QUALITY ASSURANCE ISSUES ASSOCIATED WITH DIAGNOSTIC PROTOCOLS FOR REGULATED PESTS

(Prepared by Norman BARR)

Background

The TPDP in its 2014 July meeting reviewed an earlier version of this document (Agenda Item 7.3) on Quality Assurance (QA) issues associated with DPs. The panel agreed that the document should serve as an internal resource for the TPDP members. The document compiles terminology related to protocol QA to (1) encourage consistency in usage and (2) inform discipline leads (i.e. TPDP members) of terms not common to all disciplines but useful in examination of DPs. This will aid in DP review by discipline leads across subject disciplines (e.g., Entomology, Virology) and avoid inconsistency in usage of terminology across DPs. At the 2015 June meeting the TPDP requested that the definitions include sources or references. The document has been revised to provide definitions and references. This document was presented again to the TPDP at its February 2017 meeting and TPDP members were invited to provide comments. No additional comments were provided. This document was presented again to the TPDP at its February 2018 meeting and TPDP members were invited to provide comments. The TPDP discussed if definitions provided for Validation and Verification by source ISO/IEC GUIDE99:2007 should be included or removed because these do not enhance clarity of TPDP.

**The TPDP is invited to:**

1. *review* the changes that are underlined, and decide if the text marked using strikethrough that reference the GUIDE99:2007 source should be *removed* AND if new definitions from the American Phytopathological Society (APS) should be *accepted* in the document.
2. *review* the quality assurance issues associated with DPs for regulated pests document, and see changes that are underlined, and *revise* if appropriate.

QUALITY ASSURANCE ISSUES ASSOCIATED WITH DIAGNOSTIC PROTOCOL FOR REGULATED PESTS

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Purpose

The purpose of this document is to describe Quality Assurance (QA) terms that are relevant to Diagnostic Protocols (DPs). These QA terms and concepts are not applicable to all DPs. The QA information required for a DP is indicated in the Instructions to Authors document. When QA results are available for a DP, it is advisable to include that information and its literature reference.

During the 2008 June TPDP meeting, the panel noted that many organizations had definitions for QA terms and did not want to generate new terminology in conflict with existing definitions. The panel agreed that any definitions used in the production of diagnostic protocols (DP) should be suitable for phytosanitary uses. The current document is not intended to define terms used in accreditation, certification, proficiency testing, or licensing.

QA terms and definitions used in DPs

***Analytical sensitivity***: This is often referred to as limit of detection.\*

1. Smallest amount of target that can be detected reliably [EPPO Bulletin (2014) 44: 335-337, PM7/76(3)]
2. Smallest amount of the target that can be detected reliably (target may include live organisms, antibodies, nucleic acids) (IPPC TPDP Instructions to Authors)

*\*Draft Standard ISO13484 might affect definitions for sensitivity but possibly wait until release before changing*

***Analytical specificity***:

1. Performance of a test with regard to cross-reactions with non-target or lack of reaction with target [EPPO Bulletin (2014) 44: 335-337, PM7/76(3)]
2. Characteristics of a test as concerns its performance with regard to cross-reactions with non-target (false positives) or lack of reaction with target (e.g. subgroups or individuals of the pest) (false negatives) (IPPC TPDP Instructions to Authors)

***Nucleic Acid Controls****:* There are several types of controls used to confirm sample and assay performance.

*\*Do we need to include definitions for controls of other methods?*

1. *Positive nucleic acid control.* This control is used to monitor whether or not the test performed as expected under the experimental conditions and parameters.

*Negative amplification control (no template control).* This control is necessary for PCR to rule out false positives due to contamination during preparation of the reaction mixture or to non-specific amplification. (Modified from IPPC TPDP Instructions to Authors)

*Positive extraction control.* This is used to ensure that nucleic acid from the target is of sufficient quantity and quality and that the target is detected. Nucleic acid is extracted from infected host tissue or healthy host tissue that has been spiked with the target. (IPPC TPDP Instructions to Authors)

*Negative extraction control.* This is used to monitor contamination during nucleic acid extraction or cross-reactions with the host tissue. (IPPC TPDP Instructions to Authors)

***Diagnostic sensitivity***: Proportion of infested samples testing positive compared to results from an alternative test (or combination of tests) [EPPO Bulletin (2014) 44: 335-337, PM7/76(3)]

***Diagnostic specificity***: Proportion of uninfested samples testing negative compared to results from an alternative test (or combination of tests) [EPPO Bulletin (2014) 44: 335-337, PM7/76(3)]

***Limit of Detection (LoD):***

1. the lowest amount of a target which can be reliably detected and distinguished from zero results and background signals with confidence (Hopkins & Barwick 2008, An Introduction to Methods Validation, In: Essentials of Nucleic Acids Analysis, Eds. Keer & Birch, RSC publishing)
2. measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a component in a material is β, given a probability α of falsely claiming its presence (ISO/IEC GUIDE99:2007, 4.18)

***Negative predictive value* (NPV)**: The probability that a negative test result correctly identifies the absence of a targeted pest; this can be measured as the proportion of true negatives compared to all negative results.

***Positive predictive value* (PPV)**: The probability that a positive test result correctly identifies the presence of a targeted pest; this can be measured as the proportion of true positives compared to all positive results.

***Reference Material****:* Material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties (ISO/IEC GUIDE99:2007, 5.13)

***Reference Specimen***: Specimen, from a population of a specific organism, conserved and accessible for the purpose of identification, verification or comparison [IPPC Glossary and ISPM 3:2005; revised CPM, 2009]

***Repeatability****:* A measure of Precision

1. Measurement precision under a set of repeatability conditions of measurement (ISO/IEC GUIDE99:2007, 2.21)
2. Level of agreement between replicates of a sample tested under the same conditions [EPPO Bulletin (2014) 44: 335-337, PM7/76(3)]

***Reproducibility****:* A measure of Precision

1. Measurement precision under reproducibility conditions of measurement (i.e., condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and replicate measurements on the same or similar objects) (ISO/IEC GUIDE99:2007, 2.25)
2. Ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (time, persons, equipment, location, etc.) [EPPO Bulletin (2014) 44: 335-337, PM7/76(3) and IPPC TPDP Instructions to Authors]
3. Level of agreement between aliquots of the same sample tested under different conditions. [No Known Source/Attributed] Reproducibility can be assessed at two levels:
4. in one laboratory -> intralaboratory reproducibility
5. in different laboratories -> interlaboratory reproducibility.

***Reliability*** (= precision and reproducibility)

***Ring Testing*** – See Test Performance Studies

***Robustness (or Ruggedness)***:

1. Measure of the capacity of the assay to remain unaffected by deliberate small variations in method parameters (provides an indication of assay reliability under normal use) (*Source*?)
2. The extent to which altered test conditions (e.g. temperature, volume) affect the established test performance values (e.g. analytical sensitivity, analytical specificity) [EPPO Bulletin (2014) 44: 335-337, PM7/76(3)]

***Selectivity***:

1. The extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behaviour (IUPAC)
2. Extent to which variations in the matrix affect test performance (matrix effect) [EPPO Bulletin (2014) 44: 335-337, PM7/76(3)]
3. Property of a measuring system, used with a specified measurement procedure, whereby it provides measured quantity values for one or more measurands such that the values of each measurand are independent of other measurands or other quantities in the phenomenon, body, or substance being investigated (ISO/IEC GUIDE99:2007, 4.13)

***Standard****:* Document established by consensus and approved by a recognized body, that provides, for common and repeated use, rules, guidelines or characteristics for activities or their results, aimed at the achievement of the optimum degree of order in a given context [FAO, 1995; ISO/IEC Guide 2:1991 definition]. *This term is occasionally used for biological or chemical material that has been recognized through certification by one or more organization to serve as reference material of a test method, but this can result in confusion with IPPC definition and should not be used.*

***Test Performance Studies (TPS) (also called Ring Testing)***:

1. Evaluation of the performance of one or more tests by two or more laboratories using defined samples (evaluation of a test) [EPPO Bulletin (2014) 44: 335-337, PM7/76(3)]

***Validation****:*

1. The confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled (ISO17025:2005 definition of Validation)
2. ~~Verification, where the specified requirements are adequate for an intended use (ISO/IEC GUIDE99:2007, 2.45)~~
3. Process that determines the fitness for purpose of an assay, which has been properly developed, optimized and standardized, for an intended use (APS Validatio Glossary, DOI: 10.1094/PHI-I-2018-0709-01)

***Verification***:

1. The Laboratory shall confirm that it can properly operate standard methods before introducing the tests or calibrations (ISO17025:2005 definition of Verification)
2. ~~Provision of objective evidence that a given item fulfils specified requirements (ISO/IEC GUIDE99:2007, 2.44)~~
3. Confirmation by examination of objective evidence that specified requirements have been fulfilled (APS Validation Glossary, DOI: 10.1094/PHI-I-2018-0709-01)