EFLM Opinion Paper

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PREDICT: a checklist for preventing preanalytical diagnostic errors in clinical trials

https://doi.org/10.1515/cclm-2019-1089
Received October 22, 2019; accepted October 22, 2019; previously published online November 23, 2019

Abstract: Although the importance of guaranteeing a high level of preanalytical quality in routine diagnostic testing has already been largely acknowledged over the past decades, minor emphasis is currently being placed on the fact that accurate performance and standardization of many preanalytical activities are also necessary prerogatives of clinical trials. Reliable evidence exists that clear indications on how to manage the different preanalytical steps are currently lacking in many clinical trials protocols, nor have detailed authoritative documents been published or endorsed on this matter to the best of our knowledge. To fill this gap, the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE) will provide here a specific checklist for preventing preanalytical diagnostic errors in clinical trials (PREDICT), especially focused on covering the most important preanalytical aspects of blood sample management in clinical studies, and thus encompassing test selection, patient preparation, sample collection, management and storage, sample transportation, as well as specimen retrieval before testing. The WG-PRE members sincerely hope that these recommendations will provide a useful contribution for increasing the success rate in clinical trials.

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Keywords: clinical trials; errors; laboratory medicine; preanalytical variability; studies.

Laboratory errors

Laboratory medicine is considered one of the most proactive medical disciplines in establishing a culture of quality throughout its rather long history since its beginnings, more than 100 years ago [1]. Although the many and multifaceted efforts that have been made for improving standardization and/or harmonization across various activities of the total testing process have made *in vitro* diagnostic (IVD) testing a relatively safe environment compared to other diagnostic disciplines [2], some error opportunities persist, most of which originate from extra-analytical activities. According to recent information retrieved from the current scientific literature, the error rate in laboratory diagnostics is approximately 0.3%, thus much lower than the risk of mistakes in ultrasound (i.e. ~0.8%), radiology (i.e. ~4%) and cellular pathology [2]. Needless to say that the majority of these errors (approximately 60–70%) emerge from manually intensive activities of the preanalytical phase, followed by post-analytical errors (approximately 20–30%), whilst analytical mistakes now comprise the small remainder [3, 4]. The various consequences of these potential errors encompass increased patient risk (e.g. delayed diagnoses, underdiagnoses, misdiagnoses, unnecessary follow-up diagnostics or treatment) [5, 6], waste of economic resources (e.g. phlebotomist time, new blood collection and blood tubes for recollecting unsuitable specimens) [7, 8], as well as organizational issues within (e.g. time lost for identifying and managing preanalytical problems) and outside (e.g. possible litigations for the need of suppressing laboratory data in otherwise unsuitable samples) the laboratory [9].

Although the problem of preanalytical quality in clinical diagnostic testing has been largely acknowledged and dealt with during the past decades [10], minor emphasis

has been placed on the fact that preanalytical quality shall also be a necessary prerogative in clinical trials, whereby there is a tangible risk that some clinical studies may fail to generate their true outcomes because of a variety of laboratory errors, including those arising from the preanalytical phase [11].

Laboratory testing in clinical trials

Laboratory medicine is conventionally defined as a science focused on generation of clinical information through analysis of concentration, composition and/or structure of many different analytes in various biological fluids [12]. According to this designation, laboratory testing provides irreplaceable contributions to the managed care, wherein a large part of the clinical decision-making is now strongly influenced by laboratory data [13]. Laboratory diagnostics also plays an essential role in clinical trials, since many diagnostic tests are used for defining whether or not a study participant would fulfil eligibility criteria, for assessing baseline values of many parameters that can then be modified by the clinical intervention, for demonstrating the efficacy of investigational product(s) (e.g. reflected by variation of some laboratory parameters) and, last but not least, for monitoring the safety of study participants throughout the clinical trial [14].

It therefore seems obvious that the adoption of strict preanalytical requirements would appear as a mandatory requirement for clinical diagnostic testing as in clinical trials, whereby the risk of errors in the latter scenario may generate a number of unfavorable consequences. Briefly, rejecting specimens or suppressing test results in clinical trials would require sample recollection, thus causing patient inconvenience and money waste, may have a deep impact on both the composition and the size of the study population (e.g. for inappropriate inclusion and/or exclusion of some study subjects), would also lead to inaccurate definition of baseline laboratory values that are then used for monitoring changes throughout the clinical trial, may lead to underdiagnosing or misdiagnosing possible side effects and complications, but could also lead endpoint derangement by contributing to generate either false positive or false negative outcomes (Table 1). Additionally, in most clinical trials, samples for baseline or follow-up testing have to be collected at very well defined timepoints in order to be comparable to results of other study subjects. Rejecting samples due to preanalytical unconformity could subsequently result in exclusion of not only this specific sample but the entire data of the respective individual.

Table 1: Potential consequences of preanalytical errors in clinical trials.

1. Need of repeating blood collection
2. Inappropriate inclusion/exclusion of study subjects
3. Inaccurate definition of baseline laboratory values for longitudinal monitoring of changes throughout the clinical trial
4. Missing data throughout longitudinal monitoring
5. Underdiagnosis or misdiagnosis of side effects and complications throughout the clinical trial
6. Achievement of falsely negative or falsely positive outcomes of the study based on unreliable variation of laboratory data

Failures of clinical trials

Clinical trials are conventionally defined as studies carried out in clinical research, involving human participants, and designed to answer specific questions on biomedical or behavioral interventions and hence focused on new treatments (e.g. pharmacologic agents, vaccines, dietary supplements), lifestyle changes, medical devices or new diagnostic investigations [14]. There is now consolidated evidence that the risk of obtaining a misleading outcome for a clinical trial (i.e. either positive or negative) is particularly high, an event included in the conventional concept of "lost in translation from the bench to the bedside", encompassing the lack of translation of basic research findings (i.e. at the "bench") into effective clinical interventions (i.e. at the "bedside") [15]. Although there is no official statistics on clinical trial failure and data can be very heterogeneous [16, 17], interesting evidence has emerged from the report of Wong et al. [18], who recently analyzed over 400 thousand entries of clinical trial data involving over 21 thousand potential pharmacologic agents, between the years 2000 and 2015. Overall, progression from phase 1 studies to clinical approval could only be recorded for 5.7% of all clinical trials, with rather different success rates across different specialties, e.g. as high as 13.5% for ophthalmologic drugs and as low as 2.1% for anticancer treatments. The attrition rate for certain specific human pathological diseases has then been reported as dramatically high, such as in Alzheimer's disease, with failure rates as high as 72% in phase 1, 92% in phase 2 and 98% in phase 3, thus ultimately leading to an overall failure rate as high as 99.6% in this specific clinical setting [19]. Notably, Ioannidis and Bossuyt also recently emphasized that the current biomarker pipeline

is dramatically vulnerable to failure, since only a marginal number of diagnostic tests would complete their clinical translation, thus ultimately contributing to wasting up to 85% of research investment [20].

The factors leading to clinical trial failure (beyond lack of efficacy or safety concerns with the intervention) are many, encompassing a different human response to interventions compared with that observed in preclinical models, lack of human and/or economic resources, poor study design (e.g. inappropriate eligibility criteria, sample size, endpoints and statistical methods), inaccurate site selection (involving both clinical and testing facilities), poor recruitment or large dropouts, patient safety issues, as well as poor execution of the study or inappropriate (statistical) analysis of the data [21]. Among these various factors, diagnostic errors (thus including preanalytical mistakes) are usually overlooked as a possible cause of clinical trial failure, whilst emerging evidence seemingly attests that this may not be the case. For example, Crucitti et al. reported the case of a phase 3 clinical trial carried out in five different sites in Africa and India, in which the effectiveness of the candidate microbicide cellulose sulfate was tested for prevention of HIV and other sexually transmitted infections [22]. Notably, the study failed for major discordances of test results of HIV and Amplicor CT/NG polymerase chain reaction (PCR) between the reference and the site laboratories, which were then attributed to contamination during sample preparation, thus further emphasizing the importance of preanalytical quality in molecular biology [23]. In another recent report published by Schultze and Irizarry, the major sources of uncertainty in laboratory data generated within safety assessment studies have been thoughtfully reviewed [24], concluding that these mostly encompass ignorance of standard operating procedures (SOPs), sample misidentification, instrument malfunctioning, quality control failures and test interference. Notably, the risk of clinical trial failure for delayed processing of blood specimens for glucose testing has also been highlighted. In fact, blood tubes, which cannot be centrifuged for up to 24 h after phlebotomy will incur in a gradual (spurious) decline of glucose concentration, which may finally impair data interpretation for assessing the health status of potential study participants. In multicenter trials, the use of different types of blood collection tubes or additives may be a source of diverging results, heavily impacting the statistical evaluation [25].

The critical issues of managing some preanalytical variables in bio-banking, along with potential problems emerging from inaccurate or inappropriate acquisition, preparation and preservation of biological material, have then been extensively addressed in many publications

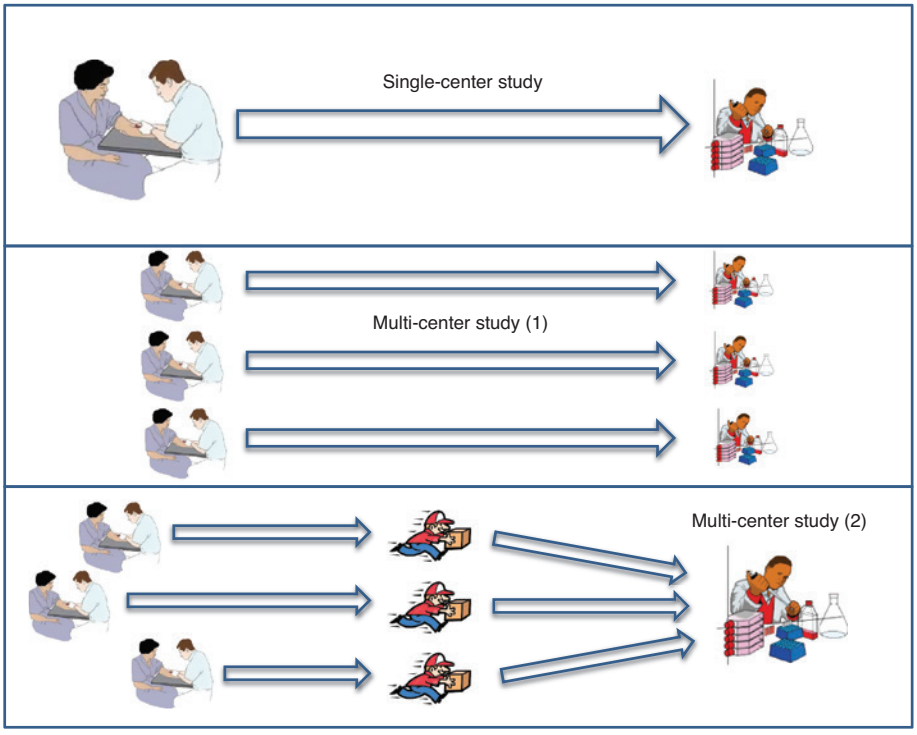

Figure 1: Blood sample management in clinical trials.

[26–32], as well as by the accreditation standard ISO 20387:2018 [33]. Irrespective of these documents, evidence has been provided that using inadequate preanalytical procedures or overlooking SOPs for collection, processing and storage of biospecimens may generate a negative bias in experimental outcomes and could also impair the scientific data reproducibility [34, 35].

A standardized collection and documentation of all preanalytical conditions during the process of patient preparation, collection and storage of biospecimens is crucial in order to be able to exclude any preanalytical bias of results in future studies. Notably, the cumulative risk of preanalytical bias gradually increases in parallel with the complexity of the study, being lower in single-center studies, intermediate in multicenter studies characterized by multiple peripheral collection sites and local testing, whilst it is predictably the highest in multicenter studies with many peripheral collection sites and a single reference laboratory (i.e. centralized testing). In this last case not only local procedures for blood sample collection and handling need to be standardized, but also local management and specimen transportation to the reference laboratories will require strict harmonization (Figure 1).

Managing preanalytical variability in clinical trials

Irrespective of the general importance of defining appropriate preanalytical requirements for all clinical diagnostic testing, no guidelines on how to manage preanalytical variability in clinical studies are available to the best of our knowledge [36]. For example, the most recent National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases Guidelines for Good Clinical Laboratory Practice (GCLP) Standards [37] embrace a number of pre-clinical and clinical aspects of Good Laboratory Practices (GLP), thus listing many organization and technical requirements, which are however almost entirely focused on analytical quality specifications and post-analytical issues. The only reference to specimen transport and management encompasses the need that "*laboratories must have a documented procedure describing methods for the following tasks associated with specimen collection, tracking, labelling, preservation, conditions for transportation, storage and destruction. Documented protocol-specific procedures for specimen preparation and analysis must be available*" [37]. Although this preamble is indeed essential, no detailed GCLP recommendations are provided to inform the laboratory to establish specific SOPs for preanalytical management of samples used in clinical trials, nor emphasis is given to standardization/harmonization of practices among the different centers. The World Health Organization (WHO) has also published a document entitled "Good Clinical Laboratory Practice (GCLP)" [38], which is again almost entirely focused on organizational and analytical issues, limiting the discussion of preanalytical sample management to the vague sentence "*trial material should be analysed and reported within a time frame consistent with patient safety issues and trial protocol, analytical plan, standard operating procedure and any contractual requirements*" [38]. What can hence be clearly assumed from these two important documents is that while well-written procedures should be in place for covering all the activities of the total testing process, no specific indications are given to standardize or harmonize the various preanalytical steps within a clinical trial, either single- or multicenter. Some other similar documents have been published, but none of these contains sufficiently detailed instructions on preanalytical sample management either [39–41].

The notable risk of obtaining spurious outcomes and jeopardizing patient safety, along with the evidence that an enormous amount of money, up to €10 million of research funds, may be lost each year in the European Union due to collection of unsuitable blood samples in clinical trials [14, 42], has persuaded us to propose some specific advices, in the form of a "checklist", which shall be taken into consideration while designing a study protocol (i.e. PREDICT; preventing preanalytical diagnostic errors in clinical trials). In keeping with the conventional structure of preanalytical phase, these recommendations have been classified into categories of test selection, patient preparation, blood sample collection, management and storage, blood sample transportation, as well as specimen retrieval before testing. In the following sections of this opinion paper, we will hence briefly discuss these various aspects and highlight the possible peculiarities related to blood samples in clinical trials.

Test selection

The most appropriate selection of laboratory tests, a clear-cut concept conventionally translating into the notions of "appropriateness" or "demand management" [43, 44], is as critical in routine clinical practice as in clinical trials. In the latter circumstance, it is quite frequent to review study protocols including obsolete, redundant and even useless tests, due to persistence of old habits while drafting protocols, along with inadequate or insufficiently

updated knowledge on test significance [45, 46]. The case of pregnancy test is paradigmatic, whereby many phase 1–3 study protocols still include urine or qualitative beta-human chorionic gonadotropin (beta-hCG) assessment within the protocol of laboratory analyses [47]. Regardless of the fact that immunochemical methods for urine beta-HCG quantification are now unavailable in many clinical laboratories around the world, this test is obsolete and inaccurate for early pregnancy identification, and may hence generate insufficient protection against embryonic or fetal drug exposure, as well as unnecessary health risks on research participants [48]. The receipt of study protocols containing a number of obsolete or inappropriate laboratory tests is then a direct experience for some of us. These basically include the presence of creatine kinase isoenzyme MB (CK-MB) rather than cardiac troponins for diagnosing myocardial infarction and/or cardiac injury [49], or the request to calculate the estimated glomerular filtration rate (eGFR) with the Modification of Diet in Renal Disease (MDRD) formula rather than using the newly recommended Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [50], and so forth. Due to their potential use for establishing participant eligibility, for identifying side effects and defining clinical outcomes, the use of the most appropriate and updated laboratory investigations in clinical trials shall hence be considered as mandatory as it is in routine clinical practice.

Although only marginally related to appropriateness, it is worthwhile mentioning here that the analytical methodology should also be selected according to the aim of the test, thus identifying in advance whether it will be used for screening, diagnosis, prognostication, therapeutic monitoring or follow-up, so that the type of analysis, the analytical technique and the test concentration cutoffs could be selected according to the diagnostic performance and customized for the intended use within the study protocol [51]. Understandably, the most convenient strategy for improving the appropriateness of laboratory testing in clinical trials encompasses the active inclusion of laboratory professionals in the panels of people in charge of developing the study protocol.

Patient preparation

Patient preparation is always a critical preanalytical issue, whereby the blood sample shall accurately reflect the conditions *in vivo*, as recently endorsed by the joint European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and Latin America Confederation of Clinical Biochemistry (COLABIOCLI) recommendations for venous blood sampling [52]. Therefore, although it is obvious that the basic advices given for routine sample collection shall also be applied to clinical trials [53], some additional precautions are necessary. Since results of the single participant are pooled with those of many other participants in the database of the clinical trial, it is mandatory that the process of patient preparation for sample collection shall be thoughtfully standardized. This entails accurate standardization of blood collection activities from one patient to another when samples are drawn in a single center, but also uniformity of blood collection activities when specimens are collected in different centers. This requires accurate collection of clinical information (e.g. use of medicines and supplements, pathologies), followed by (where appropriate) strict standardization of fasting time, time of collection, abstention from cigarette smoking and coffee intake, period of rest before drawing blood and patient position during sampling (Table 2) [54].

Blood sample collection and handling

The concepts expressed in the previous paragraph regarding patient preparation holds true also for sample labelling, collection, management and transportation. All these activities shall be standardized and accurately described in the study protocol, so that phlebotomists and other healthcare operators would always follow the same procedures. Briefly, the study protocol shall hence encompass clear indications on sample type and volume, sample matrix, blood collection device and blood collection tubes/additives, time of tourniquet application, preferred venipuncture site, order of draw and sample mixing, as indicated in Table 2 [52]. The use of identical automatic tube labeling devices is a reasonable option for improving standardization.

Blood sample preparation, transportation and/or storage

This part of the preanalytical phase applies mainly to multicenter studies based on centralized measurements within a single reference laboratory, where samples will be conveyed from remote collection facilities, or analyzing all their samples in batch [55, 56]. Although a thorough description of pros and cons of local vs. centralized testing is outside the scope of this article, and has been comprehensively reviewed elsewhere [57, 58], it is notable that the risk of analytical bias is lower with centralized testing, whilst local analysis would limit the risk of preanalytical bias arising from sample transportation. Both

Table 2: PREDICT (preventing preanalytical diagnostic errors in clinical trials) checklist.

1. Test selection
   a. The panel for designing the study protocol shall always include laboratory professionals
   b. The diagnostic investigations shall always be selected according to recent clinical evidence
   c. The diagnostic techniques shall be selected according the scope of the test (screening, diagnosis, prognostication, therapeutic monitoring or follow-up)
2. Patient preparation
   a. Record all medicines and supplements that the candidate participant is currently taking and identify potential interference with tests that will be performed
   b. Collect information on all pathologies affecting the participant and identify potential interference with the tests that will be performed
   c. Standardize patient conditions; this refers especially to the use of identical:
      i. fasting time (not less than 12 h)
      ii. time of the day for collection (preferably between 7–9 AM)
      iii. abstention from cigarette smoking and coffee intake before venipuncture
      iv. period of resting (no strenuous physical activity 48 h before collection)
      v. sampling position (patient seated for not less than 10 min)
3. Blood sample collection and handling
   a. Confirm participant identity using at least two different identifiers
   b. Uniform sample labelling procedures
   c. Define and standardize sample type (e.g. the sample matrix)
   d. Outline sampling volume for each type of sample matrix
   e. Use the same type of blood collection device
   f. Use the same brand (i.e. manufacturer) and type (material, additive, draw volume) of blood collection tubes
   g. Standardize tourniquet application (i.e. <1 min)
   h. Define the preferred venipuncture site
   i. Have venipuncture performed by expert phlebotomists
   j. Follow the order of draw
   k. Standardize sample mixing
4. Blood sample preparation, transportation and/or storage
   a. Accurately review sample stability acceptance criteria for the analytes that will be measured
   b. Standardize temperature and time passed before separation
   c. Locally centrifuge specimens when stability criteria cannot be fulfilled during transportation
   d. Standardize centrifuge conditions (time, temperature, G force, use of the brake, multiple centrifugations)
   e. Standardize post-centrifugation delay (i.e. time between centrifugation and analysis)
   f. Aliquot serum or plasma as soon as possible after centrifugation
   g. Standardize and monitor sample transportation (time and temperature)
   h. Store plasma, serum or whole blood fluffing available evidence on analyte(s) stability for temperature (i.e. refrigeration, freezing at −20 °C or −70/80 °C) and length of storage (hours, day, months or years)
   i. Avoid repeated freezing and thawing, provided that this practice can be supported by reliable evidence
5. Specimen retrieval before testing
   a. Thaw specimens according to reliable evidence on analyte stability
   b. Accurately mix sample matrix before testing, always using the same technique
   c. Check the quality of the sample before analysis (i.e. assess the presence of interfering substances by means of serum indices)
   d. Do not analyze samples that are unsuitable for testing
6. All deviations from the protocol or potential additional preanalytical biases shall always be recorded

solutions are suitable, provided that a detailed protocol, containing accurately standardized analytical or preanalytical procedures, is made available. For those clinical trials involving sample shipment from remote collection centers to the reference laboratory, it is mandatory to locally centrifuge the specimens when there is a tangible risk that the stability of analytes in serum or plasma may be jeopardized during transportation. If centrifugation is either locally performed or carried out in the reference laboratory, centrifuge conditions shall be standardized (Table 2), whilst serum or plasma shall be separated as soon as possible after centrifugation. The condition of sample transportation (i.e. time and temperature) shall then be accurately standardized, recorded and monitored.

For samples where analysis is not immediately done, they will need to be stored according to available evidence in terms of analyte stability at different temperatures and lengths of storage. Repeated freezing and thawing cycles shall usually be avoided, preferably by aliquoting samples into volumes fitting the analytic need according to the study protocol prior to storage.

Specimen retrieval before testing

In those clinical trials entailing the use of biobanks for long-term storage of biological material, sample retrieval before testing may be an additional critical issue. Reliable evidence has been provided that different approaches for mixing and/or managing thawed samples may substantially modify the results of some laboratory tests [59]. It is hence advisable that SOPs will be made available to all the participating laboratories, aimed at standardizing the procedures used for preparing samples for testing and encompassing procedures for thawing and mixing of specimens, as well as clear indications that unsuitable samples shall not be analyzed (Table 2). This last advice is supported by evidence that generating a spurious test result in a clinical trial sample not only may impact the safety of the study subject, but may also derange the study endpoints, thus leading to either failure or unfounded validation of a healthcare intervention which could then be improperly introduced into clinical practice (Table 2). This is especially important for hemolyzed samples, which are the first cause of test suppression in clinical laboratories [60, 61].

Conclusions

Although some theoretical hurdles have been identified in the translational process from the bench to the bedside, it is also undeniable that some clinical trials may actually provide spurious outcomes because of practical problems such as the use of inappropriate diagnostic tests for evaluating efficacy, effectiveness or safety of a given healthcare intervention or because of a poor quality throughout the total testing process, especially in the preanalytical phase [36]. It is now widely appreciated that preanalytical errors are largely underestimated compared to analytical and post-analytical mistakes [62], and this is clearly reflected by paucity and elusiveness of official advices given for collection and management of biospecimens in clinical trials [37, 38]. Even in real-world scenarios, clinical trial coordinators very often insist on some analytical and technical factors (i.e. temperature monitoring of freezers), thus seemingly underestimating real problems that may impair sample quality or reliability such as, for example, repeated freezing-thawing cycles despite the fact that the temperature of the freezers has been strictly monitored, or the highly accurate performance of tests in patients who should not have been enrolled (e.g. platelet aggregation studies in patients who do not admit taking antiplatelet drugs).

What is hence rather clear now is that unreliable laboratory data obtained in clinical trials, including those plagued by preanalytical problems, not only will be associated with inadequate interpretation of study findings, but may also lead to misdiagnosing side effects or complications, thus failing to generate useful information, potentially harming the study participants, as well as inappropriately inflating the cost of business for sponsors, thereby ultimately prompting research disinvestment by companies and policy-makers. In order to reduce the risk of clinical trial failure for preanalytical problems, we have developed a tentative checklist containing some key points that should be proactively assessed when designing a clinical trial and then formally implemented in its SOPs, highlighting that all deviations from the protocols be clearly recorded. We sincerely hope that these recommendations will provide a useful contribution towards increasing the reliability of laboratory medicine results in clinical trials.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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