to $\underline{a}_{14}$ (n=10).

The error in $\underline{a}_6$ ($\underline{a}_7$, $\underline{a}_8$, $\underline{a}_9$) arising from carry-over due to reagent x in method b (or c, d, e) can be neglected, if the value of $\underline{a}_6$ to $\underline{a}_9$ is less than the mean value of $\overline{\underline{a}} \pm 2\underline{s}_a$.

The sequence must be analysed for each method (a - e in the example chosen) performed on the particular multi-test analyser, and should be repeated at least once, unless the instrument design makes some reagent interactions impossible. Marked effects have been reported for carry-over, for example between reagents for the lipase and triglyceride assays (ref. 8). The

specimen used should have a mid-range concentration of all the analytes to be measured so that both inhibition and enhancement effects can be detected (ref. 11).

The reagent probe carry-over can also be expressed as a percentage (ref. 4):

$$\underline{h}'_b\ (\%) = \frac{(\underline{a}_6 - \bar{\underline{a}})}{\bar{\underline{a}}} \cdot 100 \qquad \text{(IX)}$$

Carry-over caused by insufficient washing of a stirrer, which is used for several methods, can be checked with the same specimen sequence as for reagent probe carry-over. This type of carry-over can also lead to specimen-dependent carry-over effects which are detected as described in sub-section 1.1

Carry-over from reaction mixture, or cleaning solutions to reaction mixture, can occur in cuvettes which are repeatedly used and insufficiently rinsed. The examination of such effects requires specific experimental protocols.

Carry-over effects caused by insufficient cleaning of cuvettes can be checked by three sequences with aliquots of the same specimen. Each sequence consists of at least ten determinations and is performed in the same set of cuvettes. After each assay, the cuvettes are submitted to the regular cleaning procedure. In the first and third sequence, a method should be applied (e.g. lipase assay) which may be prone to reagent carry-over from another method which is used in the second sequence (e.g. triglyceride assay using lipase). The mean values of the first and third sequences are compared by means of the Wilcoxon signed rank test.

CONCLUDING REMARKS

The concept of presenting carry-over effects as a difference of two results or its corresponding percentage value has two advantages:

(i) it is applicable to all conditions which at present are encountered in practice;

(ii) it states directly by how much a particular result deviates from the correct value, obtained in the absence of carry-over effects.

New analytical systems should be designed to avoid any type of carry-over as far as possible.

REFERENCES

1. International Organisation for Standardisation (ISO), Genève, International vocabulary of basic and general terms in metrology, p.26, (1984). ISBN 9267 010328.

2. J. Büttner, R. Borth, J.H. Boutwell, P.M.G. Broughton and R.C. Bowyer, in IFCC Recommendations and Related Documents 1978-1983 (ed. N.E. Saris) Vol. I, p.53, de Gruyter, Berlin (1984).

3. R.E. Thiers, R.R. Cole and W.J. Kirsch, Clin. Chem. 13, 451 (1967).

4. S.D. Winter, Ch. Kerr, M. McAvoy, M.D. Gallomore, C. Smith and R.B. Lepoff, Clin. Chem. 31, 1896-1899 (1985).

5. B. Passey, K.E. Blick and C.L. Bennett, Clin. Chem. 32, 701 (1986).

6. P. Henry, J. Autom. Chem. 1, 195-198 (1979).

7. R. Haeckel and J.A. Porth, J. Clin. Chem. Clin. Biochem. 10, 91-94 (1972).

8. R. Haeckel, J. Clin. Chem. Clin. Biochem. 23, 255-256 (1985).

9. P.M.G. Broughton, A.H. Gownlock, J.J. McCormack and D.W. Neill, Ann. Clin. Biochem. 11, 207-208 (1974).

10. M. Hjelm, Z. Analyt. Chem. 243, 781-790 (1968).

11. P.M.G. Broughton, J. Autom. Chem. 6, 94-95 (1984).

12. C. Naudin, A. Vassault and A. Truchaud, Information Scientifique du Biologiste 10, 373-375 (1984).