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Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition

This document provides background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interfering substances on clinical chemistry test results.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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Abstract

Clinical and Laboratory Standards Institute document EP7-A2—*Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition* is intended to promote uniformity in the evaluation of interference characteristics of clinical laboratory measurement procedures. EP7 describes procedures for manufacturers to screen potentially interfering substances, to quantify interference effects, and to confirm interference in patient samples. This document also describes procedures for clinical laboratories to verify interference claims, and to investigate discrepant results caused by unsuspected interfering substances. Detailed examples are given. EP7 also contains background information on interference testing concepts, tables of recommended test concentrations for analytes and potential interference, and data collection and analysis worksheets.

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Foreword

Interfering substances can be a significant source of error in clinical laboratory measurements.[1-3] Such errors may, in some cases, represent a hazard to the patient. While precision is routinely monitored by internal quality control, and accuracy can be verified by comparison to reference materials or procedures, laboratories cannot easily detect error caused by interfering substances. Therefore, manufacturers of *in vitro* diagnostic (IVD) analytical systems must include evaluation of the effects of the potentially interfering substances in their risk analyses at the design stage.

Although continuously improving the specificity of measurement procedures is a desirable goal, compromise is sometimes necessary to meet the needs of clinical laboratories. The purpose of this document is to enable manufacturers and laboratories to evaluate interfering substances in the context of medical needs and to inform their customers of known sources of medically significant error. This guideline identifies potential hazards to be evaluated in the risk management process described in ISO 14971.[4]

To accommodate the variety of existing and future measurement procedures, we provided guidance instead of rigid protocols. The subcommittee struck a balance between consistency of structured protocols and flexibility to accommodate the technology being evaluated. Laboratorians and manufacturers need to understand the scientific concepts, make informed choices, and work together toward the common goal of improving patient care. Clearly, identifying an interference effect, evaluating its medical significance, determining its underlying cause, and ultimately improving the measurement procedure requires close cooperation between laboratory and manufacturer.

Background information is included to explain key chemical and statistical concepts. Please note that this document focuses on interference with analytical processes. It does not address physiological effects caused by drugs and their metabolites. The IFCC has issued a series of recommendations on drug effects[5-7] that have been published as a compendium.[8] Comprehensive literature surveys of the analytical and physiological effects of drugs and other substances have been published.[9-11]

The basic substance of EP7-A2 remains unchanged. A thorough review of the exogenous and endogenous compounds recommended for testing was performed. Each drug or drug metabolite was systematically categorized into specific drug classes. This guideline was developed to inform the reader and provide a logical approach to complete the evaluation of the effects of potentially interfering compounds on the measurement procedure test results. The guideline is intended to make the decision easier by basing it on reasonable, objective criteria. We now ask the reader to give us comments and suggestions. Each comment and suggestion will be considered carefully at the next revision.

A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all obstacles to harmonization. Despite these obstacles, CLSI recognizes that harmonization of terms facilitates the global application of standards and is an area that needs immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

In order to align the usage of terminology in this document with that of ISO, the following terms are used in EP7-A2:

The term *trueness* has replaced the term *accuracy* when referring to the closeness of agreement between the *average value* obtained from a large series of test results and an accepted reference value. *Accuracy,* in its metrological sense, refers to the closeness of the agreement between the result of a *single measurement* and a true value of a measurand, thus comprising both random and systematic effects.

The term *measurement procedure* has replaced the terms *method*, *analytical method,* and *analytical system* for a set of operations used in the performance of particular measurements according to a given method. The term *assay* has been replaced by *method, measurement procedure, measurement, analyze,* and *analysis* as appropriate. At this time, due to user unfamiliarity, the term *examination* is not used in this edition of EP7.

The terms *specimen* and *sample* are both used in this document, with *specimen* reserved for material collected directly from the patient, and *sample* reserved for aliquots of the patient specimen and for processed materials (e.g., PT samples, reference materials).

The term *analyte* is used appropriately in this document. The term a*nalyte* is used to represent the particular component of interest to the patient diagnosis, while the term *measurand* is used to describe the specific quantity that is measured by a particular measurement procedure (i.e., the measurand describes what is actually causing the result of the measurement). This important difference can be subtle, since it can be due to the detection of different measurands in the procedures being compared. The term *precision* is a measure of "closeness of agreement between independent test/measurement results obtained under stipulated conditions."[12] The terms in this document are consistent with uses defined in the ISO 3534 and ISO 5725 series of standards.

At this time, due to user unfamiliarity and for the sake of the practicability of the guideline, it is important to point out that the working group has chosen not to replace the term *interfering substance* or *interferent* with the VIM (*International Vocabulary of Basic and General Terms in Metrology*) term *influence quantity* (i.e., quantity that is not the measurand but that affects the result of the measurement). The users of EP7 should understand that the fundamental meanings of the terms are identical, and to facilitate understanding, the terms are defined along with their ISO counterparts in the guideline's Definitions section.

All terms and definitions will be reviewed again for consistency with international use, and revised appropriately during the next scheduled revision of this document.

Key Words

Evaluation, hazard analysis, interference, interferent, matrix effects, performance claims, risk management, specificity, validation, verification

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1 Scope

This document is intended to serve two purposes:

1) to assist manufacturers and other developers of laboratory measurement procedures in characterizing the susceptibility of measurement procedures to interfering substances, by offering scientifically valid experimental designs, by specifying the relevant substances and concentrations to be tested, and by clarifying appropriate data analysis and interpretation, so that potential hazards can be evaluated and meaningful interference claims may be provided to users; and

2) to assist clinical laboratories in investigating discrepant results due to interfering substances, by defining a systematic investigation strategy, by specifying data collection and analysis requirements, and by promoting greater cooperation between laboratory users and manufacturers, so that new interferences can be identified, disclosed, and ultimately eliminated.

This guideline is intended for manufacturers of *in vitro* diagnostic medical devices and clinical laboratories.

Manufacturers and other developers of laboratory measurement procedures are responsible for characterizing the analytical performance of their procedures and analyzing hazards to patients caused by errors due to interfering substances. Manufacturers are required to provide information about interference susceptibility to those who use their systems. **NOTE:** The term "manufacturer," for the purpose of this document, is used to mean anyone that develops a measurement procedure for use in a clinical laboratory.

Clinical laboratories are responsible for ensuring that measurement procedures are specific enough to meet the needs of their physician clients. Laboratories should also investigate discrepant results, identify interfering substances, and provide objective feedback to the manufacturers who supply their analysis systems.

2 Introduction

2.1 Measurement Procedures

Any measurement procedure, quantitative or qualitative, may be subject to interference. This document is written for a broad spectrum of measurement procedures and analyzers. Modification may be necessary to accommodate the particular characteristics of the procedure being evaluated. Two specific method principles (i.e., separation techniques and immunochemical measurement procedures) are discussed in Appendix A.

2.1.1 Specimen Type

Interferences with measurement procedures that use serum, plasma, whole blood, cerebrospinal fluid, urine, and most other body fluids may be evaluated using this guideline.

2.1.2 Interfering Substances

Potentially interfering substances may originate from the following endogenous and exogenous sources:

- metabolites produced in pathological conditions, such as diabetes mellitus, multiple myeloma, cholestatic hepatitis, etc.;

- compounds introduced during patient treatment, such as drugs, parenteral nutrition, plasma expanders, anticoagulants, etc.;

- substances ingested by the patient, such as alcohol, drugs of abuse, nutritional supplements, various foods and drink, etc.;

- substances added during sample preparation, such as anticoagulants, preservatives, stabilizers, etc.;

- contaminants inadvertently introduced during sample handling from sources such as hand cream, powdered gloves, serum separators, collection tube stoppers, etc.; and

- the sample matrix itself, such as chemical and physical properties that differ from the ideal fresh sample.[13-16]

2.2 Concepts and Scientific Principles

2.2.1 Contribution of Interference to Inaccuracy

Inaccuracy (total analytical error) consists of three principal contributors: imprecision, method-specific bias, and sample-specific bias.[17,18] Measurement procedure evaluations frequently estimate only the first two. Sample-specific bias (i.e., interference) is often viewed as an isolated problem with specific samples, rather than as a quantifiable characteristic of the procedure. From the standpoint of an evaluation, susceptibility to interference causes both systematic and random error, both of which can be quantified statistically as components of inaccuracy (total analytical error).[19,20]

- For a given *patient population*, the average concentration of interfering substances in the samples may cause a systematic bias, which will be included in the estimate of bias. Individual deviations from this average bias contribute to the total random error observed in a comparison to a more specific measurement procedure. For some procedures, random interference effects exceed imprecision as the dominant source of random error.

- For an *individual patient*, interfering substances cause a bias dependent on their concentrations in the patient's specimen. The bias changes as the interferent concentration changes (e.g., due to clearance or metabolism). The resulting change in bias could be erroneously interpreted as a change in patient condition.

2.2.2 Clinical Relevance

In laboratory medicine, interference has to be viewed from a clinical perspective. Clinical relevance determines whether an analytical effect is considered interference. The form of the analyte intended to be measured and its concentration basis must be clearly defined.

Paradoxically, analytical results from some measurement procedures may reflect the true analyte concentrations, but not necessarily the clinically relevant values. For example, flame photometry and indirect potentiometry correctly measure the total concentration of sodium in an aliquot of plasma, regardless of the lipid concentration. However, if the lipid concentration is high, these procedures will falsely indicate hyponatremia in a patient with proper electrolyte balance. Direct potentiometry correctly reports normal sodium in this case, because it responds to sodium activity in the plasma water fraction, which is what the body regulates. Thus, overestimating the total sodium in the sample is appropriate from

a clinical standpoint. It is important to define the clinically relevant concentration before attempting to interpret interference test results.

2.2.3 Preanalytical Effects

A change in the analyte or its concentration prior to analysis is commonly termed a "preanalytical effect." While such effects may "interfere" with the clinical use of a laboratory result, they are not analytical interference. Unless specified otherwise, a measurement procedure should measure all of the analyte existing in the sample at the time of analysis, regardless of its origin.

Common examples of preanalytical effects are:

- *in vivo* (physiological) drug effects, such as a change in circulating hormone concentration in response to a drug;
- chemical alteration of the analyte by hydrolysis, oxidation, photodecomposition, etc.;
- physical alteration of the analyte, such as enzyme denaturation;
- evaporation or dilution of the sample; and
- contamination with additional analyte (e.g., salts from intravenous infusion, loss of glucose from prolonged contact with the clot, or red cell contents from hemolysis).

2.2.4 Relative Interference

Interference is calculated relative to the measurement of analyte in a control or base pool. In some cases, the control pool may contain a certain amount of endogenous interferent (i.e., the average concentration of the substance in the patient population from which the pool was obtained). Common examples are bilirubin, hemoglobin, protein, and lipids.

Some measurement procedures compensate or correct for the average concentration of interfering substances, so that the interference effect is reduced in the patient population. Typical approaches include sample pretreatment, blanking, serum-based calibration, and mathematical correction. Error is introduced when the concentration of interfering substance in patient specimen is greater than or less than the average concentration in patient population.

For example, a drug method affected by protein shows bias of 0.05 μmol/L per 1.0 g/dL protein. Since the average protein concentration in a serum sample is 7.0 g/dL, the average bias relative to a *protein-free* pool would be 0.35 μmol/L. If the average bias were eliminated by one of the measurement procedures mentioned above, however, the protein effect on an individual sample would be +0.05 μmol/L for each g/dL increase/decrease in protein concentration relative to an average protein concentration of 7.0 g/dL. The bias of a serum sample with 7.5 g/dL protein would be only +0.025 μmol/L, not 0.40 μmol/L. Unless the protein concentration in the sample were exactly 7.0 g/dL, the drug result for each patient specimen would show a small positive or negative bias, depending on its actual protein concentration.

The following information expands on the example. Assume the true value of the hypothetical drug is 25.0 μmol/L, and the method is affected by protein to the extent described above. Note that the error due to protein ranges only +0.20 μmol/L in the bias-corrected measurement procedure, while the error ranges from +0.15 to +0.55 μmol/L in the nonbias-corrected measurement procedure.

Endogenous Protein conc. (g/dL)	Method Without Bias Correction		Method With Bias Correction	
	Result (µmol/L)	Bias (µmol/L)	Result (µmol/L)	Bias (µmol/L)
3.0	25.15	0.15	24.80	-0.20
5.0	25.25	0.25	24.90	-0.10
7.0	25.35	0.35	25.00	0.00
9.0	25.45	0.45	25.10	0.10
11.0	25.55	0.55	25.20	0.20

2.2.5 Mechanisms of Interference

Analytical processes may be perturbed by interfering substances in several ways.

- Chemical artifacts. The interferent may suppress the reaction by competing for reagents or inhibiting indicator reactions. It could also alter the form of the analyte by complexation or precipitation.

- Detection artifacts. The interferent may have properties similar to the analyte, such as fluorescence, color, light scattering, elution position, or electrode response that are detected and measured.

- Physical artifacts. The interferent may alter a physical property of the sample matrix, such as viscosity, surface tension, turbidity, or ionic strength, causing an apparent change in measured analyte concentration.

- Enzyme inhibition. The interferent may alter the activity of an enzyme (analyte or reagent) by sequestering metal activators, binding to the catalytic site, or oxidizing essential sulfhydryl groups. The interferent may also compete for a key substrate in an enzyme-based measurement procedure. For example, adenylate kinase competes with creatine kinase for ADP, and thus is measured falsely as creatine kinase in some measurement procedures.

- Nonspecificity. The interferent may react in the same manner as the analyte. Although some differentiate nonspecificity from interference, its practical effects are the same to the laboratory. Some common examples: keto acids react in alkaline picrate creatinine measurement procedures; indoxyl sulfate reacts in some diazo bilirubin procedures.

- Cross-reactivity. An interferent structurally similar to an antigen may "cross-react" with the antibody in an immunochemical measurement procedure. This is a form of nonspecificity.[21] For example, caffeine is measured in some theophylline procedures. The degree of cross-reactivity is regarded as a measure of the specificity of an immunochemical method, but it is not a useful measure of its susceptibility to interference.

- Water displacement. Nonaqueous substances (protein, lipids) affect activity-based measurements by displacing aqueous plasma volume.[22,23] These effects are not considered interference if it is desired to measure the analyte concentration as the concentration in plasma water.

3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all infectious agents and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the U.S. Centers

for Disease Control and Prevention (Garner JS. Hospital Infection Control Practices Advisory Committee. Guideline for isolation precautions in hospitals. *Infect Control Hosp Epidemiol*. 1996;17(1):53-80). For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to the most current edition of CLSI document M29—*Protection of Laboratory Workers From Occupationally Acquired Infections.*

4 Definitions

accuracy (of measurement) – closeness of the agreement between the result of a measurement and a true value of the measurand (VIM93)[24]; **NOTE:** See **measurand** below.

alpha (α) error//Type I error – probability of falsely rejecting the null hypothesis that a substance does not interfere when it is true; **NOTE:** See **confidence level** below.

alternative hypothesis – in *Interference Testing*, a statement to be tested at a specified power, that a substance causes interference greater than a specified limit (d_{alt}); **NOTE:** See **power** and **beta error** below.

analyte – component represented in the name of a measurable quantity (ISO 17511)[25]; **NOTE 1:** In the type of quantity "mass of protein in 24-hour urine," "protein" is the analyte. In "amount of substance of glucose in plasma," "glucose" is the analyte (ISO 17511)[25]; **NOTE 2:** The analyte is the particular component of interest to the patient.

analytical specificity – ability of a measurement procedure to measure solely the measurand (ISO 17511).[25]

anomalous result – see **discrepant result** below.

beta (β) error//Type II error – probability of falsely rejecting the alternative hypothesis that a substance causes interference when it is true; **NOTE:** See **power** below.

bias – difference between the expectation of the test results and an accepted reference value (ISO 3534-1)[26]; **NOTE:** In this document the "accepted reference value" in Section 7 would be the result from the same measurement procedure in the absence of the interference. In Section 8, it would be the result from the comparative measurement procedure.

clinical significance – in the context of an evaluation of measurement procedure, the importance of an error due to its potential to alter a physician's diagnosis, treatment, or management of a patient.

comparative measurement procedure – a well-characterized measurement procedure that serves as the basis for assigning the true concentration of an analyte in a sample in an evaluation of a measurement procedure.

confidence level – the value (1 - α) of the probability associated with a confidence interval; **NOTE 1:** The probability is usually denoted as a percentage: 100 (1 - α) %; **NOTE 2:** See **alpha error** above.

discrepant result//anomalous result//spurious result – result that is inconsistent to a clinically significant degree, with another result obtained from the same sample, with a result from another measurement procedure or with a well-substantiated clinical diagnosis.

drug effect – term commonly used to describe the physiological influence of a drug on the *in vivo* concentration of a substance, as opposed to an *in vitro* effect on the analytical process.

endogenous interferent – physiologically occurring substance in a sample (e.g., bilirubin or hemoglobin) that causes interference with the analysis of another substance.

exogenous interferent – substance originating outside the body (e.g., a drug or its metabolites, a specimen preservative, or a sample contaminant) that causes interference with the analysis of another substance in the specimen.

factorial experiment – experimental design in which all possible treatment combinations formed from two or more factors, each being studied at two or more levels, are examined so that interactions (differential effects) as well as main effects can be estimated.

imprecision – dispersion of independent results of measurements obtained under specified conditions; **NOTE:** It is expressed numerically as "standard deviation" or "coefficient of variation."

inaccuracy – numerical difference between a value and the true value; **NOTE 1:** See **accuracy** above; **NOTE 2:** See **total analytical error** below**.**

interference – in *Clinical Chemistry*, a cause of clinically significant bias in the measured analyte concentration due to the effect of another component or property of the sample; **NOTE:** It may result from nonspecificity of the detection system, suppression of an indicator reaction, inhibition of the analyte (enzymes), or some other cause of specimen-dependent bias.

interference claim – statement describing the effect that a substance may have on the results of a measurement procedure; **NOTE:** It is typically included in the product labeling under "Limitations of the Method."

interference criteria – maximum allowable interference resulting in the bias of measured analyte concentration from the true value that has the potential to alter a physician's diagnosis, treatment, or management of a patient.

interference screen – in the evaluation of an analytical system, a series of tests performed with high concentrations of commonly occurring substances to identify those that are likely to cause interference.

interference sensitivity – susceptibility of a measurement procedure to error caused by interference from other components or properties of the sample.

interfering substance//interferent – this term is defined the way VIM defines "influence quantity" i.e., quantity that is not the measurand but that affects the result of the measurement (VIM93).[24]

matrix – all components of a material system, except the analyte (ISO 15193).[27]

matrix effect – influence of a property of the sample, other than the measurand, on the measurement of the measurand according to a specified measurement procedure and thereby on its measured value (ISO 17511)[25]; **NOTE:** Viscosity, surface tension, turbidity, ionic strength, and pH are common causes of matrix effects.

measurand – particular quantity subject to measurement (VIM93)[24]; **NOTE 1:** This term and definition encompass all quantities, while the commonly used term **analyte** refers to a tangible entity subject to measurement (i.e., the measurand describes what is causing the result of the measurement [e.g., enzyme activity], and the analyte describes the particular component of interest to the patient); **NOTE 2:** See **analyte** above.

method-specific bias – systematic error due to the characteristics and properties of the measurement procedure.

nonspecificity – reactivity of an agent in a test system to substances other than the analyte of interest; **NOTE:** Nonspecificity is usually caused by antibodies, enzymes, ionophores, or reagents binding, complexing, or reacting with substances other than the analyte.

null hypothesis – in *Interference Testing*, a statement to be tested at a specified confidence level, that a substance does *not* cause interference (d_{null}).

one-sided test – statistical test of significance that is used when the alternative hypothesis states the direction (positive or negative) of the interference effect, such as +0.2 mg/dL bias at a creatinine concentration of 1.0 mg/dL.

power – probability of not rejecting the *alternative* hypothesis that a substance causes interference when it is true; **NOTE:** The probability is usually denoted as a percentage, 100(1-β) %.

precision (of measurement) – closeness of agreement between independent test results obtained under stipulated conditions (ISO 3534-1)[26]; **NOTE:** Precision is not typically represented as a numerical value but is expressed quantitatively in terms of **imprecision**—the standard deviation (SD) or the coefficient of variation (CV%) of the results in a set of replicate measurements.

random specimen-dependent interference – variability caused by the presence of different concentrations of interfering substances in a population of patient specimens; **NOTE 1:** Random interference is quantified as the standard deviation of the biases of individual patient specimens[19]; **NOTE 2:** It is a component of $S_{y.x}$ in regression analysis, and can be a significant contributor to total random error.[17]

repeatability (of results of measurements) – closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement (VIM93)[24]; **NOTE:** Sometimes referred to as *within-run precision.*

sample – one or more parts taken from a system and intended to provide information on the system, often to serve as a basis for decision on the system or its production (ISO 15189)[28]; **NOTE:** For example, a volume of serum taken from a larger volume of serum (ISO 15189).[28]

specificity – ability of a test or procedure to correctly identify or quantify an entity in the presence of interfering phenomena/influence quantities; **NOTE 1:** In the context of QC, the probability that a QC system will indicate absence of special cause variation (i.e., process error) when special cause variation is truly absent; 1 minus the probability of "false alarms" wherein QC data points exceed tolerance limits yet no error can be identified in the test system; **NOTE 2:** In *Immunology*, specificity is an antiserum quality defining its reactivity with defined antigens and lack of specificity is the inaccuracy introduced by cross-reacting and/or interfering substances, because cross-reacting substances compete with the analyte for antibody-binding sites.

specimen (patient) – the discrete portion of a body fluid or tissue taken for examination, study, or analysis of one or more quantities or characteristics, to determine the character of the whole.

specimen matrix – milieu in which the analyte exists; **NOTE:** Clinical specimen matrices include serum, plasma, urine, cerebrospinal fluid, and other body fluids.

specimen-specific bias – difference between the measured value and the true value that results from a characteristic or property of the specimen, as opposed to a characteristic of the measurement procedure

(e.g., calibration, reagent instability); **NOTE:** It is the interference effect exhibited by an individual specimen.

spurious result – see **discrepant result** above.

statistical significance – importance due to the likelihood that an event did not occur by chance, based on a specified power and confidence level.

therapeutic concentration – concentration of a drug that is effective in producing a desired clinical effect.

total analytical error – consists of certain components and is quantified as a confidence interval with confidence level 90%, or 95%; **NOTE 1:** Conceptually the same as "inaccuracy;" **NOTE 2:** Seeks to estimate the largest likely error (of measurement) as defined by VIM: result of a measurement minus a true value (or accepted reference value); **NOTE 3:** Estimated from the distribution of differences in concentration between the test and reference measurement procedure. Example: 97.2% of the differences between the test and reference measurement procedure fell within the limits of ±4 mmol/L; hence the 95% total analytical error goal was met; **NOTE 3:** See **inaccuracy** above. (See the most current edition of CLSI/NCCLS document EP21—*Estimation of Total Analytical Error for Clinical Laboratory Methods.*)

toxic concentration – concentration of a drug or other substance that is injurious to the patient.

trueness – closeness of agreement between the average value obtained from a large series of test results and an accepted reference value; **NOTE:** The measure of trueness is usually expressed in terms of bias (ISO 3534-1).[26]

two-sided test – statistical test of significance that is used when the alternative hypothesis does not state the direction (positive or negative) of the interference effect, such as a ±0.2 mg/dL bias at a creatinine concentration of 1.0 mg/dL.

Type I error – false rejection of the *null* hypothesis; **NOTE:** See **alpha error** above.

Type II error – false rejection of the *alternative* hypothesis; **NOTE:** See **beta error** above.

validation – confirmation through the provision of objective evidence, that requirements for a specific intended use or application have been fulfilled (ISO 9000)[29]; **NOTE 1:** WHO defines validation as "the action (or process) of proving that a procedure, process, system, equipment, or method used works as expected and achieves the intended result" (WHO-BS/95.1793)[30]; **NOTE 2:** The components of validation are quality control, proficiency testing, validation of employee competency, instrument calibration, and correlation with clinical findings.

verification – confirmation through the provision of objective evidence that specified requirements have been fulfilled (ISO 9000)[29]; **NOTE:** A one-time process completed to determine or confirm test performance characteristics before the test system is used for patient testing.

within-laboratory precision – see and use **repeatability** above.

5 Decision Criteria for Interference Testing

Acceptability criteria must be decided prior to conducting an evaluation experiment to ensure objectivity. The evaluator has to decide what magnitude of analytical effect constitutes interference with the *clinical* use of the measurement results, since the appropriate experimental design for an interference test depends upon how large a discrepancy is considered clinically significant.

In establishing acceptability criteria, the distinction must be made between *clinical* significance and *statistical* significance. Both are important in establishing useful criteria.

5.1 Clinical Acceptability Criteria

The degree of allowable error caused by interference obviously depends on the medical use of the test results. Accuracy requirements (total allowable error) have been proposed for some analytes; the cited references represent a few examples.[31-39] For other analytes, accuracy criteria may be established using one of the approaches described below. Limits for allowable interference can be developed by partitioning the accuracy (total allowable error) criteria into bias, imprecision, and interference components. The portion of the total error allowed for interference is the residual error after the bias and imprecision of the measurement procedure, as well as the physiological variability of the analyte, are subtracted (as variances).

5.1.1 Criteria Based on Physiological Variability

One approach to establishing accuracy requirements is based on the physiological variability of the analyte.[40,41] In principle, error limits are set such that analytical variability is minimized relative to the inherent variability of the analyte in the individual or the population (which depends on the clinical application of the analyte). This approach works well for physiologically controlled analytes.

5.1.2 Criteria Derived From Clinical Experience

The consensus of clinical experts is frequently used to establish accuracy requirements. From their clinical experience, practitioners agree on the magnitude of an error that would influence their diagnosis or treatment decisions. Reasonable accuracy and interference criteria can be established from a cross-section of relevant clinical expertise.

5.1.3 Criteria Based on Analytical Variability

Interference criteria can also be derived from the total long-term imprecision of the measurement procedure. If the effect, with high levels of the interferent in the patient samples, is small relative to the analytical variability (e.g., less than one standard deviation of it), then the increase of the total error caused by the interferent is not likely to affect clinical decisions significantly, and the substance would not be considered an interferent.

5.2 Statistical Significance and Power

Before concluding that a substance interferes or does not interfere, the evaluator must be assured that the results are statistically significant. Adequate replication is required so that the test is performed with sufficient power to detect clinically significant interference, and with a sufficient confidence level to recognize when no clinically important bias exists.

The statistical approach used in this guideline is called "hypothesis testing." The evaluator decides in advance how much of a bias in a patient result would be clinically significant. The amount of this allowable bias will be referred to as the interference limit, or interference criterion. The *null* hypothesis that there is no interference (i.e., the bias does not exceed this limit) is then tested, as well as the *alternative* hypothesis that there is interference (i.e., the bias exceeds the limit). These statistical tests are made with predetermined statistical power ($1 - \beta$) and confidence ($1 - \alpha$) levels. See Sections 7.1 to 7.1.6 to determine sample size based on power and confidence.

5.3 Analyte Test Concentrations

Interference should be initially evaluated at two medical decision concentrations of the analyte. If cost or other practical considerations limit preliminary testing to only one concentration, be aware that it is possible to miss clinically significant interference at other analyte concentrations.[42,43]

Recommended test concentrations for many common analytes are given in Appendix B. Published critical or decision values were used when available. Selection of analyte test concentrations was somewhat arbitrary in the absence of medical consensus values, but standardization of interference claims is the important goal. The upper or lower limit of the reference range and a pathologic concentration were selected in most cases, guided by the clinical applications.

5.4 Potential Interfering Substances

For a comprehensive measurement procedure characterization, begin by compiling a list of substances that have the potential to interfere. Consider substances that are likely to be present in patient specimens, based on knowledge of the chemistry of the procedure and its intended use. The following checklist is provided as a guide.

- Common sample abnormalities, such as hemolysis, icterus, and lipemia.
- Common prescription and over-the-counter drugs.
- Abnormal biochemical metabolites expected in the patient population.
- Medications most often prescribed in the patient population for which the test is ordered.
- Drugs, including metabolites, which are likely to interfere with the measurement procedures because of their chemical or physical properties.
- Substances reported to interfere with similar measurement procedures. See the literature surveys by Young et al[9] and Tryding and Roos.[10]
- Sample additives, such as anticoagulants (heparin, EDTA, citrate, oxalate, etc.), and preservatives (NaF, iodoacetate, HCl, etc.).
- Substances that may contact specimens during collection and processing, such as serum separator devices, specimen collection containers and their stoppers, catheters, catheter flush solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powdered gloves, etc.
- Dietary substances known to affect certain tests (caffeine, beta-carotene, poppy seeds, etc.).

The list may be quite extensive. The following can be eliminated with little risk of missing an important interferent. Be sure to document the rationale when potential interferents are ruled out.

- Substances that have essentially identical composition and structure to ones already on the list. However, all structural analogs should be tested in measurement procedures, based on the affinity of an antibody, enzyme, or other specific binding protein.
- Substances that have been shown not to interfere with measurement procedures, based on the same scientific principle.

- Compounds unlikely to interfere, based on expert knowledge of their chemical properties and the chemistry of the measurement procedure.

- Drugs prescribed at a dosage too low to cause interference, based on knowledge of the measurement procedure.

- Drugs cleared or metabolized so rapidly that they would not be present at an interfering concentration at the time of analysis.

5.5 Interferent Test Concentrations

To determine if a substance would interfere under "worst case" conditions, the comprehensive interference screen should be conducted at the highest concentrations that a laboratory would expect to observe among patient specimens submitted for analysis. The guidelines below are provided to assist in selecting appropriate test concentrations.

Since both positive and negative effects might occur from different mechanisms (e.g., hemoglobin has catalase activity as well as strong absorbance in the visible spectrum), each substance should be tested at two different concentrations to avoid the possibility that competing effects might cancel at the concentrations tested. See Section 7.3 for a description of alternative experimental procedures that enable multiple concentrations of analyte and interferent(s) to be tested simultaneously.

- **Drugs and Metabolites**

For serum, plasma, and whole blood samples, test at least three times the highest concentration reported following a drug therapeutic dosage (acute peak concentration) or at the highest expected concentration, if known. If the expected blood concentration is not known, assume the therapeutic dose is distributed in 5 L of blood and test at least three times this concentration. See Appendix C for a table of recommended test concentrations for many common drugs.

For urine, determine the maximum amount eliminated in 24 hours, and test at least three times this quantity per liter of urine. If the urinary elimination is unknown, test at least three times the maximum therapeutic dosage per liter of urine.

- **Endogenous Substances**

Identify the highest concentration expected in the intended patient population, and test at this concentration. See Appendix D for a table of recommended concentrations for testing some common endogenous constituents.

- **Anticoagulants and Preservatives**

For serum, plasma, and whole blood, test at five times the recommended additive concentration to simulate a "short draw" (i.e., partially filled blood collection tube).

For urine, test at five times the amount of preservative recommended for a 24-hour collection per liter of urine.

- **Dietary Substances**

For serum, plasma, and whole blood, test at least three times the maximum expected concentration.

For urine, test at five times the amount eliminated in 24 hours per liter of urine.

- **Specimen Collection and Processing Devices**

Place the device in contact with a sample pool for 24 hours to extract any potentially interfering substances. The volume should be based on the "worst case" situation in actual use. Take precautions against sample evaporation and the loss of labile analytes, and include an appropriate control sample identical to the test sample and treated exactly the same way, except for contact with the test device.

6 Quality Assurance and Safety

Before conducting an interference experiment, verify that:

- instruments have been calibrated and maintained according to the manufacturer's instructions;
- the analytical system is in control and performing as expected;
- all operators have been trained and demonstrate acceptable proficiency; and
- laboratory safety procedures are being followed.

Document compliance with the above requirements.

6.1 Training and Familiarization

The individuals conducting the evaluation must be familiar with the operation of required instrumentation and be trained in the measurement procedure. Instruments must be properly maintained and repaired, and the manufacturer's instructions must be followed.

6.2 Precision Verification

The precision must be consistent with the manufacturer's performance specifications. An estimate of repeatability is needed to determine the number of replicates required by the experiments in Section 7. If the repeatability is not known, the preliminary experiment described in the most current edition of CLSI/NCCLS document EP5—*Evaluation of Precision Performance of Quantitative Measurement Methods* should be performed.

6.3 Trueness Verification

Bias of the measurement procedure should be determined by a suitable recovery[44] or comparison of procedures experiment (see the most current edition of CLSI/NCCLS document EP9—*Method Comparison and Bias Estimation Using Patient Samples* for more information). Although a constant bias will not affect the interference studies, a proportional bias will cause interference to be under- or over-estimated at various analyte concentration levels.

6.4 Carryover Assessment

Results could be affected by carryover from preceding samples. If carryover is present, the experiment must be designed to separate the carryover effect from the interference effect.

6.5 Quality Control

The analytical system must be shown to be in stable operation before testing is begun. Performance should be monitored during the testing period by statistical quality control procedures. Follow the manufacturer's instructions and refer to the most current edition of CLSI/NCCLS document C24—*Statistical Quality Control for Quantitative Measurements: Principles and Definitions* for further guidance.

6.6 Safety and Waste Disposal

For specific information on the safety, proper handling, and disposal of laboratory chemicals, refer to the manufacturer's labeling and Material Safety Data Sheets (MSDS). This information can be obtained from the supplier.

7 Estimation of Interference Characteristics

This section provides experimental procedures for evaluating the susceptibility of a measurement procedure to interfering substances. Although a laboratory may wish to follow these procedures as part of a thorough qualification of a new procedure, they are primarily intended for manufacturers to use in characterizing their procedures.

There are two basic approaches to evaluating the susceptibility of a measurement procedure to interference. Each has advantages and inherent limitations, but they provide complementary information and should be used together. The two approaches are:

- evaluating the effect of potentially interfering substances added to the sample of interest (see Sections 7.1 to 7.3); and

- evaluating the bias of individual, representative patient specimens in comparison to a highly specific comparative measurement procedure (see Section 8.2).

7.1 Interference Screen

Adding a potentially interfering substance to a sample pool and evaluating bias relative to a control portion of the same pool is called "paired-difference testing." Evaluating many potential interferents at relatively high concentrations to simulate "worst case" concentrations is called an "interference screen." If no clinically significant effect is observed, the bias, if any, caused by the substance is unimportant and no further testing is performed.

Substances that show a clinically significant effect are considered interferents, which are further evaluated to determine the relationship between the interferent concentration and the degree of interference.

No practical interference testing strategy can identify all interfering substances. Some interferents (e.g., drug metabolites) may not be identified in the screen; other substances may be falsely classified as interferents (e.g., the form of the substance does not represent the naturally occurring form). An interference screen provides a standardized evaluation that complements studies of actual patient specimens.

Two limitations of interference testing are recognized:

- Properties of the compounds added to a serum pool may be different from those of the compound naturally circulating *in vivo*.

- Different interference effects may offset at the concentrations of interferent and analyte tested. For this reason, hemoglobin should *always* be evaluated for interference at more than one concentration of bilirubin (see Section 5.5).

Data from authentic patient specimens can be used in conjunction with data from "spiked" samples to help ascertain the "truth."

Recommended test levels are given in Appendix B for many common analytes. Each potential interferent should be tested at two analyte concentrations. If this is not practical, Appendix B identifies the preferred concentration to test. Carefully evaluate the potential for interaction, and test suspected substances at two analyte concentrations.

7.1.1 Experimental Design

Both test and control pools are analyzed in the same manner as patient specimens, with adequate replication, within one analytical run.

Sufficient replication is required in order to minimize the possibility of falsely rejecting the null hypothesis of no interference (in statistics, a "Type I error"), or falsely rejecting the alternative hypothesis that there is interference (a "Type II error").

The number of times each sample should be replicated depends on four factors:

- magnitude of the smallest difference between the analyte test results that is considered clinically significant;
- confidence level with which the null hypothesis is tested;
- power with which the alternative hypothesis is tested; and
- repeatability of the measurement procedure.

7.1.2 Test Materials

Sample preparations of test solutions for interference testing are provided in Appendix G.

7.1.2.1 Base Pool

Prepare the base pool as follows:

(1) Obtain fresh specimens of the appropriate type (serum, urine, etc.) from several healthy individuals who are not taking medications. The pool should reflect, insofar as possible, the specimen matrix that is typically submitted for the analyte of interest.

(2) If suitable fresh specimens are not available, substitute frozen or lyophilized samples *with due caution.* Processed control fluids, which may contain preservatives and stabilizers, as well as unrealistic analyte combinations, may demonstrate interference effects that differ from fresh human serum.[45,46] The evaluator is responsible for validating that the test materials adequately simulate fresh clinical specimens. The most current edition of CLSI/NCCLS document EP14—*Evaluation of Matrix Effects* may be used for this purpose.

(3) Calculate the required pool volume, considering the measurement procedure's sample volume requirements, the number of substances to be tested, and the replication requirements.

(4) Determine the concentration of analyte in the base pool and adjust the test pools to the medical decision concentrations of the analyte, using suitably pure material. Avoid introducing other substances along with the analyte. See Appendix B for recommended analyte test concentrations.

7.1.2.2 Stock Solution

Prepare a stock solution of each potential interferent as follows:

(1) Obtain a suitably pure form of the potential interferent, or the form that best approximates the circulating form of the substance. If pharmaceutical-grade preparations must be used, keep in mind that they may contain excipients, preservatives, bactericides, fungicides, antioxidants, colorants, flavorings, metallic oxides, counter-ions, and fillers, any of which may be the true cause of an observed effect.

(2) Choose a solvent in which the test substance is sufficiently soluble. Check the *Handbook of Chemistry and Physics*[47] or the Merck Index[48] for solubility of the test substances in these solvents. Verify that the solvent does not cause interference with the measurement procedure under evaluation. Some possible solvents are listed in order of general preference.

- reagent grade water (see the most current edition of CLSI document C3—*Preparation and Testing of Reagent Water in the Clinical Laboratory* for detailed information);
- diluted HCl or NaOH;
- ethanol or methanol;
- acetone;
- dimethyl sulfoxide (DMSO);
- other organic solvents.

(3) Dilute the sample matrix as little as possible, preferably no more than 5%, solubility permitting, by preparing a concentrated stock solution at least 20 times the intended test concentration.

(4) Organic solvents require special consideration. Volatile solvents must be protected against evaporation. The stock solution should be prepared at the highest practical concentration. Many have very low solubility in water or can introduce artifacts by affecting the reagents or the reaction itself. Chloroform requires at least a 1:100 dilution in serum because of its low solubility. Ethanol at a concentration greater than 1 to 2% can denature antibodies.

Thoroughly document the preparation of the stock solution. **NOTE:** In some cases, interference may increase as the concentration of an endogenous substance (e.g., CO_2, H^+ [pH] or protein) decreases. To evaluate this effect, the concentration of potential interferent in the base pool must be lowered while maintaining the analyte concentration, and with minimal perturbation of the matrix. The control is prepared from the base pool, taking into account any dilution or additions. The approach used will depend on the nature of the analyte and interferent and must be validated by the evaluator.

7.1.2.3 Control Pool

Prepare the control pool exactly as the test pool in all respects, except the test interferent is replaced with the same volume of solvent used to prepare the stock test pool.

(1) If the test substance is present in the control pool (e.g., bilirubin), determine its concentration using a suitable analytical measurement procedure.

(2) If the apparent analyte concentration in the control pool is unexpected compared to the base pool, evaluate the solvent as a potential interferent.

7.1.3 Replication Requirements

The number of replicates required for the desired confidence and power depends on the statistical hypothesis being tested.

- A two-sided test is used when the alternative hypothesis does not state the direction (positive or negative) of the interference, such as ±0.2 mg/dL bias at a creatinine concentration of 1.0 mg/dL.

- A one-sided test is used when the direction of interference (positive or negative) is included in the alternative hypothesis, such as α-ketobutyrate causes +0.2 mg/dL bias at a creatinine concentration of 1.0 mg/dL.

7.1.3.1 Two-Sided Test

For a two-sided test, reasonably assuming normal distribution of the measurement errors, a good approximation of the number of replicates required can be calculated from the following equation:

$$n = 2[(z_{1-\alpha/2} + z_{1-\beta})s / d_{\max}]^2 \quad (1)$$

where:

$z_{1-\alpha/2}$ is the percentile from the standardized normal distribution corresponding to the confidence level 100(1-α) % for a two-sided test;

$z_{1-\beta}$ is the percentile from the standardized normal distribution corresponding to the power 100(1-β)%;

s is the repeatability standard deviation of the measurement procedure; and

$d_{\max}$ is the maximum allowable interference to be detected at the analyte test concentration.

7.1.3.2 One-Sided Test

For a one-sided test, replace in the equation $z_{1-\alpha/2}$ with $z_{1-\alpha}$ (2)

where: $z_{1-\alpha}$ is the percentile from the standardized normal distribution corresponding to the confidence level 100(1-α) % for a one-sided test.

7.1.3.3 *z*-values

For convenience, the *z*-values values for some commonly used confidence and power levels are shown below.

Table 1. Commonly Used Percentiles for Confidence Level and Power

Confidence (Power)	**0.900**	**0.950**	**0.975**	**0.990**	**0.995**
z-percentile	1.282	1.645	1.960	2.326	2.576

For example, the evaluator needs to detect an effect of ±1.5 mg/dL, which has been established as the degree of acceptable interference, at the 95% confidence level ($\alpha = 0.05$) and 95% power ($\beta = 0.05$). This requires a two-sided test. The repeatability is 1.0 mg/dL. To calculate the number of replicates required, substitute these values in Equation (1).[49]

$$n = 2[(z_{0.975} + z_{0.95})]s / d_{max}]^2$$

$$= 2[(1.960 + 1.645)1.0 / 1.5]^2$$

$$= \underline{11.6}$$

Since the number of replicates must be an integer, the number is always rounded up, in this case to 12. This is the number of replicates required for each sample (test and control).

7.1.3.4 Number of Replicates

The number of replicates needed to detect various interference effects with 95% confidence and power are shown below. For convenience, the interference criteria are expressed as multiples of the repeatability (within-run) standard deviation (d_{max}/s) in Table 2.

Table 2. Number of Replicates Needed to Detect Various Interference Effects With 95% Confidence and Power

d_{max} /s	No. of replicates	d_{max}/s	No. of replicates
0.8	41	1.5	12
1.0	26	1.6	10
1.1	22	1.8	8
1.2	18	2.0	7
1.3	16	2.5	5
1.4	14	3.0	3

7.1.3.5 The Effect of Replication

An example illustrates the importance of an adequate number of replicates. Physicians interpret small changes in serum creatinine as an indication of potential kidney rejection. Sometimes they may react to a change of as little as 0.2 mg/dL. Laboratorians, however, know that a variety of biochemical metabolites and medications interfere with alkaline picrate creatinine measurement procedures and could be responsible for an apparent rejection.

In one situation, a recent kidney recipient showed a repeatable change from 1.0 to 1.2 mg/dL. The physician wants to know if the change could be caused by a cephalosporin antibiotic.

At 1 mg/dL creatinine, the repeatability standard deviation is 0.075 mg/dL. The laboratory considers 0.1 mg/dL to be a significant interference. With adequate replication, the effect of imprecision can be reduced so that a possible interference of 0.1 mg/dL would be detected.

First, express the imprecision as a multiple of the repeatability standard deviation (d_{max}/s): 0.1 mg/dL / 0.075 mg/dL = 1.33.

Then, rounding down to 1.3, use Table 2 in Section 7.1.3.4 to determine the required number of replicates. It shows that detecting an effect of this magnitude with 95% confidence and power requires 16 replicates each for the control and test conditions.

If a larger interference were considered acceptable, such as an effect of 0.2 mg/dL (d_{max}/s = 2.7), fewer replicates would be needed to achieve the same degree of confidence. The table shows that only four replicates would be required for the control and test conditions, instead of 16.

7.1.4 Experimental Procedure

The protocol for a paired-difference interference test is as follows:

(1) Determine the appropriate analyte concentration.

(2) Establish the criterion for a "clinically significant" difference (d_{max}).

(3) Determine the number of replicates (*n*) needed for each pool. See Section 7.1.3.4 to determine *n*.

(4) Prepare a base pool of clinical samples (see Section 7.1.2.1).

(5) Prepare a 20x stock solution of the substance to be tested (see Section 7.1.2.2).

NOTE: If another concentration is used, adjust the dilutions in Steps 6 and 8 accordingly.

(6) Pipette 1/20 volume fraction of the stock solution into a volumetric flask. This is the "test" pool. Example: Add 0.5 mL of 20x stock solution to a 10-mL volumetric flask.

(7) Make up to volume with the base pool. Mix well.

(8) Pipette 1/20 volume fraction of the solvent used to prepare the stock solution into a second volumetric flask. This is the "control" pool.

(9) Make up to volume with the base pool. Mix well.

(10) Prepare *n* aliquots of the test sample and *n* aliquots of the control sample. The number of replicates *n* was determined in Step 3.

(11) Analyze the test (T) and control (C) samples in alternating order (e.g., $C_1T_1C_2T_2C_3T_3....C_nT_n$).

NOTE: If the system is affected by sample carryover, include additional samples to protect the control samples from carryover from the test samples, e.g., $C_1T_1C_xC_xC_2T_2C_xC_xC_3T_3...C_xC_xC_nT_n$, where the additional control sample (C_x) results are discarded.

(12) Record the results for data analysis. A worksheet is provided in Appendix E.

7.1.5 Data Analysis

Compute the "point estimate" of the observed interference effect, d_{obs}, as the difference between the means of the test and control samples.

$$d_{obs} = Interference = \bar{x}_{test} - \bar{x}_{control} \quad (3)$$

Compute the cut-off value, d_c, to determine which hypothesis to accept by using the following equation, where n is the actual sample size from Equation (1) or Table 2 in Section 7.1.3.4. The cut-off, d_c, can be computed for a two-sided test using the following equation:

$$d_c = \frac{d_{null} + sz_{1-\alpha/2}}{\sqrt{n}} \quad (4)$$

where d_{null} is the value stated in the null hypothesis, usually = 0.

For a one-sided test, replace 1 - α/2 with 1 - α.

The 95% confidence interval for the interference effect may be calculated, if desired, according to the following equation.

95% Confidence Interval =

$$\left(\bar{x}_{test} - \bar{x}_{control}\right) \pm t_{0.975,n-1}\, s\sqrt{\frac{2}{n}} \quad (5)$$

The standard deviation of the mean difference of measurements on n test and n control samples, indeed, is

$$s\sqrt{\frac{2}{n}}$$

reasonably assuming that imprecision of measurement of the analyte concentration is the same for both test and control samples,

where:
s is the standard deviation of repeatability of the measurement procedure,
n is the number of replicates per sample,
$t_{0.975,\,n-1}$ is taken from a Student t-table as the 97.5th percentile of a t-distribution with n - 1 degrees of freedom. (For n > 30, substituting 2.0 for $t_{0.975,\,n-1}$ is a reasonable approximation.)

7.1.6 Interpretation of Results

If the point estimate, d_{obs}, is less than or equal to the cut-off value, d_c, conclude the bias caused by the substance is less than d_{max}; otherwise, accept the alternative hypothesis that the substance interferes.

Consider the following caveats when interpreting interference testing results:

- The actual interference may differ from the observed "point estimate" due to sampling error. However, if the null hypothesis is true, there is 100(1-α)% confidence in accepting it; and if the alternative hypothesis is true, there is 100(1-β)% confidence in accepting it. Conversely, the confidence in rejecting either is 100α% and 100β%, respectively.

- The artificial nature of the samples tested may have introduced artifacts.

  — The actual interfering substance may not be the parent drug, but rather a metabolite.

— The test sample matrix may not represent the typical pathological samples for the analyte in question, and may introduce a matrix effect.

— The substance added may not be identical to the interferent in pathological samples due, for example, to protein binding, metal complexation, precipitation, or analyte heterogeneity.

- The arbitrary choice of test concentrations may not reveal interference.

— An effect may only be expressed in synergy with other compounds.

— Interference may exist at other concentrations of analyte and interferent, but not at the specific concentrations tested.

7.2 Characterization of Interference Effects

If an interference effect is found at one or more analyte concentrations tested in Section 7.1, carry out a dose-response series to determine the degree of interference as a function of the interferent concentration. A dose-response series of interferent concentrations is prepared from admixtures of the highest interferent concentration pool and the control pool.

7.2.1 Experimental Design

The dose-response experiment determines the relationship between the interferent concentration and the magnitude of interference, which permits estimation of the effect at any interferent concentration within the range tested.

A series of test samples, systematically varying only in the concentration of interferent, is prepared by making quantitative volumetric admixtures of two pools, one at the highest concentration to be tested and the other at the lowest. All samples are analyzed together, in random order, under repeatability conditions. This is necessary to avoid run-to-run variables, such as calibration or reagent lot change, which would confound interpretation of the results.

An advantage of testing multiple concentrations of interferent is that fewer replicates are required at each concentration to detect interference with the same statistical confidence. This is because the repeatability information obtained from all the samples is pooled in determining the confidence intervals.

It is generally sufficient to conduct the dose-response series in triplicate at each test concentration. For those who wish to calculate the number of replicates required at each concentration to assure 95% confidence and power, the formula is given in Appendix F.

7.2.2 Test Materials

7.2.2.1 Base Pool

Prepare a base pool as described in Section 7.1.2.1.

7.2.2.2 Stock Solution

Prepare a stock solution of the potential interferent as described in Section 7.1.2.2.

7.2.2.3 High Pool

Prepare a high pool to contain the concentrations of potential interferents specified in Section 5.5. Dilute the stock solution with the base pool, as described in Section 7.1.4, to achieve this concentration.

NOTE: If lower concentrations of endogenous substances cause interference, see the Note in Section 7.1.2.2.

7.2.2.4 Low Pool

Prepare a low pool that contains the average concentration of the interferent in the pool of clinical samples. In most cases, it will be negligible (e.g., in the case of a therapeutic drug) or low (e.g., in the case of hemoglobin or bilirubin) and the low pool may be prepared according to directions for the "control pool" as described in Section 7.1.2.3.

7.2.2.5 Test Pools

Prepare a series of test pools to contain intermediate concentrations of the interferent. This procedure for preparing pools provides for higher relative accuracy and precision of the interferent concentrations in different pools, as shown in the paper by Vaks.[50] These are prepared quantitatively as admixtures of the high and low pools, as indicated below. Five concentrations are sufficient to determine a linear dose-response relationship.

(1) Mix equal volumes of the low and high pools to create a concentration midway between the two extremes.

(2) Mix equal volumes of the low- and midconcentration pools to create a concentration a quarter of the way between the two extremes.

(3) Mix equal volumes of the mid- and high-concentration pools to create a concentration three quarters of the way between the two extremes.

7.2.2.6 Preparation Scheme

Figure 1 illustrates the preparation scheme for a hypothetical interferent, normally present at an average of 5 mg/dL in patient specimens, which may reach 20 mg/dL in pathological serum. The high pool should therefore be made up to 40 mg/dL, and the low pool concentration is measured at 5.0 mg/dL.

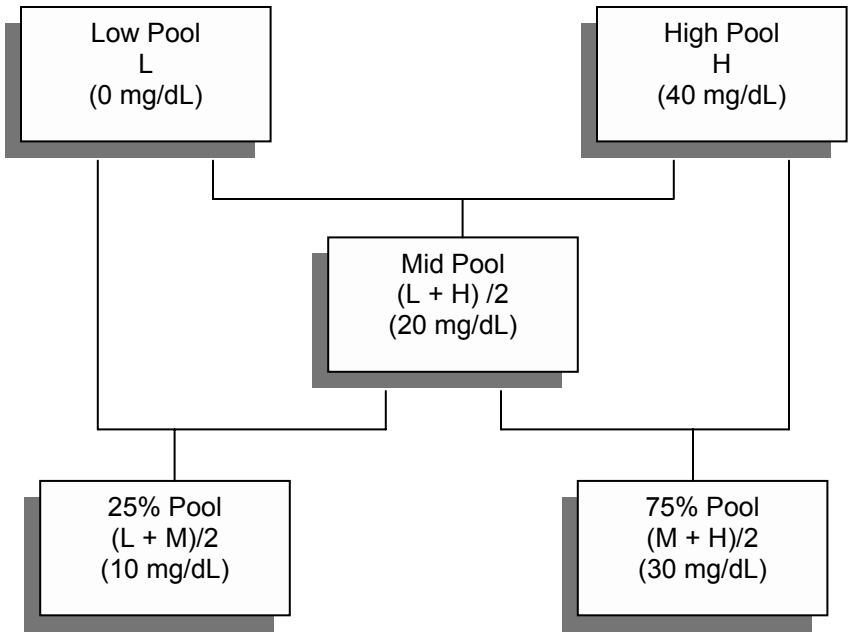

Figure 1. Preparation Scheme for Five-Level Series

7.2.3 Experimental Procedure

The protocol for a dose-response interference test is as follows.

(1) Determine the highest and lowest concentrations to be tested.

(2) Determine the difference that would be considered "clinically significant." This has already been done if the "paired-difference" experiment was carried out (see Section 7.1.4).

(3) Determine the number of replicates, *n,* to be run at each concentration (see Appendix F).

(4) Prepare the high and low pools.

(5) Prepare a midconcentration pool by pipetting equal volumes of the high and low pools into a suitable flask. Gently mix well.

(6) Prepare a 25% pool by pipetting equal volumes of the low- and midconcentration pools into a suitable flask. Gently mix well.

(7) Prepare a 75% pool by pipetting equal volumes of the mid- and high-concentration pools into another suitable flask. Gently mix well.

(8) Prepare *n* aliquots of each pool as determined in Step 3 above.

(9) Analyze the series of five pools within the same analytical run. The first set of replicates should be analyzed in ascending order, the second set in descending order, the third set in ascending order, etc., in order to average out any systematic drift effects.

(10) Another approach to minimizing drift effects is to run all samples and replicates in random order; the sequence is assigned using a random number generator or a table of random numbers.

(11) Calculate the average concentration for the low pool and subtract it from all other results. Tabulate the net results for data analysis.

(12) If the laboratory has ready access to a measurement procedure for the interferent, it may be useful to verify its concentration by measurement.

7.2.4 Data Analysis

Plot the results, with the observed effect on the *y*-axis and the interferent concentration on the *x*-axis, and examine the shape of the dose-response relationship.

7.2.4.1 Linear Effects

If the data appear randomly distributed about a straight line, apply linear least squares regression analysis.[48] Determine the slope, intercept, and residual error ($s_{y.x}$), from the individual observations (not averages). Draw the regression line on the graph, and confirm that it fits the data and that the response is linear. An example of interference linearly related to the interferent concentration is illustrated in Table 3.

Table 3. Summary of Results From a Five-Level Dose-Response Series Showing a Linear Relationship (All results are in mmol/L.)

Pool	Interferent	Observed Effect		
		Rep 1	Rep 2	Rep 3
1	5.00	4.82	5.85	2.89
2	14.5	5.86	11.05	10.41
3	24.0	14.77	14.11	12.70
4	33.5	16.34	18.43	21.08
5	43.00	28.21	24.35	22.44

The data are plotted and the linear regression equation calculated, as shown in Figure 2.

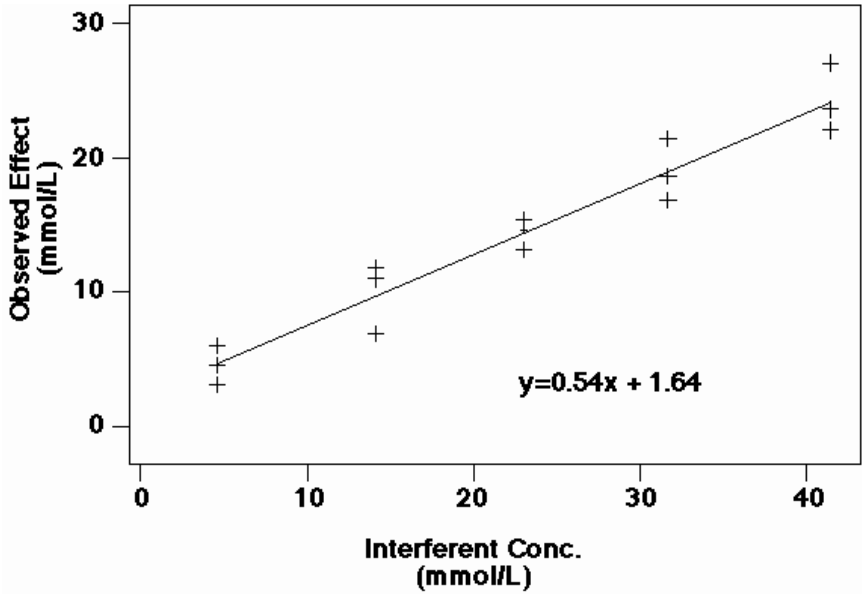

Figure 2. Plot of Results From the Dose-Response Experiment Described in Table 3

A 95% confidence band can be computed around the dose-response line, from which the 95% confidence interval for the interference can be determined at any interferent concentration. A graphical illustration, using the data from Figure 2, is given below.

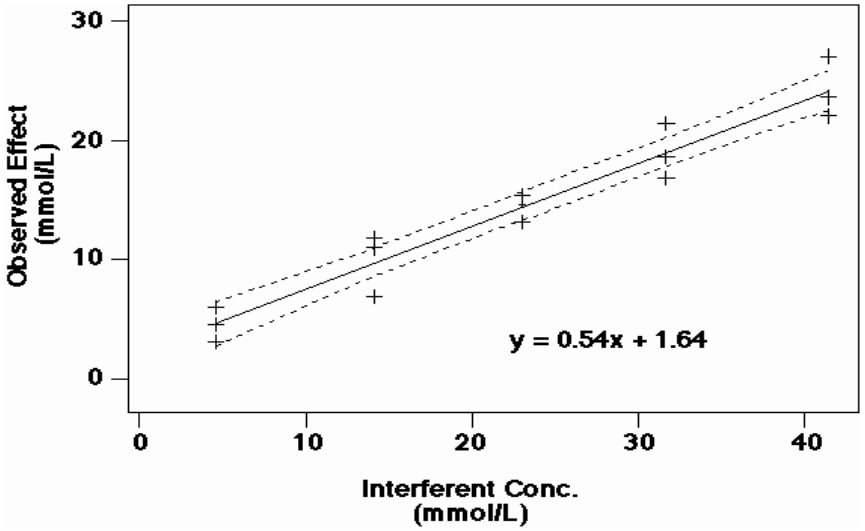

Figure 3. Plot Illustrating the 95% Confidence Band About the Regression Line

Note that the size of the confidence interval changes as a function of the interferent concentration, with the greatest confidence in the results from the middle of the interferent concentration range. Statistical calculators and computer programs are available that will calculate regression statistics and confidence intervals. For a procedure for calculating confidence intervals around the regression line, see a standard statistical textbook such as Draper.[51]

7.2.4.2 Nonlinear Effects

Interference may not be a linear function of the interferent concentration. If the plotted data show curvature, an adequate estimate of the degree of interference at a given interferent concentration may often be determined graphically. The data in Table 4 will be used to illustrate the procedure.

Table 4. Summary of Results From a Five-Level Dose-Response Series Showing a Nonlinear Relationship (All results are in mmol/L.)

Pool	Interferent	Observed Effect		
		Rep 1	Rep 2	Rep 3
1	5.00	-1.42	1.54	0.06
2	14.5	8.76	13.95	10.31
3	24.0	19.87	19.21	17.83
4	33.5	20.24	22.38	24.95
5	43.00	29.51	25.65	23.74

When the data are plotted, as in Figure 4, the degree of interference at any interferent concentration can be estimated from the graph. It can also be calculated by nonlinear regression analysis using a quadratic polynomial model.

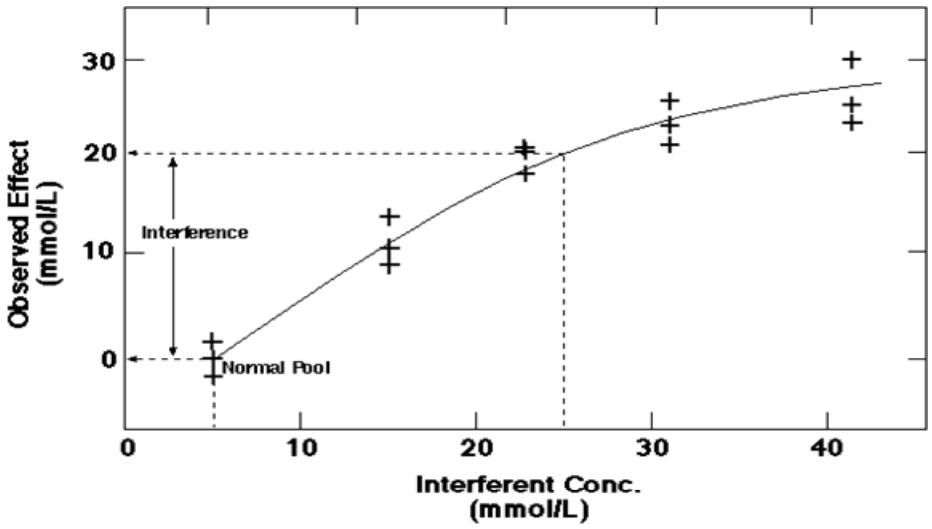

Figure 4. Plot of Results From the Dose-Response Experiment Described in Table 4

To determine the interference expected at 25 mmol/L, draw the best fit curve through the data and read the interference on the *y*-axis corresponding to an interferent concentration of 25 mmol/L. In this case, the interference is estimated to be 20 mmol/L.

Confidence intervals may be computed using a suitable nonlinear regression analysis program, which is available in most statistical analysis packages.

7.2.5 Interpretation of Results

If the relationship is linear, the regression *slope* represents the bias per unit of interferent. The *y-intercept* represents the correction for the endogenous interferent concentration, if any. The degree of interference can be estimated at any interferent concentration from the regression equation, or from the graph, whether the relationship is linear or nonlinear.

Referring back to the data in Figure 2 for an example, since the slope is positive, the experiment showed the substance causes a positive interference. What is the magnitude of interference when the interferent is present at 25 mmol/L?

From the regression equation, we determine that

$$y = 0.82 \times 25 \text{ mg/dL} - 4.1 = 16.4 \text{ mmol/L} \quad (6)$$

7.3 Evaluating Combinations of Analyte and Interferent(s)

Two (or more) potential interferents can be tested more efficiently in a single experiment, in which the concentrations of the test substances and the concentrations of analyte are varied systematically. The effects of the individual constituents are estimated by factorial analysis.

The advantages are increased efficiency and more information; fewer analyses are required than for one-at-a-time testing, and interaction among interfering substances—as well as the analyte—can be evaluated. A potential disadvantage is that sample preparation is more complex, increasing the chance of human error.

Application of factorial analysis to interference testing has been described by Kroll et al.[52,53] For a more detailed description of multifactorial experimental designs, see Box, Hunter, and Hunter.[54]

8 Evaluating Interference Using Patient Specimens

The interference screen described in Section 7.1 has obvious limitations. No matter how comprehensive, unexpected interferences may be encountered in patient specimens. To minimize the likelihood of this happening, specimens from relevant patient populations should be analyzed to evaluate inherent sample-to-sample variability. A reproducible "outlier" result associated with an individual sample gives a clear indication of an unknown interfering substance. A high degree of "scatter" caused by reproducible sample-related biases is also a good indication that interfering substances are present.

Patient specimen results may also be used to confirm interference demonstrated in spiked pool testing. If bias is not observed in samples known to contain the substance in question, further investigation should be undertaken to reconcile the conflicting observations.

8.1 Experimental Design

The experiment is based on analyzing two groups of patient specimens (i.e., a test group and a control group) on (1) the measurement procedure being evaluated, and (2) on a reference procedure[27] or other qualified comparative measurement procedure. Biased results from patient subgroups relative to a control group indicate interference.

NOTE: Detailed statistical procedures are not provided in this section.

8.2 Comparative Measurement Procedure

A well-characterized measurement procedure with low susceptibility to interferences is employed to establish the "true values" in the comparison study. Ideally, a reference procedure should be used for this purpose.[27] If a reference procedure is not available, another qualified comparative measurement procedure (i.e., a procedure with good precision and specificity, preferably a different principle of measurement) may be used (see the most current edition of CLSI/NCCLS document EP9—*Method Comparison and Bias Estimation Using Patient Samples* for more information). If the comparative measurement procedure lacks sufficient specificity, the ability to draw definitive conclusions is compromised. The following situations are possible:

- An observed bias in certain patient specimens could be due to interference with either measurement procedure.

- Lack of bias between the two measurement procedures could be due to (1) similar sensitivity to the same interferent, or (2) neither procedure being affected by the interferent.

The relationship between the two measurement procedures (systematic bias) is determined from analysis of the control samples.

8.3 Patient Populations

8.3.1 Test Specimens

Test specimens are selected from the patient populations of interest. They are known to contain one or more potential interferents (e.g., therapeutic drugs), are taken from patients diagnosed with specific conditions or diseases, or both.

For example, patient specimens may be selected based on the following criteria:

- relevant diseases (e.g., specimens from patients with cardiac, liver, or renal disorders);

- relevant medications (e.g., specimens from patients known to be taking the drugs of interest);

- uremic patients (e.g., predialysis), whose blood is likely to contain high concentrations of endogenous metabolites or drugs; and

- other identifiable constituents (e.g., abnormal concentrations of bilirubin, hemoglobin, protein, lipids).

8.3.2 Control Samples

Control samples must span the same range of analyte concentrations. They are selected because they are known not to contain the substance(s) or because they include substances associated with the diseases being tested. Control samples may be selected:

- from patients not taking the drugs of interest;

- with normal concentrations of the potentially interfering substance;

- with the same or similar diagnosis; and/or

- with an analyte distribution similar to the test samples.

Samples from the control group must be included in every run.

8.4 Experimental Procedure

Each sample is run in duplicate by each measurement procedure. The number of test and control samples required depends on three factors:

- the precision of the two measurement procedures;
- the magnitude of the interference effect to be detected; and
- the level of confidence required.

If the effect is large and both measurement procedures have good precision, 10 to 20 samples in each group are sufficient. If more samples are needed to quantify the effect with desired level of confidence (i.e., the bias is so small that it is masked by imprecision) the effect is not likely to be of clinical significance. Please refer to the most current editions of CLSI/NCCLS document EP9—*Method Comparison and Bias Estimation Using Patient Samples* and CLSI document EP14—*Evaluation of Matrix Effects*, for additional statistically based procedures for determining the number of samples for this design.

- Select the groups of test and control samples.

- Select an appropriate reference or qualified comparative measurement procedure.

- Analyze each sample in duplicate by both measurement procedures within as short a time span as possible, usually within two hours. The time span must be justified (e.g., by analyte and procedure stability criteria), and the rationale must be documented. Follow these precautions:

  — Timing is especially important if the analyte or potential interferent is labile, if the matrix is unstable (e.g., whole blood), or if microvolumes are used (because of sample evaporation). Special precautions are required in these cases.

  — Spread the runs over several days to reduce the contribution of day-to-day imprecision. Alternate the sequence of the two runs each day; alternate (or randomize) control and test samples within each run.

  — Carefully set up the sequence of samples for testing if the measurement procedure is subject to carryover.

  — Be alert to any systematic differences that might lead to a false indication of interference.

- If a bias is observed, measure the concentration of the drug or other potential interferent in the samples, if possible, to establish the relationship between bias and interferent concentration.

8.5 Data Analysis

Visual inspection of plotted data will usually indicate if interference is present. Review the data from each group of selected patient specimens in comparison to its control group and assess whether there is systematic bias. If there is, evaluate the range of the differences between the selected patient results and the mean of the control results and compare it to the interference criteria. From this, decide whether interference is ruled out or further investigation is required. The procedure and examples below provide

additional guidance, but determination of the cause of the interference is beyond the scope of this guideline.

8.5.1 Plotting Bias Versus the Comparative Measurement Procedure Value

Steps to be followed when plotting bias versus the comparative measurement procedure value:

(1) Tabulate the results for data analysis. Average the duplicate results for each sample.

(2) For each sample, calculate and record the average bias (test measurement procedure result minus comparative measurement procedure result).

(3) Plot each point, with bias on the vertical axis and the comparative measurement procedure concentration on the horizontal axis. Use different plotting symbols for the test and control samples.

(4) Determine the *Sy.x* statistic for each group from linear regression analysis (with the comparative measurement procedure = *x*). This can be used to compute the 95% confidence intervals (see examples below).

8.5.2 Evaluating Bias for Possible Interference

Some typical outcomes of this type of experiment are illustrated in Figure 5.

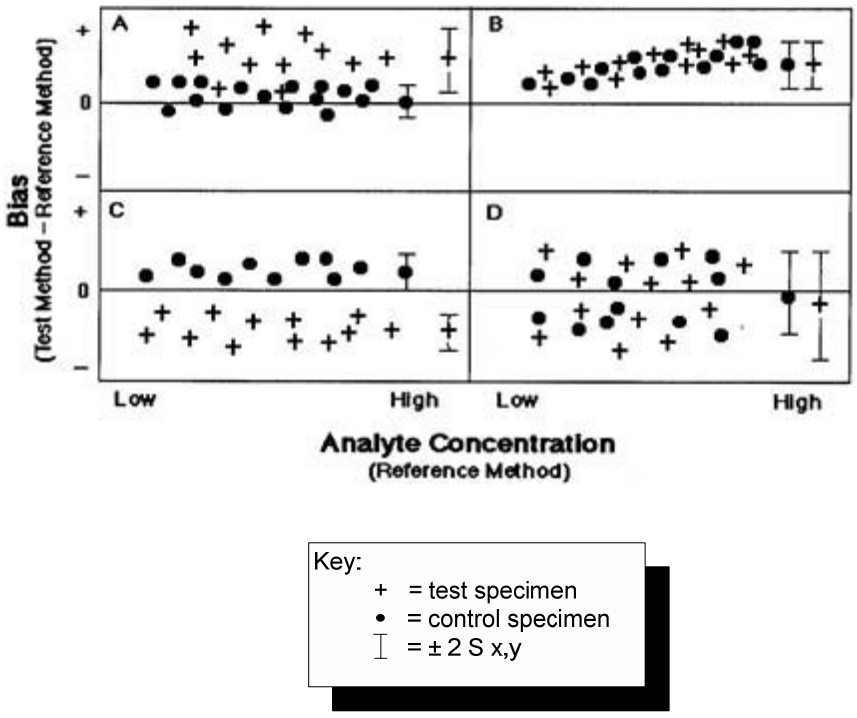

Figure 5(A-D). Four Possible Outcomes of Different Interference Tests Based on Patient Specimens (The variables are discussed in the text.)

8.5.3 Positive Bias Relative to the Control Group

In Figure 5(A), the test group data (+) show a bias and are more variable than the control group data (•), which show a tighter scatter pattern and negligible bias to the comparative measurement procedure. In this example, the results suggest interference by some constituent of the test samples, but are not

conclusive because the confidence intervals overlap each other (plotted to the right of the data points; calculated as average bias ±2 $S_{x.y}$). These results could have occurred by chance. Further investigation is needed.

8.5.3.1 No Bias Relative to the Control Group—Proportional Bias

In Figure 5(B), both the test group and control group show a positive proportional bias. The confidence intervals are nearly superimposed. No difference attributable to interference is indicated.

8.5.3.2 Negative Bias Relative to the Comparative Measurement Procedure

In Figure 5(C), the data show a clear negative interference. Confidence intervals are widely separated. The control group shows a positive bias. Note that the effect is significantly larger than might have been suspected if a control group had not been included in the experiment to correct for systematic bias unrelated to the potential interferent. The difference between upper limit of test group biases and the average bias of the control group can be compared to the interference criteria to estimate whether there may be clinically significant interference.

The following situations can take place:

- If the mean difference between the biases of the control and test groups is both clinically and statistically significant, then the conclusion is that clinically significant interference has been detected.

- If the above difference is statistically but not clinically significant, no clinically significant difference has been detected.

- If the difference is clinically but not statistically significant, a larger sample size is needed.

8.5.3.3 No Bias Relative to the Control Group

In Figure 5(D), the average bias in the test group is slightly negative relative to the control group. However, interference of this magnitude must be considered relative to the large degree of variability shown in the data from the control group. The confidence intervals show no statistical difference in the results.

8.5.4 Plotting Bias Versus Potential Interferent

If the concentration of a suspected interferent can be measured, determine if its concentration can be correlated to the observed bias.

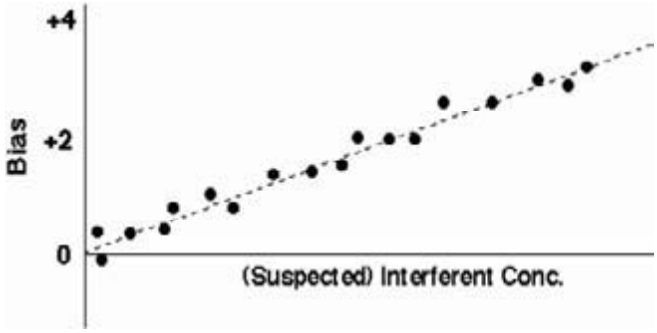

Figure 6. Plot Demonstrating Good Correlation of Bias (Interference) as a Function of the Concentration of Suspected Interferent

(1) Plot the bias (test measurement procedure result minus comparative measurement procedure result) on the vertical axis against the concentration of the potential interferent on the horizontal axis. Figure 6 illustrates an observed effect that correlates well with the potential concentration. Construction and interpretation of "bias plots" is found in the most current edition of CLSI/NCCLS document EP9—*Method Comparison and Bias Estimation Using Patient Samples.*

(2) Examine the plot of bias vs. suspected interferent concentration. If the relationship is linear and the scatter is relatively constant over the range, then all the data can be analyzed together. The relationship between interference effect and interferent concentration can be determined by linear regression analysis, as described in Section 7.2.4.

An alternative approach, if the relationship is not linear: subset the data into smaller concentration ranges and calculate the average bias (interference) and average interferent concentration for each subset of data. This indicates the magnitude of interference due to the substance being tested.

8.6 Interpretation of Results

Limitations of using patient specimens are chiefly related to the lack of control over test variables, and the requirement for a highly specific comparative measurement procedure for definitive interpretation of the results.

- **CAUTION: This experiment only demonstrates *correlation* of bias with a specific substance; it does not prove a cause-effect relationship. The actual interferent could be a substance coincidentally present with the suspected interferent. For example, interference by a biochemical metabolite appearing as the consequence of a disease may be mistakenly attributed to a drug used to treat the disease.**

- Labile constituents (e.g., acetoacetate, CO_2) can be lost if the samples are not fresh.

- Hospitalized patients usually receive multiple drugs (or multiple drug regimens) and may have elevated concentrations of endogenous metabolites.

- Prospectively grouping patients by disease and medication can be very difficult to accomplish.

- The interferent may not be present in the sampled patient specimens.

- The comparative measurement procedure may not be sufficiently well characterized with respect to interference. It could also be affected by the same interferent.

Nevertheless, this approach has proven valuable in providing clues to interfering substances that otherwise might be missed, and it may be the only approach that detects unsuspected interference by a drug metabolite. It also provides a means of confirming suspected interference in actual patient specimens. See Appendix A for special considerations for measurement procedures using separation techniques or immunochemical measurement procedures.

9 Establishing, Validating, and Verifying Interference Claims

These guidelines may be used by manufacturers to characterize and verify specificity and to establish interference claims, and by clinical laboratories to validate manufacturers' claims and validate that the specificity of their measurement procedures satisfies medical requirements. A well-characterized procedure allows a clinical laboratory to leverage manufacturers' data to satisfy its own validation and

verification requirements. This section describes the essential requirements that must be met for an interference evaluation to conform to this protocol.

Validation and *verification* are similar concepts that have been applied in slightly different ways in the clinical laboratory, medical device, and software industries. This has led to some confusion. This guideline uses the terms as defined in ISO 9001—*Quality management systems—Requirements*,[55] ISO 15189—*Medical laboratories – Particular requirements for quality and competence*,[56] and CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*. Both terms refer to the provision of objective evidence that certain requirements have been met. *Validation* means that *users' (or regulatory) requirements* have been met (e.g., accuracy requirements for patients' results), while *verification* means that *specified criteria* have been met (e.g., interference criteria or interference claims).

9.1 Establishing Interference Claims

Interference is a limitation of the measurement procedure with respect to its intended use. For commercial procedures, substances known to interfere shall be disclosed in the instructions for use. Substances tested that do not interfere should also be disclosed so that the laboratory can verify the suitability of the procedure for the patient population it serves.

The clinical laboratory requires the following information from the manufacturer:

- the analyte and interferent concentrations covered by the claim;
- the names of the substances evaluated for potential interference;
- the chemical and/or generic names of known interfering substances;
- the criteria used to define clinically significant interference;
- the concentrations above which the substances interfere or below which they do not interfere;
- the interference observed with 95% confidence at specified analyte concentration(s); and
- the evaluation protocol (if CLSI document EP7 is not cited, describe the approach and specify the power and confidence level used to determine interference).

Three acceptable approaches are used in stating interference claims.

(1) The interference claim may state the concentration of a substance above which it causes a bias exceeding the interference criteria (power = 95%).

(2) The interference claim may state the concentration of a substance below which no interference was observed (confidence level = 95%).

(3) The interference claim may state only that a substance has been reported to interfere with the measurement procedure. This statement is appropriate when quantitative information is not available. For example, a published report contains evidence that results from patients taking a certain medication do not agree with the true values. If the degree of error is clinically significant, the manufacturer should (1) investigate further to characterize and disclose the interference; or (2) disclose that the substance has been reported to interfere and cite the journal reference or other source of data.

Information about substances tested and found not to interfere should be summarized and provided to users. It may be provided in the form of a "specificity" claim (see Section 9.1.2).

Model statements for interference and specificity claims are given below. Other approaches are acceptable, but consistency is encouraged to facilitate interpretation by laboratories.

9.1.1 Interference Claims

Example 1. Results of Interferent Concentration Series:

The AST measurement procedure was evaluated for interference according to CLSI document EP7. The following common substances, when added to serum, interfered at the concentrations indicated. A bias exceeding 10% is considered a significant interference.*

Substance Tested	Interfering concentration at AST 25 U/L	Interfering concentration at AST 200 U/L	Comments
Hemoglobin	250 g/dL	325 g/dL	As hemolysis
N-Acetylcysteine	150 mg/mL	300 mg/mL	Therapeutic IV dose is 180 mg/mL

*Upper limit of 95% confidence interval.

CAUTION: Do not attempt to correct analyte results based on these results. The relationship between analyte and interferent has not been determined.

Example 2. Bilirubin Measurement Procedure —Two-Level Interference Screen:

The bilirubin measurement procedure was evaluated for interference according to CLSI document EP7. The following commonly occurring substances caused interference when added to serum at the analyte and interferent concentrations indicated. Bias exceeding 0.2 mg/dL is considered interference.

Interferent	Interferent concentration	Analyte (mg/dL)	Bias* (mg/dL)	Comments
Hemolysis (Hemoglobin)	300 g/dL	1.2	- 0.4	Gross hemolysis
	"	16.5	+ 0.5	
N-Acetylcysteine	90 mg/dL	1.2	< 0.2	Therapeutic IV dose
	"	16.5	-0.6	
Acetylsalicylic Acid	50 mg/dL	1.2	< 0.2	Toxic dose
	"	16.5	+ 0.3	

*Upper limit of 95% confidence interval.

CAUTION: Do not attempt to correct analyte results based on these results. The relationship between analyte and interferent has not been determined.

9.1.2 Specificity Claim

The following substances, when tested in serum at AST activities of 25 and 200 U/L according to this CLSI protocol, were found not to interfere at the concentrations indicated. A bias less than 10%* is not considered a significant interference. The manufacturer should report, upon request, the observed bias found, the bias corresponding to the upper 95% confidence limit, the SD of the experiment, and the sample size.

Substance Tested	Test Conc.	Comments
Hemoglobin	300 mg/dL	As hemolysis
N-Acetylcysteine	180 ng/dL	2x therapeutic IV dose
Tolazamide	55 µg/dL	2x therapeutic dose

*Upper limit of 95% confidence interval.

9.2 Verifying Analytical Specificity

Verification means objectively demonstrating that specified criteria for use are met. Acceptability criteria should be established based on medical requirements (see Section 5).

Manufacturers must verify that the specificity of their measurement procedures meets design criteria derived from their immediate customers—clinical laboratories.

Clinical laboratories must verify that the performance of their procedures, including specificity, meets the manufacturer's claims, or they need to validate that the procedures meet the accuracy requirements derived from their immediate customers—the physicians.

Obviously, these activities are closely linked. Manufacturers' requirements must satisfy medical needs. However, laboratories may impose more stringent performance requirements, since discrepant results may lead to unnecessary troubleshooting and erode physicians' confidence in the laboratory.

Additional criteria may be established for the allowable frequency of discrepant results, or "outliers," in the intended patient population. Because of the potential for unpredictable interference, accuracy criteria are often established such that 99% or 95% of individual results must fall within a specified allowable error limit. The criteria must be justified by the medical requirements of the measurement procedure.

9.2.1 Manufacturers

Verification of measurement procedure specificity means the manufacturer has objective evidence that pre-established interference criteria are met. Interference testing should begin early in the development of a new procedure, so that design changes can be implemented prior to design transfer, if necessary.

The essential elements of this CLSI protocol for a manufacturer's verification activities are listed below. A manufacturer may declare conformance to CLSI document EP7 when all of the elements are included:

- potential interferents to be evaluated are systematically identified (see Section 5.4);
- interference criteria are established based on user requirements (see Section 5.1);
- comprehensive interference screen is conducted (see Section 7.1);

- concentrations that cause interference are determined (see Section 7.2); and
- interference and specificity information required by laboratories is described in the product labeling/instructions for use (see Section 9.1).

9.2.2 Clinical Laboratories

Verification of the measurement procedure's specificity means the laboratory has objective evidence that its criteria for interference are met. The manufacturer's criteria and data may be accepted by the laboratory for this purpose, if they apply to the patient population served by the laboratory.

If a laboratory cannot rely on a manufacturer's verification data or other sources of interference information, the laboratory must establish its own criteria and conduct its own evaluation of the most likely sources of interference. Section 9.2.1 lists the steps to follow to evaluate in order to declare conformance to this guideline.

A laboratory may also need to verify that a particular interference claim is met. This subject is covered in Section 9.4.

9.3 Validating Analytical Specificity

Validation means objectively demonstrating that customer requirements are met. The degree of validation should be commensurate with the risk of discrepant results caused by interfering substances.

9.3.1 Manufacturers

Validation means providing objective evidence that the method's specificity, including any limitations described in labeling claims, meets the functional needs of their immediate customers (e.g., clinical laboratories). Customer needs are typically related to the medical requirements for accuracy.

The essential elements of this guideline for a manufacturer's validation include evaluation of relevant patient populations. Section 7 describes how to confirm observed effects in natural patient specimens and how to evaluate relevant patient populations for unanticipated interfering substances. These validation activities are typically combined with the comparison of methods experiment in the most current edition of CLSI/NCCLS document EP9—*Method Comparison and Bias Estimation Using Patient Samples* during internal and external performance trials.

9.3.2 Clinical Laboratories

Validation means providing objective evidence that the specificity of the method, including any limitations described in labeling claims, meets the medical needs of their immediate customers (e.g., physicians).

Interference is a characteristic of the method and the clinical specimens, and a comprehensive interference evaluation may be beyond the capability of the laboratory. The laboratory may accept the manufacturer's criteria and data if it can show that: 1) the substances tested by the manufacturer are relevant to its own population; 2) the criteria used to define interference are appropriate for the medical needs of its clients; and 3) the interference evaluation was conducted using scientifically valid experimental protocols. The rationale for using manufacturer's criteria and data should be documented.

If a laboratory cannot rely on a manufacturer's validation data, it must conduct its own evaluation of relevant patient populations. Demonstration that results from representative patient specimens agree between the new measurement procedure and another commercial procedure may provide sufficient

validation. The evaluation protocol (e.g., CLSI/NCCLS document EP9— *Method Comparison and Bias Estimation Using Patient Samples*) and acceptance criteria (e.g., the percentage of individual patient results that must fall within a specified bias limit) must be established in advance. Discrepant results should be investigated as described in Section 10.

9.4 Verifying Interference and Specificity Claims

Interference and specificity claims can be verified experimentally. The appropriate approach depends on the type of claim.

9.4.1 Maximum Interference Claim

The interference may be claimed to be less than a stated maximum value. Example: The effect of 1 mg/dL magnesium on calcium results in the range 8 to 14 mg/dL is less than 0.2 mg/dL.

To verify this statement, use the procedure in Section 7.1.4 to conduct a paired-difference experiment at the appropriate concentrations of magnesium and calcium. Compute the mean effect (x_d). If it is less than 0.2 mg/dL, the claim is accepted; otherwise, it is rejected.

9.4.2 Observed Interference Claim

The results of an interference test may be given. Example: *In the presence of 1 mg/dL magnesium, calcium results in a normal serum pool were +0.14 mg/dL higher than the control value.*

To verify this claim, conduct a paired-difference experiment to test the null hypothesis that interference is less than or equal to 0.14 mg/dL. The alternate hypothesis is that it is greater.

9.4.3 Nonquantitative Interference Claims

When interference is reported without quantitative information (for example, “Methotrexate has been reported to interfere with this measurement procedure”), statistical verification is not necessary. An experiment to characterize the degree of interference is described in Section 7.2.

9.4.4 Specificity Claim

The statement, “Salicylate does not interfere with this measurement procedure,” can be verified experimentally. Conduct a paired-difference test (see Section 7.1.4) of the null hypothesis at the medical decision concentration of the analyte, setting reasonable criteria for interference (Section 5.1), and analyzing and interpreting the results as described in Sections 7.1.5 and 7.1.6.

10 Investigating Discrepant Patient Results

Every laboratory occasionally encounters discrepant results. A result may be reported by physicians as inconsistent with a diagnosis or a previous result, or a discrepancy between two measurement procedures may be discovered in the laboratory. If a value for a specific patient is repeatable, and the procedure is properly standardized, the probable cause is interference.

The following troubleshooting strategy may be followed to investigate a discrepant result. If interference is confirmed and the interfering substance can be identified, the laboratory should report its findings to the manufacturer and include the information in its procedure manual.

NOTE: It is not possible to anticipate all of the possible scenarios a laboratory might encounter. These recommendations are intended as guidelines. They should be modified as necessary to accommodate particular circumstances.

10.1 Verify System Performance

Before beginning the investigation, verify that the system is performing acceptably. Look for any indication of sporadic system malfunctions that could have caused the discrepant result.

- Check quality control records and verify that the system has been operating consistently within control limits.
- Analyze freshly prepared quality control samples to verify that system performance is still within control limits.
- Confirm that the measurement procedure is properly calibrated and maintained.

10.2 Evaluate Sample Quality

Next, check the sample for obvious problems. Look for any indication of abnormal characteristics that could explain the discrepant result.

- Visually examine the sample for fibrin clots, hemolysis, elevated bilirubin, lipemia, turbidity, and other visible abnormalities. If present, determine if it is consistent with the observed bias.
- Verify that the specimen was collected, transported, and stored properly, using a recommended collection procedure, compatible preservatives, anticoagulants, etc. If not, determine if this could be the probable cause.
- Rule out specimen mix-up and other specimen handling errors. If an error occurred, determine if it explains the discrepancy.

10.3 Confirm the Original Result

Confirm that the sample demonstrates sample-specific bias before proceeding further. Develop an investigation plan that makes the best use of the rest of the valuable sample remaining.

- Repeat the analysis on the same sample to rule out random error (imprecision) or incidental error (outlier) as a cause.
- Check previous laboratory results from the same patient, if available. They may show a trend that correlates with a specific medical intervention or other patient change.
- Dilute and reanalyze the sample. If the calculated result after dilution is higher or lower than the one from the undiluted sample, a positively or negatively interfering substance may be present.
- Analyze the sample by a different principle of measurement, including other samples as controls. If necessary, send them to another laboratory for analysis.
- Collect and reanalyze another specimen from the same patient, and/or from patients with the same or similar diagnosis and medications. Follow the investigation path suggested by the results.

10.4 Identify Potentially Interfering Substances

If the discrepant result is confirmed, and the system is functioning properly, attempt to identify the interfering substance.

- Review the product labeling for known interfering substances that may have been present.
- Determine the patient's diagnosis and medical condition. Check for recent diagnostic procedures and treatments, such as surgery, anesthesia, transfusions, radiological procedures, and physical manipulations, such as prostatic massage.
- Review the patient's medication records. Check for recently prescribed drugs, hyperalimentation, radioisotopes, as well as over-the-counter medications and vitamins.
- Determine if the patient is on an unusual diet, and if so, whether it is likely to be responsible for the discrepant result.
- Call the manufacturer and inquire whether other similar reports have been received. Report the findings and request assistance in determining the cause.

10.5 Determine the Probable Interferent

Once potentially interfering substances have been identified, test the most likely candidates. A quick, low-power experiment is suitable for detecting large effects and zeroing in on the probable interferent.

(1) Collect 2 mL of serum from a healthy, drug-free person for each substance to be tested.

(2) If the analyte is not normally present in healthy individuals, add a sufficient quantity to represent a typical concentration.

(3) Divide the fresh pool into 1-mL portions.

(4) Prepare a concentrated stock solution of the substance to be tested. Aim for 50 to 100x the expected serum concentration.

(5) Add 50 µL of the stock solution to 1.0 mL of serum. Label it, "test sample."

(6) Add 50 µL of the solvent used to prepare the stock solution to another 1.0 mL of serum. Label it, "control sample."

(7) Analyze each sample in duplicate in the same analytical run.

(8) Calculate the difference between the test and control results.

(9) If the difference exceeds the laboratory's criteria for interference, rule out a chance occurrence due to imprecision by comparing it to the repeatability (within-run precision) of the measurement procedure at that concentration (see calculation below). If the result exceeds the expected uncertainty due to imprecision, it suggests interference is the probable cause. If the results are negative, however, interference by yet unidentified substances (e.g., drug metabolites) cannot be ruled out.

The uncertainty due to imprecision can be approximated from the known repeatability standard deviation at or near the analyte concentration tested. This assumes similar repeatability for the control and test samples. For duplicate measurements, use two standard deviations for 95% confidence.

$$2s_{test\text{-}control} = 2\sqrt{(s^2_{test} / n) + (s^2_{control} / n)}$$

$$\approx 2\sqrt{s^2_{control} / 2}$$

$$\approx 2\sqrt{s^2_{control}}$$

$$\approx 2\sqrt{s^2_{control}}$$

10.6 Characterize the Interference

Once the probable interfering substance is determined, the laboratory should attempt to work with the manufacturer to confirm it and characterize its effect on the measurement procedure. The procedure in Section 7.2 is used for this purpose. Manufacturers have an obligation to investigate reports of clinically significant discrepancies[57] and consequently depend on obtaining relevant data from customers. If a new interference is substantiated, the manufacturer is required to include the information in its product labeling for the benefit of all users.

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Appendix A. Guidelines for Specific Measurement Procedures

A1. Guidelines for Measurement Procedures Based on Separation Techniques

Separation techniques, such as chromatography and electrophoresis, are designed to be relatively free from interferences because the separation step resolves the analyte from potential interferents. Nevertheless, a systematic investigation of potentially interfering substances must be made during development of all separation techniques. Interference effects will usually fall into one of two categories:

(1) The interferent is not separated from the analyte, and it enhances the signal, causing a falsely increased result; or it quenches the signal, causing a falsely decreased result.

(2) The interferent may affect the readout obtained for the internal standard, which ultimately affects the final, calculated, analytical result.

Testing and reporting of the effect of interfering substances on separation techniques should include:

- processing the appropriate sample "blank" (water, serum, solvent, etc.) through each of the steps of the separation and detection technique. Sometimes an interferent will be manifested as a spurious signal (spot, peak, readout, etc.) at the location where the analyte or internal standard is usually found;

- testing solvent, reagents, containers or support media from different manufacturers or from different lots or batches to define the conditions under which interferences may or may not be detected; and

- noting migration time, elution pattern, location of spot or peak, speed and direction of movement, or other physical manifestation, as appropriate, for any drugs, metabolites, or other exogenous or endogenous substances that can be detected within the time or space defined by the analyte and internal standard.

The choice of internal standard will sometimes be dictated by the location and intensity of any interfering substances in the system. By including two internal standards and checking the relative ratios of each to the other, interferents that masquerade as an internal standard may be detected.

A2. Guidelines for Measurement Procedures Based on Immunochemical Principles

Interference with immunochemical measurement procedures may generally be classified in a manner similar to that of other chemical reactions. As discussed in this document, various mechanisms may operate to cause either a falsely increased or decreased analytical result. Special attention must be given to the possibility that cross-reactivity or affinity of the antibody for compounds other than the analyte may exist. The specificity of the entire method depends upon the specificity of the antibody for the analyte in the environment where the analytical reaction(s) takes place.

In addition to characterizing the cross-reactivity between analyte and interferent when a measurement procedure is first evaluated and developed, manufacturers should check cross-reactivity for each lot of antibody marketed. Because of the biological systems used in producing the antibody, changes in antibody affinity may be introduced after the initial assessment. It is important that this information be provided to users.

In addition to analyte cross-reactive substances, the sample may contain endogenous human antibodies against the analyte or against the reagent antibodies used in the measurement procedures. For example, autoantibodies against thyroid can bind T4 or T3 and compete with the reagent antibody to produce an

Appendix A. (Continued)

interference in the apparent measurement result.[1] Circulating human heterophile antibodies may be produced against animal antibodies used therapeutically, such as in vaccines or used in specific tissue targeting of pharmacologic or radiographic agents. For example, heterophile antibodies against a therapeutically administered mouse antibody can react with mouse antibodies used in an immunochemical measurement procedure, alter the reactivity of the reagent antibodies, and alter the apparent measurement result. Heterophile antibodies frequently cause positive interference in sandwich-type methods by bridging of capture and label antibodies. However, the interference with a method will vary with details of the measurement procedure, and both spurious increases and decreases in analyte values have been reported.[2–5]

The presence of endogenous heterophile antibodies in a patient's serum can be detected by serial dilution of the suspect serum with a serum negative for heterophile antibody and evaluation of parallelism to a second part of sera. A second detection method is to preincubate the sample in question with nonimmune serum or an antibody of irrelevant specificity from the species in question to bind any heterophile antibody that may be present. Repeat measurement of the preincubated sample will have a different result if heterophile antibodies were present. Additional detection methods include separation of the heterophile antibody from the serum by Protein-A absorption, heat treatment sample, or other separation technique, as appropriate. Measurement procedures to specifically measure human antimouse antibodies are available.[6]

Recommendations:

- Cross-reactivity of an interferent should be tested both in the absence and in the presence of analyte at a concentration near the upper limit of the therapeutic range.

- Cross-reactivity of an antibody should be reported for all drugs or metabolites that might be expected to interfere, or that are commonly coadministered with the analyte. For example, cross-reactivity of a "phenobarbital" antibody should be checked by challenging the preparation with each of the barbiturates in use for the population of patients for whom the kit is to be used, and with drugs structurally similar to phenobarbital.

- Whenever possible, cross-reactivity should be checked by analyzing serum from patients with high therapeutic concentrations of related drugs which might be expected to interfere. Thus, a "phenobarbital" measurement system should be used to analyze serum from patients receiving no phenobarbital, but receiving secobarbital, butabarbital, pentobarbital, etc. Special attention should be given to those drugs that have been previously reported to have significant cross-reactivity with the antibodies to the analyte being evaluated. Calculation of cross-reactivity is shown in Equation (A1), and calculation of % interference is shown in Equation (A2) below.

$$\%\ \text{Cross-reactivity} = 100^{*}\left(\frac{\text{measured value - true value}}{\text{Concentration of interferent}}\right) \quad \text{(A1)}$$

$$\%\ \text{Interference} = 100^{*}\left(\frac{\text{measured value - true value}}{\text{true value}}\right) \quad \text{(A2)}$$

where the measured value is the result when both analyte and interferent are present in the sample analyzed. The concentration units for both analyte and interferent must be the same.

- Interference due to the possible presence in some patients' serum of human heterophile antibodies against the reagent antibodies should be investigated and documented by a technique appropriate to the analytical system.

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Appendix B. Analyte Test Concentrations

This appendix provides recommended test concentrations for many common analytes.

Analyte	MW	Test Concentration – Low		Test Concentration – High	
		(SI Units)	(Conv. Units)	(SI Units)	(Conv. Units)
Acetaminophen	151.16	33 µmol/L	5 µg/mL	199 µmol/L	30 µg/mL
Acetone	58.08	0.34 mmol/L	2 mg/dL	3.4 mmol/L	20 mg/dL
Albumin	66 000	35 g/L	3.5 g/dL	50 g/L	5 g/dL
Aldosterone	360.44	0.2 nmol/L	8 ng/dL	1.1 nmol/L	40 ng/dL
Alpha-fetoprotein	66 000	300 ng/L	30 ng/dL	150 µg/L	150 ng/mL
Ammonia	17.03	10 µmol/L	14 µg/dL	80 µmol/L	112 µg/dL
Apolipoprotein A1	28 000	0.8 g/L	80 mg/dL	1.8 g/L	180 mg/dL
Apolipoprotein B	549 000	0.6 g/L	60 mg/dL	1.3 g/L	130 mg/dL
Bilirubin, conjugated	842.9	2.4 µmol/L	0.2 mg/dL	86 µmol/L	5 mg/dL
Bilirubin, unconj.	584.67	17 µmol/L	1 mg/dL	257 µmol/L	15 mg/dL
C-reactive protein	114 000	0.01 g/L	1 mg/dL	0.04 g/L	4 mg/dL
Calcium, ionized	40.08	1 mmol/L	4 mg/dL	2 mmol/L	8 mg/dL
Calcium, total	40.08	2 mmol/L	8 mg/dL	3 mmol/L	12 mg/dL
Carbamazepine	236.26	12.7 µmol/L	3 µg/mL	50.8 µmol/L	12 µg/mL
Chloride	35.5	90 mmol/L	90 mEq/L	110 mmol/L	110 mEq/L
Cholesterol, HDL	386.66	0.9 mmol/L	35 mg/dL	1.8 mmol/L	70 mg/dL
Cholesterol, total	386.66	3.88 mmol/L	150 mg/dL	6.47 mmol/L	250 mg/dL
CO_2 (total)	44.01	20 mmol/L	20 mEq/L	35 mmol/L	35 mEq/L
Cortisol	362.47	138 nmol/L	5 µg/dL	828 nmol/L	30 µg/dL
Creatinine	113.12	133 µmol/L	1.5 mg/dL	442 µmol/L	5 mg/dL
Digoxin	780.92	0.5 nmol/L	0.4 ng/mL	2.6 nmol/L	2 ng/mL
Epinephrine	183.21	218 pmol/L	40 pg/mL	546 pmol/L	100 pg/mL
Estriol	288.39	139 nmol/L	40 ng/mL	1040 nmol/L	300 ng/mL
Ethanol	46.07	2.17 mmol/L	10 mg/dL	21.7 mmol/L	100 mg/dL
Ferritin	474 000	45 pmol/L	20 ng/mL	449 pmol/L	200 ng/mL
Folic acid	441.4	11 nmol/L	5 ng/mL	34 nmol/L	15 ng/mL
Follicle-stimulating hormone (FSH)	30 000	5 IU/L	5 mIU/mL	40 IU/L	40 mIU/mL
Glucose	180.16	4.4 mmol/L	80 mg/dL	6.7 mmol/L	120 mg/dL
Hemoglobin	64,456	100 g/L	10 g/dL	200 g/L	20 g/dL
Human chorionic gonadotropin (hCG)	36 700	5 IU/L	5 mIU/mL	50 IU/L	50 mIU/mL

Appendix B. (Continued)

Analyte	MW	Test Concentration – Low		Test Concentration – High	
		(SI Units)	(Conv. Units)	(SI Units)	(Conv. Units)
Iron	55.84	7.2 µmol/L	40 µg/dL	26.9 µmol/L	150 µg/dL
Iron binding capacity	NA	44.8 µmol/L	250 µg/dL	80.6 µmol/L	450 µg/dL
Lactate (as lactic acid)	90.08	0.7 mmol/L	6.3 mg/dL	2.6 mmol/L	23.4 mg/dL
Lead	207.19	0.48 µmol/L	10 µg/dL	4.83 µmol/L	100 µg/dL
Lithium	6.939	0.2 mmol/L	0.14 mg/dL	1.5 mmol/L	1.0 mg/dL
Luteinizing hormone (LH)	30 000	5 IU/L	5 mIu/mL	110 IU/L	110 mIu/mL
Magnesium	24.31	1.6 mmol/L	3.9 mg/dL	2.6 mmol/L	6.3 mg/dL
N-acetylprocainamide (NAPA)	277.37	18 mol/L	5 µg/mL	108 mol/L	30 µg/mL
Norepinephrine	169.18	0.65 nmol/L	110 pg/mL	4.14 nmol/L	700 pg/mL
Phenobarbital	232.24	215 mol/L	5 µg/mL	1722 mol/L	40 µg/mL
Phenylalanine	165.19	61 µmol/L	1 mg/dL	1211 µmol/L	20 mg/dL
Phenytoin	252.28	12 µmol/L	3 µg/mL	79 µmol/L	20 µg/mL
Phosphorus (inorganic)	30.97	0.8 mmol/L	2.5 mg/dL	2.1 mmol/L	6.5 mg/dL
Potassium	39.1	3 mmol/L	3 mEq/L	5 mmol/L	5 mEq/L
Prealbumin	30 000	100 g/L	10 mg/dL	400 g/L	40 mg/dL
Primidone	218.26	14 mol/L	3 µg/mL	55 mol/L	12 µg/mL
Procainamide	235.33	17 mol/L	4 µg/mL	42 mol/L	10 µg/mL
Progesterone	314.47	3.2 nmol/L	1 ng/mL	64 nmol/L	20 ng/mL
Protein (total serum)	NA	60 g/L	6 g/dL	80 g/L	8 g/dL
Quinidine	324.42	6.2 µmol/L	2 µg/mL	15 µmol/L	5 µg/mL
Salicylate (as salicylic acid)	138.12	0.14 mmol/L	2 mg/dL	1.45 mmol/L	20 mg/dL
Sodium	22.99	130 mmol/L	130 mEq/L	150 mmol/L	150 mEq/L
Testosterone	288.43	1.7 nmol/L	50 ng/dL	10.4 nmol/L	300 ng/dL
Theophylline	180.17	33.3 µmol/L	6 µg/mL	111 µmol/L	20 µg/mL
Thyroid stimulating hormone (TSH)	28 000	0.3 mIU/L	0.3 µIU/mL	8 mIU/L	8 µIU/mL
Thyroxine, free (FT4)	776.87	13 pmol/L	1 ng/dL	32.5 pmol/L	2.5 ng/dL
Transferrin	77 000	2 g/L	200 mg/dL	4 g/L	400 mg/dL
Triglycerides (total)	NA	1.7 mmol/L	150 mg/dL	5.6 mmol/L	500 mg/dL

Appendix B. (Continued)

Analyte	MW	Test Concentration – Low		Test Concentration – High	
		(SI Units)	(Conv. Units)	(SI Units)	(Conv. Units)
Triiodothyronine, free (FT3)	650.98	3.9 pmol/L	250 pg/dL	10.8 pmol/L	700 pg/dL
Tyrosine	181.19	221 µmol/L	4 mg/dL	1104 µmol/L	20 mg/dL
Urea	60.06 (Urea) 28.01 (Urea N)	3 mmol/L	9 mg/dL	7 mmol/L	40 mg/dL
Uric acid	168.11	0.2 mmol/L	3 mg/dL	0.5 mmol/L	9 mg/dL
Valproic acid	144.21	35 mol/L	5 µg/mL	693 mol/L	100 µg/mL
Vitamin B12 (cyanocobalamin)	1355.42	148 pmol/L	200 pg/mL	740 pmol/L	1000 pg/mL

Analytes not listed:

Enzymes: 2x and 10x the upper limit of the population reference interval.

Therapeutic drugs: Zero and upper limit of the therapeutic interval.

Other analytes: Low and high, or "normal" and "abnormal" values, as appropriate for the clinical use of the test.

Appendix C. Interferent Test Concentrations

This appendix provides recommended test concentrations for many common drugs, and some drug metabolites and anticoagulants.

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Acetaminophen	151	µmol/L	1324	66 – 200	>1324	28:08:92 Miscellaneous Analgesics and Antipyretics	
Acetazolamide	222	µmol/L	270	45.0 – 67.5	90 – 135	52:10 EENT Carbonic Anhydrase Inhibitors	Acetazolamide is a carbonic anhydrase inhibitor.
Acetylcysteine N-Acetylcysteine	163	mmol/L	10.2	3.4	–	48:24 Mucolytic Agents	Test concentration based on acetaminophen antidote. Rapidly metabolized to cysteine.
Acetylprocainamide N-Acetylprocainamide	277	mol/L	144	7.2 – 108	90.2 – 144	72:00 Local Anesthetics	Metabolite of procainamide
Acetylsalicylic acid	180	mmol/L	3.62	0.72 – 2.17	> 3.62	28:08:04 Nonsteroidal Anti-Inflammatory Agents	Also evaluate active metabolite salicylic acid.
Albuterol (Salbutamol)	239	µmol/L	1.67	0.08 – 0.84	–	12:12 Sympathomimetic (Adrenergic) Agents	
Allopurinol	136	µmol/L	294	36.8 – 147	–	92:00 Unclassified Therapeutic Agents	Also evaluate active metabolite aloxanthine.
Alprazolam	309	µmol/L	6.48	0.16 – 3.24	2.43	28:24:08 Anxiolytic, Sedative, Hypnotic Benzodiazepines	
Amikacin	586	µmol/L	136.8	17.1 – 68.4	42.8 – 68.4	8:12:02 Aminoglycosides	
Aminocaproic acid	131	µmol/L	6.11	0.76 – 3.05	3.05	20:12:16 Hemostatics	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Aminophylline (see Theophylline)	–	–	–	–	–	86:16 Respiratory Smooth Muscle Relaxants	Aminophylline is a modified form of theophylline.
5-Aminosalicylic acid	153	µmol/L	26.1	13.1	–	56:00 Gastrointestinal Drugs	Metabolite of sulfasalazine
Aminosalicylic acid p-Aminosalicylic acid	153	mmol/L	5.22	0.2 – 2.62	–	8:16:04 Antituberculosis Agent	
Amiodarone	682	µmol/L	8.92	0.74 – 4.46	3.71 – 5.20	24:08 Misc. Cardiac Agents	Also evaluate metabolite N-desethylamiodarone.
Amitriptyline	277	µmol/L	3.61	0.27 – 0.90	1.81 – 3.61	28:16:04 Antidepressants	Also evaluate metabolite 10-hydroxynortriptyline.
Amlodipine	409	nmol/L	245	49 – 123	–	24:02 Calcium Channel Blockers	
Amobarbital	226	µmol/L	88.4	4.42 – 22.1	66.3 – 88.4	28:24.04 Anxiolytic, Sedative, and Hypnotic Barbiturates	
Amoxapine	314	µmol/L	3.19	0.32 – 1.60	1.6	28:16:04 Antidepressants	
Amoxicillin	365	µmol/L	206	8.22 – 68.5	–	08:12:16 Penicillins	
Amphetamine (d- and l-)	135	µmol/L	7.4	1.48	7.4	28:20 Respiratory and Cerebral Stimulants	
Ampicillin	349	µmol/L	152	7.59 – 50.6	–	08:12:16 Penicillins	
Ascorbic acid (Vitamin C)	176	µmol/L	342	23 – 114	–	88:12 Vitamins	
Astemizole	459	µmol/L	0.65	0.22	–	04:00 Antihistamine Drugs	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Atenolol	266	µmol/L	37.6	0.75 – 7.52	37.6	24:01 Beta-Adrenergic Blockers	
Atorvastatin	1209	µg Eq/L	600	300	–	24:06 Antilipemic Agents	Measured using enzyme inhibition biological measurement procedure; results reported as "Atorvastatin" equivalents, which include parent compound and active metabolites (ortho- and para-hydroxylated metabolites).
Azathioprine	277	µmol/L	10.8	3.6	–	92:00 Unclassified Therapeutic Agents	Also evaluate active metabolite 6-Mercaptopurine.
Azithromycin	785	µmol/L	15.3	0.4 – 5.1	–	08:12:12 Macrolides	
Benazepril	461	µmol/L	48	0.24 – 23.6	–	24:03 Angiotensin Converting Enzyme (ACE) Inhibitors	
Benzoate, Sodium	144	mmol/L	2.9	0.97	5.0	92:00 Misc. Therapeutic Agents	Active metabolite of Benzyl alcohol
Bisoprolol	326	µmol/L	0.92	0.31	–	24:24 β-Adrenergic blocker	
Bromide	79.9	mmol/L	37.5	1.25 – 25	6.25 – 37.5	92:00 Misc. Therapeutic Agents	
Bupivacaine	288	µmol/L	57.3	2.8 – 19.1	0	72:00 Local Anesthetics	
Buproprion	240	µmol/L	12.5	0.21 – 0.42	12.5	28:16:04 Antidepressants	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Buspirone	386	nmol/L	38.9	13	–	28:24:92 Miscellaneous Anxiolytics, Sedatives, and Hypnotics	
Caffeine	194	µmol/L	308	25.7 – 103	103 – 308	28:20 Respiratory and Cerebral Stimulants	
Captopril	217	µmol/L	23	4.6	23	24:03:04 Angiotensin Converting Enzyme (ACE) Inhibitors	Also evaluate captopril disulphide (active metabolite).
Captopril disulphide	433	µmol/L	3.5	1.2	–	24:03:04 Angiotensin Converting Enzyme (ACE) Inhibitors	Active metabolite of captopril
Carbamazepine	236	µmol/L	127	12.7 – 50.8	50.8 – 127	28:12:92 Miscellaneous Anticonvulsants	Also evaluate carbamazepine-10, 11-epoxide (active metabolite of carbamazepine).
Carbamazepine-10, 11-epoxide	252	µmol/L	59.4	0.08 – 23.8	31.7 – 59.4	28:12:92 Miscellaneous Anticonvulsants	Active metabolite of carbamazepine; anticonvulsant activity similar to the parent drug
Carisoprodol	260	µmol/L	115	19.2 – 26.9	26.9 – 115	12:20 Skeletal Muscle Relaxants	Also evaluate active metabolite meprobamate.
Cefaclor	386	µmol/L	194	13 – 65	–	08:12:06 Cephalosporins	
Cefadroxil	381	µmol/L	276	26.2 – 91.9	–	08:12:06 Cephalosporins	
Cefazolin	454	µmol/L	2643	37.4 – 881	–	08:12:06 Cephalosporins	
Cefotaxime	455	µmol/L	673	92 – 224	–	08:12:06 Cephalosporins	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Cefoxitin	427	µmol/L	1546	47 – 515	–	08:12:07 Misc β Lactams	
Ceftriaxone	555	µmol/L	1460	90 – 486	–	08:12:06 Cephalosporins	
Cefuroxime	424	µmol/L	1416	236 – 472	–	08:12:06 Cephalosporins	
Cephalexin	347	µmol/L	337	11.5 – 112	–	08:12:06 Cephalosporins	
Cephalothin	396	µmol/L	760	51 – 253	–	08:12:06 Cephalosporins	
Cephapirin sodium	445	µmol/L	876	79 – 292	–	08:12:06 Cephalosporins	
Cetirizine	389	µmol/L	7.7	2.6	–	04:00 Antihistamine Drugs	
Chloral Hydrate (see metabolite Trichloroethanol)	165	NA	NA	NA	NA	28:24:92 Miscellaneous Anxiolytics, Sedatives, and Hypnotics	Evaluate active metabolite 2,2,2-trichloroethanol
Chloramphenicol	323	µmol/L	155	30.9 – 77.3	77.3 – 124	08:12:08 Chloramphenicol 52:04:04 EENT Antibiotics	
Chlordiazepoxide	300	µmol/L	33.3	3.33 – 16.7	16.7 – 33.3	28:24:08 Anxiolytic, Sedative, Hypnotic Benzodiazepines	
Chlorothiazide	296	µmol/L	67.6	6.76 – 33.8	–	40:28 Diuretics	
Chlorpromazine	319	µmol/L	6.3	0.03 – 1.57	2.35 – 6.27	28:16:08 Antipsychotics/Tranquilizers	
Chlorpropamide	277	mmol/L	2.7	0.1 – 1.3	0.7 – 2.7	68:20:20 Sulfonylureas	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Cimetidine	252	µmol/L	79.2	1.98 – 29.7	79.2	56:40 Miscellaneous GI Drugs	
Ciprofloxacin	331	µmol/L	30.2	1.51 – 15.1	–	08:22 Quinolones (systemic) 52:04:04 EENT Antibiotics	
Citalopram	324	µmol/L	1.85	0.92	–	28:16:04 Antidepressants	
Clarithromycin	748	µmol/L	26.7	13.4	–	08:12:12 Macrolides	Also evaluate metabolite 14 (R)-hydroxyclarithromycin).
Clavulanate potassium	237	µmol/L	29.5	14.8	–	08:12:16 Penicillins	Based on a typical dosage.
Clindamycin	505	µmol/L	89.1	4.0 – 29.7	–	08:12:28 Miscellaneous Antibiotics 84:04:04 Topical Antibiotics	
Clonazepam	316	nmol/L	507	31.7 – 254	254 – 317	28:12:08 Anticonvulsant Benzodiazepines	Based on typical dosage.
Clonidine	230	nmol/L	43.5	0.87 – 8.70	8.70 – 43.5	24:05 Misc. Hypotensive Agents	
Clorazepate	315	µmol/L	6.36	0.32 – 3.18	3.18	28:24:08 Anxiolytic, Sedative, Hypnotic Benzodiazepines	Also evaluate active metabolite nordiazepam and oxazepam.
Codeine	299	µmol/L	5.34	0.03 – 1.00	5.34	28:08:08 Opiate Agonists 48:08 Antitussives	Significant tolerance can develop with chronic use of opiates. "Lethal" concentrations may be therapeutic in some patients.

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Cotinine	176	µmol/L	10.8	0.05 – 0.54	–	12:92 Miscellaneous Autonomic Drugs	Metabolite of nicotine. Levels may be 5 to 20x higher in cirrhosis.
Cyclobenzaprine	275	µmol/L	1.45	0.15	1.45	12:20 Skeletal Muscle Relaxants	
Cyclophosphamide	261	µmol/L	1437	479	–	10:00 Antineoplastic Agents	Test level is based on typical dosage.
Descarboethoxyloratadine (Desloratadine)	311	µmol/L	0.97	0.32	–	04:00 Antihistamine Drugs	Metabolite of loratadine
Desethylamiodarone [N-Desethylamiodarone (Noramiodarone)]	645	µmol/L	5.4	1.5 – 3.9	3.1 – 5.4	24:08 Misc. Cardiac Agents	Metabolite of Amiodarone
Desipramine	266	µmol/L	3.75	0.19 – 1.13	1.5 – 3.75	28:16:04 Antidepressants	Active metabolite of imipramine
Dexamethasone	393	µmol/L	1.53	0.51	–	52:08 EENT Anti-Inflammatory Agents 68:04 Adrenals	
Dextran 40	40 000	g/L	60	10 – 20	–	40:12 Replacement Agents	
Dextromethorphan	271	µmol/L	3.7	0.74 – 1.48	3.7	48:08 Antitussives, Expectorants, and Mucolytic Agents	
Diatrizoate, sodium	636	µmol/L	314	–	–	36:68 Diagnostic Agent for Roentgenography	Test level is based on typical dosage.

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Diazepam	285	µmol/L	18	0.7 – 8.8	11 – 18	12:20 Skeletal Muscle Relaxants 28:12:08 Anticonvulsant Benzodiazepines 28:24:08 Anxiolytic, Sedative, Hypnotic Benzodiazepines	
Diclofenac	296	µmol/L	169	8.45	169	52:08 EENT Anti-Inflammatory Agents	
Diethyldithiocarbamate (Dithiocarb)	148	µmol/L	30	2.0 – 10.1	–	92:00 Misc. Therapeutic Agents	Metabolite of Disulfiram
Digoxin	781	nmol/L	7.8	1.0 – 2.6	>3.8	24:08 Misc. Cardiac Agents	
Dihydrocodeine	301	µmol/L	3.3	0.23 – 0.83	3.32	28:08 Opiate Agonist	Also known as alpha-6-hydrocodol
Diltiazem	415	µmol/L	15	0.1 – 1.0	8.9 – 15	24:02 Calcium Channel Blockers	
Diphenhydramine	255	µmol/L	19.6	0.78 – 1.96	19.6	04:00 Antihistamine Drugs	
Diphenoxylate	453	nmol/L	66.2	22.1	–	56:08 Antidiarrheal Agent	Rapidly cleared or metabolized; not normally detectable in plasma after three hours. Evaluate metabolite diphenoxylic acid.
Diphenoxylic acid	415	nmol/L	1157	386	–	56:08 Antidiarrheal Agent	Metabolite of diphenoxylate
Disopyramide	340	µmol/L	29.5	5.90 – 14.8	20.7 – 23.6	24:08 Misc. Cardiac Agents	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Disulfiram	297	µmol/L	16.9	1.7 – 8.4	16.9	92:00 Unclassified Therapeutic Agents	Also evaluate active metabolite diethyldithiocarbamate (dithiocarb).
Dopamine	153	µmol/L	5.87	1.96	–	12:12 Sympathomimetic (Adrenergic) Agents	Metabolized rapidly; half-life is on the order of a few minutes.
Doxazosin	452	µmol/L	1.33	0.44	–	24:04 Alpha Adrenergic Blockers	
Doxepin	279	µmol/L	3.22	0.32 – 1.07	1.79 – 3.58	28:16:04 Antidepressants	Also evaluate nordoxepin (active metabolite).
Doxycycline	445	µmol/L	67.5	22.5	67.5	08:12:24 Tetracyclines	
EDTA	292	µmol/L	3.4			20:12:04 Anticoagulants	Test level is based on typical dosage. Also evaluate simulated "short draw" with prefilled heparin collection tubes.
Enalapril maleate – see Enalaprilat	492	NA	NA	NA	NA	24:32:04 Angiotensin Converting Enzyme (ACE) Inhibitors	Evaluate active metabolite enalaprilat.
Enalaprilat	349	µmol/L	0.86	0.29	–	24:32:04 Angiotensin Converting Enzyme (ACE) Inhibitors	Active metabolite of enalapril.
Epoetin alfa	30,400	mU/L	15	3 – 5	–	20:16 Hematopoietic Agents	Recombinant human erythropoietin
Equilin	268	µmol/L	56	18.7	–	68:16:04 Estrogens	Equine hormone - component of conjugated estrogens

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Erythromycin	734	µmol/L	81.6	2.72 – 27.2	–	08:12:12 Macrolides 52:04:04 EENT Antibiotics 84:04:04 Topical Antibiotics	
Estazolam	295	µmol/L	2.03	0.68	–	28:24:08 Anxiolytic, Sedative, Hypnotic Benzodiazepines	
Estradiol	272	nmol/L	4.41	0.03 – 1.47	–	68:16 Estrogens	Component of conjugated estrogens
Estrone	270	nmol/L	2.77	0.04 – 0.92	–	68:16 Estrogens	Component of conjugated estrogens
Ethambutol	204	µmol/L	58.7	4.9 – 29.3	48.9	08:16 Antituberculosis Agents	
Ethanol	46.1	mmol/L	86.8	21.7 – 43.4	43.4	40:20 Caloric Agents 72:00 Local Anesthetics 84:04:16 Miscellaneous Topical Anti-Infectives	
Ethchlorvynol	145	µmol/L	692	13.8 – 138	138 – 692	28:16:04 Antidepressants	
Ethosuximide	141	µmol/L	1770	283 – 708	708 – 1770	28:12 Anticonvulsants	
Ethyl ether	74	mmol/L	2.7	1.22 – 1.35	–	92:00 Misc. Therapeutic Agents	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Ethylene glycol	62.1	mmol/L	4.83	–	0.32 – 2.42	92:00 Misc. Therapeutic Agents	Also evaluate metabolite oxalate.
Famotidine	337	µmol/L	1.78	0.59	–	56:40 Miscellaneous GI Drugs	
Felodipine	384	nmol/L	78	26	–	24:02 Calcium Channel Blockers	
Fenofibrate	361	µmol/L	125	41.6	–	24:06 Antilipemic Agents	
Fenoprofen	242	µmol/L	805	82.6 – 268	–	28:08:04 Nonsteroidal Anti-Inflammatory Agents	
Flecainide	414	µmol/L	5.3	0.48 – 2.65	1.21 – 3.62	24:08 Misc. Cardiac Agents	
Fluconazole	306	µmol/L	245	65.2 – 81.5	–	08:12:04 Antifungal Antibiotics	
Fluorescein	376	µmol/L	1064	213 – 426	–	36:96 Miscellaneous Diagnostic Agent	
Flucytosine	129.1	mmol/L	2.33	0.16 – 0.78		8:14:32 Antifungal Agents	
Fluoride	19	µmol/L	105	0.7 – 21	–	92:00 Misc. Therapeutic Agents	
Fluorouracil 5-Fluorouracil	130	mmol/L	3	0.1 – 1.0	–	10:00 Antineoplastic Agents 84:36 Miscellaneous Topical Agents	Rapidly cleared or metabolized; not normally detectable in plasma after three hours.

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Fluoxetine	309	µmol/L	11.2	0.3 – 1.3	–	28:16:04 Antidepressants	Lethal 4 to 20 µmol/L. Also evaluate metabolite norfluoxetine.
Flurbiprofen	244	µmol/L	184	61.4	–	52:36 Miscellaneous EENT Drugs	
Furosemide	331	µmol/L	181	30.2 – 90.6	90.6	40:28 Diuretics	
Gabapentin	171	µmol/L	526	5.84 – 175	–	28:12:92 Miscellaneous Anticonvulsants	
Gemfibrozil	250	µmol/L	300	100	–	24:06 Antilipemic Agents	
Gentamicin	478	µmol/L	21	10.5 – 20.9 (peak)		08:12:02 Aminoglycosides 52:04:04 EENT Antibiotics	
Gentisic acid	154	µmol/L	117	13.0 – 39.0	–	28:08:04 Nonsteroidal Anti-Inflammatory Agents	A salicylate metabolite
Glipizide	446	µmol/L	4.48	2.24	4.48	68:20:20 Sulfonylureas	
Glutethimide	217	µmol/L	138	9.2 – 27.6	92 – 138	28:24 Sedative-hypnotic	
Glyburide	494	µmol/L	3.89	1.3	–	68:20:20 Sulfonylureas	
Guaifenesin	198	mmol/L	15.2	5.05	–	48:16 Expectorants	Rapidly cleared or metabolized; not normally detectable in plasma after three hours.
Haloperidol	376	µmol/L	2.66	0.01 – 0.53	0.13 – 2.66	28:16:08 Antipsychotics/Tranquilizers	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Halothane	197	µmol/L	759	101 – 253	–	Anesthetic (inhalation)† (USP 2000)	
Heparin	Varies	U/L	3000	350 – 1000	–	20:12:04 Anticoagulants	Test level is based on typical dosage. Also evaluate simulated "short draw" with prefilled heparin collection tubes.
Hydrochlorothiazide	298	µmol/L	20.2	1.68 – 6.72	–	40:28 Diuretics	
Hydrocodone	299	µmol/L	0.67	0.07 – 0.17	0.67	28:08:08 Opiate Agonists	
Hydromorphone	285	nmol/L	702	3.51 – 176	351 – 702	28:08:08 Opiate Agonists	
Hydroxyclarithromycin 14 (R)-Hydroxyclarithromycin	764	µmol/L	19.6	6.54	–	08:12:12 Macrolides	Metabolite of clarithromycin
Hydroxynortriptyline 10-Hydroxynortriptyline	279	µmol/L	2.47	0.82	–	28:16:04 Antidepressants	Metabolite of amitryptiline and nortriptyline
Hydroxyzine	375	µmol/L	2.67	0.27	2.67	04:00 Antihistamine Drugs 28:24:92 Miscellaneous Anxiolytics, Sedatives, and Hypnotics	
Ibuprofen	206	µmol/L	2425	48.5 – 340	485 – 2425	28:08:04 Nonsteroidal Anti-Inflammatory Agents	
Imipramine	280	µmol/L	2.5	0.71 – 1.25	1.79	28:16:04 Antidepressants	Also evaluate active metabolite desipramine.

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Indomethacin	358	µmol/L	100	14.0 – 50.2	–	28:08:04 Nonsteroidal Anti-Inflammatory Agents	
Iodide	127	mmol/L	2.99	–	–	48:16 Expectorants 68:36:08 Antithyroid Agents	
Isoniazid	137	µmol/L	292	7.29 – 146	146 – 292	08:16 Antituberculosis Agents	Also evaluate metabolite N-acetylisoniazid.
Isosorbide dinitrate (Isosorbide 2,5-dinitrate)	236	nmol/L	636	212	–	24:12:08 Vasodilating Drugs	
Isosorbide mononitrate	190	µmol/L	1.58	0.53	–	24:12:08 Vasodilating Drugs	
Kanamycin	485	µmol/L	124	28.8 – 61.8	51.5	08:12:02 Aminoglycosides	
Ketamine	238	µmol/L	42.1	16.8 – 21.1	42.1	28:04 General Anesthetics	
Levofloxacin	370	µmol/L	48.6	16.2	–	08:22 Quinolones (systemic)	
Levothyroxine	777	µmol/L	1.29	0.06 – 0.15	1.29	68:36:04 Thyroid Agents	
Lidocaine	234	µmol/L	51.2	5.1 – 25.6	25.6 – 38.4	24:08 Misc. Cardiac Agents 72:00 Local Anesthetics 84:08 Topical Antipruritics and Local Anesthetics	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Lisinopril	406	µmol/L	0.74	0.25	–	24:32:04 Angiotensin Converting Enzyme (ACE) Inhibitors	
Lithium	6.94	mmol/L	3.2	0.6 – 1.4	2	28:28 Antimanic Agents	
Lofepramine	419	µmol/L	71.7	23.9	–	Antidepressant† (USP 2000)	Also evaluate active metabolite desipramine.
Loratadine	383	µmol/L	0.78	0.26	–	04:00 Antihistamine Drugs	Also evaluate metabolite descarboethoxyloratadine.
Lorazepam	321	µmol/L	3.11	0.16 – 0.93	0.93 – 3.11	28:24:08 Anxiolytic, Sedative, Hypnotic Benzodiazepines	
Medroxyprogesterone acetate	387	µmol/L	2.09	0.03 – 0.70	–	68:32 Progestins	Test concentrations based on treatment of breast cancer (400 to 2000 mg/day). Typical doses for other uses are 2.5 to 10 mg/day.
Meperidine	247	µmol/L	20.2	1.21 – 4.04	20.2	28:08:08 Opiate Agonists	
Mephenytoin	218	µmol/L	91.6	4.58 – 22.9	18.3 – 91.6	28:12:12 Anticonvulsant Hydantoins	Also evaluate metabolite normephenytoin.
Mephobarbital	246	µmol/L	60.9	28.4	32.5 – 60.9	28:24:04 Anxiolytic, Sedative, and Hypnotic Barbiturates	Also evaluate metabolite Phenobarbital.
Mepivacaine	246	µmol/L	81.2	4.06 – 40.6	24.4 – 40.6	72:00 Local Anesthetics	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Meprobamate	218	µmol/L	458	13.7 – 119	183 – 458	28:24:92 Miscellaneous Anxiolytics, Sedatives, and Hypnotics	
Mercaptopurine 6-Mercaptopurine	152	µmol/L	13.1	1.31 – 6.57	6.57	10:00 Antineoplastic Agents	Metabolite of azathioprine
Mesoridazine	387	µmol/L	13	0.26 – 3.63	–	28:16:08 Antipsychotics/Tranquilizers	Metabolite of thioridazine
Metformin	129	µmol/L	310	31	38.7 – 310	68:20:92 Miscellaneous Antidiabetic Agents	
Methadone	310	µmol/L	6.46	0.16 – 3.23	3.23 – 6.46	28:08:08 Opiate Agonists	
Methicillin Sodium	402	µmol/L	597	44.8 – 199	–	08:12:28 Miscellaneous Antibiotics	Test level is based on typical dosage.
Methocarbamol	241	µmol/L	1038	108 – 208	1038	12:20 Skeletal Muscle Relaxants	
Methohexital Sodium (Methohexitone)	262	µmol/L	114	7.62 – 38.1	38.1	28:24:04 Anxiolytic, Sedative, and Hypnotic Barbiturates	
Methotrexate	455	mmol/L	2	> 1.10	–	10:00 Antineoplastic Agents	Test level based on high dose regimen; plasma may exceed 1000 µmol/L.
Methoxynaphthyl-acetic acid 6-Methoxy-2-naphthylacetic acid	216	µmol/L	1386	462	–	28:08:04:92 Nonsteroidal Anti-Inflammatory Agents	Active metabolite of nabumetone

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Methyldopa	211	µmol/L	71	4.73 – 35.5	33.1 – 47.3	24:08:04 Hypotensive Agent	
Methylphenidate	233	µmol/L	1.29	0.43	–	28:20 Respiratory and Cerebral Stimulants	
Methyprylon	183	µmol/L	273	43.7 – 54.6	273	28:24: Anxiolytic, Sedative	Not commercially available in the U.S.
Metoclopramide	300	µmol/L	1.5	0.5	–	56:40 Miscellaneous GI Drugs	
Metoprolol	267	µmol/L	18.7	0.28 – 1.87	18.7	24:01 Beta-Adrenergic Blockers	
Metronidazole	171	µmol/L	701	35 – 234	–	08:40 Miscellaneous Anti-Infectives 84:04:04 Topical Antibiotics	
Mexiletine	179	µmol/L	22.3	2.8 – 11.2	8.37 – 22.3	24:08 Misc. Cardiac Agents	
Morphine	285	µmol/L	1.75	0.04 – 0.35	0.70 – 1.75	28:08:08 Opiate Agonists	Significant tolerance can develop with chronic use of opiates. "Lethal" concentrations may be therapeutic in some patients.
Nabumetone - see metabolite 2-(6-Methoxynaphthyl)acetic acid	NA	NA	NA	NA	NA	28:08:04 Nonsteroidal Anti-Inflammatory Agents	Evaluate active metabolite 2-(6-methoxynaphthyl)acetic acid
Nadolol	309	µmol/L	3.88	1.29	–	24:24 β-Adrenergic blocker	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Naproxen	230	µmol/L	2170	130 – 521	–	28:08:04 Nonsteroidal Anti-Inflammatory Agents	
N-Desethylamiodarone (Noramiodarone)	645	µmol/L	7.75	1.55 – 3.87	3.10 – 5.42	24:08 Misc. Cardiac Agents	Metabolite of amiodarone
Nefazodone	470	µmol/L	6.39	2.13	–	28:16:04 Antidepressants	
Netilmicin	476	µmol/L	42	1.0 – 21	31.5	08:12:02 Aminoglycosides	
Nicotine	162	µmol/L	6.2	0.15 – 0.31	6.16	12:92 Miscellaneous Autonomic Drugs	
Nifedipine	346	nmol/L	1156	145 – 578	289	24:02 Calcium Channel Blockers	
Nitrofurantoin	238	µmol/L	16.8	8.4	12.6	08:36 Urinary Anti-Infectives	
Nizatidine	332	µmol/L	9.06	3.02	–	56:28:12 Antiulcer agents	
Nordiazepam	271	µmol/L	18.5	0.22 – 7.38	18.5	28:24:08 Anxiolytic, Sedative, Hypnotic Benzodiazepines	Active metabolite of diazepam, N-desmethyldiazepan and chlordiazepoxide. Also evaluate active metabolite oxazepam. Test level is based on common dosage amounts.
Nordoxepin	265	µmol/L	3.77	0.34 – 1.13	1.89 – 3.77	28:16:04 Antidepressants	Active metabolite of doxepin
Norfluoxetine	295	µmol/L	7.46	0.17 – 3.73	3.39	28:16:04 Antidepressants	Active metabolite of fluoxetine

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Norpropoxyphene	326	µmol/L	13.5	0.61 – 6.75	9.21	28:08:92 Miscellaneous Analgesics and Antipyretics	Active metabolite of propoxyphene
Nortriptyline	263	µmol/L	3.80	0.19 – 0.57	0.76 – 3.80	28:16:04 Antidepressants	Also evaluate metabolite 10—Hydroxynortriptyline. Noramitriptyline also occurs as metabolite of Amitriptyline.
Norverapamil	441	µmol/L	4.54	0.11 – 0.45	0.91 – 4.54	24:02 Calcium Channel Blockers	Active metabolite of verapamil
Olanzapine	312	µmol/L	0.96	0.32	–	28:16:08 Antipsychotics/Tranquilizers	
Omeprazole	345	µmol/L	17.4	5.8	–	56:28:36 Antiulcer agents Proton pump inhibitor	
Oxazepam	287	µmol/L	17.5	0.52 – 4.89	6.98 – 17.5	28:24:08 Anxiolytic, Sedative, Hypnotic Benzodiazepines	Also occurs as metabolite of nordiazepam.
Oxycodone	315	µmol/L	1.59	0.03 – 0.32	0.63 – 1.59	28:08:08 Opiate Agonists	
Paroxetine	329	µmol/L	3.04	0.3	3.04	28:16:04 Antidepressants	
Penicillamine	149	µmol/L	161	26.8 – 53.7	–	64:00 Heavy Metal Antagonist	
Pentazocine	285	µmol/L	17.5	0.35 – 3.5	3.5 – 17.5	28:08:12 Opiate Partial Agonists	
Pentobarbital	226	µmol/L	354	4.42 – 177	44.2 – 133	28:24:04 Anxiolytic, Sedative, and Hypnotic Barbiturates	Pentobarbital is also the major accumulating metabolite of thiopental.

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Perphenazine	404	nmol/L	223	1.98 – 74.4	–	28:16:08 Antipsychotics/Tranquilizers	
Phenelzine	136	µmol/L	3.67	0.15	3.67	28:16:04:12 MAO Inhibitor	
Phenmetrazine	177	µmol/L	5.64	1.69	5.64	28:20 Anorexigenic Agent Respiratory and Cerebral stimulant	
Phenobarbital	232	µmol/L	431	43.1 – 172	215 – 431	28:12:04 Anticonvulsant Barbiturates 28:24:04 Anxiolytic, Sedative, and Hypnotic Barbiturates	Also evaluate active metabolite pentabarbital. Also occurs as a metabolite of primidone.
Phenytoin	252	µmol/L	198	19.8 – 79.2	79.2 – 198	28:12:12 Anticonvulsant Hydantoins	
Pindolol	248	µmol/L	4.03	0.4	4.03	24:24 β-Adrenergic blocker	
Piroxicam	331	µmol/L	181	3.02 – 60.4	–	28:08:04 Nonsteroidal Anti-inflammatory Agents	
Prednisolone	361	µmol/L	8.31	2.77	–	52:08 EENT Anti-inflammatory Agents 68:04 Adrenals	Metabolite of prednisone
Prednisone	358	µmol/L	0.84	0.28	–	68:04 Adrenals	Also evaluate metabolite prednisolone.

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Primidone	218	µmol/L	183	9.2 – 87.1	68.7 – 183	28:12:04 Anticonvulsant Barbiturates	Also evaluate active metabolite Phenobarbital.
Probenecid	285	µmol/L	2100	350 – 700	–	40:40 Uricosuric Agents	
Procainamide	235	µmol/L	102	17 – 51	42.5 – 68.0	24:08 Misc. Cardiac Agents	Also evaluate active metabolite N-Acetylprocainamide (NAPA).
Procaine	236	µmol/L	169	46.5 – 84.6	84.6	72:00 Local Anesthetics	
Prochlorperazine	374	µmol/L	2.67	0.13 – 0.80	0.80 – 2.67	28:16:08 Antipsychotics/Tranquilizers 56:22 Antiemetics	
Promethazine	284	µmol/L	4.22	0.35 – 2.11	3.52	04:00 Antihistamine Drugs 28:24:92 Miscellaneous Anxiolytics, Sedatives, and Hypnotics	
Propoxyphene	326	µmol/L	4.91	0.15 – 2.46	3.07	28:08:92 Miscellaneous Analgesics and Antipyretics	
Propranolol	259	µmol/L	7.71	0.02 – 3.86	3.86 – 7.71	24:01 Beta-Adrenergic Blockers	
Protriptyline	263	µmol/L	3.8	0.19 – 0.91	0.76 – 3.80	28:16:04:28 Tricyclics and Other Norepinephrine-reuptake Inhibitors	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Pseudoephedrine	165	µmol/L	60.5	1.82 – 6.05	60.5	12:12 Sympathomimetic (Adrenergic) Agents	
Quinidine	324	µmol/L	37	4.62 – 18.5	24.6 – 30.8	24:04:04 Antiarrhythmic Agents	
Quinine	324	µmol/L	148	6.16 – 49.3	–	08:20 Antimalarial Agents	
Ranitidine	314	µmol/L	19.1	0.32 – 6.36	–	56:40 Miscellaneous GI Drugs	
Rifampin	823	µmol/L	78.1	4.88 – 39.0	61	08:16 Antituberculosis Agents	
Risperidone	411	µmol/L	0.88	0.02 – 0.29	–	28:16:08 Antipsychotics/Tranquilizers	
Salbutamol - see Albuterol	NA	NA	NA	NA	NA	12:12 Sympathomimetic (Adrenergic) Agents	
Salicylamide	137	mmol/L	0.7	0.02 – 0.23	–	28:08:92 Miscellaneous Analgesics and Antipyretics	
Salicylic acid	138	mmol/L	4.34	0.72 – 2.17	2.9	28:08:04 Nonsteroidal Anti-Inflammatory Agents	Metabolite of acetylsalicylic acid (aspirin)
Secobarbital Sodium	260	µmol/L	84	4.2 – 21	63 – 84	28:24:04 Anxiolytic, Sedative, and Hypnotic Barbiturates	
Sertraline	306	µmol/L	1.96	0.10 – 0.98	1.64	28:16:04 Antidepressants	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Sildenafil citrate	667	pmol/L	12.9	0.67 – 1.80	–	92:00 Misc. Therapeutic Agents	
Spironolactone	417	µmol/L	1.44	0.48	–	40:28:10 Potassium-Sparing Diuretics	Also evaluate active metabolite canrenone.
Sulfadiazine (see Sulfasalazine)	NA	NA	NA	NA	NA	08:12:20 Sulfonamides	
Sulfamethoxazole	253	mmol/L	1.58	0.20 – 0.59	1.19 – 1.58	08:40 Miscellaneous Anti-Infectives	
Sulfapyridine	249	mmol/L	1.2	0.30 – 0.36	1.20	8:12:20 Sulfonamides	Metabolite of Sulfasalazine (Sulfadiazine)
Sulfasalazine/Sulfadiazine	398	µmol/L	754	50.3 – 302	754	56:40 Miscellaneous GI Drugs	Also evaluate metabolite sulfapyridine.
Sulfisoxazole	267	mmol/L	1.12	0.34 – 0.37	1.12	8:12:20 Sulfonamides	
Sumatriptan	295	nmol/L	1017	61.0 – 339	–	28:92 Misc. CNS Agents	
Tacrolimus	804	nmol/L	50	3.7 – 24.8	18.7 – 24.8	92:00 Unclassified Therapeutic Agents	
Tamoxifen	372	µmol/L	4.00	1.35	–	10:00 Antineoplastic Agents	
Temazepam	301	µmol/L	16.7	3.33	16.7	28:24:08 Anxiolytic, Sedative, Hypnotic Benzodiazepines	
Terazosin	387	µmol/L	7.8	0.26 – 2.58	–	24:04 Alpha Adrenergic Blockers	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Terfenadine	472	nmol/L	85	21.2	84.8	4:00 Antihistamines	No longer commercially available in the U.S. Also evaluate terfenadine 'acid metabolite' (active metabolite).
Terfenadine 'acid metabolite'	504	µmol/L	2	0.8	1.99	4:00 Antihistamines	Active metabolite of terfenadine
Tetracycline	444	µmol/L	34	4.5 – 11.3	–	08:12:24 Tetracyclines	
Theophylline	180	µmol/L	222	27.8 – 111	111 – 222	86:16 Respiratory Smooth Muscle Relaxants	Also evaluate active metabolite caffeine. Aminophylline is a modified form of theophylline.
Thiocyanate	58.1	µmol/L	6880	1720 – 3440	2064 – 3440	24:08:20 Direct Vasodilators	Metabolite of nitroprusside
Thiocyanate (Thiocyanic acid)	59	µmol/L	1720	51.6 – 499	860 – 1720	24:08:20 Direct Vasodilators	Metabolite of nitroprusside
Thiopental (Thiopentone)	242	µmol/L	248	4.13 – 20.6	82.5 – 248	28:24:04 Anxiolytic, Sedative, and Hypnotic Barbiturates	Also evaluate active metabolite pentobarbital (Pentobarbitone).
Thioridazine	371	µmol/L	14	0.27 – 7.02	5.4	28:16:08 Antipsychotics/Tranquilizers	Also evaluate active metabolites mesoridazine and sulphoridazine.
Timolol	316	µmol/L	1.9	0.16 – 0.63	–	52:36 Miscellaneous EENT Drugs	
Tobramycin	468	µmol/L	51.4	8.56 – 25.7	25.7	08:12:02 Aminoglycosides	
Tocainide	192	µmol/L	130	20.8 – 62.4	52 – 130	24:04:04 Antiarrhythmic Agents	
Tolbutamide	270	mmol/L	2.37	0.2 – 0.4	–	68:20:20 Sulfonylureas	Lethal 2.4 mmol/L

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Tramadol	263	µmol/L	11.4	3.8	–	28:08:12 Opiate Partial Agonists	
Tranylcypromine	133	µmol/L	2.25	0.75	2.25	28:16:04:12 Monoamine Oxidase Inhibitors	
Trazodone	372	µmol/L	13.5	1.35 – 6.73	8.61 – 10.8	28:16:04 Antidepressants	
Triamterene	253	µmol/L	35	1.19 – 11.9	–	40:28:10 Potassium-Sparing Diuretics	
Triazolam	343	nmol/L	116	58.2	116	28:24:08 Anxiolytic, Sedative, Hypnotic Benzodiazepines	
Trichloroethanol 2, 2, 2-Trichloroethanol	149	µmol/L	335	13.4 – 80.3	134 – 335	28:24:92 Miscellaneous Anxiolytics, Sedatives, and Hypnotics	Active metabolite of chloral hydrate
Trifluoperazine hydrochloride	480	µmol/L	2.08	0.10 – 0.63	0.10 – 2.08	28:16:08:04 Phenothiazines	
Trimethobenzamide	389	µmol/L	15.4	2.57 – 5.14	–	56:22 Antiemetics	
Trimethoprim	290	µmol/L	138	17.2 – 68.8	68.8	08:36 Urinary Anti-Infectives	
Troglitazone	442	µmol/L	20.4	2.26 – 6.80	–	68:20:92 Miscellaneous Antidiabetic Agents	Not commercially available in the U.S.
Valproic acid	144	µmol/L	3467	347– 867	693 – 3467	28:12:92 Miscellaneous Anticonvulsants	
Vancomycin	1449	µmol/L	69	12.4 – 27.6	27.6 – 69.0	08:12:28 Miscellaneous Antibiotics	

Appendix C. (Continued)

Generic Name	MW	Units	Test Concentration	Therapeutic Concentration	Toxic Concentration	AHFS* Drug Class	Comments
Venlafaxine	277	µmol/L	1.08	0.36	–	28:16:04 Antidepressants	
Verapamil	455	µmol/L	4.4	0.11 – 1.32	0.88 – 4.40	24:02 Calcium Channel Blockers	Also evaluate metabolite norverapamil.
Warfarin	308	µmol/L	32.5	3.2 – 10	32.5	20:12:04 Anticoagulants	
Zolpidem	307	µmol/L	9.75	0.26 – 0.98	9.75	28:24:92 Miscellaneous Anxiolytics, Sedatives, and Hypnotics	

*** American Hospital Formulary Service (AHFS) Therapeutic Class**

† USP 2000

Appendix D. Interference Test Concentrations for Endogenous Analytes

Compound	Molecular Weight	Reference Interval [1]	Pathological Values[2]	Recommended Test Concentration[3] (common pathological value)	Comments
Acetoacetate	102	< 0.1 mmol/L	2 mmol/L	2.0 mmol/L	
Acetone	58	0.05 – 0.34 mmol/L	12 mmol/L	12 mmol/L	
Ammonia	80	11 – 32 µmol N/L	107 µmol N/L	107 µmol N/L	
L-Ascorbic Acid	176	23 – 85 µmol/L	< 11 µmol/L >114 µmol/L	170 µmol/L	
B-Carotene	537	0.19 – 1.58 µmol/L	3.7 – 11.2 µmol/L	3.7 µmol/L	
Bicarbonate	84	21 – 28 mmol/L	10 – 45 mmol/L	35 mmol/L	
Bile Acids	390 – 513	0 – 10 µmol/L		30 µmol/L	
Bilirubin, conj	778 – 971	0 – 3.4 µmol/L (adult)	513 µmol/L	342 µmol/L	
Bilirubin, unconj	585	5 – 21 µmol/L (adult)	684 µmol/L	342 µmol/L	
Calcium (total)	40	1.90 – 2.55 mmol/L	5.0 mmol/L	5.0 mmol/L	
Chenodeoxycholic Acid	392	0 – 1.61 µmol/L	2.5 µmol/L	30 µmol/L	
Chloride	35	98 – 107 mmol/L	85 – 160 mmol/L	120 mmol/L	

Appendix D. (Continued)

Compound	Molecular Weight	Reference Interval [1]	Pathological Values[2]	Recommended Test Concentration[3] (common pathological value)	Comments
Cholesterol	387	2.95 – 5.2 mmol/L[4]	13 mmol/L	13 mmol/L	
Cholic Acid	408	0.07 – 0.91 µmol/L		2.67 µmol/L	
Creatinine	113	53 – 115 µmol/L	115 µmol/L	442 µmol/L	Corresponds to 5 mg/dL, Critical Value
Free fatty acids, total		0 – 0.92 mmol/L		2.76 mmol/L	
Fructose	180	56 – 333 µmol/L		1000 µmol/L	
Galactose	180	< 0.28 mmol/L		0.84 mmol/L	
D-glucose	180	4.1 – 5.9 mmol/L	1.7 – 55 mmol/L	55 mmol/L	55 mmol/L corresponds to 1000 mg/dL.
Glutathione, reduced	613/307	0.79 – 1.05 mmol/L		3 mmol/L	
Glycerol, free	92	0.032 – 0.232 mmol/L		0.696 mmol/L	
Hemoglobin	64,000	1 – 2 g/L	> 2 g/L	2 g/L	
Lactate	90	0.5 – 2.2 mmol/L	6.6 mmol/L	6.6 mmol/L	
Magnesium	24	0.65 – 1.05 mmol/L	5 mmol/L	15 mmol/L	Critical Value = 5 mmol/L
Oleic Acid	282	< 0.4 mmol/L		1.4 mmol/L	
Oxalic Acid	126	11 – 27 µmol/L	>27	81 µmol/L	

Appendix D. (Continued)

Compound	Molecular Weight	Reference Interval [1]	Pathological Values[2]	Recommended Test Concentration[3] (common pathological value)	Comments
Palmitic Acid	256	0.2 – 2.0 mmol/L		6.0 mmol/L	
Phospholipids (as Lecithin)	678	2.6 – 3.7 mmol/L		11.1 mmol/L	
Phosphorus (inorg)	31	0.7 – 3.0 mmol/L		9.0 mmol/L	
Potassium	39	3.6 – 5.0 mmol/L	7.0 mmol/L	7 mmol/L	ECG shows peaked T waves at concentrations > 7 mmol/L.
Protein (total)	NA	60 – 80 g/L	< 60 g/L > 90 g/L	120 g/L	
Protein (albumin)	NA	39 – 51 g/L		60 g/L	
Protein (γ globulin)	NA	6 – 13 g/L		60 g/L	
Pyruvate	88	34 – 103 µmol/L		309 µmol/L	
Sodium	23	135 – 145 mmol/L	180 mmol/L	180 mmol/L	
Stearic acid	284	0.1 mmol/L		0.4 mmol/L	
Sulfate (inorg)	142	1 mmol/L	2 mmol/L	2 mmol/L	
Triglycerides	NA	0.34 – 3.7 mmol/L		37 mmol/L	

Appendix D. (Continued)

Compound	Molecular Weight	Reference Interval [1]	Pathological Values[2]	Recommended Test Concentration[3] (common pathological value)	Comments
L-Tyrosine	181	44 – 1325 mmol/ L		4000 mmol/L	
Urea	60	1.1 – 14.3 mmol/L		42.9 mmol/L	
Uric acid	168	150 – 476 µmol/L		1.4 mmol/L	
pH	NA	7.11 – 7.45	< 6.80 > 8.0	8.0	pH compatible with life is 6.80 – 7.80

[1] Reference interval taken from Burtis CA, Ashwood ER, eds. *Tietz Textbook of Clinical Chemistry.* 3rd ed. Philadelphia: W.B. Saunders, 1999, unless otherwise noted.

[2] Typical range, including highest expected blood concentration.

[3] In serum and plasma (upper limit of normal, common pathologic concentration).

[4] Reference interval taken from http://cclnprod.cc.nih.gov/dlm/testguide.nsf/HRRAll?OpenForm&Count=920

Appendix E. Worksheets

E1. Interference Investigation Worksheets

E1.1 Worksheet 1-1

Date:		
DESCRIBE THE ANALYTICAL SYSTEM:		
Analyte:	**Comparative Measurement Procedures**:	
Method A:	**Method B**:	
Reagent Lot #:	**Method C**:	
Instrument:	**Specimen Type**:	

VERIFY ACCEPTABLE SYSTEM PERFORMANCE:

	Comments
Precision acceptable?	
Accuracy acceptable?	
QC acceptable?	
Specimen acceptable?	

SUMMARY OF OBSERVED RESULTS:

	Method A	***Method B***	***Method C***
Original result			
Repeat results-same specimen			
Repeat results-diluted specimen			
Previous results-same patient			
Repeat results-new specimen			

Appendix E. (Continued)

E1.2 Worksheet 1-2

LIST POTENTIAL INTERFERENTS:

Source	Relevant Information
Product labeling	
Other literature	
Manufacturer's customer service center	
Patient diagnosis/condition	
Recent procedures/treatments	
Recent medications	
Laboratory contaminants	
Patient diet	

CONCLUSIONS:

REPORTED:

Name:	**Date:**

Appendix E. (Continued)

E1.3 Worksheet 1-3

DATA SHEET

RECORD TEST DETAILS:

Date:	**Technologist:**
Analyte:	**Test Substance:**
Concentration:	**Concentration:**
Precision (%):	**Acceptable Limit:**
Method:	**Instrument:**
Reagent Lot #:	**Calibrators:**

RECORD RESULTS:

Control Pool		Test Pool	
C1		**T1**	
C2		**T2**	
C3		**T3**	
C4		**T4**	
C5		**T5**	
C6		**T6**	
C7		**T7**	
C8		**T8**	

CALCULATE STATISTICS:

	Control Pool		Test Pool	
Mean	**X=**		**X=**	
Std. Dev.	**SD=**		**SD=**	
C.V.	**CV=**		**CV=**	

CALCULATE DIFFERENCE:

Test pool mean – Control pool mean =

Appendix E. (Continued)

E1.4 Worksheet 1- 4

DATA SHEET				
DETERMINE 95% CONFIDENCE LIMITS:				
	Control Pool		**Test Pool**	
Variance	**S^2=**		**S^2=**	
Average variance =				
Pooled standard deviation =				
Upper confidence limit [d+1.96 x $(2s^2/n)^{-1/2}$] =				
Lower confidence limit [d-1.96 x $(2s^2/n)^{-1/2}$] =				
CONCLUSIONS:				
APPROVED:				
Name:		**Date:**		

Appendix E. (Continued)

E2. Interference Investigation Worksheets (With Example Data)

E2.1 Worksheet 1-1: Example

Date: 3/15/95	
DESCRIBE THE ANALYTICAL SYSTEM:	
Analyte: *Creatinine*	**Comparative Measurement Procedures:**
Method A: *Kinetic Jaffe*	**Method B**: *GRC Enzymatic*
Reagent Lot #: *271-857-445*	**Method C**: *HPLC*
Instrument: *Atlas RD2 analyzer*	**Specimen Type**: *Plasma*

VERIFY ACCEPTABLE SYSTEM PERFORMANCE:

	Comments
Precision acceptable?	*√ Control data show long-term SD is within the manufacturer's claim.*
Accuracy acceptable?	*√ CAP result on 3/1/95 demonstrated acceptable accuracy.*
QC acceptable?	*√ System has been in control for the past four months.*
Sample acceptable?	*√ Clear, pale yellow appearance. No obvious abnormalities.*

SUMMARY OF OBSERVED RESULTS:

	Method A	***Method B***	***Method C***
Original result	*4.6*	*N/A*	*N/A*
Repeat results-same sample	*4.6, 4.8*	*1.1, 1.1*	*1.0, 1.1*
Repeat results-diluted sample	*4.6, 4.7*	*Not done*	*Not done*
Previous results-same patient	*Not available*	*Not done*	*Not done*
Repeat results-new sample	*3.8, 3.8*	*1.0, 1.1*	*Not done*

Appendix E. (Continued)

E2.2 Worksheet 1-2: Example

LIST POTENTIAL INTERFERENTS:

Source	Relevant Information
Product labeling	*Cephalosporin antibiotics, keto-acids, bilirubin, hemoglobin.*
Other literature	*Same as above.*
Manufacturer's customer service center	*Called Customer Service hotline; they are unaware of any other interferents.*
Patient diagnosis/condition	*Admitted for abdominal surgery. Patient has a history of diabetes.*
Recent procedures/treatments	*None*
Recent medications	*Seldane, Nasalide, Ketoconizole, Acetaminophen*
Laboratory contaminants	*None apparent*
Patient diet	*Nothing unusual*

CONCLUSIONS:

Sample tested positive for keto-acids. Suspect beta-hydroxybutyrate interference.

REPORTED *to manufacturer*

Name: *Robert H. Fleming*	**Date:** *3/3/95*

Appendix E. (Continued)

E2.3 Worksheet 1-3: Example

DATA SHEET

RECORD TEST DETAILS:

Date: *3/2/95*	**Technologist:** *R. Doyle*
Analyte: *Creatinine*	**Test Substance:** *Cephaloxin*
Concentration: *1.5 mg/dL*	**Concentration:** *10 mg/dL*
Precision (%): *0.8%*	**Acceptable Limit:** *0.15 mg/dL or 3%*
Method: *Jaffe*	**Instrument:** *Autochem 101*
Reagent Lot #: *B2106-44*	**Calibrators:** *Set # C1812*

RECORD RESULTS:

Control Pool		Test Pool	
C1	*1.49 mg/dL*	**T1**	*5.25 mg/dL*
C2	*1.42 mg/dL*	**T2**	*5.41 mg/dL*
C3	*1.55 mg/dL*	**T3**	*5.34 mg/dL*
C4	*1.55 mg/dL*	**T4**	*5.34 mg/dL*
C5	*1.55 mg/dL*	**T5**	*5.34 mg/dL*

CALCULATE STATISTICS:

	Control Pool		Test Pool	
Mean	**X=**	*1.49 mg/dL*	**X=**	*5.33 mg/dL*
Std. Dev.	**SD=**	*0.065 mg/dL*	**SD=**	*0.080 mg/dL*
C.V.	**CV=**	*4.38%*	**CV=**	*1.50%*

CALCULATE DIFFERENCE:

Test pool mean – Control pool mean = *3.85 mg/dL*

Appendix E. (Continued)

E2.4 Worksheet 1-4: Example

DATA SHEET

DETERMINE 95% CONFIDENCE LIMITS:

	Control Pool		Test Pool	
Variance	**S^2=**	*0.0042*	**S^2=**	*0.0064*
		Average variance =		*0.0053*
		Pooled standard deviation =		*0.0730*
		Upper confidence limit [d+1.96 x $(2s^2/n)^{-1}$] =		*3.91 mg/dL*
		Lower confidence limit [d-1.96 x $(2s^2/n)^{-1}$] =		*3.78 mg/dL*

CONCLUSIONS:

The lower confidence limit (3.78 mg/dL) greatly exceeds the acceptable limit (0.15 mg/dL). This demonstrates that Cephaloxin interferes with Autochem 101 Jaffe creatinine method. Reported to the manufacturer 4 March 1995.

APPROVED:

Name: *D.M. Wilson, PhD*	**Date:** *3/4/95*

Appendix F. Calculation of Replicates for Dose-Response Tests

F1. Planning the Statistical Test

Figure 1 in Section 7.2.2.6 shows graphically how a high pool and a low pool are mixed to produce pools having intermediate concentrations of the potential interferent.

In a dose-response test, the appropriate number of replicates must be determined for each concentration of potential interferent studied to achieve a suitable degree of precision. Several approaches can be taken, depending on whether emphasis is placed on estimating the slope, intercept, or some other parameter of interest. The approach taken here is to focus on estimating the slope.

Let the concentration of the potential interferent in the high pool be denoted H, and let that of the low pool be L; then the midpool, being a 50-50 mixture of the low and high pools, will have concentrations $(L+H)/2$; the 25% pool, being a 50-50 mixture of the low and midpools, will have concentration $(L+((L+H)/2))/2 = (3L+H)/4$; the 75% pool, being a 50-50 mix of the mid- and high pools, will have concentration $(H+((L+H)/2))/2 = (L+3H)/4$. Denote these five concentrations of the potential interferent as Xi, $i = 1, 2, 3, 4, 5$: $X1 = L$, $X2 = (3L+H)/4$, $X3 = (L+H)/2$, $X4 = (L+3H)/4$, and $X5 = H$.

Let the null hypothesis be the proposition that the slope (defined here as b) is equal to zero. Let alpha (α) be the probability (that the test is designed to have) of declaring the null to be false when it is actually true. In practice, α is usually set at 0.01, 0.05, or 0.10. Next define d such that if the slope were truly equal to d, the test would have a low probability of concluding the slope equals zero (i.e., a high probability of rejecting the null hypothesis). Let β be the probability of incorrectly accepting the null hypothesis when it in fact equals d. In practice, β is usually set at 0.01, 0.05, 0.10, or 0.20.

It can be shown that the optimum number of replicates, n, is determined as follows:

$$n = \left[\frac{1.26\left(Z\frac{\alpha}{2} + Z_{\beta} \right) X_{\sigma rep}}{\left(H - L \right)\sigma_x} \right]^2 \qquad \text{(F1)}$$

where:

σ_{rep} = estimate of repeatability standard deviation of the analyzer, assumed to be constant over the range of interferent concentrations being considered

σ_x = standard deviation of the concentrations of the potential interferent = $(5/32)\ (H\text{-}L)^2$ where H=Highest interferent concentration and L=Lowest interferent concentration

$Z[\alpha/2]$ = $\alpha/2$ fractiles of the standard unit normal distribution

$Z[\beta]$ = β fractile of the standard unit normal distribution

NOTE: Documentation of the mathematical proof is on file at CLSI.

Appendix F. (Continued)

The following are standard unit normal fractile values for typical α and β levels.

α	$Z[\alpha/2]$
0.01	2.58
0.05	1.96
0.10	1.64

β	$Z[\beta]$
0.01	2.33
0.05	1.64
0.10	1.28
0.20	0.84

Since the objective is to fit a straight line to the *Y* versus *X* data, the following linear relationship is adopted as the working model:

$$Y_{ij}=a + b\,X_i + E_{ij} \qquad \text{(F2)}$$

$$i = 1, 2, 3, 4, 5,$$
$$j = 1, 2,.....,n,$$

where Y_{ij} is the analyte concentration measured at the j^{th} replicate at the i^{th} interferent level, *a* and *b* are constants, and E_{ij} is the error in the model prediction at the j^{th} replicate at the i^{th} interferent level.

If Y_i is regressed upon X_i, $i = 1, 2,...n$, by ordinary least squares, then we will get estimates $\hat{a}$ and $\hat{b}$ with *a* and *b*, respectively. The slope estimates, $\hat{b}$, will be given by:

$$\hat{b} = \frac{\sum_{i=1}^{5} \overline{Y}_i \left(X_i - \overline{X} \right)}{4\sigma_x^2} \qquad \text{(F3)}$$

where $\overline{X} = (L+H)/2$.

This completes the experimental design (except for such operational matters as the randomization of the orders in which the different samples' replicates are to be measured).

In subsequent descriptions, the slope estimate, $\hat{b}$, is assumed to be positive. This is to simplify the discussion and does not affect the generality of the procedure.

Appendix F. (Continued)

F2. Doing the Statistical Test

When we have selected a value of n that is consistent with (E1) and with the specified values for α, β, and ∗, then b* (the cutoff on $\hat{b}$, for the test), can be computed as follows:

$$b^* = \frac{|z(\alpha/2)|\,\sigma_{rep}}{2n\sigma_x^2} \qquad \text{(F4)}$$

Using b* as the cutoff value for $\hat{b}$, it is concluded, on the basis of this test, that the null hypothesis (which asserts that $b = 0$) is true if $\hat{b}$, i, the magnitude of the regression-estimated slope, is less than or equal to b*, and we will declare that the null is false if $\hat{b}$ is greater than b*. If the null is not rejected, then, in reporting the results, it should be pointed out that the test was capable of detecting, with the probability of 1-β, a slope of magnitude ∗. If the null is rejected, then it should be pointed out that the probability that the null would have been erroneously declared to be false, when it is in fact true, is α.

But this can be done only if the actual number of replicates for each of the test samples is equal to the planned value of n. In practice, some replicates may be omitted, lost, or eliminated as outlier observations. When this occurs, in principle, ordinary least squares should not be used to run the regression of $\hat{Y}_i$ on X_i. Instead, a weighted least squares analysis is more appropriate. (For details of weighted least squares, see Box, Hunter, and Hunter, *Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building*. 2nd ed. London: Longman Group, Ltd; 1956:505-508).

In practice, however, the n_I's will not usually differ by very much, from each other or from n, so the results of weighted regression will be very nearly the same as the results of ordinary, unweighted regression; to do weighted least squares would usually not be worth the trouble. Therefore, the slope estimate, $\hat{b}$ given by (E3) can still be used. It may be necessary to insert an approximately reasonable n (i.e., an n approximately equal to the average number of replicates actually taken in the experiment) into the expression (E4).

Appendix G. Preparation of Test Solutions for Interference Testing

G1. Hemolysis: Osmotic Shock Procedure

High Test Level: 500 mg/dL Hemoglobin

Caution: **Paired-difference testing cannot differentiate between interference effects of hemoglobin and other constituents of red blood cells, such as K^+, NH_4+, LDH, AST, etc.**

Stock Solution*:

(1) Collect 5 mL heparinized blood.

(2) Centrifuge ten minutes to pack cells.

(3) Discard plasma and replace with 10 mL isotonic saline.

(4) Invert tube slowly ten times. Centrifuge ten minutes and discard saline wash. Repeat wash with saline twice again.

(5) Dilute the cells with an equal volume of distilled water. Invert tube and mix well ten times. Freeze overnight.

(6) Thaw cells and bring to room temperature.

(7) Centrifuge 30 minutes to remove the stroma. Save supernatant (hemolysate). Discard red cell debris in pellet.

(8) Analyze hemolysate to determine the hemoglobin concentration.

Test Pool: Add measured volume of hemolysate to 10 mL serum to make 500 mg/dL hemoglobin.

Control Pool: Add equivalent volume of saline to 10 mL of same serum pool. Determine actual hemoglobin levels in the pools by analysis.

Stability: Keep no more than one week at 4 °C. Longer storage may result in conversion to methemoglobin, which may not have the same interference characteristics as hemoglobin.

Reference: Meites S. Reproducibly simulating hemolysis for evaluating its interference with chemical methods. *Clin Chem.* 1973;19:1319.

G2. L-Ascorbic Acid

High Test Level: 3 mg/dL

Stock Solution: Prepare 300 mg/dL in cold (0 to 5 °C) water, deoxygenated previously by nitrogen bubbling. Keep stock and serum solutions on ice before testing. Determine actual concentration spectrophotometrically (aM 15 000 1/mol-cm at 265 nm).

Test Sample: Add 0.1 mL to 10 mL serum. Minimize exposure to air.

Appendix G. (Continued)

Control Sample: Add 0.1 mL water to 10 mL serum.

Stability: Stock and test solutions are unstable. Test all solutions within two hours after preparation of original stock solution.

Reference: Nealon D, PhD, Ortho-Clinical Diagnostics, Personal communication, 1995.

G3. Free Fatty Acids

High Test Level: 3 mmol/L total

Test Materials: Palmitic acid, stearic acid, oleic acid

Test Pool: Procedure involves transfer of free fatty acids (FFA) from particulate absorbent (Celite) to serum.

(1) Spread dried Celite evenly in beaker to thickness of 5 mm.

(2) Add 1 mmol FFA dissolved in hexane per 10 g Celite. Particles should be completely immersed in hexane.

(3) Evaporate with gentle warming under N_2 stream.

(4) Transfer 200 mg/dL dried particles to screw-cap vial, mix thoroughly, and add 4 mL fresh human plasma (EDTA anticoagulant).

(5) Gently stir for 30 minutes (under N_2 for unsaturated FFA).

(6) Remove particles by decantation and centrifugation.

(7) Adjust pH to match control pool with NaOH.

(8) Determine exact amount by analysis. Expect 3 to 4 mmol/L.

Control Pool: Repeat the above procedure with a portion of the same fresh human plasma but omitting FFA from the hexane. Expect 0.5 to 0.8 mmol/L endogenous FFA. Determine exact amount by analysis.

Stability: Use fresh.

Reference: Spector AA, Hoak JC. An improved method for the addition of long-chain fatty acid to protein solutions. *Anal Biochem*. 1969;32:297-302.

G4. Lipemia: Ultracentrifugation Procedure

High Test Level: 3000 mg/dL triglycerides

Test Material: Lipemic serum pool with triglyceride level 3000 mg/dL.

Test Pool: Lipemic serum pool (untreated).

Appendix G. (Continued)

Control Pool: Clarify the lipemic sample using an ultracentrifuge. Perform the analysis and use the clear portion of the sample as the control pool.

Stability: Use fresh. Do not freeze.

Caution: For analytes measured in plasma water volume, (e.g., electrolytes by direct potentiometry), results may be affected by water displacement effects.

Reference: Novros J, PhD, Ortho-Clinical Diagnostics, Personal communication, 1995.

G5. Drugs

High Test Level: See Appendix C.

Test Material: Obtain drug in pure form. Pharmaceutical preparations may be used, but effects may be due to other ingredients. Specify form (acidic, basic, salt) and purity when results reported.

Stock Solution: Dissolve in order of preference, in water, 50 mmol/L phosphate (pH 7), ethanol/methanol, chloroform. Prepare stock at 20 x the final concentration of the test pool. Be sure to take into account accompanying salts, water of hydration, etc., when calculating the concentration of the compound of interest.

Test Pool: Quantitatively make up 0.1 to 0.5 mL stock to 10 mL with fresh human serum pool.

Control Pool: Quantitatively make up equivalent volume of solvent used to 10 mL with same human serum pool.

Stability: Depends on drug.

Reference: Baer DM, Jones RN, Mullooly JP, Horner W. Protocol for the study of drug interferences in laboratory tests: Cefotaxime interference in 24 clinical tests. *Clin Chem.*1983;29:1736-1740.

G6. Bilirubin

Caution: **Bilirubin is sensitive to exposure to certain wavelengths of light. Do all preparation in yellow or subdued light.**

High Test Level: 20 mg/dL

Unconjugated Bilirubin

Stock Solution: Dissolve 20 mg unconjugated bilirubin in 2 mL 0.1N NaOH.

Test Pool: Add 0.1 mL of stock solution to 5 mL serum pool with stirring.

Control Pool: Add 0.1 mL 0.1N NaOH to 5 mL of the same serum pool.

Appendix G. (Continued)

Conjugated Bilirubin

Stock Solution: Dissolve 20 mg ditaurobilirubin in 2 mL water. This is a commercially available synthetic bilirubin derivative with solubility and spectral characteristics similar to naturally occurring conjugated bilirubin.

Test Pool: Add 0.1 mL of stock to 5 mL serum with stirring.

Control Pool: Add 0.1 mL H_2O to 5 mL starting serum pool.

Stability: Use test solutions on the day they are prepared. Keep refrigerated; avoid exposure to white light.

Reference: Nealon D, PhD, Ortho-Clinical Diagnostics, Personal communication, 1995.

G7. Bicarbonate/$C0_2$

High Test Level: 40 mM

Bicarbonate Addition Method

Test Pool: Weigh 6.5 mg $NaHCO_3$ into 5 mL serum.

Control Pool: Starting serum pool (CO_2 25 mM).

Reference: Nealon D, PhD, Ortho-Clinical Diagnostics, Personal communication, 1995.

CO_2 Addition Method

Test Pool: Bubble CO_2 into sample. Measure CO_2 periodically until desired CO_2 level is reached. Measure pH and cap tightly with minimum air space.

Control Pool: Starting serum pool. Measure CO_2 and pH.

Stability: Unstable; use promptly.

Reference: Nealon D, PhD, Ortho-Clinical Diagnostics, Personal communication, 1995.

G8. pH

Test Levels: pH 6.8 and pH 8.8

Test Pools:

For pH 6.8 Add 30 µL 2N HCl to 5 mL serum pool. Measure pH immediately before testing.

For pH 8.8 Add 20 µL 2N LiOH to 5 mL serum pool. Measure pH immediately before testing.

Control Pool: Add 20/30 µL distilled water to 5 mL starting serum. Measure pH. Fresh serum pool should be about 7.8.

Appendix G. (Continued)

Stability: Solutions are unstable; prepare immediately before use.

Reference: Nealon D, PhD, Ortho-Clinical Diagnostics, Personal communication, 1995.

G9. Protein

High Test Level: 12 g/dL

Concentrated Serum Method

Test Pool: Prepare concentrate from fresh serum using ultrafiltration.

Control Pool: Save ultrafiltrate. Use to make intermediate concentrations.

Stability: Not determined.

Reference: Nealon D, PhD, Ortho-Clinical Diagnostics, Personal communication, 1995.

Specific Protein Addition Method

Test Pool: Add 0.50 g gamma-globulin to 10 mL fresh serum.

Control Pool: Starting serum pool

Stability: Not determined.

Reference: Nealon D, PhD, Ortho-Clinical Diagnostics, Personal communication, 1995.

G10. Bile Acids

Test Level: 6 mg/dL

Test Materials: Trihydroxy bile acids - as cholic acid

Test Pool: Prepare a solution of 300 mg cholic acid in 100 mL methanol. Add 100 mL water. Add 0.4 mL of this solution to 10 mL serum.

Control Pool: Add 0.4 mL of a 1:1 methanol-water solution to 10 mL serum.

Stability: Use fresh

Reference: Nealon D, PhD, Ortho-Clinical Diagnostics, Personal communication, 1995.

G11. Keto-acids (acetoacetate, pyruvate, etc.)

High Test Level: See Appendix C.

Stock Solution: Dissolve keto acids in cold water to make 20x test level.

Test Pool: Add 0.1 mL stock solution to 10 mL serum (pH of serum should be adjusted to between 6.0 to 7.0 using 1N HCl, taking care not to precipitate proteins).

Appendix G. (Continued)

Control Pool: Add 0.1 mL water to 10 mL starting serum.

Stability: Unstable; prepare immediately before use.

Reference: Nealon D, PhD, Ortho-Clinical Diagnostics, Personal communication, 1995.

CLSI consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact CLSI or visit our website at www.clsi.org.

Summary of Consensus Comments and Committee Responses

EP7-A: *Interference Testing in Clinical Chemistry; Approved Guideline*

Section 9.1.2, Specificity Claim (Formerly Section 8.1.2)

1. The form of the specificity claim for a manufacturer allows one to state "no interference," provided that the conditions of Section 8.1.1 are met. The information that the user gets is the concentration(s) at which the assay was tested; the concentration at which the interference was tested; and the point at which interference is considered significant. In the example, 10% is used as a level above which interference is considered significant. This is not enough information for the user, because:

   (1) If several substances were tested in this way and reported not to interfere, they could nevertheless each have interferences less than 10% so that their combined effect is greater than 10%.
   (2) A user might desire a different level at what is considered a significant problem (e.g., less than 5%). With the way the results are presented, the user cannot determine if the 5% criteria were met.
   (3) In some countries, uncertainty intervals must be calculated. This requires knowing the effects of all biases. The interference results are not reported in a way that allows the uncertainty intervals to be calculated.

- **A recommendation has been added to Section 9.1.2 (formerly Section 8.1.2) stating: "The manufacturer should report, upon request, the observed bias found, the bias corresponding to the upper 95% confidence limit, the SD of the experiment, and the sample size." The distribution of most interferents in the patient population is generally unknown, and assumptions would have to be made with inadequate information. For ubiquitous metabolites and very common drugs, it might be possible to estimate standard deviations of the net biases due to sample-specific factors, but interference from drugs that are only present in occasional patients has to be treated as a special cause of error.**

Appendix C, Interferent Test Concentrations

2. EP7-P contains a listing for recommended serum/plasma test levels for endogenous substances (Appendix A on pages 326-327). There is a more updated list for exogenous substances in EP7-A (Appendix C). Endogenous substances previously on the list in EP7-P do not appear in EP7-A. Are the levels for endogenous substances previously listed in EP7-P still relevant and can it be assumed that these levels remain unchanged?

- **Parts II and III of Appendix A in EP7-P were inadvertently omitted in EP7-A. These tables are included in Appendix C of EP7-A2.**

Appendix A, Guidelines for Specific Measurement Procedures (Formerly Guidelines for Specific Methodologies)

3. I have a question about the cross-reactivity calculation on page 43 in EP7-A. The calculation of cross-reactivity is written as % Cross-reactivity = 100 (measured value – true value/concentration of interferent). However, I think, the (measured value – true value) should be divided by true value. This way, you are calculating the extent of cross-reactance of the substance in the assay at the tested concentration.

- **The cross-reactivity is calculated by: % Cross-reactivity = 100 (measured value – true value/concentration of interferent). The % interference is calculated by: % Interference = 100 (measured value – true value/true value). Calculations for both "cross-reactivity" and "% interference" are included in EP7-A2 as shown below:**

$$\%\ \text{Cross-reactivity} = 100 * \left(\frac{\text{measured value - true value}}{\text{Concentration of interferent}} \right)$$

$$\% \text{ Interference} = 100^* \left(\frac{\text{measured value - true value}}{\text{true value}} \right)$$

Appendix B, Analyte Test Concentrations

4. We have a question regarding the hemoglobin level for interference testing for our glucose testing systems. The low and high recommended hemoglobin serum/plasma test levels listed in Appendix B on page 45 of EP7-A are 10 and 20 g/dL. These levels would be consistent with levels found in whole blood samples. In Appendix A on page 326 of EP7-P, since a normal plasma level is around 2.5 mg/L (note unit differences), the recommended test level was 500 mg/dL. It is a huge jump from 500 mg/dL (reported in EP7-P) to 20 g/dL (reported in EP7-A). Was this change intentional?

- **Hemoglobin test levels were reduced to 100 and 200 mg/dL to simulate hemolysis in EP7-A, and the correct table entries in Appendix D should have been 1 and 2 g/L. The correct values (i.e., 1 g/L [10 g/dL] and 2 g/L [20 g/dL]) have been included in EP7-A2.**

5. I am currently revising SOPs for interference testing and have a question. In EP7-A, it is recommended on page 47, that enzymes be tested at 2x and 10x the upper limit of the population reference ranges. So, we take the top of our reference interval for our enzyme methods and test vs. interferents at 2x and 10x that concentration. Why are enzymes tested above the reference interval as indicated?

- **The committee recommended testing each analyte at two different concentrations (activities) because of the possibility that positive and negative effects due to different causes could cancel each other. Since some enzymes are present in very low levels, the low test levels were set at 2x the upper limit of the population reference to allow inhibition effects to be observed. The high test level was set at 10x the upper limit of the population reference range to represent a high activity.**

6. We are formulating our new procedures based on the new EP7-A and would like some clarifications. For bilirubin interference—for conjugated bilirubin (ditaurobilirubin)—why are we using the same MW as the unconjugated bilirubin (584.67) as shown in Appendix B? The MW of ditaurate is 842.9.

- **The following corrections have been made for conjugated bilirubin (ditaurobilirubin) in Appendix B: MW = 842.9; Low test concentration = 2.4 µmol/L; and High test concentration = 86 µmol/L.**

7. Sometimes weighing 20 mg of ditaurobilirubin does not necessarily yield 20 mg of conjugated bilirubin measured as "direct bilirubin."

- **Direct bilirubin methods vary in their recovery of conjugated bilirubin. The concentration of the ditaurobilirubin solution should be verified with an accurate total bilirubin method.**

Appendix B, Analyte Test Concentrations, and Appendix C, Interferent Test Concentrations

8. In examining the analyte and interferent test concentrations in Appendixes B and C, respectively, we noted a discrepancy in the units reported for acetaminophen in Appendix C, and were wondering if you could clarify this for us. In Appendix B, the acetaminophen test concentrations (low and high) are reported as 33 to 199 µmol, whereas in Appendix C, they are reported as 0.03 to 0.20 µmol. It appears that the Appendix C entry is lower by a factor of 1000, and it appears that the units reported in the Appendix C entry should be millimolar rather than micromolar.

- **The appropriate units are µmol/L. Values are corrected in Appendixes B and C.**

Appendix C, Interferent Test Concentrations

9. Please explain the differences in therapeutic and testing concentrations between CLSI/NCCLS guidelines EP7-P and EP7-A. I am currently reviewing my company's interferent testing list and am using EP7-A as a reference point. The testing concentrations for most drugs listed in EP7-A are significantly lower than those listed in EP7-

P. I have been told that quoting the new figures from EP7-A is not enough and I would have to provide a reason for the change.

- **The recommended test concentrations in EP7-P set at 10x the highest expected concentration were based on an earlier IFCC guideline. Experience showed that preparing concentrated solutions of many analytes was difficult if not impossible, and introduced unnecessary risk of artifacts. Based on comments received on EP7-P, the subcommittee decided that a 3x upper therapeutic range margin was sufficient to simulate worst case scenario for interference testing purposes.**

10. I need clarification for the differences in therapeutic concentrations of drugs in Appendix C of the new guidelines (EP7-A) and the old guidelines (EP7-P). In the previous documents, they are referenced as 'mg/dL' and the most recent documents reference these as mmol/L. When we convert these values to mg/dL and compare, they differ significantly; e.g., acetaminophen: EP7-P states the therapeutic concentration is 1 to 2 mg/dL and test concentration is 20 mg/dL. However in EP7-A, the therapeutic concentration for acetaminophen is 0.03 to 0.2 µmol/L and test concentration is 1.66 µmol/L. When these figures are converted to mg/dL, these differ to those in EP7-P, i.e., the test concentration of 1.66 µmol/L = 0.025 mg/dL which is significantly lower than the previous figure. There are also numerous other drugs which when comparing, there is a significant difference. It would be very helpful to us if the reasoning behind this difference could be clarified.

- **Most of the inconsistencies were due to the use of different molecular weights for the drug substances, which affected the conventional to SI conversions (e.g., EP7-P listed free base, USP listed HCl). All of the drug molecular weights in the current table have been verified against the USP or other sources.**

11. I was reviewing the recommended test and therapeutic concentrations for common drugs in the table in Appendix C. Interferent Test Concentrations on page 48 of EP7-A (2002, vol. 22 no. 27) document, I noticed that the Unit for Acetaminophen is listed as µmol/L, but the values for the test, therapeutic, and toxic concentrations appear to be in mmol/L. I consulted *Tietz Textbook of Clinical Chemistry* (1999) to confirm the correct concentrations. *Tietz* lists the therapeutic concentration in SI units as 66 to 199 µmol/L, and the toxic concentration is listed as greater than 1324 µmol/L. The correct reporting unit in SI for acetaminophen is µmol/L (as confirmed with our local hospital here in Ottawa, Ontario, Canada), and therefore the table in Appendix C on page 48 of the CLSI document should be revised as follows: For acetaminophen in units = µmol//L: test concentration = 1667; therapeutic concentrations = 33- 200; and toxic concentrations = 330-1667.

- **The appropriate units are µmol/L. The values for acetaminophen in Appendix C: test concentration (1324 µmol/L), therapeutic concentrations (66 to 200 µmol/L), and toxic concentration (>1324) are from the *Tietz Textbook of Clinical Chemistry* (1999).**

12. What is the source of the information regarding the therapeutic and test concentrations of N-Acetylcysteine (NAC) in Appendix C on page 48 of EP7-A? The test level of 16.6 mmol/L and therapeutic level of 5.52 mmol/L seem quite high compared to the values in the literature, which are in the µmol/L range for patients receiving NAC as a mucolytic agent. I would expect that levels of NAC are higher in patients receiving bolus and maintenance doses of NAC for an acetaminophen overdose, but I have a difficult time believing that the levels would be in the mmol/L range.

- **Patients treated with N-acetylcysteine for acetaminophen poisoning have been shown to reach plasma concentrations in the millimolar range. Randall Baselt (*Disposition of Toxic Drugs and Chemicals in Man*, 6th Edition. Foster City, CA: Biomedical Publications, 2002. ISBN 0-9626523-5-0) reported an average peak plasma value of 3.4 mmol/L immediately after the loading dose in a study of 17 patients. Since this is the "worst case" situation for emergency patients, the recommended test concentration has been set at 10.2 mmol/L. NOTE: Donald Young reports values up to 200 µmol/L (Values in healthy persons in SI Units. *SI Units for Clinical Measurement.* 1998:191).**

13. Since N-acetylcysteine is quickly metabolized to cysteine, shouldn't cysteine also be tested as an interferent?

- **N-acetylcysteine is metabolized to a number of compounds, including cysteine. The selection of compounds to test as potential interferents is the responsibility of the evaluator (see Section 9 for guidelines).**

14. We are trying to use Appendix C concentrations listed in EP7-A to update our package inserts for cross-reactants and interferents. When we calculate the test concentration based on maximum dosage, why are we getting concentrations significantly higher than those listed in Appendix C? One example: Amitriptyline is dosed maximally at 300 mg/day which is 60 mg/L; times 3 would then give CLSI calculated test concentration of 180 000 ng/mL, but the Appendix lists 1000 ng/mL. Why is there a significant difference?

- **It is not appropriate to use dosage to determine the levels to test for drug interference. Dosage may not translate into serum/blood levels due to drug uptake kinetics, volume of distribution of the drug, half life, etc. It is recommended that the therapeutic range be the starting point to determine interference testing.**

15. Is there a more recent appendix that is further updated with more recent tricyclic antidepressants or co-administered compounds, as we have a number of other compounds that are not listed?

- **Specific recommendations will be considered at the next revision.**

16. Where do you find your serum levels or toxic concentrations, as we are trying to find information on drugs of abuse?

- **The information was taken from various sources, including textbooks, published reviews, scientific literature, the manufacturer's labeling, and recommendations of committee members, advisors, and consultants. It is intended only as a guide in selecting concentrations for interference testing and should not be used for any other purpose.**

Appendix D, Interference Test Concentrations for Endogenous Analytes (Formerly Appendix C), and Appendix G, Preparation of Test Solutions for Interference Testing (Formerly Appendix F)

17. I have a question as to the recommended testing levels for bilirubin interference (both conjugated and unconjugated). Bilirubin does not appear in Appendix C, as stated in the response to comment 71 in EP7-A. In Appendix F on page 78, directions are given for the preparation of interference testing solutions for bilirubin and the high-test level is indicated to be 20 mg/dL for both unconjugated and conjugated bilirubin. Should we make the assumption that 20 mg/dL is the recommended test level for both unconjugated and conjugated bilirubin?

- **The recommended test level for both conjugated and unconjugated bilirubin is 342 µmol/L (20 mg/dL), as shown in Appendix D.**

Appendix D, Interference Test Concentrations for Endogenous Analytes, and Appendix E, Worksheets (Formerly Appendix D)

18. Although Appendixes D and E are a great help, I would like to see more "worked out" examples.

- **Specific recommendations will be considered at the next revision.**

Appendix G, Preparation of Test Solutions for Interference Testing (Formerly Appendix F)

19. It is important that the actual interferent present in the "spiked" samples be quantitated to ensure that accurate amounts of the "claimed" interferents are indeed present in the samples.

- **It is up to the manufacturer to justify that the test solutions are sufficiently accurate for interference testing. Gravimetric preparation of most solutions should be sufficient when compounds are of known purity.**

20. In Appendix F, Section F1, Hemolysis: if one is examining the effects of hemoglobin (hemolysate) on assays such as Na+, K+, and Cl-, is there a way to correct for the concentration of these during the testing, or do you just accept those concentrations as part of the interference?

- **Paired difference testing assumes the analyte concentration is not changed by the "spiked" sample. If the analyte is added along with the test solution, paired difference testing is not appropriate. In such cases,**

the amounts of analyte in the "spiked" sample and the control must be determined by a measurement procedure known not to be affected, such as a reference measurement procedure.

21. If one adds deionized water as part of the osmotic shock treatment of red cells, shouldn't deionized water be added to the control pool—not saline? This is especially in reference to assays for Na^+ and Cl^-.

- **The last step involves a 50:50 suspension of the red cells, so the final sodium and chloride concentrations will be somewhere in between water and saline. However, saline will not compensate for the additional potassium or other red cell constituents. For analytes that are major constituents of red cells, lack of interference should be demonstrated by parallel analysis of naturally hemolyzed patient specimens by the measurement procedure being evaluated and a procedure known not to be affected, such as a reference procedure.**

22. We've been using a MOPS/TRIS buffer (isotonic) to wash the cells instead of saline. Do you see any issues with that?

- **The evaluator is responsible for providing scientific justification or validating any changes to the procedure.**

23. What is the typical concentration of hemoglobin following the protocol after centrifugation?

- **The hemoglobin concentration after centrifugation should be 90 to 110 g/L, according to Meites S. (Reproducibly simulating hemolysis for evaluating its interference with chemical methods. *Clin Chem.* 1973;19:1319).**

24. In EP7-P, it was indicated to be OK to freeze.

- **In Appendix G, Section G1, Hemolysis (formerly Appendix F1), freezing is an option, according to Meites S. (Reproducibly simulating hemolysis for evaluating its interference with chemical methods. *Clin Chem.* 1973;19:1319).**

25. In Appendix F4, Lipemia, what speed (rpm) is required for the ultracentrifugation method described?

- **Lipids can be cleared from serum by ultracentrifugation at ~200 000 to 600 000 g. The time needed to centrifuge the lipids will depend on the centrifuge. Check with the manufacturer of the centrifuge for exact details.**

26. In Appendix F6, Bilirubin (now Appendix G6), it indicates that the high test level is 20 mg/dL. However, it indicates preparation of 20 mg/dL of unconjugated bilirubin and 20 mg/dL of conjugated bilirubin. Should testing be done with the unconjugated and conjugated species mixed (1:1 to provide 10 mg/dL of each species = 20 mg/dL t-Bili) or are these two separate test series each at 20 mg/dL?

- **Testing should be done with separate solutions of conjugated and unconjugated bilirubin, both at concentrations of 20 mg/dL.**

27. Our system is very sensitive to pH. In the case of the unconjugated bilirubin, the final concentration of 2 mM NaOH could pose a problem and cause variations. Any options?

- **Unconjugated bilirubin is soluble in chloroform (10 mg/mL), yielding a hazy solution. It is also soluble in benzene, chlorobenzene, carbon disulfides, acids, and alkalies; slightly soluble in alcohol and ether; and practically insoluble in water. An appropriate control sample must be run to show that any effect is due to bilirubin and not to the solvent.**

Summary of Consensus/Delegate Comments and Committee Responses

EP7-A2: *Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition*

General

1. EP7-A2 appears to be inconsistent with its use of terms. On page viii, it states "The terms specimen and sample are both used in this document, with specimen reserved for actual patient materials, and sample reserved for processed materials (e.g., PT samples, reference materials)." Yet on many pages (e.g., page 37), "sample" is used in referring to both patient material and control material. Also, the use of the term "assay" instead of "analysis" or "test" throughout the document (e.g., pages 1, 3, and 14) does not appear appropriate in contrast to the great attention made to differentiate the terms method and measurement procedure.

- **Text in the Foreword was revised to clarify the differences in meaning between the terms "specimen" and "sample" and the terms were reviewed throughout the document to ensure accepted usage. The term *assay* has been replaced by *method*, *measurement procedure, measurement, analyze,* and *analysis* as appropriate. At this time, due to user unfamiliarity, the term *examination* is not used in this edition of EP7.**

Foreword

2. What is IVD?

- **The last sentence of the first paragraph has been revised to include "*in vitro* diagnostic" before the abbreviation (IVD).**

Section 4, Definitions

3. Add "Type I" as a synonym for "alpha error."

- **As suggested, "Type I error" has been added as a synonym for "alpha (α) error" as noted: alpha (α) error//Type I error.**

4. Add "Type II" as a synonym for "beta error."

- **As suggested, "Type II error" has been added as a synonym for "beta (β) error" as noted: beta (β) error//Type II error.**

Section 5.1, Clinical Acceptability Criteria

5. Under "Clinical Acceptability Criteria," one additional criterion used to evaluate the degree of allowable error due to interference is that due to both analytical and physiological variability. Thus, I think that there should be another subsection titled "Criteria Based on Analytical and Physiological Variability."

- **This recommended revision will be considered during the next revision of this document.**

Section 5.5, Interferent Test Concentrations

6. Use of the phrase 'worst case': can a less colloquial phrase be located?

- **The phrase "worst case" is commonly used and understood by professionals in laboratories and manufacturers, and the committee believes that this is the appropriate terminology as used in the document.**

7. Define "short draw."

- **For clarification, the sentence has been revised to read: "For serum, plasma, and whole blood, test at five times the recommended additive concentration to simulate a "short draw" (i.e., partially filled blood collection tube)."**

Appendix A, Guidelines for Specific Measurement Procedures

8. Equations (A1) and (A2): I think the original equation may be right for the basic concept of % cross-reactivity, which measures the binding of the substance to the antibody. However, when we test interference in the clinical assay system, we are measuring not only binding of substances to the antibody, but also suppression of an indicator reaction, inhibit of the analyte, or any other cause of specimen-dependent bias as is written in the definition of "interference" in Section 4, Definitions. Therefore, I think the second equation may be more appropriate in the interference testing in the clinical assay.

- **For clarity, Equation A2 has been changed to % interference.**

Appendix B, Analyte Test Concentrations

9. For blood lead, low level is too high. Should be 0.5 μmol/L if not lower (see CDC permitted exposure limits for children).

- **The committee has reviewed the CDC recommendations and has revised the low test concentration values for lead to 0.48 μmol/L (10 μg/dL) in Appendix B.**

NOTES

The Quality System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care.* The quality management system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any healthcare service's path of workflow (i.e., operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The quality system essentials (QSEs) are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Service & Satisfaction
Personnel	Process Control	Assessment	Facilities & Safety

EP7-A2 addresses the quality system essentials (QSEs) indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI/NCCLS Publications section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
					X C3 C24 EP5 EP9 EP14						

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care.*

Related CLSI/NCCLS Publications*

C3-P4 **Preparation and Testing of Reagent Water in the Clinical Laboratory; Proposed Guideline—Fourth Edition (2005).** This document provides guidance on water purified for clinical laboratory use; methods for monitoring water quality and testing for specific contaminants; and water system design considerations.

C24-A2 **Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline—Second Edition (1999).** This guideline provides definitions of analytical intervals, planning of quality control procedures, and guidance for quality control applications.

EP5-A2 **Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition (2004).** This document provides guidance for designing an experiment to evaluate the precision performance of quantitative measurement methods; recommendations on comparing the resulting precision estimates with manufacturers' precision performance claims and determining when such comparisons are valid; as well as manufacturers' guidelines for establishing claims.

EP9-A2 **Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (2002).** This document addresses procedures for determining the bias between two clinical methods, and the design of a method comparison experiment using split patient samples and data analysis.

EP14-A2 **Evaluation of Matrix Effects; Approved Guideline—Second Edition (2005).** This document provides guidance for evaluating the bias in analyte measurements that is due to the sample matrix (physiological or artificial) when two measurement procedures are compared.

HS1-A2 **A Quality Management System Model for Health Care; Approved Guideline—Second Edition (2004).** This document provides a model for providers of healthcare services that will assist with implementation and maintenance of effective quality management systems.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most recent editions.

NOTES

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