Guidance for Industry

Document for Special Controls for Erythropoietin Assay Premarket Notifications [510(k)s]

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U.S. Department Of Health And Human Services
Food and Drug Administration
Center for Devices and Radiological Health

Immunology Branch
Division of Clinical Laboratory Devices
Office of Device Evaluation

Preface

Public Comment

Comments and suggestions may be submitted at any time for Agency consideration to Nina Chace, Center for Devices and Radiological Health, HFZ-440, 9200 Corporate Boulevard, Rockville, MD 20850. Comments may not be acted upon by the Agency until the document is next revised or updated. For questions regarding the use or interpretation of this guidance contact Dr. Joseph Hackett at (301) 594-3084.

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I. Summary

This document presents current approaches and concerns regarding the use of Erythropoietin Assay *in vitro* diagnostic devices. It is based on 1) current science; 2) clinical experience; 3) previous submissions by manufacturers to the Food and Drug Administration (FDA); 4) the FDA Modernization Act of 1997 (FDAMA); and 5) FDA regulations in the Code of Federal Regulations (CFR). As advances are made in science and technology, and as changes in implementation of legislation occur, these Points to Consider will be re-evaluated and revised as appropriate.

NOTE: As stated in the final rule reclassifying the Erythropoietin Assay *in vitro* diagnostic devices from Class III to Class II, this guidance document is the special control for this device.

This draft guidance document describes a means by which Erythropoietin Assay *in vitro* diagnostic devices may comply with the requirement of special controls for class II devices. Designation of this guidance document as a special control means that manufacturers attempting to establish that their device is substantially equivalent to a predicate device should demonstrate that the proposed device complies with either the specific recommendations of this guidance or some alternative control that provides equivalent assurances of safety and effectiveness.

II. Purpose of Guidance:

The purpose of this document is to provide guidance and clarification regarding the types of information and data that should be submitted to the Food and Drug Administration (FDA) for its evaluation of premarket notification submissions [510(k)s] for erythropoietin assays.

A manufacturer is expected to demonstrate that the device is substantially equivalent to a device legally marketed in the United States and is as safe, effective and reasonably accurate.

This document is an adjunct to the CFR and FDA 87-4224, The *In Vitro* Diagnostic Devices: Guidance for the Preparation of 510(k) Submissions Manual. This document does not supersede those publications, but is included to provide additional guidance and clarification on what information is helpful for FDA to clear a device for marketing. It is hoped that this will lead to more reliable, reproducible, and standardized commercial tests.
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*This guidance document represents the agency's current thinking on erythropoietin (EPO) assays. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations or both.

III. Definition of Device:

This generic type of device is intended for use in clinical laboratories as an *in vitro* diagnostic test for the qualitative or quantitative measurement of erythropoietin (an hematopoietic growth factor that regulates the production of red blood cells) in serum or plasma. This device provides diagnostic information to aid in the evaluation of erythrocytoses (increased total red cell mass) and anemias. With the advent of the administration of recombinant erythropoietin as a biologic therapy to increase red blood cell mass, an erythropoietin assay may be used also to aid in the prediction and monitoring of response to recombinant erythropoietin treatment of anemias.

Product Codes: GGT

Classification: Class II

Panel: Hematology (81)

Review Required: Premarket Notification 510(k)

Regulation Section: 21 CFR Part 864.7250 Erythropoietin assay

Identification: An erythropoietin assay is a device that measures the concentration of erythropoietin (an hematopoietic growth factor that regulates the production of red blood cells) in serum or urine. This device provides diagnostic information to aid in the evaluation of erythrocytoses (increased total red cell mass) and anemias. With the advent of the administration of recombinant erythropoietin as a biologic therapy to increase red blood cell mass, an erythropoietin assay may be used also to aid in the prediction and monitoring of response to recombinant erythropoietin treatment in persons with anemias.

Introduction

In the **Federal Register** of September 12, 1980 [45 FR 60611], FDA issued a final rule classifying the erythropoietin (EPO) assay into class III [21 CFR 864.7250]. This classification was based on the following risks to health identified by the Hematology and Pathology Devices Panel (the Panel). Misdiagnosis caused by inaccurate test results might lead to inappropriate therapy of a potentially fatal disease, polycythemia vera. The FR notice also stated that 1) the then current EPO assays were not sufficiently accurate, precise, sensitive, or specific; 2) a performance standard providing reasonable assurance of safety and effectiveness could not be established; and 3) an acceptable reference material was not available to standardize EPO assays. The Panel recommended a medium priority for initiating a proceeding to require PMAs under section 515(b) of the act [21 U.S.C. 360e(b)]. In the FR of August 14, 1995 [60 FR 41986], FDA published a notice requiring

manufacturers of 31 group 2, class III devices, including erythropoietin assays, to submit to the FDA summaries of, and citations to, safety and effectiveness information known for these devices. After reviewing the submitted information and searching the scientific literature, FDA, on its own initiative, has reclassified this device from class III to class II based on new information with respect to the device.

There are three different types of erythropoietin assays, each with its own principle of operation: (1) *in vivo* bioassays, (2) *in vitro* bioassays, and (3) immunological assays.

(1) *In vivo* Bioassay Principle of Operation

EPO *in vivo* bioassays measure the biologic activity of EPO in an intact animal in which endogenous erythropoietin production has been suppressed by fasting, induction of polycythemia with transfusion of packed red cells or exposure to atmospheric hypoxia with subsequent maintenance at normal oxygen tension. Such animals are sensitive to erythropoietic stimuli. The effect of the test material on the rate of red cell production, as estimated by the reticulocyte count or by percentage of radioactive iron (^{59}Fe) incorporated into peripheral red blood cells is directly related to the amount of erythropoietin injected. However, this assay will not detect normal or subnormal amounts of erythropoietin in human serum. Despite the primitive nature of the test, its expense, and laboriousness, this is the standard reference assay against which all others are compared because it is the only test that measures the true *in vivo* biologic activity of EPO.[6]

(2) *In vitro* Bioassay Principle of Operation

In *in vitro* bioassays, erythropoietin activity is assessed in short-term cultures of erythroid tissue taken from rats or mice, either from bone marrow, spleen or 12-15 day fetal mouse liver. One of the following three end points have been used for evaluation: (a) ^{59}Fe-incorporation into heme, (b) incorporation of precursors into RNA, DNA or glycosylated side chains, e.g., ^{125}I-deoxyuridine, ^{3}H-thymidine or ^{14}C-glucosamine uptake, or (c) growth of erythroid colonies from marrow erythroblasts (erythroid colony-forming units, CFU-Es). Although *in vitro* bioassays are less laborious and more sensitive than the *in vivo* bioassay, they are not very accurate. Their reactivity is modulated by numerous factors such as the concentration of iron and transferrin, the type of fetal calf serum, prostaglandins, and other inducing factors, and by inhibitors in serum and plasma such as complement-dependent antibodies. They also can detect deglycosylated erythropoietin, which is inactive *in vivo*. Their use has been gradually displaced by immunoassays that are both more sensitive and more specific.[6]

(3) Immunological Assay Principle of Operation

Many different kinds of immunological assays for erythropoietin have been used for investigations reported in the scientific literature. These include agar double diffusion, hemagglutination-inhibition, radioimmunoassay, enzyme immunoassays,

immunoradiometric, enzyme-linked immunoabsorbent assays (ELISA), and chemiluminescence immunoassays. Commercial EPO assays have employed the principles of hemagglutination-inhibition, radioimmunoassay, enzyme immunoassay and chemiluminescence immunoassay.

All immunoassays have the same basic principles of operation. A polyclonal antiserum or monoclonal clone of antibody is raised against and made to react specifically with erythropoietin. This antibody gives the test specificity for erythropoietin. Next, the erythropoietin detected by the antibody in a patient serum or plasma sample must be separated from contaminants and/or measured with some detection methodology. The antibody/erythropoietin complexes may be precipitated, washed or separated by gravity, either alone by precipitation, or by complexing to some solid phase like plastic trays or beads, magnetic particles, or red cells, etc. These complexes are then measured by inhibition of precipitation (agglutination) of red cells, radioactive counting (radioimmunoassay), production of colored chromogen by enzymatic reaction (enzyme immunoassay), or chemiluminescence of attached molecules.

For all of these methods, the amount of erythropoietin in a sample is determined by comparison to reference standards. Erythropoietin activities are usually expressed in international units (IU). One unit of erythropoietin was originally defined as the activity that produced the same erythropoietin-stimulating effect as 5 micromoles of cobalt. The first International Reference Preparation (IRP) of Erythropoietin, Human, Urinary, for Bioassay became depleted around 1970. The availability of the Second International Reference Preparation of Erythropoietin, Human, Urinary, for Bioassay (SIRP) was reported in 1972. It was derived from the urine of anemic humans and was standardized by the Committee on Biological Standards of the World Health Organization. It is still available in ampoules containing 10 IU.[6] Internal standards or commercial preparations that have been standardized against the SIRP are also suitable for use in EPO assays. In 1991, the International Standard for Recombinant DNA-derived Erythropoietin 87/684 was reported to be 86 IU Erythropoietin r-DNA derived per ampoule. Significant differences in reactivity were seen between *in vivo, in vitro* biological, and various immunological assays with each of the above standards. These discrepant results suggested that a rDNA EPO standard would be more suitable for assays for therapeutic doses of rDNA EPO, while SIRP would be more appropriate as the reference for diagnostic assays for naturally occurring human EPO.

IV. Administrative 510(k) Requirements

The criteria for a premarket notification submission are given in 21 CFR Part 807, Subpart E and should be consulted before filing an application with the FDA. These criteria include:

1. A 510(k) summary of safety and effectiveness information as described in § 807.93 or a 510(k) statement stating that such information would be made available to interested individuals upon request as described in § 807.93. Safety and effectiveness

information refers to information in the premarket notification submission, including adverse safety and effectiveness information, that is relevant to an assessment of substantial equivalence. The information could be descriptive information about the new and predicate device(s), or performance or clinical testing information.

2. A statement that the submitter believes, to the best of his/her knowledge, that all data and information submitted are truthful and accurate, and that no material fact has been omitted as set forth in § 807.87(j).

3. An indications-for-use statement with all of the proposed clinical indications and intended uses of the device described. A separate form is available for this.

4. A table of contents and accurate pagination with consecutive numbering.

V. Data Considerations

FDA may request different types of data and statistical analyses in premarket notification applications to market *in vitro* diagnostic devices. The types of data and analyses that may be requested depend on the intended use, technological characteristics of the new device, and claims made by the manufacturer. The performance of the device can be established by comparison to any legally marketed medical device with the same intended use and/or by other studies to determine the operating characteristics of the device.

FDA will be looking for data to back all claims for substantial equivalence and specific performance characteristics of the device. It is helpful if all protocols for in house and external testing are well documented and analyses and conclusions accompany test data results. Summaries of results and explanations of unexpected results, charts (scatter grams, histograms, etc.) may be helpful, also. Raw unprocessed laboratory data may be requested.

It is important to characterize the performance of the erythropoietin assay. Following are suggested types of data and or performance characteristics to be considered for inclusion in the 510(k) submission.

A. Non-Clinical Laboratory Studies

1. Reagent Characterization

a. It is important to characterize the antibody(ies) and antigens(s) used in the assay.

b. If any recombinant/monoclonal technology was used in the preparation of the antibody (ies) or antigen(s), it is important to describe the methods used.

2. Assay Interference

It is important to characterize any interference with or cross-reactivity[1] of the test. The following substances or samples are suggested for testing for possible interference: icteric, hemolyzed, hyperlipidemic, or hyperproteinemic serum or plasma, drugs, alpha-2-macroglobulin, alpha and gamma globulins, albumin, heterophilic antibodies, as appropriate, e.g., human anti-mouse (HAMA), human anti-rabbit, and any blocking reagents recommended for their removal.

3. Performance Characteristics

The following performance characteristics are suggested for determination.

a. Analytical Sensitivity

The analytical sensitivity or detection limit may be defined as the lowest quantity differentiated from Zero. (95% confidence intervals or 2 standard deviations (SD) above the mean of the Zero control are commonly used).

b. Reportable Range

It is useful to validate the reportable range of the assay.

c. Precision

It is important to estimate precision and reproducibility at medically relevant EPO levels.[2]

d. High-Dose Hook Effect for Immunoassays

The high-dose hook (prozone) effect is confined to one-stage immunometric (sandwich) assays, giving a decrease in signal at very high concentrations of analyte. It is caused by excessively high concentrations of analyte simultaneously saturating both capture and detecting antibodies. It is helpful to characterize assay values of samples with the highest EPO values obtainable and their dilutions to check the validity of results of high level samples.[27]

e. Accuracy/Recovery Studies for Immunoassays:

It is useful to determine the spiking recovery [3,4,5,6] of known amounts of erythropoietin. Analytical recovery studies involve analyses after known amounts of analyte are added to the biological fluid on which the

determination will be performed. It is important to include samples from patients with grossly abnormal and pathological conditions, as well as samples from healthy individuals. The percent recovery may be calculated by the formula: amount detected/amount added x 100.[6]

f. Dilution/Parallelism Matrix Effect Studies:

Rarely different EPO tests have been reported to give different dose-response curves with certain patient samples.[7] The slope of the calibration curve should be compared to the slopes of observed vs. expected values for dilutions of several patient samples containing elevated EPO levels. These patients should represent a variety of diseases and abnormal conditions.

4. Comparison Studies

The new device is compared to a legally marketed predicate device to determine substantial equivalence. The package insert for the legally marketed device should be included in the document file also.

a. Quantitative Methodologies

Linear regression analysis may be used for comparison of two devices based on quantitative methodology.[8] When the use of linear regression is not appropriate for comparing two devices, other statistical evaluations, such as measures of concordance, McNemar's Test, etc., may be employed.

It is important to use samples free from interfering substances from normal persons and well characterized patients covering the whole reportable assay range (from low to normal to high) for the quantitative comparison studies. The statistical theory of linear regression analysis is concerned with independent data (i.e., only one sample pair from each patient). Identification of medically relevant decision points and an error analysis performed at each of these points may be helpful. Reasonable test results/values from some of the following diagnoses may help to characterize the accuracy of test results: normal persons; polycythemia vera; anemia, both renal and non renal; aplastic anemia; tumors; lymphomas; myelomas.

If linear regression is performed, the slope, intercept, correlation coefficient, range tested and a description of the numbers and the diagnoses of the samples tested should be reported in the package insert.

b. Qualitative Methodologies

Where a legally marketed device or a recognized reference method is available, it is important that the relative sensitivity and specificity of the new assay be determined and reported in the Performance Characteristics section of the package insert.

5. Specimen Collection and Handling Conditions

a. Two or More Matrices

If two or more types of specimens are recommended for use, it is important to consider reference ranges for each type of specimen unless it can be demonstrated that different specimen matrices, preservatives, anticoagulants, etc., do not affect assay results differentially.

b. Heat inactivation of serum samples at 56°C

It has been reported that heat inactivation of serum samples at 56°C destroyed EPO in specimens tested by the mouse spleen cell assay.[16] If heat inactivation is recommended for an EPO assay, data to demonstrate that EPO test results are valid under the recommended conditions should be provided.

6. Stability

According to Good Manufacturing Practices (GMPs), a manufacturer maintains files on the stability of all of the components of the device. The manufacturer does not have to submit this data to the FDA, but can provide the data in summary form if it is requested to establish safety and effectiveness of the device.

7. Reference Range for Normal Individuals

A reference range for an assay is useful information. If the device results correlate well using linear regression (slope close to 1.0 and intercept close to zero) with a method that has a published reference range for healthy individuals, 40-60 subjects can be sufficient to confirm agreement.[9,10] If the device results do not correlate well, it is important to establish a reference range for the new assay using samples from 120 to 200 normal persons. The population tested should be described by age, sex, geographic location, and any symptoms of disease or other factors that might influence the values obtained, e.g., pregnancy, smoking, etc. It is helpful to state in the package insert the statistical method used to characterize the population.

8. Summary of Information Published and Unpublished Supplementary Data

A summary of all published and unpublished information and/or published clinical data pertinent to the specific device should be included.

VI. Other Considerations

A. Devices Used for Generating Data for Submission

It is important to perform all studies either with a product which is representative of the final product that will be marketed or one that can be related to that product through concurrent testing.

B. Statistical Methods for Evaluation of Device

It should be noted that all statistical methods in a 510(k) are appropriate for the study protocol, type of data collected and intended use of the device. They should be selected from recognized sources and be properly referenced in the submission.[11]

VII. Labeling Considerations

A. Instructions

It is important to assure that the labeling complies with section 502(a) of the act (21 USC 352(a)), that the directions for use are not false or misleading, and that according to section 502(f)(1) of the act (21 USC 352(f)(1)), directions for use are adequate. (Section 201(n) of the act (21 USC 321(n)) defines misbranding due to misleading labeling.)

It is important also to follow 21 CFR § 809.10 for the requirements for labeling of in vitro diagnostic products. As stated in § 801.119, this will meet the regulations for compliance with sections 502(a) and 502 (f)(1) of the act and 21 CFR Part 801, relating to labeling.

B. The following are additional details for some of the points cited above in the statutes and regulations.

1. The Intended Use Statement [§ 809.10(b)(2)],

It is important to include the following information in the intended use statement.

a. Manufacturer's name.

b. Product name.

c. Whether the assay is quantitative or qualitative.

d. Analyte.

e. Test methodology.

f. Special instrumentation requirements.

g. Specimen type(s).

h. Clinical significance

i. Typical Intended Use Statement:

A typical intended use statement is: "ABC company's Erythropoietin Assay is an *in vitro* device intended for the quantitative determination of erythropoietin levels in human serum or plasma. The test system is intended as an aid in the diagnosis of anemias and polycythemias. With the advent of the administration of recombinant erythropoietin as a biologic to increase red blood cell mass, an erythropoietin assay may be used also to aid in the prediction and monitoring of response to recombinant erythropoietin treatment of anemias.

j. Conditions for Use

It is important to describe any special applications of the device or specific contraindications or indications for use not addressed in the Intended Use Statement. These special conditions for use also may be addressed further in either the Summary and Explanation, Limitations, or Performance Characteristics sections of the package insert.

2. Specimen collection and preparation for analysis [§809.10(b)(7)]

It is important to include a description of:

a. The type of specimen to be collected, e.g., plasma, serum, urine.

b. The amount of specimen required, both optimum and minimum.

c. Collection precautions:

Special precautions for collection of specimens including temperature and condition of the specimen, special preparation of the patient, as it bears on the validity of the test are beneficial. For example:

i. Diurnal Variation

There are several reports of diurnal variation of erythropoietin in the literature.[12,13,14] It is useful to recommend that samples be collected at a

consistent time of day. Morning samples taken between 7:30 am and 12:00 noon have been recommended.[13]

ii. Clotting Temperature Variation

It was reported that serum samples clotted at room temperature caused a decrease in EPO value of about 30% over clotting on ice as assessed by radioimmunoassay.[24] The package insert should warn users about the variability caused by clotting temperature and recommend a specific temperature for consistent clotting of samples. This should be the same temperature used for the samples assayed for the determination of the assay normal reference range (and hence the cutoff between positive and negative results).

d. Known Interfering Substances or Conditions.

It has been reported that certain filtration-sterilization membranes bind erythropoietin[21] to cause falsely low or negative results in assays requiring sterile filtration of samples, e.g. *in vitro* bioassays. It is helpful to validate the use of any recommended filtration sterilization membrane before recommendation for use.

3. Procedure: Directions for Use [809.10(b)(8)(vi)]

a. Individual Variation in *in vivo* bioassays.

The EPO response of individual animals used in *in vivo* bioassays is quite variable. For this reason it is important to include many animals in a study to obtain a reasonably correct average measurement of EPO activity.[22]

b. Production of endogenous EPO in fetal liver cell assays.

Test samples may trigger fetal liver cells to produce endogenous erythropoietin.[21] It is important to include several parallel control fetal liver cell cultures in each assay to establish baseline EPO activity.

c. Toxicity of plasma samples to cultured cells

Plasma samples are often toxic to cultures. The product labeling of any cultured cell assays should warn users to this possibility and inform them that toxins may be partly removed from plasma samples by prolonged dialysis or heat inactivation.[21] Any heat inactivation procedure recommended should be validated not to inactivate any EPO present in the specimen.

d. Test Values Falling Below the Limit of Detection of the Test.

The reference range of normal persons extends below the limit of detection of many EPO tests. A statement in the interpretation section of the package insert that EPO test values falling below the test limit of detection be reported as "less than X (the limit of detection)" is beneficial.

e. Inaccurate Positive Results Caused by heterophile antibodies or non specific activators.

Users should be warned in the product labeling to assay at least three dilutions of any elevated and/or suspect positive result to detect possible non-parallel results compared to reference standards caused by heterophile antibodies or non specific activators in patient samples.[22]

4. Quality Control [§809.10(b)(8)(vi)]

It is important to include the following information about quality control in the product labeling:

a. Controls:

Users should be informed about the types of specimens or commercially available products that may be used for positive and negative controls including recommended levels of analyte, if control materials are not provided as a part of the kit.

b. Quality control:

Recommendations for quality control parameters other than positive and negative controls, may also be given as appropriate.

c. Interpretation of quality control:

It is important to give users directions for interpretation of the results of quality control samples (satisfactory limits of performance).

d. Discrepancy of control results:

The quality control section of the package insert should conclude with a statement like the following: "If control results do not fall within stated parameters, assay results are invalid."

5. Limitations of the Procedure [§809.10(b)(10)]

It is important to list in the package insert all known test limitations and contraindications, with references. It is suggested that it be stated that results should be used only in conjunction with other clinical and laboratory data. All patient and clinical factors that may affect marker levels should be listed, as well as any factors that should be considered when interpreting test results. Following are suggested examples:

a. General Limitations for all Assays

i. Because results obtained with one commercial EPO assay may differ significantly from those obtained with any other, it is recommended that any serial testing performed on the same patient over time should be performed with the same commercial EPO test. In addition, it is recommended that all published results be clearly identified with regard to the EPO assay system employed.[18]

ii. If applicable, include a statement to the effect that: "This test is incapable of discriminating abnormally low EPO values from normal levels of EPO."

iii. Lower EPO levels than expected have been seen with anemias associated with the following conditions: rheumatoid arthritis, acquired immunodeficiency syndrome, cancer, and ulcerative colitis[25], sickle cell disease, and in premature neonates.[20]

iv. After allogeneic bone marrow transplant, impaired erythropoietin response may delay erythropoietin recovery.[25]

v. Patients with hypergammaglobulinemia associated with multiple myeloma or Waldenstrom's disease have impaired production of erythropoietin in relation to hemoglobin concentration. This has been linked to increased plasma viscosity.[25]

vi. EPO levels of persons living at high altitudes with erythrocytosis may

rapidly fall to normal after returning to low altitudes.[26]

b. Limitations for *In Vivo* Bioassays

i. *In vivo* bioassays are affected by the same factors that affect erythropoiesis *in vivo* and may be present in the injected sample.[20] Erythropoietic agents such as cobalt or androgens and contaminants such as endotoxin can interfere with the assay.[21]

ii. If assay precision is insufficient, it is important to state "This test is unable to detect slight changes in EPO concentration found in mild or moderate anemias or in mild or moderate secondary polycythemias.[22]"

iii. It has been shown that some serum samples contained proteins and polypeptides that stimulated *in vitro* erythropoiesis by bioassay, but were distinct from erythropoietin.[21]

c. Limitations for *In Vitro* Bioassays

i. Inaccurate mouse spleen cell bioassay results have been observed at normal levels[16.] These inaccuracies may have been caused by non specific inhibitors in patient serum samples or by differences between patient's serum and the artificial solution in which assay calibrators were dissolved (matrix effects).

ii. The reactivity of *in vitro* bioassays may be modulated by some inhibitors that are poorly defined or by non specific stimulators that may be present in the patient sample. Examples of such substances are transferrin-bound serum iron,[21] unknown factors in fetal calf serum used as sample diluent, or inhibiting complement-dependent antibodies.[23] To be sure positive results are valid, assaying at least three dilutions of patient sample to establish parallelism of results compared to reference standards is useful.

iii. *In vitro* bioassays can detect deglycosylated erythropoietin, which is inactive *in vivo.*[21]

iv. Endotoxin, which may be present ubiquitously in erythropoietin preparations, significantly inhibited erythropoiesis in *in vitro* bioassays.[21]

v. Plasma samples from patients with chronic renal failure contained substances which inhibited erythropoiesis in *in vitro* bioassays.[21]

d. Limitations for Immunological Assays

Heterophile Antibodies

The presence in patient samples of anti-species antibodies caused by allergic reactions to animals or by treatment with mouse monoclonal antibodies or rabbit anti-lymphocyte globulin have been shown to cause falsely elevated results in many immunoassays, including EPO tests.[15] It

is recommended that dilutions of any elevated and/or suspect positive results be assayed to detect non-parallelism compared to reference standards.[17]

6. Expected Values [§809.10 (b)(11)]

a. Quantitative tests:

i. Reference Range for normal persons

It is helpful to describe the population tested by geographic location, range of results, numbers of each sex tested, age range, any symptoms of disease and other factors that might influence the values obtained, e.g., pregnancy, smoking, etc. It is useful also to describe the statistical method used to characterize the population. It is suggested to conclude this discussion by stating that each laboratory should establish reference ranges for its own patient population.

ii. Sampling Variables

If applicable, how test results may vary depending on age, sex, diurnal variation, etc., should be described.

iii. Abnormal Results

It is suggested that this section of the package insert summarize from a literature survey how results may vary for different diseased populations: primary and secondary anemias and polycythemias.
For example: In patients with erythrocytosis due to uncompensated hypoxia, serum immunoreactive EPO is elevated; in those with compensated hypoxia, the serum immunoreactive EPO level is usually within the range of normal, and in patients with polycythemia vera, serum immunoreactive EPO is either normal or low. Thus, while an elevated serum EPO level suggests that erythrocytosis is a secondary phenomenon and a low EPO level supports the possibility of autonomous

erythropoiesis, a normal serum EPO level excludes neither hypoxia nor autonomous EPO production as the cause of erythrocytosis.[19]

b. Qualitative tests:

It is suggested to explain and justify the cutoff or threshold levels recommended for use of the device, including also criteria for borderline or equivocal results.

i. It is useful to include a description of the clinical studies performed to establish the specificity and sensitivity of the device. It is suggested that the results be presented in a 2 x 2 table.

ii. Also in this section of the package insert it should be discussed how test results may have varied depending on geographical location, age, and sex of the study population, and diurnal variation, etc.

7. Performance Characteristics [§ 809.10(b)(12)]

Summarize the data upon which the performance characteristics are based, e.g., accuracy (comparison and recovery), precision (repeatability), specificity (interference, cross-reactivity and matrix effects/parallelism), and sensitivity (limits of detection).

VIII. References

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