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EFLM Opinion Paper

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EFLM WG-Preanalytical phase opinion paper: local validation of blood collection tubes in clinical laboratories

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Abstract: The selection or procurement of blood collection devices in healthcare facilities is often an underestimated issue. This is probably due to different factors including the lack of knowledge of policymakers, hospital administrators and even laboratory managers about the importance of preanalytical quality and phlebotomy process, as well as to the absence of reliable guidelines or recommendations on how to precisely assess the quality of blood collection devices around the globe. With the awareness that a gap remains between manufacturers' and local validation of blood collection devices, the Working Group for Preanalytical Phase (WG-PRE) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) has drafted a consensus document aimed to provide a set of essential requisites, technical criteria (e.g. presence of physical defects, malfunctioning, safety problems) and clinical issues for supporting laboratory professionals in organization blood collection tubes tenders and validating new devices before local routine implementation. The laboratory professionals should also make sure that the tenders accurately and strictly define the responsibilities for validation experiments and the potential consequences in the case the validation outcome shows that tubes due not fulfill the expectations.

Keywords: blood collection; blood tubes; errors; preanalytical variability; venipuncture.

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Introduction

Preanalytical variability plays a crucial role in laboratory diagnostics [1]. Several lines of evidence, accumulated over the past decades, attest that most errors throughout the testing process emerge from manually intensive activities related to collection and management of biological samples [2]. The use of high quality blood collection devices is an aspect of utmost importance in routine laboratory practice, wherein inappropriate or even different sample containers may be a source of preanalytical bias, which can ultimately impact results of testing both in clinical and research settings [3]. It is also noteworthy that both the International Organization for Standardization (ISO) 9001:2008 and the ISO 15189:2012 certification and accreditation procedures include standards encompassing all laboratory activities, including preanalytical procedures, which should be standardized and monitored according to evidence-based practices.

Despite accumulating evidence about preanalytical quality assurance, selection and procurement of blood

collection devices in healthcare facilities is often an underestimated issue. National, regional and local tenders are frequently plagued by policies, guided primarily by the price rather than by quality of devices. This is probably due to different factors, including the lack of knowledge of policymakers, hospital administrators and even laboratory managers about the importance of preanalytical quality and the phlebotomy process, as well as to the absence of reliable guidelines or recommendations on how to precisely assess the quality of blood collection devices around the globe.

Validation studies are crucial activities for generating reliable evidence that a novel instrument, method, reagent or device is fit for purpose and satisfies the particular requirements for its specific intended use [4]. In 2010, the Clinical and Laboratory Standards Institute (CLSI) released a specific GP-34A guideline, aiming to detail the procedures for validation and verification of tubes for venous or capillary blood specimen collection [5]. However, this document is mainly orientated towards validation of blood collection tubes from a manufacturer's perspective to ensure that design goals and performance claims are met. With the awareness that a gap remains between manufacturers validation and clinical laboratories implementation, the Working Group for Preanalytical Phase (WG-PRE) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) has drafted the present consensus document, which aims to provide a set of simple elements and criteria specifically for laboratory professionals, to verify whether the introduction of new blood collection tubes in clinical laboratories fulfills basic criteria of technical and clinical acceptability.

Operative definitions

In agreement with the CLSI guideline GP-34A [5], the "comparative tube" is defined as the blood collection tube currently used by the clinical laboratory, the "control tube" is defined as the blood collection tube that is to be introduced and replace the current. The "desirable quality specifications for bias" are conventionally derived from biological variation. The "validation" is finally defined according to the current ISO 9000:2005 specifications [6], as "confirmation, through the provision of objective evidence that the main requirements for a specific intended use or application have been fulfilled".

Essential requisite for purchasing blood collection devices

Blood collection systems are considered as integrated in vitro diagnostic (IVD) medical devices and are thereby regulated by a number of national and supranational bodies and organizations such as the European Community (EC) or the US Food and Drug Administration (FDA) [7]. A key characteristic, highlighted by virtually all regulatory documents, is that the whole blood collection device (i.e. safety needle, butterfly needle, holder and blood tube) must be regarded as an integrated system. Therefore, the combination of the different parts must be safe and should not impair the performance of the individual components [8]. Manufacturers are responsible for assuring the full compatibility between the components of the system, to subside the risk of impairing the quality of testing and jeopardizing (both operator and patient) safety. Importantly, tenders allowing acquisition of devices from different manufacturers may end up with combinations that are not validated for clinical use. The manufacturers themselves also typically include specific claims in their product datasheets, stating that "devices (needles, single-use holders, safety devices) are designed to be used as a system of products, and the integration of other manufacturer's products is solely the responsibility of the user". However, according to the EFLM WG-PRE, it is outside the role and duty of laboratory professionals to perform a thoughtful validation study to establish whether or not an integrated system is safe and does not impair the quality of testing. Therefore, the possibility of using separate parts of the blood collection system obtained or purchased from different manufacturers is strongly discouraged by the EFLM WG-PRE except when the integration has been previously validated by the manufacturer(s) or by national or supranational regulation bodies.

Apart from research and development, tube manufacturers should be able to also demonstrate usability studies of their products following study subject recruitment according to Good Clinical Practice/International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (GCP/IHC) guidelines and independent Ethical Committee requests. Demonstration of ease of use, sustained plasma quality, perceived value compared to the comparative devices and potential performance risk should be included. User (named sites) feedback on collection, transport (foot, pneumatic tube, courier, etc.), reception and analysis

results are especially valuable. The tubes/devices should also be analyzed by the manufacturer in different clinical settings, on major instrument platforms and investigated for potential test result bias and/or imprecision of analytes especially where this may be important for clinical decision making, including serum indices and analytes with known instability over time. The reasons for failures including missing and excluded data should be stated. For new suppliers evidence of factory capacity over time should also be supplied. The EFLM WG-PRE does however, also recommend that a laboratory performs a local validation of all new blood collection tubes (i.e. control tubes) estimating the potential bias and imprecision of test results compared to the previously used material (i.e. comparative tubes) to verify the manufactures claims. This approach has been proven by the constantly growing number of studies in all areas of diagnostic testing [9–12], including molecular biology [13, 14].

The costs attributable to using an appropriate number of tubes for a local validation (as described in the following parts of this article) should be charged to the manufacturers participating in the tender. More specifically, the details of the validation process should be included in the tender specification, with a specific request to the manufacturers to supplement the laboratory with a number of tubes and cost of reagents that is sufficient to complete each part of the validation. Consideration should also be given to the process of submitting the validation to an Ethical committee and/or Institutional review board for approval (as for CLSI recommendations) [5]. Last but not least, the commission of a tender for purchasing blood collection devices should always include not less than one laboratory professional among the members (Table 1).

Table 1: Essential requisites for purchasing blood collection devices.

1. Components of the blood collection system in use (i.e. safety needle, butterfly needle, holder and blood tube) should be produced by the same manufacturer or else the combination/integration of separate parts should be validated by accredited regulation organizations such as the European Community (EC) or the US Food and Drug Administration (FDA).
2. Manufacturers should demonstrate performance studies of their products following study subject recruitment.
3. Manufacturers should demonstrate ease of use, sustained plasma quality, perceived value compared to the comparative devices on the market and the risk associated with the use of their product.
4. Failure rates per 10,000 tubes should be stated for each tube type. The reasons for failures, including missing and excluded data, should also be stated during usability studies.
5. New suppliers should provide evidence of capacity to produce the product over longer period of time (at least 2 years).
6. The cost for the appropriate number of tubes and reagents for local validation should be charged to the manufacturers participating in the tender.
7. The validation study should be submitted for Ethical committee and/or Institutional review board approval.
8. The committee for a tender for blood collection devices should always include not less than one laboratory professional.

Validation of blood tubes

In the following sections of this article, the EFLM WG-PRE suggest a consensus protocol and some pertinent indicators that may be used for the local validation, both technical and clinical, of new (i.e. "control") blood collection tubes, to be compared with the current system in use by the same laboratory (i.e. "comparative" blood tubes).

Local technical validation of blood collection tubes

The local (user) technical validation of blood collection tubes should be intended to verify whether the manufacturer claims about structure, assembly, functionality and safety of the new (i.e. "control") blood collection tubes are fulfilled, as verified by using local practices. Preferably, the sample size should include not <240 blood collections randomized to both the control (n=120) and the comparative (n=120) blood tubes, as recommended by the CLSI guidelines EP28-A3 to meet the minimum requirements for reliability and usefulness [5]. As an alternative, the collection of two paired tubes from the same patient with the two different systems may be advisable for a more stringent comparison, although not strictly necessary for this technical validation. Patients who are difficult to bleed should be excluded, as they may skew the data. For the technical validation, the EFLM WG-PRE supports recording the following information:

1. Tubes with physical defects of manufacturing (calculate percentage)
2. Tubes with no vacuum or that fail to form a vacuum (calculate percentage)
3. Tubes not properly fitting into the blood collection device (calculate percentage)
4. Tubes under filling after blood collection (i.e. 10% lower than the nominal filling volume; calculate percentage) [15]
5. Leaking from tube caps (calculate percentage)
6. External surface contamination with blood at the end of venipuncture (calculate percentage)

7. Hemolyzed specimens, with significant hemolysis (e.g. 0.5 g/L) defined according to local practices (calculate percentage)
8. Undue clotting in (a) EDTA and (b) sodium citrate blood tubes (calculate percentage of undue clotting in each type of blood tubes)
9. Tubes broken or spilling blood after manufacturer-specific centrifugation (calculate percentage)
10. Inappropriate positioning of gel separator after manufacturer-specific centrifugation (calculate percentage)
11. Serum tubes with incomplete clotting after manufacturer-specific handling (i.e. time for clotting, centrifugation conditions; calculate percentage)

For the calculation of maximum allowable deviation, the EFLM WG-PRE is in support of estimating the percentage of each indicator for 120 tubes of both the control and comparative blood tubes according to the formula reported in Table 2. When the difference between the comparative and control blood tubes is higher than the acceptability criteria consensually agreed by the EFLM WG-PRE for each of the selected indicators (i.e. 1%), then consideration that the comparative blood tubes have failed to pass the validation process should be raised. Importantly, the EC Directive 93/42/EEC appoints that any instrument, apparatus, appliance, material or other article, whether used alone or in combination that is intended by the manufacturer to be used for human beings for the purpose of diagnosis, prevention, monitoring, treatment or alleviation of disease, must be considered a medical device (MD). Therefore, besides informing tube manufacturers about potential issues emerged during the validation process, the major safety and quality problems should also be reported to the pertinent regulatory national or supranational agency (i.e. FDA, EC or UK medicines and healthcare products regulatory).

The technical problems of blood tubes may also be investigated by means of objective approaches of risk analysis such as the Failure Mode and Effects Analysis (FMEA). This systematic technique was originally developed in the late 1950s to investigate problems emerging from military systems malfunctions. However, the FMEA approach may either be reliably used for identifying failure patterns of blood tubes, their causes and consequences, and registering the information in specific FMEA worksheets [16]. Some previous experience in the field of preanalytical activities including blood collections have already been published [17].

Local clinical validation of blood collection tubes

The local (user) clinical validation of blood collection tubes should be intended to verify whether the new (i.e. "control") blood collection tubes may be a source of bias in test results, as verified using local instrumentation and reagents. Therefore, the validation of new devices prior to routine introduction should entail statistical analysis of laboratory data obtained with the existing and locally validated blood collection tubes. The sample size should include between 20 and 100 (the higher the better) paired and sequential blood collections, using both the control and the comparative blood tube systems, by means of two

Table 2: Acceptability criteria for technical validation of new blood collection tubes.

Item	Acceptable difference
Tubes with physical defects of manufacturing	<1%
Tubes with no vacuum or that fail to form a vacuum	<1%
Tubes not properly fitting into the blood collection device	<1%
Tubes under filling	<1%
Tubes leaking from the cap before and after centrifugation	<1%
Blood contamination of collection device	<1%
Hemolyzed specimens	<1%[a]
Undue clotting	
EDTA blood tubes	<1%
Sodium citrate blood tubes	<1%
Tubes broken or spilling blood after centrifugation	<1%
Inappropriate positioning of gel separator	<1%
Serum blood tubes with incomplete clotting	<1%

Difference: $\left[\frac{\text{number of comparative tubes}}{120}\right]*100-\left[\frac{\text{number of control tubes}}{120}\right]*100$. [a]When causes other than the blood tube (e.g. blood collection device, phlebotomists, sample transportation or patient population) can be excluded.

different venipunctures, preferably on the opposite arms, as recommended by the CLSI guidelines EP28-A3 to meet the minimum requirements for reliability and usefulness [18]. For the clinical validation, the EFLM WG-PRE supports the paired measurement of all laboratory parameters for which the comparative blood tubes are to be implemented.

For calculation of the maximum allowable deviation, the EFLM WG-PRE recommends comparing and analyzing results obtained with the two different tube systems by Passing and Bablok regression (and/or Deming fit) and Bland and Altman plots, using values obtained with the control blood tubes as reference. When the regression is not acceptable and the mean percentage bias between the two blood tube systems is found to be greater than the previously defined desirable quality specifications for bias for each of the analyte tested, then the EFLM WG-PRE suggests that either (i) previous blood collection tube system is kept in use, or (ii) the laboratory implements new tubes, but modifies local reference ranges for parameters for which there is a clinically significant difference between old and new tubes. Quality specifications for validation experiments should be defined taking into consideration the Milan EFLM Strategic conference hierarchy [19]. The final evaluation should remain dependent upon the clinical decisions the results are used for, differences between health and disease and biological variation.

Conclusions

The validation of new laboratory equipment, instrumentation, methods and IVD devices by manufacturers and local users is a necessary part of clinical laboratory accreditation. Failure to comply with good manufacturing or good laboratory practices may have adverse consequences on operator and patient safety. Several lines of evidence now attest that the implementation of new blood collection devices, including blood tubes, may modify local practices and also influence the measured concentration of the analytes. Indeed, the selection and acquisition of systems for blood collection should be considered a critical aspect for assuring quality, safety and efficiency of the preanalytical phase of laboratory diagnostics and, therefore, of total testing process. This issue is expected to become even more important as long as innovative molecular biomarkers make it through the translational process, and are introduced into routine clinical practice [20, 21], as this emerging arena is particularly vulnerable to preanalytical issues [22–24].

Although the CLSI document GP34-A is an useful tool for verifying tubes for venous and capillary blood drawing, the real impact of blood collection tubes on local quality and safety of testing is often overlooked, and laboratory professionals often fail to recognize the need to accurately assess the reliability of new devices or perform continuous monitoring of ongoing performance [25]. Due to the fact that it is unfeasible for manufacturers to establish the impact of their devices on all instruments and reagents, the EFLM WG-PRE has drafted this consensus document with the aim of supporting laboratory professionals planning blood collection tube tenders and validating the devices before routine implementation. The laboratory professionals should also make sure that the tenders accurately and strictly define the responsibilities for validation experiments and the potential consequences in the case the validation outcome shows that tubes due not fulfill the expectations.

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