Opinion Paper

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Understanding and managing interferences in clinical laboratory assays: the role of laboratory professionals

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Abstract: The recently raised concerns regarding biotin interference in immunoassays have increased the awareness of laboratory professionals and clinicians of the evidence that the analytical phase is still vulnerable to errors, particularly as analytical interferences may lead to erroneous results and risks for patient safety. The issue of interference in laboratory testing, which is not new, continues to be a challenge deserving the concern and interest of laboratory professionals and clinicians. Analytical interferences should be subdivided into two types on the basis of the possibility of their detection before the analytical process. The first (type 1) is represented by lipemia, hemolysis and icterus, and the second (type 2), by unusual constituents that are not undetectable before analysis, and may affect the matrix of serum/plasma of individual subjects. Type 2 cannot be identified with current techniques when performing the pre-analytical phase. Therefore, in addition to a more careful evaluation and validation of the method to be used in clinical practice, the awareness of laboratory professionals should be raised as to the importance of evaluating the quality of biological samples before analysis and to adopt algorithms and approaches in the attempt to reduce problems related to erroneous results due to specific or non-specific interferences.

Keywords: analytical interferences; biotin; immunoassays; patient safety; quality; quality assurance.

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Introduction

Several recently published papers underline the relevance of biotin as an "emerging" interferent in immunoassays [1–3]. Manufacturers, regulatory agencies and laboratory professionals are striving to find concrete solutions to overcome this challenging issue, although it will take time and money to find a solution and a clinical validation, to overcome this problem. However, interferences in laboratory medicine constitute a more general problem, widely discussed by laboratory professionals keen to adopt procedures and algorithms to prevent "clinical episodes" due to inconsistent/spurious results, to suspect "analytical" causes of inconsistent/spurious results and to properly interact with clinical teams to avoid patient harm.

Available evidence on the overall laboratory error rate (0.012%–0.6%) [4–6] indicates that a minor contribution is ascribable to analytical errors (0.078%) [7–9] and, in particular, to interferences in the measurement methods, but the real frequency and severity of interferences represents a "moving target" related to assays conformation, as well as changes in laboratory organization, innovative therapies for some diseases, and in the lifestyle of the population [10, 11]. Therefore, several tasks should be performed by laboratory professionals for interferences to be suspected and further investigated in specific circumstances, also when suggested by clinicians if the result provided for a specific patient fails to fit with the clinical picture.

Frequency and type of interferences

Table 1 shows the main causes of interference in current laboratory testing. The reported interferences should be subdivided into two types on the basis of the possibility of their detection before the analytical process. The first (type 1), easy to detect, is represented by lipemia,

Table 1: Main causes of interference in laboratory tests.

– Lipids, hemoglobin and other serum constituents
– Anti-reagent antibodies (heterophilic Ab, HAMA, rheumatoid factor, biotin)
– Anti-analyte Ab (e.g. anti-thyroglobulin, anti-insulin)
– Macrocomplexes (macroprolactin, macro-TSH, macroenzymes)
– Paraproteins

hemolysis and icterus, and the second (type 2), by unusual constituents that are not undetectable before analysis, and may affect the matrix of serum/plasma of individual subjects. Yet type 2 cannot be identified with current techniques when performing the pre-analytical phase.

Pre- and post-analytical interferences

The process of laboratory testing has three recognized phases, the pre-and post-analytical phases being well understood and acknowledged at an international level [12, 13]. Recent decades have seen a greater awareness of the interplay and interconnection between the pre- and post-analytical phase with the inter-analytical process [6]. In fact, in the pre-analytical phase, type 1 interferences influencing the accuracy of results (such as hemolysis, icterus, lipemia) have been rigorously addressed, with a well-developed quality indicator system that allows in reliable monitoring of their rate [14, 15]. The manufacturers of modern biochemistry analyzers have developed instruments able to objectively quantify hemoglobin, bilirubin and turbidity (HIL indices) in serum or plasma samples by adopting multiple spectrophotometric measurements, and are therefore able to identify HIL interference much more accurately than with the traditional visual approach [16–18]. On the other hand, professional laboratories using biochemistry analyzers from multiple manufacturers, as well as laboratory networks in which a variety of analytical platforms are used, face an additional layer of complexity when they attempt to harmonize their handling of HIL interference [19]. In such scenarios, consideration first needs to be given to how HIL index results from the different platforms compare and, second, to how HIL interference affects results from an individual analyzer. Some papers report, for example, satisfactory agreement between the hemolytic index calculated from different platforms and the reference method [16, 20]. Instead, a lack of harmonization is recognized when reporting the results in samples with HIL indices above the alert levels: in fact some laboratories report the results along with an accompanying comment and others consider a "hold index" above which the results should not be reported [21, 22]. Finally, it appears of value to use the HIL indices as an additional diagnostic tool as demonstrated by some authors [23, 24]. Therefore, even if the harmonization process may improve the management of some pre-analytical interferences, the state-of-the-art emphasizes the need for both adequate and accurate professional knowledge and valuable technical support for monitoring potential errors arising from these specific problems.

Analytical interferences

Although rare, type 2 interferences are responsible for inaccurate, sometimes grossly incorrect, test results, some of which are known to translate into a potential cause of patient harm [25]. The frequency rate of these interferences is difficult to ascertain because no studies in the literature have been specifically designed to evaluate whether false-positive or false-negative results due to this type of analytical interference have been progressively modified over time [26]. In addition, as the frequency of these interferences may change according to the type of clinical workload (e.g. the ratio between inpatients and outpatients), changes in dietary habits and medical treatments, the phenomenon should be considered widespread. In fact, interferences may affect particular measurement techniques [27] and in particular immunoassays (heterophilic antibodies, HAMA, paraproteins, immunocomplexes, rheumatoid factors, etc.) [28, 29], whole analysis platforms (e.g. assays based on biotin-streptavidin principles) [1], and/or single specimens in spectrophotometric methods (such as macroenzymes) [30, 31].

A reported test result that is grossly "abnormal", is not comparable with previous results from the same patient, or does not fit with the clinical picture may immediately raise the suspicion of inconsistent results ascribable to interferences. In other cases, a suspected interference may be confirmed only after several instrumental and laboratory investigations have been performed to rule out the possible diseases and conditions responsible for those abnormal results [32]. Consequently, time and resources should be invested for a definitive comprehension and classification of the analytical problem thus incurring possible delays in results reporting and patient discomfort.

The methodological approach

Various technical algorithms and methods have been suggested in order to start the investigation procedure if an erroneous result from interference is suspected [33]. Although internal quality assessment (IQA) and external quality assessment (EQA) control programs are fundamental tools for monitoring the quality performances of laboratory methods, they are only able to evaluate the "overall" analytical process, but do not enable the detection of erroneous results in an individual sample [34]. The adequate understanding of the limitations, and of the specific analytic characteristics and performances of the method used, may thus facilitate the starting investigation: first of all from the professional point-of-view, an "irregular" result should be confirmed by repeating the assay on the same specimen, or on the original sample if an aliquoting procedure has been carried out, in order to rule out the possibility of an analytical/pre-analytical error. Furthermore, in most cases it is advisable for the investigating laboratory to send an aliquot of the specimen to other laboratories to confirm the result by one or more different methods, ideally an alternative methodology. If, after taking into account the method-related differences in the bias as reflected in EQA and other available data, the results differ significantly, this provides convincing evidence of the interference although the "correct" result will not necessarily be identified [35]. However, these basic investigations may confirm the presence of interference and, from this starting point, different and sometimes more complex studies should be carried out depending on the measure and platform or assay involved [33, 36]. The algorithm adopted and the type of investigation mainly used in routine daily practice are reported in Figure 1.

Professional reasoning

Several approaches have been recommended in the attempt to reduce problems related to erroneous results due to specific or non-specific interferences that are undetected by routine laboratory quality control procedures and reproducible within the test system; these errors are relatively rare, but sometimes clinically plausible, and any individual analytical error may seriously compromise patient care [37]. Furthermore, as shown by daily experience of test results, any type of assay or test may be susceptible to analytical interference as "a perfect assay" is non-existent. Consequently, greater concern should be paid by laboratory professionals to the validation/monitoring procedures on obtained analytical results. Even if the first line approach should be an exact understanding of the robustness of the system and the assay adopted, as well as inherent limitations associated with it, professional reasoning supported by specific algorithms and objective criteria are of utmost importance in evaluating results [38]. In particular, an unusual result probably due to interferences, may be suspected if one or more of the issues listed in Table 2 are observed.

In addition, recent papers [34] suggest an algorithm based on the Bayesian approach, which could include several specific quality indicators (e.g. prevalence/incidence of some specific clinical conditions, demographic parameters, interrelationship between immunoassay results and some laboratory and functional tests). However, this and other described models should be considered only as a starting point, no final decision being made as to whether or not an analytical result should be accepted and reported. Accordingly, further strategies are needed to demonstrate or exclude that an individual sample value is actually a false-positive or false-negative result. Some authors [33] have suggested that a reliable flow chart showing the sequence of laboratory tests can be performed to investigate individual samples with suspected interferences, others [38] have discussed in detail the most commonly used investigations, which are inexpensive, pragmatic and quick to perform and therefore relevant for all clinical laboratories. Prompted by these suggestions, in daily routine practice we have adopted a "standard algorithm", which is simpler than that proposed elsewhere [33–38]: based on a clinical or laboratory professional suspicion, it allows us to search for the more frequent interferences.

For some well-known interferences, such as macroprolactin [39], in samples showing concentrations higher than 30 μg/L at the first observation, we implemented an automatic rule on the laboratory information system (LIS) that produces a second level test, allowing the monomeric form to be determined, after PEG 6000 precipitation. The concentration of the monomeric form obtained, in association with the specific reference interval and the percentage with respect to total prolactin, is reported with an additional interpretative comment. In our experience, analogous types of interference (immune/macrocomplex) have been observed in troponin I assays (in some cases irrespective of the method, particularly in patients presenting the IgG-TnI immunocomplex), as well as in vitamin B12 and calcitonin assays. In addition, several cases of immunocomplex involving different enzymes (alfa-amylase, creatinphosphokinase, lactate dehydrogenase, aspartate aminotransferase) have been correctly identified by adopting the standard algorithm described in Figure 1.

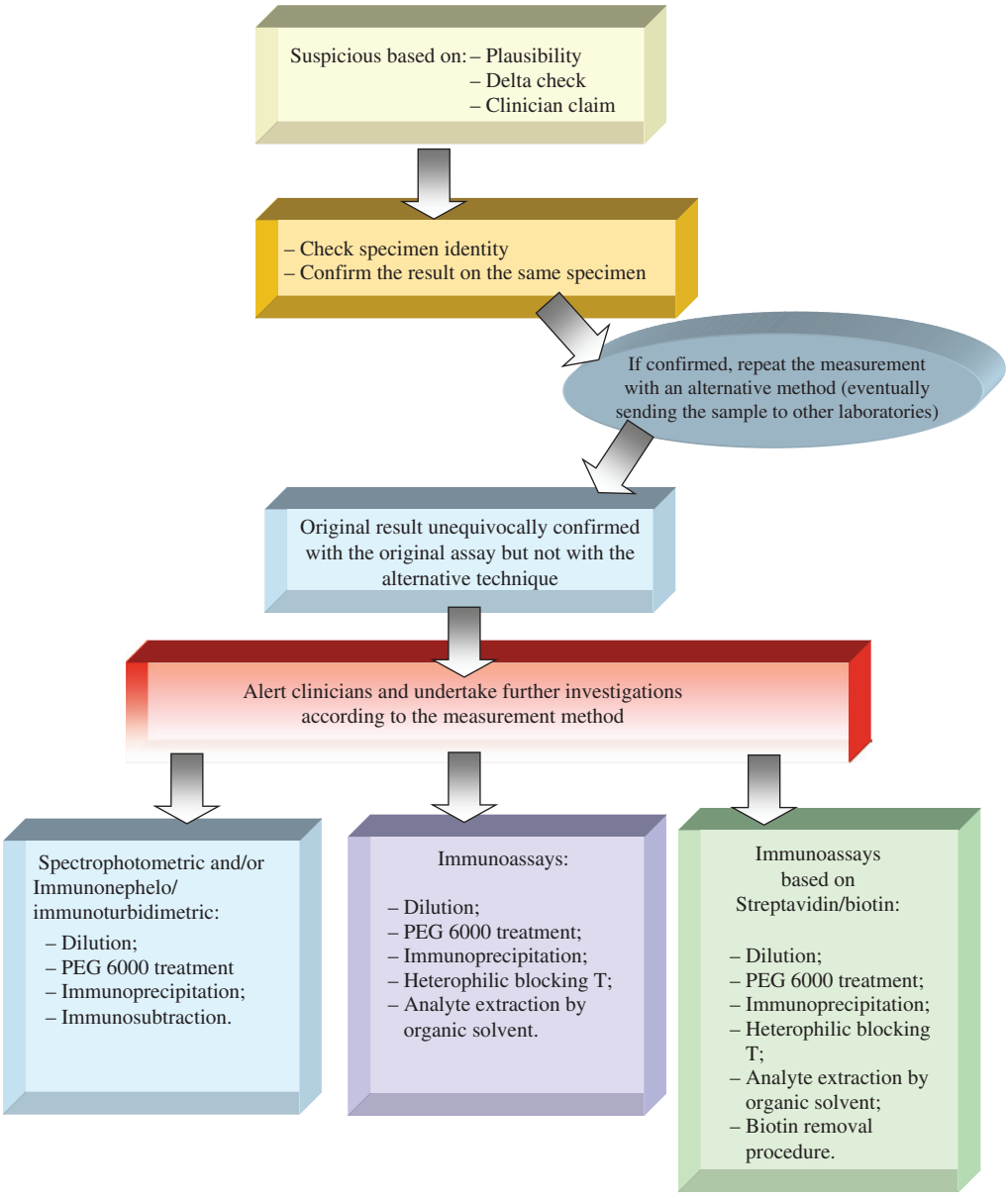

Figure 1: Flow-chart and main types of investigation suggested for assessing suspected interferences.

Table 2: Characteristics of "suspicious analytical results" due to interferences.

– Inconsistent with results of other biochemical parameters
– Unusual in more than one assay (in particular immunoassay)
– Significantly and inexplicably changed in comparison to previous results (delta-check, transversal and longitudinal data assessment, plausibility checks)
– Grossly and permanently "abnormal" (in an apparently health subject)
– Clinically unexpected (after the communication with physician)
– Inconsistent with other clinical correlates

Appropriate interaction with clinical teams and patients

The last step is continuous, effective communication and exchange of information between clinicians and laboratorians in order to minimize the risk of clinical errors arising from erroneous analytical results [33]. This can be achieved mainly by informing clinicians when immunoassay results may be particularly vulnerable to interference, and always encouraging them to question results that, on the basis of the clinical picture, are unexpected [40].

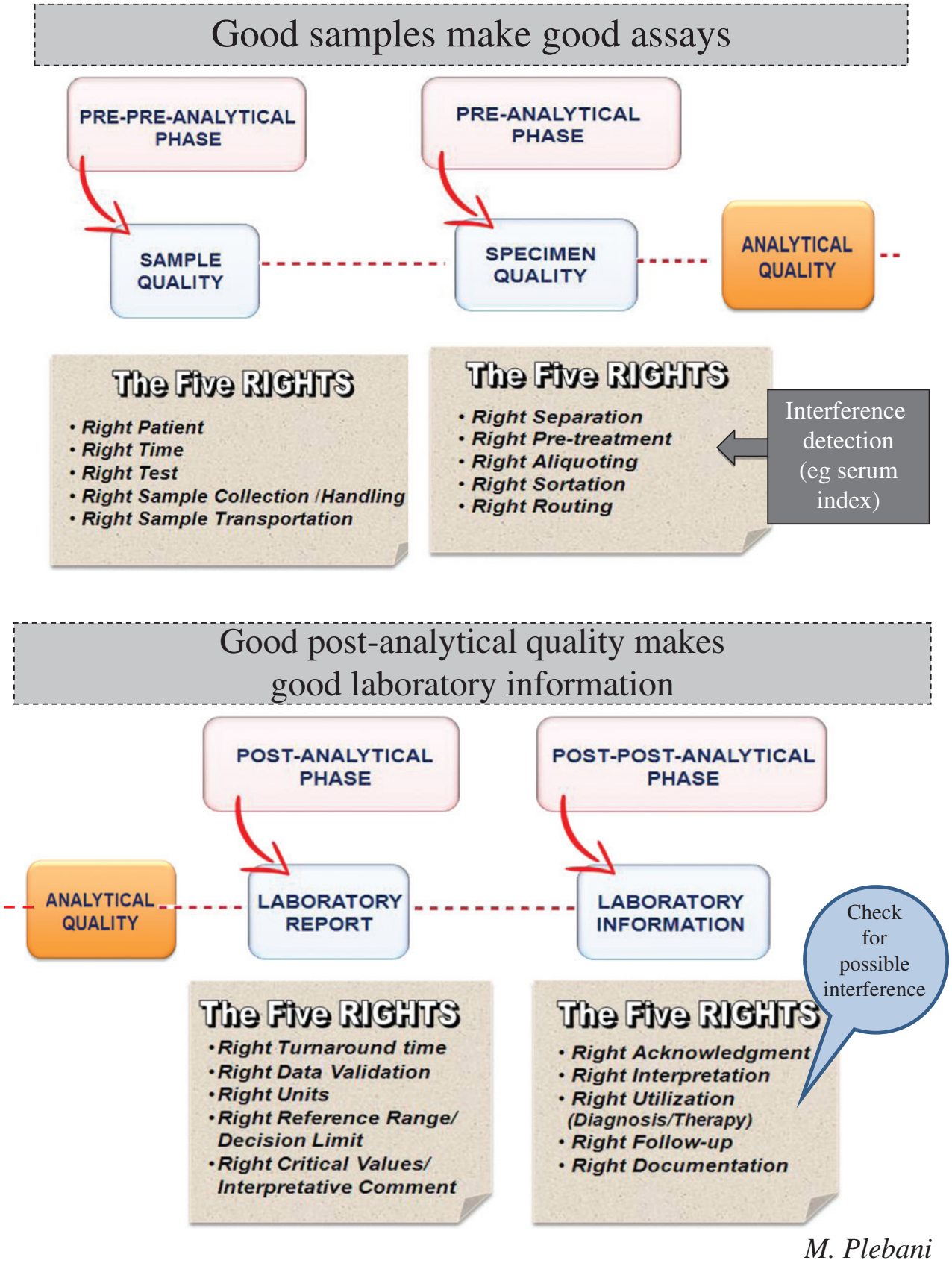

Figure 2: The "five rights paradigm" integrated by managing the interferences in pre- and post-analytical phases (from [6], modified).

Furthermore, patients for whom there is evidence of endogenous assay interference should be informed that they are at risk of future false-positive results, and should be encouraged to report this risk whenever they have a blood specimen taken. This information should also be contained in the patient's personal medical record (HER), as well as in a letter to the family doctor, in particular when the wrong result might be attributed to an endogenous component that might give rise to the same interference in other tests.

Conclusions

The recently raised concerns regarding biotin interference in immunoassays has increased the awareness of laboratory professionals and clinicians of the evidence that the analytical phase is still vulnerable to errors. Although the state-of-the-art highlights the vulnerability of extra-analytical phases, the metric adopted in many studies may lead to overconfidence in analytical quality. Indeed, each and every individual sample potentially presents a specific matrix, sometimes due to an altered ratio between different measurands (e.g. in end-stage renal disease patient samples) or to the presence of cross-reactants, anti-reagent and anti-analyte antibodies. While IQA and EQA programs evaluate the overall quality of an analytical series, they cannot adequately allow an evaluation of the risk of errors in all patient samples. In some groups of patients and individuals, even well-standardized reference measurement procedures and results validated by traditional statistical control procedure, may still be affected by interferences thus generating erroneous results.

In the case of type 1 interferences, improvement in pre-analytical phase thanks to the introduction of workstations able to identify the presence of excessive endogenous components, such as hemoglobin, bilirubin and lipids, is an effective tool for obviating the risk of erroneous results. However, in the case of type 2 interferences, the presence and nature of the interfering substance cannot be identified and predicted before analysis. In the case of biotin, the risk of error should be predicted more easily than in the case of other interferences, such as HAMA and heterophilic antibodies, as the intake should be reported by the patient, thus leading to the withdrawal of its introduction for the recommended timeframe [40–42]. In the case of HAMA and heterophilic antibodies, the presence of an interferent should be suspected only if previously detected and reported.

The issue of interference in laboratory testing, which is not new, continues to be a challenge deserving the concern and interest of laboratory professionals and clinicians and, even some technologies appear less vulnerable (e.g. mass-spectrometry assays), cannot be fixed only by technological approaches. In addition to a more careful evaluation and validation of the method to be used in clinical practice, the awareness of laboratory professionals should be raised as to the importance of evaluating the quality of biological samples before analysis; they should be encouraged to mainly adopt HIL indices to identify type 1 interferences in the pre-analytical phase. After an analytical result has been obtained, plausibility and delta-check analyses should be used in the post-analytical phase to identify possible interferences. Finally, in the post-post-analytical phase any request by a clinician to verify an analytical result should be carefully considered, and should pave the way to further investigations, as shown in Figure 1. The true remedy for the issue of interference in laboratory medicine, therefore, starts from improving the appropriateness of test requests and information about the sample specimen, and may end by discovering and identifying possible rare and obscure causes of implausible results.

On the basis of the discussed issues and, as highlighted in Figure 2, the "five rights" paradigm describing the total quality process in laboratory medicine [6] should be integrated with the management of the interferences that may influence the pre-analytical as well as the post-post analytical phases.

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