WADA Technical Document – ISL TD2027VAL

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Minimum Requirements for Validation of Analytical *Testing* Procedures for *Doping Control*

1.0 Introduction

This *Technical Document* (*TD*), which constitutes an integral part of the *International Standard* for Laboratories (ISL [1]), establishes minimum requirements for the validation of Analytical *Testing* Procedures (ATPs) for *Doping Control* purposes (see also *TD* ATP [2]).

Validation, in this context, refers to the process of ensuring that an Analytical Method produces consistent, reliable, and reproducible results for its intended purpose. By adhering to the specified validation requirements and criteria established in this ISL *TD*, the Laboratory ensures that its ATPs are Fit-for-Purpose and, therefore, suitable for the analysis of representative Analytes or other relevant for anti-doping purposes non-prohibited analytical targets (*e.g.*, non-prohibited confounding factors of the Steroidal Module of the *Athlete Biological Passport* (*ABP*) or non-prohibited substances that share common *Metabolites* with *Prohibited Substances*).

a) Any ATP, whether newly developed or based on the literature, shall be validated before being applied to the analysis of *Samples*.

   However, if this is a standard Test Method that has already been validated by a manufacturer or by a well-recognized organization (*e.g.,* by a National Measurement Institute), the Laboratory needs to perform only a verification of the performance of the Analytical Method.

b) Based on risk assessment, the Laboratory shall define within its Management System the conditions that would trigger Test Method revalidation or verification (for example, when changes in the ATP are needed or after a periodic review or update, or when moving into a new Laboratory facility with no impactful change of analytical instrumentation).

c) The inclusion of a validated ATP within the Laboratory's Scope of ISO/IEC 17025 [3] Accreditation establishes that the Analytical Method has been validated as Fit-for-Purpose.

This *TD* is applicable to all ATPs performed by a Laboratory [2]. However, additional specific validation requirements and considerations, which complement the requirements outlined in this ISL *TD*, can be found in other applicable ISL *TDs* (*e.g.*, ISL *TD* CG/LH [4], ISL *TD* DL [5], ISL *TD* IRMS [6], ISL *TD* USM [7], ISL *TD* BSM [8], ISL *TD* NA [9], ISL *TD* EPO [10], ISL *TD* GH [11]) or Laboratory Guidelines (LGs).

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2.0 Validation Reports

Validation records for ATPs shall be summarized in a Validation Report and supported by traceable documentation and the necessary analytical data. The Validation Report shall be approved by, at least, the Certifying Scientist in charge of the validation and the Laboratory Director (or authorized delegate).

The Validation Report shall include all information to support the results of the Laboratory's validation experiments, demonstrating the Fitness-for-Purpose of the ATP, including:

a) The purpose of the Analytical Method: Initial *Testing* Procedure (ITP) or Confirmation Procedure (CP), including whether it is a Qualitative Procedure or a Quantitative Procedure.

b) A signed statement that the Analytical Method is Fit-for-Purpose.

c) The scope (*e.g.*, full validation, complementary (re)validation, extension to method scope, etc.).

d) Code of the Standard Operating Procedure (SOP) of the Analytical Method or, if an SOP is not available, details of the applied Analytical Method:

   i. Sample preparation procedure and instrumental conditions.

   ii. Analytes and Internal Standards (ISTDs).

e) The type of the Analytical Method (*e.g.* standard, non-standard or Laboratory-developed, with references to the literature, manufacturer's method description or, alternatively, a reference to the SOP where this information can be found).

f) A summary of the relevant material information and results for each validation requirement, including:

   i. Certified Reference Materials (CRMs), where available, or Reference Materials (RMs), for Analyte(s) and ISTDs (*e.g.,* internal codes), and information that allows full traceability to origin, batch number, statements of measurements and/or Certificate of Analysis (CoA), stability, and storage conditions (whenever applicable).

   ii. Samples/Quality Controls (QCs) used for validation experiments, including information and traceability to content and preparation protocols (*e.g.*, source, characterization, storage conditions, stability).

   iii. Indication where experimental data can be found.

   iv. Acceptance criteria and conclusion.

   v. Relevant considerations for the use of the Analytical Method in routine analyses.

g) Unexpected results obtained during validation, with a description of the actions taken, if applicable.

h) The Validation Report shall ensure traceability to all relevant working documents, including files related to the Management System.

i) Files related to the Validation Report shall be retained for as long as *Sample* records, including routine analytical data derived from the application of the validated Test Method, are stored in the Laboratory (in accordance with ISL requirements [1]).

3.0 Validation Requirements

The validation process shall include the following key elements, as applicable, which may vary due to the nature of certain Analytical Methods (see also Table 1 in Article 4.0).

3.1. Selectivity [1]

[Comment to Article 3.1: For commercially available, standard Test Methods, if the Selectivity has already been evaluated by the manufacturer, the Laboratory shall at least verify that the manufacturer's Selectivity information can achieve the required performance. The manufacturer's declared Selectivity shall be relevant to the matrix of analysis.]

3.2. Limit of Detection (LOD) [1]

a) The LOD is estimated as the minimum concentration of the Analyte that can be routinely detected (but not necessarily identified or quantified) in representative samples at a 95% detection rate.

[Comment to Article 3.2 a): If the LOD cannot be directly established due to unknown Analyte concentrations (i.e., due to the reliance on a Reference Collection (RC) in the absence of RM), the Laboratory may estimate the LOD using a substance with similar physicochemical properties – for instance, the LOD for a Metabolite could be estimated based on data from its parent compound or other structurally related Metabolites.]

b) For chromatographic-mass spectrometric ITP, the Laboratory's method validation shall include the estimation of the LOD for all relevant Analyte(s).

i. For Non-Threshold Substances, the estimated LOD of the ITP shall be not higher than (≤):

- 50% of the corresponding Minimum Required Performance Level (MRPL) [12]

$$\text{LOD} \leq 0.5 \times \text{MRPL} \quad (1)$$

OR

- the corresponding *Minimum Reporting Level* (*MRL*) [13], where applicable.

$$\text{LOD} \leq MRL \quad (2)$$

[Comment to Article 3.2 b)-i.: Equation (2) is not applicable in the following cases, for which the LOD shall meet condition (1) LOD ≤ 0.5 · MRPL:

- *Those substances for which an MRL has been established to determine the concentration above which the finding shall be reported as an Adverse Analytical Finding (AAF) without the need to conduct GC/C/IRMS analysis (e.g., 19-NA, 19-NE, boldenone, boldenone Metabolite, formestane) [6, 9].*
- *Those Analytes with specific requirements established in relevant ISL Technical Letters (TLs), for example, substances classified under class S1.2 that may be used as growth promoters for livestock [14].*
- *In case of target Analytes with specific LOD requirements established in other relevant TDs.]*

ii. For Threshold Substances, the estimated LOD of the ITP shall be not higher than (≤):

- 50% of the corresponding Threshold [5]

$$\text{LOD} \leq 0.5 \times \text{Threshold} \quad (3)$$

[Comment to Article 3.2 b)-ii.: This is not applicable to those exogenous Threshold Substances which, if identified in a Sample in conjunction with a diuretic or a masking agent, shall be reported without quantification [4]. In these cases, the LOD shall be not higher than 50% of the corresponding MRPL [12] for the applicable substance class.]

iii. For *Markers* of the *ABP* that are analyzed with quantitative ITP procedures, as established in a relevant ISL *TD (e.g.* for the urinary and blood *Markers* of Steroidal Module of the *ABP*, see *TD* USM [7] or *TD* BSM [8], respectively), the LOD shall be not higher than (≤):

- 50% of the corresponding Limit of Quantification (LOQ)

$$\text{LOD} \leq 0.5 \times \text{LOQ} \quad (4)$$

c) For ITP based on PolyAcrylamide Gel-Electrophoretic (PAGE) procedures, the LOD, as estimated in the matrix of analysis during Test Method validation, shall be not higher than (≤) 50% of the corresponding MRPL (see ISL *TD* EPO [10]).

3.3. Limit of Identification (LOI) [1]

a) Chromatographic-Mass Spectrometric Test Methods

i. The Laboratory shall document that the chromatographic-mass spectrometric confirmatory Qualitative Procedure allows the identification of the relevant target Analyte(s) in compliance with the ISL *TD* IDCR [15].

ii. The Laboratory shall estimate, during method validation, the LOI of the CP, at a maximum 5% false negative identification rate, for a target Analyte for which an RM is available.

[Comment 1 to Article 3.3 a)-ii.: If the LOI cannot be directly established due to unknown Analyte concentrations (i.e., due to the reliance on an RC in the absence of RM), the Laboratory may estimate the LOI using a substance with similar physicochemical properties – for instance, the LOI for a Metabolite could be estimated based on data from its parent compound or other structurally related Metabolites.]

[Comment 2 to Article 3.3 a)-ii.: Since the LOI is an estimation of the identification rate at 95% probability obtained by the Laboratory during Test Method validation, the Laboratory may report a finding below the validated LOI as an AAF or an Atypical Finding (ATF), as applicable, when the Analyte is identified in the Sample according to the criteria established in the ISL TD IDCR [15]).]

iii. The Retention Time, Reference Diagnostic Ions, and Relative Abundances of all Diagnostic Ions/Ion Transitions shall be evaluated for LOI determinations according to the criteria established in the ISL *TD* IDCR [15].

iv. For the confirmation of Non-Threshold Substances (subject or not to an *MRL*), the LOI shall be less than (<):

- The corresponding MRPL [12]

$$LOI < MRPL \quad (5)$$

[Comment to Article 3.3 a) iv.: This may not be applicable for some specific target Analytes, as specified in a relevant ISL TD).

v. For the confirmation of Threshold Substances, the LOI evaluation is only applicable for the ATP applied for the qualitative identification of the Analyte(s), in compliance with the ISL *TD* IDCR [15].

For Threshold Substances, the LOI shall be less than (<):

- the corresponding Threshold [4, 5, 11].

$$LOI < T \quad (6)$$

[Comment to Article 3.3 a) v.: This is not applicable for those exogenous Threshold Substances which, if identified in a Sample in conjunction with a diuretic or a masking agent, shall be reported without quantification [5]. In those cases, the LOI shall be less than the corresponding MRPL for the applicable substance class.]

vi. For confirmation of *Markers* of the *ABP (e.g.,* for the urinary and blood *Markers* of the Steroidal Module of the *ABP*, see *TD* USM [7] and *TD* BSM [8], respectively), the LOI shall be less than (<) the corresponding LOQ.

$$LOI < LOQ \quad (7)$$

b) PAGE Analytical Methods

For PAGE Analytical Methods, the LOI is replaced by the LOD of the CP.

3.4. Limit of Quantification (LOQ):

The LOQ of Quantitative Procedures is estimated as the minimum concentration of the Analyte that can be measured with acceptable Measurement Uncertainty ($u_c \leq u_{c\_Max}$), as established in a relevant ISL *TD* (*e.g.*, ISL *TD* CG/LH [4], ISL *TD DL* [5], ISL *TD* IRMS [6], ISL *TD* USM [7], ISL *TD* BSM [8], ISL *TD* GH [11]).

3.5. Reliability of Detection

Capability of the ITP to consistently detect (at a 100% detection rate) Presumptive *Adverse Analytical Findings* (PAAFs) and indicate the need to conduct a CP on the *Samples*:

a) At the MRPL or below for Non-Threshold Substances without an *MRL* [12]: The capability to consistently detect the target Analyte at or below (≤) MRPL.

b) At the *MRL* or below for Non-Threshold Substances with an *MRL* [13]: The capability to consistently detect the target Analyte at ≤ *MRL* and properly estimate the concentration that should trigger a CP.

To account for a possible underestimation of concentrations of Non-Threshold Substances with an *MRL* during the ITPs, the Laboratory shall establish, and document in the Test Method's SOP, criteria determined during the ITP method validation (*e.g.*, concentration levels set as cut-offs) to evaluate initial results as PAAFs and ensure that all potentially positive *Samples* are subjected to CPs.

[Comment to Article 3.5. b): Unless otherwise specified in a ISL TD, ISL TL or LGs, the Laboratory may also choose to forward a Sample containing a Non-Threshold Substance with an MRL to confirmation analysis irrespective of the substance concentration in the Sample – therefore, establishing ITP concentration levels as cut-offs would not be applicable.]

c) At 50% Threshold or below for Threshold Substances [4, 5]: The capability to consistently detect the Analyte at concentrations equal to or lower than (≤) 50% Threshold and properly estimate the concentration that should trigger the relevant confirmatory Quantitative Procedure.

To account for a possible underestimation of concentrations of Threshold Substances during non-quantitative ITPs, the Laboratory shall establish, and document in the Test Method's SOP, criteria determined during the ITP method validation (*e.g.*, concentration levels set as cut-offs) to evaluate initial results as PAAFs and ensure that all potentially positive *Samples* are subjected to confirmatory Quantitative Procedures.

[Comment to Article 3.5. c): The Laboratory may also choose to forward a Sample containing an exogenous Threshold Substance to confirmation analysis, irrespective of the substance concentration in the Sample – therefore, establishing ITP concentration levels as cut-offs would not be applicable.]

3.6. Carryover

The potential transfer of Analytes or other materials from one *Sample* and/or QC to the other during the analysis.

3.7. *Sample* Extract Stability

The stability of Aliquots or any relevant extract of the analysis (after *Sample* preparation) while waiting for analysis.

3.8. Working Range

The range of concentrations of an Analyte that can be measured with an acceptable MU ($u_c \leq u_{c\_Max}$).

a) For Threshold Substances [4, 5, 11], the working range shall be documented from at least 50% to 200% of the Threshold value.

b) For Quantitative Procedures of substances without an associated Threshold value [6-8], the Working Range of the Test Method shall be appropriate for its intended use (*e.g.*, for the *Markers* of the Steroidal Module of the *ABP*, the working range should be investigated at the concentrations normally found in *Samples* analyzed by the Laboratory).

3.9. Repeatability (s_r)

Variability of results obtained within a Laboratory using the same method, over a short time, using a single operator, item of equipment, etc. It is also referred to as intra-batch / intra-run precision.

3.10. Intermediate Precision (s_w)

Variation in results observed when one or more factors, such as time, equipment, or operator are varied within a Laboratory. It is also referred to as inter-batch / inter-run precision.

3.11. Bias (*b*)

Systematic deviation of a measured result from the expected or reference value when using the complete measurement procedure.

3.12. Measurement Uncertainty (MU) [5]

For specific MU requirements, see the applicable ISL *TD* (*e.g.*, ISL *TD* CG/LH [4], ISL *TD DL* [5], ISL *TD* IRMS [6], ISL *TD* USM [7], ISL *TD* BSM [8], ISL *TD* GH [11] or LGs).

4.0 Applicability of the Validation Requirements

Table 1 below specifies the minimum applicable validation requirements across different types of ATP (ITPs and CPs, including Qualitative Procedures and Quantitative Procedures) and according to substance category (*i.e.*, Non-Threshold Substance without an *MRL*, Non-Threshold Substance with an *MRL*, and Threshold Substance) where needed.

[Comment to Article 4.0: The Laboratory may implement additional validation requirements beyond those specified in Table 1 for a given ATP. Furthermore, supplementary criteria might be necessary to ensure compliance with specific ISL TDs or ISL TLs, such as verifying the completeness of hydrolysis or derivatization.]

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Table 1. Summary of Validation Requirements by Procedure Type

Validation Requirement	**ITP** (all substances)	**CP** **(Qualitative Procedure)**	**CP** **(Quantitative Procedure)** [1]
Selectivity	Required	Required	Required
LOD	Required	Required [2]	N/A
Reliability of Detection	At ≤ MRPL (Non-Threshold Substance without *MRL*) At ≤ *MRL* (Non-Threshold Substance with *MRL*) At ≤ 50% Threshold (Threshold Substance)	N/A	N/A
LOI	N/A	Required [3]	N/A
Carryover	Required	Required	Required
Sample Extract Stability	Required	Required	Required
Working Range	N/A [4]	N/A	Required
LOQ	N/A [4]	N/A	Required
Repeatability (s_r)	N/A [4]	N/A	Required
Intermediate Precision (s_w)	N/A [4]	N/A	Required
Bias (b)	N/A [4]	N/A	Required
MU (u_c)	N/A [4]	N/A	Required

[1] Applicable to all procedures with an associated MU, except Specific Gravity (SG) determinations.

[2] Only required for non-chromatographic mass spectrometric CPs such as PAGE Analytical Methods [10]

[3] Only required for chromatographic-mass spectrometric confirmatory Qualitative Procedures.

[4] Unless the ITP classifies also as a Quantitative Procedure.

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5.0 References

[1] The World Anti-Doping *Code International Standard* for Laboratories (ISL).

[2] *WADA Technical Document* ISL *TD* ATP: Analytical *Testing* Procedures

[3] ISO/IEC 17025:2017 - General requirements for the competence of testing and calibration laboratories.

[4] *WADA Technical Document* ISL *TD* CG/LH: Analysis, Reporting & Management of Urinary Human Chorionic Gonadotrophin (hCG) and Luteinizing Hormone (LH) Findings in Male *Athletes.*

[5] *WADA Technical Document* ISL *TD DL*: *Decision Limits* for the Confirmatory Quantification of Exogenous Threshold Substances.

[6] *WADA Technical Document* ISL *TD* IRMS: Detection of Synthetic Forms of *Prohibited Substances* by GC/C/IRMS.

[7] *WADA Technical Document* ISL *TD* USM: Analytical and Reporting Requirements for the Urinary *Markers* of the Steroidal Module of the *Athlete Biological Passport*.

[8] *WADA Technical Document* ISL *TD* BSM: Analytical and Reporting Requirements for the Blood *Markers* of the Steroidal Module of the *Athlete Biological Passport*.

[9] *WADA Technical Document* ISL *TD* NA: Harmonization of Analysis and Reporting of 19-Norsteroids.

[10] *WADA Technical Document* ISL *TD* EPO: Harmonization of Analysis and Reporting of Erythropoietin (EPO) and other EPO-Receptor Agonists (ERAs) by Polyacrylamide Gel Electrophoretic (PAGE) Analytical Methods.

[11] *WADA Technical Document* ISL *TD* GH: Human Growth Hormone (hGH) Isoform Differential Immunoassays for *Doping Control* Analyses.

[12] *WADA Technical Document* ISL *TD* MRPL: Minimum Required Performance Levels for Non-Threshold Substances.

[13] *WADA Technical Document* ISL *TD MRL*: *Minimum Reporting Levels* Applied in *Doping Control*.

[14] *WADA* ISL *Technical Letter TL*23: *Minimum Reporting Level* for Certain Substances Known to be Potential Meat Contaminants.

[15] *WADA Technical Document TD* IDCR: Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the Identity of Analytes for *Doping Control* Purposes.

[Current versions of WADA's ISL, Technical Documents and Technical Letters may be found at https://www.wada-ama.org/en/what-we-do/international-standards]