Reference Intervals in Coagulation Analysis

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Abstract

Blood coagulation analysis is characterized by the application of a variety of materials, reagents, and analyzers for the determination of the same parameter, or analyte, by different laboratories worldwide. Accordingly, the application of common reference intervals, that, by definition, would represent a "range of values (of a certain analyte) that is deemed normal for a physiological measurement in healthy persons," is difficult to implement without harmonization of procedures. In fact, assay-specific reference intervals are usually established to allow for the discrimination of normal and abnormal values during evaluation of patient results. While such assay-specific reference intervals are often determined by assay manufacturers and subsequently adopted by customer laboratories, verification of transferred values is still mandatory to confirm applicability on site. The same is true for reference intervals that have been adopted from other laboratories, published information, or determined by indirect data mining approaches. In case transferable reference intervals are not available for a specific assay, a direct recruiting approach may or needs to be applied. In comparison to transferred reference interval verification, however, the direct recruiting approach requires a significantly higher number of well-defined samples to be collected and analyzed. In the present review, we aim to give an overview on the above-mentioned aspects and procedures, also with respect to relevant standards, regulations, guidelines, but also challenges for both, assay manufacturers and coagulation laboratories.

Keywords
► coagulation analysis
► reference intervals
► direct approach
► indirect approach

Zusammenfassung

Die Gerinnungsdiagnostik ist durch die Anwendung verschiedenster Reagenzien und Assays zur Bestimmung einzelner Analyten gekennzeichnet. Dementsprechend bedarf die Anwendung übergreifender, generischer Referenzintervalle, die per Definition

einen "Wertebereich (eines bestimmten Analyten), der für eine physiologische Messung bei gesunden Personen als normal angesehen wird," darstellen würden, einer entsprechenden Harmonisierung von Testverfahren. Somit werden in der Regel Assay-spezifische Referenzintervalle festgelegt, um eine Unterscheidung zwischen normalen und abnormalen Messwerten im Rahmen der Analyse von Patientenproben zu ermöglichen. Während entsprechende Referenzintervalle häufig von den Testherstellern ermittelt und anschließend von den anwendenden Laboren übernommen werden, bleibt eine Verifizierung der übertragenen Werte obligatorisch, um deren Anwendbarkeit vor Ort zu bestätigen. Das gleiche gilt für Referenzintervalle, die aus Veröffentlichungen oder von anderen Labors übernommen, oder aber durch indirekte (Data-Mining) Verfahren ermittelt wurden. Falls für einen bestimmten Assay keine übertragbaren Referenzintervalle verfügbar sind, kann (oder muss) ein direktes Rekrutierungsverfahren angewandt werden. Im Vergleich zur Verifizierung von übertragenen Referenzintervallen erfordert dieser direkte Ansatz jedoch eine wesentlich höhere Anzahl an zu analysierenden Proben gesunder Probanden. In der hier vorliegenden Arbeit soll ein Überblick über die vorgenannten Aspekte und Verfahren der Erhebung von Referenzintervallen gegeben werden. Dies auch im Hinblick auf einschlägige Normen, Vorschriften und Richtlinien, aber auch bezüglich der hiermit verbundenen Herausforderungen sowohl für Hersteller als auch Labore.

Schlüsselwörter
► Gerinnungsdiagnostik
► Referenzintervalle
► Direkter Ansatz
► Indirekter Ansatz

Introduction

A reference interval (RI) represents the variation of test results from samples taken from normal healthy individuals and is usually defined as the central 95% interval of values delimited by the 2.5th and 97.5th percentiles.[1] Assay-specific RIs must be reported with each laboratory report to allow for assessment of normal versus abnormal results.[2] Importantly, however, laboratory results that fall outside the corresponding RI do not necessarily indicate the presence of a disease. This important aspect differentiates RIs from clinical decision limits (CDLs) that have been established to predict the presence of a specific disease or a higher risk of adverse clinical outcome.[3] Furthermore, both terms, RI and CDL, need to be distinguished from (assay) "target ranges," which set the limits for the desired effects of certain drugs.

Given the high variability of reagents/assays available for blood coagulation analysis, establishment of assay-specific RIs is an important and also elaborate task that, however, may include transference of already established/published RIs or provided by the assay manufacturers.[4] While adaption of corresponding values still requires verification on site, transference of RIs is less laborious and cost-intensive when compared with direct recruiting approaches at which a significantly higher number of samples taken from a well-defined reference population needs to be analyzed.[1] Another strategy for the determination and/or verification of RIs is the so-called indirect approach at which statistical methods are applied to identify a "healthy" core population within the overall patient data available from the laboratory information system.[5] Indeed, the latter procedure may be especially useful for the establishment or verification of age-dependent RIs in which the availability of samples from healthy donors is limited.[6]

The need for reporting of assay-specific RIs is defined in relevant standards and regulations, including RiliBÄK (Guideline of the German Medical Association on Quality Assurance in Medical Laboratory Examinations), DIN EN ISO 15189, and the European In Vitro Diagnostic Medical Regulation (IVDR 2017/746).[7–9] On the other hand, guidance on the establishment of RIs was and still is given by corresponding publications of the International Federation of Clinical Chemistry (IFCC) and the Clinical and Laboratory Standards Institute (CLSI).[1,10–16] The aim of the present article is to give a general overview on the need for and establishment of RIs by assay manufacturers and in the coagulation laboratory.

Reference Intervals versus Clinical Decision Limits

RIs and CDLs are important parts of the information communicated by laboratories. They help in the interpretation of clinical pathology results. RIs are derived from a reference population whereat the result given between two reference limits (typically the 2.5th and 97.5th percentiles) define the RI.[1] In a direct approach, individuals from a healthy population are selected for sampling based on defined criteria, while the indirect approach is used in situations where healthy subjects are difficult to recruit.[1,2] Routine clinical pathology databases include results from individuals without disease and the underlying distribution may be distinguished from pathological results.[5] In addition to assay-/laboratory-specific RIs, there are also two (more) common types of RIs. The first are objective RIs with many

prerequisites, like assay harmonization, and a background of well-conducted multicenter studies. The second are subjective convention RIs which are defined by surveys and expert opinion.[3] In general, it is important to realize that laboratory results that fall outside a RI do not necessarily indicate the presence of a disease.

Indeed, in contrast to RIs, CDLs are commonly used to interpret laboratory test results and are based mainly on clinical outcome studies, guidelines, and consensus values. The values above or below the threshold are associated with a significantly higher risk of adverse clinical outcomes or for the presence of a specific disease.[3] Three identifying approaches are used. First, the Bayesian approach is the most evidence-based approach to guide the management of patients. It includes the clinical sensitivity and specificity of a diagnostic test and the relative distribution of individual test results.[3] The second approach, the epidemiological approach, is based on the clinical outcome derived from population-based studies. In a third approach, the pathophysiological method, critical values are associated with a pathophysiological state. The term "critical value" (result) represents a pathological state different from normal that poses a risk of dying or of major patient harm unless immediate action is taken.[17] It is important to know that any result outside the RI is associated with a relative increase in the risk of mortality, but the odds ratio for death varies from analyte to analyte.[18] In conclusion, RIs focus on optimizing sensitivity, while CDLs focus on optimizing specificity for the detection of a disease.[3,19]

Guidelines for and Reference Intervals in Guidelines, Standards, and Regulations

Since 1979, the IFCC deals with the matter of reference values and corresponding RIs, resulting in a series of recommendations on the implementation of RIs in the medical laboratory published between 1987 and 1991.[10–16] While these concepts and recommendations have been widely adopted and also continuously developed further, the C28-A3 guideline, first published in 2008 by the CLSI and the IFCC, represented the most significant step in the development of RIs and, currently in its 3rd edition, is still the benchmark for both, medical laboratories and manufacturers.[1,20] This guideline provides the necessary information on background, strategies, selection of donors, preanalytical and analytical considerations, as well as analysis of gathered reference values for the establishment of a corresponding RI.[1]

Concerning the medical laboratory, according to RiliBÄK, documentation of applied test procedures as well as the medical reports must contain RIs or other guidance on interpretation of test results (RiliBÄK 2019, sections 6.2.3 and 6.3.2).[7] Also with respect to the current International Organization for Standardization (ISO) standard 15189:2012, RIs or CDLs must be established and corresponding information communicated to users (section 5.5.2). In contrast to the RiliBÄK, however, section 5.5.2 of ISO 15189:2012 further states that if a laboratory modifies an investigational procedure or a preanalytical procedure, it must review the associated RIs and CDLs, as appropriate.[8] Indeed, it may be elaborate for laboratories to comply with the latter requirement, considering the high number of different types of tests and the rapid evolution of analytical technology.[21]

Obligations of the manufacturers of CE-IVD-marked assays are given in the IVDR 2017/746. Regarding RIs, the IVDR requires in chapter III on the "requirements regarding information supplied with the device" to include, "where relevant, reference intervals in normal and affected populations" (section 20.4.1, statement aa).[9] Interestingly, this wording is different from that given in the preceding European Directive on in vitro diagnostic medical devices (IVDD 98/79/EC) that states in Annex I "Essential requirements," part B, section 8.7, statement l, to include in the supplied information "the reference intervals for the quantities being determined, including a description of the appropriate reference population."[21,22] Indeed, instructions for use typically contain information on RIs determined in a study with ostensibly healthy subjects.

Activities of Manufacturers Regarding Reference Intervals for New Assays or Assays Transferred to a New Instrument

General Standards and Regulations

Approval of medical devices for the European market requires compliance with a couple of international standards and regulations. A typical process of design, development, verification, and validation follows ISO 134585:2016 (Medical devices—Quality management systems), DIN EN 13612 (Performance evaluation of in vitro diagnostic medical devices), and the IVDR 2017/746 for CE-IVD marking.[9,23,24] As part of the required verification activities, two aspects are crucial: compliance to the international standard ISO 17511:2020 (In vitro diagnostic medical devices—Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials, and human samples)[25] and enabling the clinical use of the device by establishing or verifying a RI for a healthy population. With respect to the second task, the process typically follows the recommendations given in the guideline EP28-A3 of the CLSI.[1]

Establishment and Verification of Reference Intervals

The clinical interpretation of results from an in vitro diagnostic test requires knowledge of expected values for the analyte in a disease-free, healthy population and of how such expected values can change due to the presence of a pathological condition/disease. In addition, other healthy populations might be defined for some analytes where the expected values depend on such factors as age, gender, ethnicity, diurnal or other biorhythm-related variation, or geographical location. The CLSI guideline EP28-A3 addresses the definition and derivation of RIs. It is largely based on earlier work by the IFCC Expert Panel on Reference Ranges.[26] When appropriate, CLSI recommendations are followed relative to

the selection of the reference sample group, use of nonparametric statistics to derive new RIs, and verification of method performance against existing RIs. Preceding the study, the target population is defined with a list of inclusion and exclusion criteria. If no specific subgroups have to be defined, "healthy donor" is the primary inclusion criterion and intake of medications, that are known to interfere with the analyte of interest, are on the list of exclusion criteria. Because the limits of the RIs are often established using nonparametric statistics, the size of the sampling is at least 120 if the 2.5th and 97.5th percentile should be claimed.[1] Because the establishment of a RI typically is performed on fresh samples, these studies are performed in cooperation with external clinical laboratories that have access to enough samples of the target population. To allow efficient control of the study, verification of precision and accuracy is typically included in the protocol for the external study site.[27] Because these studies are also intended to investigate the lot-to-lot variation of a new method, they are typically conducted using three independent batches of reagents and calibrators.

Age- and Pregnancy-Dependent Reference Intervals and Biological Variation

The concept of adjusted RIs was introduced early on when it became clear that different conditions could affect coagulation reference values in the healthy population. Indeed, from fetus to adult, the hemostatic system evolves and matures, making hemostatic equilibrium a dynamic process. Andrew et al. demonstrated more than 25 years ago that coagulation factors including fibrinogen, factor (F)V, FVIII, and FXIII are similar or enhanced at birth when compared with adults, whereas plasma concentrations of vitamin K–dependent factors and contact factors are found to be decreased.[28,29] It should be noted that the components of the hemostatic system are generated in the early stages of fetal development and do not cross the placenta from mother to fetus. Regarding the fibrinolytic system, plasma concentrations of plasminogen are lower at birth, although tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) are higher.
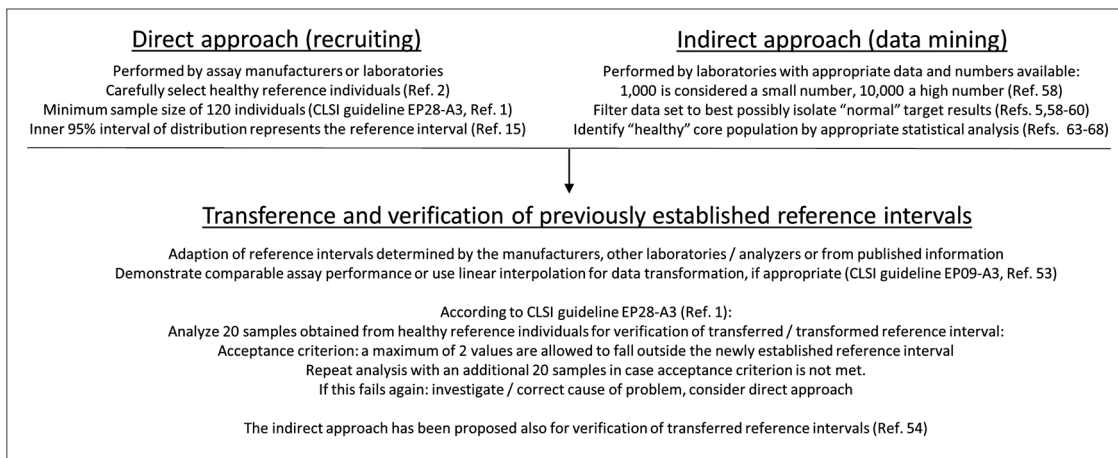
Developmental hemostasis has been confirmed by several studies in which different patient populations were examined under different methodological conditions. For instance, coagulation tests were performed on 218 healthy children aged 1 month to 18 (and >19) years. According to the results, FII, FIX, FXI, and FXII were markedly reduced in the younger. In addition, protein C (PC) and protein S (PS) were found to be decreased in early childhood.[2,30] In contrast, the highest levels of von Willebrand factor (VWF) were found in the youngest children, but without an increase in levels of FVIII. In this study, the differences were most pronounced in children less than 12 months of age. Furthermore, a larger study ($n = 902$) in children aged 7 to 17 years found the prothrombin time (PT) to be significantly prolonged at younger ages (age 7–15), whereas the activated partial thromboplastin time (aPTT) showed no differences. In the same study, age-dependent differences were also ob-

served for other factors, including FVIII, FIX, FXI, VWF activity, and VWF antigen.[2,31]

The above-described observations were confirmed in further studies, highlighting the need to establish age-related RIs. Along these lines, Klarmann et al. published pediatric reference data for PT, aPTT, fibrinogen, PC, antithrombin, FVIII, and VWF.[32] Of note, there have also been earlier studies publishing pediatric reference data for a panel of 23 coagulation parameters.[33] All of these studies showed that plasma levels of most coagulation factors at birth were only about half of those of adults, with preterm infants having lower levels than full-term neonates. Adult levels are reached from a few months to over 16 years of age for certain parameters. This understanding is critical to ensure optimal prevention, diagnosis, and treatment of thrombotic and hemorrhagic diseases in children. Accordingly, the Subcommittee on Perinatal and Pediatric Hemostasis of the ISTH Scientific and Standards Committee recommends that each laboratory defines age-dependent RIs in light of its own technical possibilities.[34]

Not only children but also the elderly population is affected by changes in laboratory values. For example, patients with mild hemophilia A have been shown to have increasing levels of FVIII over time.[35] Furthermore, also age-adjusted D-dimer cutoff values have been established.[36] However, with respect to the determination of geriatric RIs, it appears to be difficult to differentiate the effects of age and (general) pathological conditions in the elderly.[19] Nevertheless, several efforts have been made to establish such RIs for various assays and parameters.[19,37–39]

Another physiological condition that significantly influences certain parameters of hemostasis is pregnancy, which is characterized by changes in blood coagulation and fibrinolysis leading to a thrombotic predisposition, often referred to as physiological hypercoagulability. The results of numerous studies have shown that the increased thrombotic activity during pregnancy is characterized by significant hyperfibrinogenemia; an increase in the activity of plasma coagulation factors, especially FVII, FVIII, FIX, FX, and FXII; a decrease in the concentration of PS; and by an intensification of the processes of adhesion and platelet aggregation.[40] The changes in the hemostatic system occur gradually during normal pregnancy, reach the highest degree of hypercoagulability in the third trimester, and slowly regress in the puerperium. The consequence of high procoagulant activity is increased fibrin turnover, indicated by increasing concentrations of D-dimers, which are considered the most sensitive marker of secondary fibrinolytic activation.[41] A meta-analysis found that the most common complication, venous thromboembolism (VTE), has a relatively low incidence, estimated at 1.2 per 1,000 births.[42] However, the risk of pulmonary embolism is higher, occurring four to six times more frequently than in nonpregnant women of the same age. Thus, there is a need to establish specific RIs for the different stages of pregnancy to allow for better identification of pathological conditions. Accordingly, several studies on this subject have been and are continued to be published.[43–47]

Direct approach (recruiting)
Performed by assay manufacturers or laboratories
Carefully select healthy reference individuals (Ref. 2)
Minimum sample size of 120 individuals (CLSI guideline EP28-A3, Ref. 1)
Inner 95% interval of distribution represents the reference interval (Ref. 15)

Indirect approach (data mining)
Performed by laboratories with appropriate data and numbers available:
1,000 is considered a small number, 10,000 a high number (Ref. 58)
Filter data set to best possibly isolate "normal" target results (Refs. 5,58-60)
Identify "healthy" core population by appropriate statistical analysis (Refs. 63-68)

Transference and verification of previously established reference intervals

Adaption of reference intervals determined by the manufacturers, other laboratories / analyzers or from published information
Demonstrate comparable assay performance or use linear interpolation for data transformation, if appropriate (CLSI guideline EP09-A3, Ref. 53)

According to CLSI guideline EP28-A3 (Ref. 1):
Analyze 20 samples obtained from healthy reference individuals for verification of transferred / transformed reference interval:
Acceptance criterion: a maximum of 2 values are allowed to fall outside the newly established reference interval
Repeat analysis with an additional 20 samples in case acceptance criterion is not met.
If this fails again: investigate / correct cause of problem, consider direct approach

The indirect approach has been proposed also for verification of transferred reference intervals (Ref. 54)

Fig. 1 Summary of opportunities and selected references for the establishment of reference intervals in the coagulation laboratory.

Also (general) biological variation, that is the intra- or interindividual variability of a certain analyte over time, may influence the utilization of the respective RI.[2] For instance, in case intraindividual variability is significantly lower than interindividual variability, the corresponding RI will not be sensitive to changes within the range of biological variation in individuals.[19,48] On the other hand, if a high variation in results during establishment of a RI is seen, it may be true that this is caused by high (intraindividual) biological variation for that analyte.[2] With respect to blood coagulation testing, though on a generally low level, intraindividual variations of screening tests are generally lower when compared with the determination of single factor activities or antigen levels.[2,49,50] However, fibrinolytic parameters appear to show a higher biological variability, what should be considered accordingly during both, establishment of RIs and results interpretation.[51]

Establishment of Reference Intervals in the Coagulation Laboratory

A recent survey initiated by the Standing Commission Labor (STAEKOLA) of the Society of Thrombosis and Haemostasis Research (GTH) among coagulation laboratories in Germany revealed that most laboratories transfer published RIs or those provided by the manufacturer. Furthermore, albeit less common, also data mining approaches are applied. These findings reflect the fact that it is indeed a costly and laborious task for laboratories to establish RIs by direct recruiting approaches.[52] An overview on these different strategies, which are also discussed in detail below, is given in ►**Fig. 1**.

Transference and Verification of Reference Intervals
Transference of RIs may include adaption of corresponding values given by the manufacturer, other laboratories, or published information. According to the CLSI C28-A3 guideline, RIs may be directly transferred if applied test systems and conditions are comparable.[1] Thus, there is need to demonstrate similar preanalytical processes, comparability
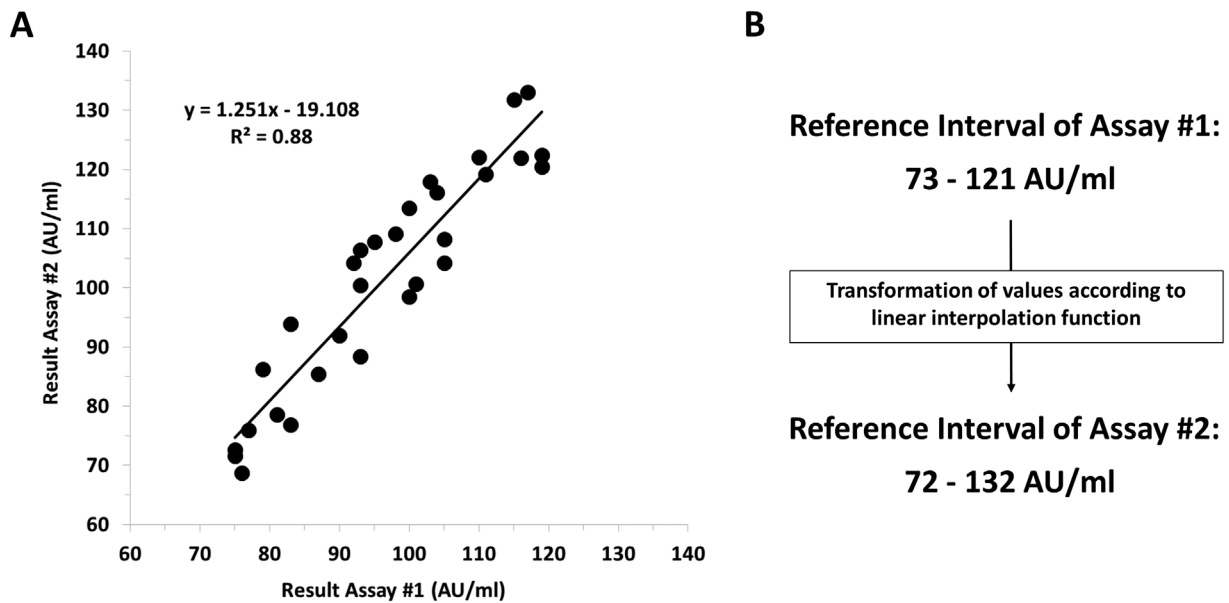
of applied methods, including precision and bias, and, in addition, no relevant differences in tested populations in these cases.[4]

When transferring RIs from studies that have been performed using different assays or analyzers, it needs to be demonstrated that gathered results are comparable to those obtained in the local laboratory. Otherwise, transformation of the RI according to CLSI guideline EP09-A3 is needed.[53] In brief, leftover patient samples with concentrations across the RI to be transferred should be used for method comparison with the assay used in the study. If correlation of data is linear and appropriate (coefficient of determination [r^2] > 0.70), slope and y-intercept values are used for adaptation of the RI to the local method. A brief overview, also addressing publications that apply this strategy, has been published elsewhere.[4] A simplified example is presented in ►**Fig. 2**.

Independent of the principle used for transference, verification of the established RIs is needed. According to CLSI guideline EP28-A3, as also summarized in ►**Fig. 1**, 20 samples (60 samples may be used for more robust analysis) obtained from healthy reference individuals should be analyzed with a maximum of 2 values being allowed to fall outside the newly established RI. In case more than 2 samples exceed the limit, analysis is repeated with an additional 20 samples using the same acceptance criterion. If this fails again, a corresponding investigation may identify the cause of the problem (e.g., reagents or analyzers). If this is not the case, a direct approach may be applied to successfully establish the RI.[1]

Alternatively, indirect (data mining) approaches may be applied for the verification of transferred RIs.[54] This is especially interesting with respect to age-dependent RIs where the availability of samples from corresponding healthy individuals is limited. However, according to the CLSI, successful verification of one partition of a RI may lead to acceptance of the others, thus allowing declaration of validity of pediatric RIs based on analysis of samples taken from adults. However, the feasibility of this strategy may depend on the characteristics of the investigated analytes

A

B

Reference Interval of Assay #1:

73 - 121 AU/ml

Transformation of values according to
linear interpolation function

Reference Interval of Assay #2:

72 - 132 AU/ml

Fig. 2 (**A, B**) Example for the transformation of a reference interval according to CLSI guideline EP09-A3. The reference interval for assay 1 (73–121 arbitrary units [AU]/mL, **B**) was originally established by a direct approach. Leftover patient samples ($n = 30$) with concentrations across this reference interval were also analyzed using assay 2. Linear interpolation of plotted data reveals an absolute as well as relative shift of values at, however, acceptable linear correlation ($r^2 > 0.7$) (**A**). Accordingly, the formula of linear interpolation can be used for adaptation of the reference interval to assay 2 (**B**). Note that this transferred reference interval is still subject to verification as described in the text as well as shown in ►**Fig. 1**.

and the potential influence of sample matrix effects, especially at lower analyte concentrations or activities.[4,55]

Direct Approach to Establish a Reference Interval

Actually, the direct sampling approach is the recommended method to establish a RI. It is thereby of great importance to carefully select the healthy reference individuals.[2] According to CLSI guideline EP28-A3, as already described earlier, a minimum sample size of 120 individuals is recommended, whereat equal numbers of male and female subjects should be recruited.[1] Laboratories should be aware of institutional policies regarding the need for approval by the ethics committee. Besides written informed consent, a questionnaire should be used to gather relevant information on individuals including health status and medications. Furthermore, physical examination or laboratory tests to exclude a certain clinical condition or disease may be applied.[1] The RI is finally derived from the values obtained from analysis of samples from the chosen reference population and usually reflects the central 95% interval as delimited by the 2.5th percentile and 97.5th percentile of the distribution of results.[2,3] As recommended by the CLSI, this calculation should be done using a nonparametric approach (e.g., the rank-based method).[1,15] However, given a normal distribution of (transformed) values, the parametric method also may be applied. A comprehensive overview on corresponding calculations, including examples, also on the determination of 90% confidence intervals, is given elsewhere.[15]

Indirect Approaches (Data Mining)

Reviewing RIs rather than just applying them from literature is especially important for coagulation parameters due to the increased reagent and batch dependency of coagulation tests in comparison to, for instance, biochemical analyses.[56] Data mining is a valuable tool for validation or verification of RIs because the data are obtained under the local analytical and preanalytical conditions.[57] Further advantages of indirect approaches are lower costs and missing ethical issues with sample collection or incidental findings.[57] However, this strategy appears not to be applicable for only rarely ordered tests.

Selection and Data Preprocessing

The first step of indirect RI estimation is to select which data to include. The number of datasets needed for a robust estimation of RIs depends on the statistical method used. As a rule of thumb, Jones et al. suggested that 1,000 patients may be considered a small number and above 10,000 as a large number.[58] Data obtained from patients treated in the intensive care unit or in emergency departments are usually excluded as well as data from obstetrics if pregnancy is considered an influencing factor (e.g., for D-dimer or PS). As pathologic values are likely to trigger retesting, in many RI studies only one (preferably the last) test result per patient is included.[5] Indirect estimation of RIs offers the possibility to filter the dataset "biochemically."[58] The dataset can be filtered based on other tests requested, the time between two requests, or other test results indicating a pathological state.[59] For instance, aPTT values can be omitted if lupus anticoagulant or clotting factors within the intrinsic coagulation pathway were requested. If clinical data are available, samples obtained from patients with certain diagnosis could be excluded, based either on expert opinion or on an unbiased machine learning approach, which has been

successfully demonstrated in the LIMIT study.[60] To review medians and other percentiles is a useful way to prove the stability of values over time. Deviations can result from analytical changes as well as from changes in preanalytical processes or in the population addressed.[5] A big advantage of indirect methods is that partitioning by age and sex can be adjusted during the process of RI determination.

Statistical Techniques and Tools

Indirect approaches identify a "healthy" core population within a dataset by statistical methods. Data mining for indirect estimation of RIs reaches back to the precomputer era. The pioneers in the field, Hoffmann[61] and Bhattacharya,[62] developed graphical methods for parameters that follow a Gaussian or near-Gaussian distribution. For some parameters, skewed distributions can be transformed into a Gaussian distribution by log-, Box-Cox, or Manly's exponential transformation.[57] However, to validate whether or not the transformation is appropriate is challenging. It is therefore recommended to also check the literature for the expected nature of distribution of data for a certain parameter.[58]

Regarding calculation of RIs from correspondingly primed datasets, the so-called Hoffmann method uses a QQ-plot, the linear part of which identifies the Gaussian component of a dataset.[63] The RIs can then be calculated by extrapolation (median ± 1.96 times the slope). Further applicable methods comprise the modified Hoffmann method,[63,64] the modified Bhattacharya method,[65] the truncated minimum chi-square (TMC) approach,[66] the Kairisto method,[67] or the Arzideh method.[68] For the Bhattacharya method, an Excel spreadsheet provided by Graham Jones is available online (http://www.sydpath.stvincents.com.au/). The DGKL (German Society for Clinical Chemistry and Laboratory Medicine) offers a tool called "reference limit estimator" (available at https://www.dgkl.de/en/activities/sections/entscheidungsgrenzen-richtwerte), based on the method described by Arzideh et al.[68] In general, RIs that are estimated by indirect approaches need to be validated. This can be done either in a "direct" approach by testing 20 apparently healthy controls of which at least 18 should be within the newly established RI (see ►Fig. 1). Another possibility is to monitor the percentage of abnormal results ("flagging rate") which should not exceed a predefined expected value.[58]

Limitations

Statistical methods differ in their susceptibility to interference by a secondary population of significant size.[69] Sometimes overlapping distributions cannot be separated adequately. One study for instance reported age-dependent RIs for fibrinogen which comprise pathologic values, with upper limits above 700 mg/dL for patients aged 70 years or older.[56] This is also a paradigm for the problem of estimating RIs for acute phase parameters like FVIII or VWF based on inpatient data. Samples obtained from unbiased screening are ideal for indirect estimation of RIs (e.g., pre-OP screening). On the other hand, it may be difficult to obtain enough sample material to obtain representative values for age-

dependent RI estimation. Especially for infants, pathological test results are probably overrepresented in clinical databases. In summary, data mining is a valuable tool for estimating and validating RIs. As the present overview cannot cover the aspects of generating RIs by data mining in detail, the excellent reviews by the IFCC[58] and Farrell and Nguyen[5] are recommended for further information.

Conclusions

The establishment of RIs in the coagulation laboratory is a key element for proper interpretation of patient results. The importance of this is reflected by corresponding references in relevant guidelines and standards (e.g., RiliBÄK and DIN EN ISO 15189).[7,8] Furthermore, according to the IVDR, also manufacturers of assays that are finally applied in costumers' laboratories are required to provide respective data where appropriate.[9] Thus, transference and verification of such given RIs according to CLSI guideline C28-A3 appears to be a convenient approach for operating laboratories.[1,4] Furthermore, also the transference of RIs that have been established by the use of different assays/analyzers is described to be possible. Within certain limits of acceptance, according to CLSI guideline EP09-A3, this is even feasible if there is a need for prior transformation of data by linear interpolation.[53] Taken together, these prospects highlight the transference and verification of previously established RIs as an economic alternative to the "fully validated" direct approach that, however, is deemed to be the preferred method but requires a minimum sample size of 120 individuals to be analyzed.[1] On the other hand, once these samples have been collected and properly stored (aliquoted), it may be a smaller matter to introduce them for (parallel) analysis by different assays. Indeed, in case at least one assay is not applicable for RI transference, one may consider to extend the then needed direct approach also to other assays (run on the same platform). The indirect approach appears to be especially interesting to determine e.g. age-dependent RIs for parameters analyzed by already established test systems.[6] However, careful selection and filtering of datasets is needed before statistical analysis to allow for correct identification of the "healthy" core population.[5,58]

Conflict of Interest

The authors declare that they have no conflict of interest.

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