

13.0 GLOSSARY¹

Accuracy²: (a) The closeness of agreement between a test method result and an accepted reference value. (b) The proportion of correct outcomes of a test method. Accuracy is a measure of test method performance and one aspect of relevance. The term is often used interchangeably with concordance (see two-by-two table). Accuracy is highly dependent on the prevalence of positives in the population being examined.

Amebocytes: The blood cells of the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*) that contain the active components of the reagent used in the BET.

Assay²: The experimental system used. Often used interchangeably with "test" and "test method."

Bacterial endotoxin test (BET)³: A test used to quantify endotoxins of Gram-negative bacterial origin using amebocyte lysate from the horseshoe crab. Two types of techniques exist: the gel-clot techniques, which are based on gel formation and the photometric techniques. The photometric techniques include the turbidimetric technique, which is based on the development of turbidity after cleavage of an endogenous substrate and a chromogenic method, which is based on the development of color after cleavage of a synthetic peptide-chromogen complex.

Coded substances: Substances labeled by code rather than name so that they can be tested and evaluated without knowledge of their identity or anticipation of test results. Coded substances are used to avoid intentional or unintentional bias when evaluating laboratory or test method performance.

Coefficient of variation (CV): A statistical representation of the precision of a test. It is expressed as a percentage and is calculated as follows:

$$\left(\frac{\text{standard deviation}}{\text{mean}} \right) \times 100\%$$

Concordance²: The proportion of all substances tested that are correctly classified as positive or negative. It is a measure of test method performance and one aspect of relevance. The term is often used interchangeably with accuracy (see two-by-two table). Concordance is highly dependent on the prevalence of positives in the population being examined.

Endogenous pyrogens: Various cytokines including interleukins (e.g., IL-1 α , IL-1 β), tumor necrosis factor (i.e., TNF- α , TNF- β), and interferon (IFN- γ) released from leukocytes in response to external stimuli (e.g., endotoxin) capable of causing an increase in body temperature above the normal level.

¹The definitions in this Glossary are restricted to the RPT, the *in vitro* pyrogen test methods included in this BRD, and the BET.

²From ICCVAM (2003)

³From USP (2005)

Endotoxin limit concentration (ELC): The concentration at which endotoxin is considered to be pyrogenic. It is expressed as the ratio of the threshold pyrogen dose (K) and the RPT dose or the maximum human dose administered on a weight (kg) basis in 1 hr (M) defined as K/M. The ELC varies based on M.

- The U.S. Food and Drug Administration (FDA) ELC for non-intrathecal medical devices is 0.5 EU/mL.
- The FDA ELC for intrathecal medical devices is 0.06 EU/mL.

Endpoint²: The biological or chemical process, response, or effect assessed by a test method.

False negative²: A substance incorrectly identified as negative by a test method.

False negative rate²: The proportion of all positive substances falsely identified by a test method as negative (see two-by-two table). It is one indicator of test method accuracy.

False positive²: A substance incorrectly identified as positive by a test method.

False positive rate²: The proportion of all negative substances that are falsely identified by a test method as positive (see two-by-two table). It is one indicator of test method accuracy.

Fever: Elevation of body temperature above the normal level.

Good laboratory practices (GLP)²: Regulations promulgated by the FDA and the U.S. Environmental Protection Agency, principles and procedures adopted by the Organization for Economic Cooperation and Development, and Japanese authorities that describe record keeping and QA procedures for laboratory records that will be the basis for data submissions to national regulatory agencies.

Hazard²: The potential for an adverse health or ecological effect. A hazard potential occurs only if an exposure occurs that leads to the possibility of an adverse effect being manifested.

Interlaboratory reproducibility²: A measure of whether different qualified laboratories using the same protocol and test substances can produce qualitatively and quantitatively similar results. Interlaboratory reproducibility is determined during the prevalidation and validation processes and indicates the extent to which a test method can be transferred successfully among laboratories.

Intralaboratory repeatability²: The closeness of agreement between test results obtained within a single laboratory when the procedure is performed on the same substance under identical conditions within a given time period.

Intralaboratory reproducibility²: The first stage of validation; a determination of whether qualified people within the same laboratory can successfully replicate results using a specific test protocol at different times.

In vitro: In glass. Refers to assays that are carried out in an artificial system (e.g., in a test tube or petri-dish) and typically use single-cell organisms, cultured cells, cell-free extracts, or purified cellular components.

In vivo: In the living organism. Refers to assays performed in multi-cellular organisms.

Lipopolysaccharide (LPS): A complex of lipid and carbohydrate (i.e., endotoxin) released from the cell walls of Gram-negative organisms that is pyrogenic and capable of producing septic shock.

Lipoteichoic acid: A polyol phosphate polymer bearing a strong negative charge that is covalently linked to the peptidoglycan in Gram-positive bacteria. It is strongly antigenic, but is generally absent in Gram-negative bacteria. Therefore, it is considered the primary pyrogenic component of Gram-positive bacteria.

Minimum valid concentration (MVC): The concentration of a product when it is diluted to the MVD expressed as $\lambda M/K$, where:

- λ = The sensitivity of the *Limulus* Amebocyte Lysate (LAL) reagent used expressed as EU/mL. The value varies with the method employed. For the gel-clot method, it is the labeled LAL sensitivity (EU/mL). For the chromogenic, turbidometric, or kinetic-turbidometric methods, it is the lowest point used in the standard curve.
- **M** = The maximum human dose for pyrogenicity administered on a weight basis (kg) in 1 hr, or the RPT dose (whichever is larger). It is one of the variables used to define the ELC defined as the ratio of K/M , where K is the threshold pyrogen dose in rabbits or humans.
- **K** = See threshold pyrogen dose.

Maximum valid dilution (MVD): When a U.S. Pharmacopeia (USP) ELC is defined, the MVD is the ratio of the product of the ELC and the product potency to the LAL reagent sensitivity (λ) expressed as $([ELC \times \text{Product Potency}]/\lambda)$. If there is no official USP ELC defined, then the MVD is the ratio of the Product Potency/MVC.

Monocytoid cells: Cells obtained from peripheral blood or grown in culture that phenotypically resemble monocytes or macrophages.

Negative control: An untreated sample containing all components of a test system, except the test substance solvent, which is replaced with a known non-reactive material, such as water. This sample is processed with test substance-treated samples and other control samples to determine whether the solvent interacts with the test system.

Negative predictivity²: The proportion of correct negative responses among substances testing negative by a test method (see two-by-two table). It is one indicator of test method accuracy. Negative predictivity is a function of the sensitivity of the test method and the prevalence of negatives among the substances tested.

Parenteral: Introduction into the body by some means other than through the gastrointestinal tract; referring particularly to intravenous (i.v.), intramuscular, subcutaneous, or intrathecal injection.

Performance²: The accuracy and reliability characteristics of a test method (see accuracy and reliability).

pH: A measure of the acidity or alkalinity of a solution. A pH of 7.0 is neutral; higher pHs are alkaline, lower pHs are acidic.

Positive control: A sample containing all components of a test system and treated with a substance known to induce a positive response, which is processed with the test substance-treated and other control samples to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay over time.

Positive predictivity²: The proportion of correct positive responses among substances testing positive by a test method (see two-by-two table). It is one indicator of test method accuracy. Positive predictivity is a function of the sensitivity of the test method and the prevalence of positives among the substances tested.

Prevalence²: The proportion of positives in the population of substances tested (see two-by-two table).

Protocol²: The precise, step-by-step description of a test method, including a list of all necessary reagents and criteria and procedures for evaluation of the test data.

Pyrogen: A substance that causes a rise in body temperature above normal or that produces a fever. Gram-negative, Gram-positive, and acid-fast bacteria, molds, viruses, and yeast and some of their cellular constituents are pyrogenic.

Quality assurance (QA)²: A management process by which adherence to laboratory testing standards, requirements, and record keeping procedures is assessed independently by individuals other than those performing the testing.

Rabbit pyrogen test (RPT)³: A test designed to limit to an acceptable level the risks of febrile reaction in the patient to the administration, by injection, or the product concerned. The test involves measuring the rise in temperature of rabbits following the i.v. injection of a test solution.

Reduction alternative²: A new or modified test method that reduces the number of animals required.

Reference test method²: The accepted *in vivo* test method used for regulatory purposes to evaluate the potential of a test substance to be hazardous to the species of interest.

Refinement alternative²: A new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal well-being.

Relevance²: The extent to which a test method correctly predicts or measures the biological effect of interest in humans or another species of interest. Relevance incorporates consideration of the accuracy or concordance of a test method.

Reliability²: A measure of the degree to which a test method can be performed reproducibly within and among laboratories over time. It is assessed by calculating intra- and inter-laboratory reproducibility and intralaboratory repeatability.

Replacement alternative²: A new or modified test method that replaces animals with non-animal systems or one animal species with a phylogenetically lower one (e.g., a mammal with an invertebrate).

Reproducibility²: The consistency of individual test results obtained in a single laboratory (intralaboratory reproducibility) or in different laboratories (interlaboratory reproducibility) using the same protocol and test substances (see intra- and inter-laboratory reproducibility).

Sensitivity²: The proportion of all positive substances that are classified correctly as positive in a test method. It is a measure of test method accuracy (see two-by-two table).

Specificity²: The proportion of all negative substances that are classified correctly as negative in a test method. It is a measure of test method accuracy (see two-by-two table).

Test²: The experimental system used; often used interchangeably with “test method” and “assay.”

Test method²: A process or procedure used to obtain information on the characteristics of a substance or agent. Toxicological test methods generate information regarding the ability of a substance or agent to produce a specified biological effect under specified conditions. Used interchangeably with “test” and “assay” (see validated test method and reference test).

Test method component: Structural, functional, and procedural elements of a test method that are used to develop the test method protocol. These components include unique characteristics of the test method, critical procedural details, and quality control measures.

Threshold pyrogen dose: The dose level at which a product is considered to be pyrogenic or non-pyrogenic. It is one of the variables (K) used to calculate the ELC defined as K/M, where M is the RPT dose or the maximum human dose administered in 1 hr (whichever is larger).

- The threshold pyrogen dose for non-intrathecal use in rabbits and humans is 5.0 EU/kg.
- The threshold pyrogen dose for intrathecal use in rabbits and humans is 0.2 EU/kg.

Tiered testing: A testing strategy where all existing information on a test substance is reviewed, in a specified order, prior to *in vivo* testing. If the irritancy potential of a test substance can be assigned, based on the existing information, no additional testing is required. If the irritancy potential of a test substance cannot be assigned, based on the existing information, a step-wise animal testing procedure is performed until an unequivocal classification can be made.

Transferability²: The ability of a test method or procedure to be accurately and reliably performed in different, competent laboratories.

Two-by-two table²: The two-by-two table can be used for calculating accuracy (concordance) ($(a+d)/(a+b+c+d)$), negative predictivity ($d/(c+d)$), positive predictivity ($a/(a+b)$), prevalence ($(a+c)/(a+b+c+d)$), sensitivity ($a/[a+c]$), specificity ($d/[b+d]$), false positive rate ($b/[b+d]$), and false negative rate ($c/[a+c]$).

		NEW TEST OUTCOME		
		Positive	Negative	Total
Reference Test Outcome	Positive	a	c	a + c
	Negative	b	d	b + d
	Total	a + b	c + d	a + b + c + d

Validated test method²: An accepted test method for which validation studies have been completed to determine the relevance and reliability of this method for a specific proposed use.

Validation²: The process by which the reliability and relevance of a procedure are established for a specific purpose.

Weight of evidence (process): The strengths and weaknesses of a collection of information are used as the basis for a conclusion that may not be evident from the individual data.