



Designation: E3395 – 23

Standard Test Method for Characterizing Performance of Field Screening Devices for the Identification of Biological Agents¹

This standard is issued under the fixed designation E3395; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 General:

1.1.1 This test method provides a procedure for characterizing the performance of nucleic acid-based field screening devices (FSDs) for the detection and identification of biological agents, when utilizing the test samples and statistical considerations described in Specification E3394.

1.1.2 This test method describes sample preparation and analysis protocols to use when characterizing the performance of nucleic acid-based field screening devices for the detection and identification of biological agents.

1.1.3 The intent of this test method is to provide a methodology to analyze samples in a manner that is analogous to how they are to be analyzed in the field by federal and state/local/tribal/territorial (SLTT) law enforcement and first responders, but under more controlled and reproducible conditions than those generally achievable when conducting field testing. The analysis of testing results as described in this test method and in Specification E3394 allow for a systematic way of measuring the statistical performance of FSDs.

1.2 Units:

1.2.1 The values stated in SI units are to be regarded as standard in this document.

1.2.2 When creating test sample mixtures, all concentrations are stated as copies/mL or genome equivalents/mL (GE/mL).

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 ASTM Standards:²

E2458 Practices for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biological Agents and Toxins from Nonporous Surfaces

E2771 Terminology for Homeland Security Applications

E3131 Specification for Nucleic Acid-Based Systems for Bacterial Pathogen Screening of Suspicious Visible Powders

E3289 Guide for Using Equipment and Assays for Field Detection of Fentanyl and Fentanyl-Related Compounds

E3290 Test Method for Establishing Performance of Equipment and Assays for Field Detection of Fentanyl and Fentanyl-Related Compounds

E3394 Specification for Field Screening Devices Used for Identification of Biological Agents

2.2 Federal Standards:³

18 US Code 178 Definitions

3. Terminology

3.1 Definitions:

3.1.1 *accuracy, n*—closeness of agreement between a test result and the accepted reference value. **E2771**

3.1.2 *assay, n*—quantitative or qualitative test used to determine the presence or absence of a chemical or biological material.

3.1.2.1 *Discussion*—Amended the definition from Specification E3131 to include chemical as well as biological material. **E3131**

3.1.3 *biological agent, n*—any microorganism (including, but not limited to bacteria, viruses, fungi, rickettsia, or protozoa); infectious substance; or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance capable of causing: (1) death, disease, or other biological malfunction in a human, an animal,

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² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

³ Available from U.S. Government Publishing Office (GPO), 732 N. Capitol St., NW, Washington, DC 20401, <http://www.gpo.gov>.

a plant, or other living organism; (2) deterioration of food, water, equipment, supplies, or material of any kind; or (3) deleterious alteration of the environment. **18 USC 178**

3.1.3.1 *Discussion*—Also termed bioterror agent.

3.1.4 *calibration, n*—set of operations that establish, under specified conditions, the relationship between the values of quantities indicated by a measurement instrument or measuring system or values represented by a material measure or a reference material and the corresponding values realized by standards.⁴

3.1.5 *confidence interval, CI, n*—range of values created using a procedure that, when repeated many times, on distinct data sets, generated from the same underlying stochastic process, will bracket the true measure of performance, such as probability of detection (POD), the proportion of times stated in the confidence level. **E3131**

3.1.5.1 *Discussion*—This definition differs slightly from the one in Specification **E3131** by explicitly linking the interval to the confidence level.

3.1.6 *confidence level, CL, n*—probability value association with a CI; the percentage of intervals that can be expected to include the true population parameter in the long run. **E3131**

3.1.7 *environmental panel, n*—collection of materials and compounds that can be found in the environment (indoor or outdoor).

3.1.7.1 *Discussion*—The environmental panel is determined by the entity directing testing and will include samples representative of aerosol backgrounds in the locations of interest. Note that this test method does not include discussion of aerosol filter sample collection, sample preparation conditions, or methods that may be necessary to extract collected material into a suitable liquid for analysis using nucleic acid-based methods. The environmental panel is used as a more “real-world” test of a detection system’s performance when spiked with a whole organism. The environmental panel should not result in any false positive or negative results and should not cause failure of any control samples.

3.1.8 *exclusivity panel, n*—collection of near-neighbor biological agents, viruses, or nucleic acids used during testing. **E3131**

3.1.8.1 *Discussion*—Results from tests involving members of the exclusivity panel, if carried out, shall follow the methodology presented in this test method and in Specification **E3394**.

3.1.9 *false negative, n*—failure to detect a compound within a sample when it is present at a concentration well above the limit of detection. **E3289**

3.1.9.1 *Discussion*—This definition differs slightly from the one in Guide **E3289** by emphasizing concentrations above the limit of detection.

3.1.10 *false positive, n*—detection of a compound within a sample when it is not present. **E3289**

3.1.11 *genome, n*—the complete set of genetic material of a human, animal, plant, or other living thing.⁵

3.1.12 *genome equivalents, GE, n*—number of genome copies present in a given mass of nucleic acid (deoxyribonucleic (DNA) or ribonucleic acid (RNA)) that can be calculated by converting the size of a genome in base pairs to micrograms of DNA or RNA.

3.1.13 *inclusivity panel, n*—collection of closely related biological agents, viruses, or nucleic acids used during testing. **E3131**

3.1.13.1 *Discussion*—Tests involving members of the inclusivity panel, if carried out, shall follow the methodology presented in this test method and in Specification **E3394**.

3.1.14 *inhibition, n*—undesirable effect that can result in a false negative result and is typically caused by the presence of compounds that interfere with the assay or detection process. **E3131**

3.1.15 *limit of detection, LOD, n*—lowest amount of analyte in a sample that can be detected with stated probability. **E3131**

3.1.16 *lower confidence bound, LCB, n*—lowest value of a one-sided CI created using a procedure that, when repeated many times on distinct data sets generated from the same underlying stochastic process, will include the true measure of performance a proportion of times equal to the stated probability. **E3131**

3.1.16.1 *Discussion*—The LCB ensures that the POD attains a satisfactory value for the CL selected and determines the minimum number of samples that shall be analyzed. Slightly modified from Specification **E3131**.

3.1.17 *measurement process, n*—step, or series of systematic steps, used to detect a material or determine if a system or instrument performs as intended. **E3289**

3.1.18 *multiplex assay, n*—assay that is capable of measuring multiple biological agents or multiple targets for a single agent in a single test sample. **E3131**

3.1.18.1 *Discussion*—This definition differs slightly from the one in Specification **E3131** by including multiple targets.

3.1.19 *near neighbor, n*—organism, virus, or nucleic acid that is similar to a desired target biological agent but should not result in a positive detection result. **E3131**

3.1.19.1 *Discussion*—Exclusivity panel members include near neighbors.

3.1.19.2 *Discussion*—Testing involving near neighbors, if carried out, shall follow the methodology presented in this test method and in Specification **E3394**.

3.1.20 *panel, n*—collection of samples used during testing.

3.1.20.1 *Discussion*—Examples of panels include inclusivity panel, exclusivity panel, and environmental panel.

3.1.21 *pooling, v*—act of creating a single test sample (the pooled sample) that contains strains from different biological agents. **E3131**

⁴ Eurachem Selection, Use and Interpretation of Proficiency Testing (PT) Schemes by Laboratories, 2nd edition, 2011. www.eurachem.org.

⁵ From <https://dictionary.cambridge.org/us/dictionary/english/genome>.