WADA Technical Document – ~~TD2022DL~~ISL TD2027DL

Document ~~Number~~number:	~~TD2022DL~~ISL TD2027DL	Version ~~Number~~number:	1.0
Written by: Reviewed by:	*WADA* Science~~/~~; *DL* / MU Working Group *WADA* Laboratory Expert Advisory Group	Approved by:	*WADA* Executive Committee
Date:	~~6 October 2021~~DD month YYYY~~17~~ March 2027	Effective ~~Date~~date:	1 January ~~2022~~2027

DECISION LIMITS FOR THE CONFIRMATORY QUANTIFICATION OF EXOGENOUS THRESHOLD SUBSTANCES ~~BY CHROMATOGRAPHY-BASED ANALYTICAL METHODS~~

1.0 Introduction

~~The objective of this~~ This *Technical Document* (*TD*~~) is~~), which constitutes an integral part of the *International Standard* for Laboratories (ISL [1]), has been established to harmonize the reporting of results for exogenous Threshold Substances ~~(as listed in Table 1) when~~ analyzed in urine *Samples* ~~using chromatography-based quantitative Confirmation Procedures~~ (CP(as listed in Table 1), with particular regard to the *Decision Limits* (~~*DL*~~*DLs*) that shall be applied to determine whether the confirmed quantitative analytical result ~~indicates~~shall be reported as an *Adverse Analytical Finding* (*AAF*). ~~It also describes the situations where the *DL* shall be corrected by the specific gravity (SG) of the urine *Sample*, as well as the use of Measurement Uncertainty (MU) information in the establishment of such *DL*.~~

[Comment~~: Decision Limits~~ to Article 1.0: DLs for endogenous Threshold Substances ~~([~~e.g., human Chorionic Gonadotropin ~~–(~~hCG~~);~~); human Growth Hormone ~~–(~~hGH~~)~~), are defined in specific ISL TDs [2, 3]]

This ISL *TD* ~~[1, 2] or Laboratory Guidelines [3].]~~

~~This document~~ provides requirements ~~on~~for the following:

- ~~Target~~ Analytes~~;~~
  a) of exogenous Threshold Substances targeted in confirmatory Quantitative Procedures (see Article 2.0).
  b) applicable Thresholds (T) and ~~*DL*;~~*DLs* (see Table 1).
  c) Maximum allowed values of ~~MU;~~Measurement Uncertainty (MU) – see Table 1.
  d) Adjustment of the *DL* for the ~~SG;~~urinary Specific Gravity (SG) - (see Article 7.0).
  e) Reporting of quantitative results~~.~~ - (see Article 8.0).

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Further guidance is provided in Annex A, including:

f) ~~Estimating~~Estimation of MU~~:~~.

g) Verification of MU by a Laboratory.

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~~**Table 1**~~

Table 1. Exogenous Threshold Substances and Applicable Thresholds, *Decision Limits*, Maximum Allowed Measurement Uncertainties and Target Analytes

Substance Class	**Threshold Substance**	**Threshold (~~T~~)**	~~**Maximum Relative Combined Standard Uncertainty at T**~~ u_{c_Max} (%) [a]	***Decision Limit*** ~~***(DL)***~~ [a,b]	**Target Analyte(s)**
S2.1.2. Hypoxia-Inducible Factor Activating Agents	~~Salbutamol~~**Cobalt**	~~**1.00 µg**~~60.0 ng/mL	~~10~~20	~~**1.20 µg**~~**80.0 ng/mL**	Inorganic Cobalt (Co^{2+})
S3. Beta-2 Agonists	**Formoterol**	40.0 ng/mL	15	**50.0 ng/mL**	Total content of formoterol, including: • Free (non-conjugated) form of formoterol AND • Its glucuronidated phase-II *Metabolite*, expressed as formoterol equivalent

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	Salbutamol	1.00 µg/mL	10	**1.20 µg/mL**	Total content of salbutamol, including: • Free (non-conjugated) form of salbutamol AND • Its glucuronidated phase-II *Metabolite*, expressed as salbutamol equivalent
~~S6.~~S6b. Specified Stimulants	**Cathine** [c]	5.00 µg/mL ~~b~~	10	**6.00 µg/mL** ~~b~~	Total content of the free (non-conjugated) form of the target substance, including both levo- (*l*-) and dextro- (*d*-) enantiomers.
	Ephedrine	10.0 µg/mL	5.0	**11.0 µg/mL**	
	Methylephedrine	10.0 µg/mL	5.0	**11.0 µg/mL**	
	Pseudoephedrine	150 µg/mL	5.0	**170 µg/mL**	
S7. Narcotics	**Morphine (M)**	1.00 µg/mL	15	**1.30 µg/mL**	Total content of Morphine, including: • Free (non-conjugated) form of Morphine AND • The phase-II M-3-glucuronidated *Metabolite* (M3G), expressed as M equivalent AND • The phase-II M-6-glucuronidated *Metabolite* (M6G), expressed as M equivalent
Substance Class	**Threshold Substance**	**Threshold**	$u_{c\ Max}$ (%) [a]	***Decision Limit*** [b]	**Target Analyte(s)**
S8. Cannabinoids	**Tetrahydrocannabinol (THC)** • Carboxy-THC	150 ng/mL	10	**180 ng/mL**	Total content of COOH-THC, including: • Free (non-conjugated) form of COOH-THC AND

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	(COOH-THC; 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid)				• Its glucuronidated phase-II *Metabolite*, expressed as COOH-THC equivalent

a. ~~-~~Maximum Allowed Relative Combined Standard Uncertainty (at levels close to the Threshold).

~~a.~~b. The *DL*, expressed to three (3) significant figures, is obtained after adding a guard band *g* to the T, which accounts for the corresponding u_{c_Max} and ensures that any value above the *DL* obtained with the ~~quantitative Analytical Method~~Quantitative Procedure is higher than (>) the T with a statistical confidence of at least 95% (see Article ~~3~~5.0).

~~b. The Threshold of 5.00 µg/mL and *DL* of 6.00 µg/mL are applicable to cathine and its *l*-enantiomer (also referred to as 1S,2S- and 1R,2R-norpseudoephedrine, respectively).~~

~~1.0 Target Analytes~~

- ~~Quantitative result~~

c. ~~The *International Standard* for Laboratories (ISL) [4] requires that results from quantitative CP applied to~~ Phenylpropanolamine (also know as norephedrine) is a diastereoisomer of cathine that is not prohibited and, therefore, shall be adequately chromatographically separated from cathine.

2.0 Confirmation Procedure for Exogenous Threshold Substances

2.1 "A" *Sample* Confirmation Procedure

As per the ISL [1], the "A" Confirmation Procedure (CP) for a Threshold Substance requires the application of:

a) A Quantitative Procedure to measure the property value (*e.g.*, concentration) of relevant target Analyte(s) of the Threshold Substance (see Table 1), and

b) A Qualitative Procedure (where applicable) for the identification of the Analyte(s) of the Threshold Substance.

2.1.1 Quantitative Procedure

a) The quantitative Confirmation Procedure (CP) for a Threshold Substance shall be based on the determination of the mean of measured property values (*e.g.*, concentrations) in three (3) ~~independent determinations. The~~"A" *Sample* Aliquots. If there is not enough *Sample* volume to analyze three (3) Aliquots, the maximum number of Aliquots that can be prepared should be analyzed.

[Comment to Article 2.1.1 a): The use of fewer than three (3) Aliquots due to insufficient Sample volume shall not invalidate the CP nor the resulting ~~relative standard deviation (RSD, %) shall be consistent with the~~ quantitative ~~CP method validation data.~~ determination.]

~~a)~~b) The Laboratory shall demonstrate the Fitness-for-Purpose of the ~~quantitative CP through method~~Quantitative Procedure Test Method validation, including the estimation of the MU~~.~~ (also refer to the ISL *TD* VAL [4]). Compliance with the criteria presented in Table 1 for u_{c_Max} (%) ensures a harmonized reporting of *AAF*s at concentration levels exceeding the applicable *DL*.

~~• Qualitative result~~

c) ~~In one~~The standard deviation of the ~~three (3) independent~~replicate determinations shall be consistent with the combined standard uncertainty of the measurement $u_c(y)$. This consistency can be evaluated using The Standard Error of the Mean (SEM) of the replicate determinations using Eq. 1 below.

$$\text{(Eq. 1)} \quad SEM = \sqrt{\frac{SD^2}{n}} \leq k \times u_c(y)$$

where:

SD: standard deviation of the replicate determinations
n: number of replicates
k: factor associated with the uncertainty of *SD* (for $n = 3$, $k = 1$; for $n = 2$, $k = 1.4$)

d) The Quantitative Procedure shall include the analysis, in the same analytical run and with the same number of replicates as the *Sample*, of appropriate QC sample(s) prepared in the matrix of analysis. The evaluation of the measured concentration(s) of the QC(s), for example, through the use of QC-charts (for frequently performed

procedures. *i.e.*, $n \geq 6$ per year) or through comparison with the expected QC concentration value (for not frequently performed procedures)[1] may serve to determine if the Test Method is Fit-for-Purpose or if the results should be rejected and the analysis repeated.

2.1.2 Qualitative Procedure

When applying a chromatographic-mass spectrometric Qualitative Procedure, the target Analyte(s) shall be identified in compliance with the ~~prevailing~~ISL *TD* IDCR [5]~~.~~ in one (1) of the three (3) Aliquots used for the quantification. The Limit of Identification (LOI) of the confirmatory Qualitative Procedure shall be not higher than (≤) the corresponding Threshold.

~~2.1 Beta-2 Agonists - Formoterol and Salbutamol~~

~~The concentration level is based on content of formoterol or salbutamol, defined as the combination~~ *[Comment to Article 2.1.2: When multiple replicates of a QC sample are analyzed, the Laboratory's Management System should specify which replicate will be used for evaluating qualitative identification criteria (e.g., in conformity with the TD IDCR [12]).]*

2.2 "B" *Sample* Confirmation Procedure

[1] The Laboratory shall verify whether the measured Analyte concentration in the positive QC sample ($\bar{X}, n \geq 3$) is compatible with the expected value (X_{ref}).

a) If the absolute difference $|\bar{X} - X_{ref}|$ is less than or equal to (≤) the expanded uncertainty of that difference ($U_{95\%}$, $k = 2$), the Test Method is performing as expected.

$$\text{(Eq. 2)}\quad |\bar{X} - X_{ref}| \leq \sqrt{U_{\bar{X}}^2 + U_{xref}^2} = 2 \cdot \sqrt{u_c^2(\bar{X}) + u_c^2(X_{ref})}$$

b) If the absolute difference $|\bar{X} - X_{ref}|$ exceeds (>) the expanded uncertainty of that difference, the Test Method performance is outside acceptable limits; therefore, the *Sample* results should be rejected and the analysis repeated.

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For the "B" CP of ~~free substance and its glucuronide conjugated forms, expressed as substance equivalent.~~

~~If either of these~~ an exogenous Threshold ~~Substances is identified in a *Sample* in conjunction with a diuretic subject to a *Minimum Reporting Level* (*MRL*) (at an estimated concentration higher than (>) the corresponding *MRL*, as defined in the TD MRPL [6]), or in~~Substance, only the Qualitative Procedure to confirm the presence of ~~any other diuretic or a masking agent (at any concentration),~~ the ~~confirmation~~relevant Analyte(s) of the ~~Threshold~~*Prohibited Substance* ~~requires only~~reported in the ~~identification of~~"A" *Sample* is required for the ~~compound, not its quantification. In such cases, the Laboratory shall:~~*AAF* to be valid.

~~• As per ISL 2021 Article 5.3.6.2.2, when there is a Presumptive *Adverse Analytical Finding* (PAAF) for a diuretic, the Laboratory may contact the *Testing* Authority (or *Results Management* Authority, if different) to enquire whether an approved *Therapeutic Use Exemption* (*TUE*) exists for the diuretic detected. If there is no approved *TUE* for the diuretic, the Laboratory shall perform the CP and report the result as an *AAF* for the diuretic in compliance with the TD MRPL [6] and the TD IDCR [5];~~

~~• In addition, as per ISL 2021 Article 5.3.6.2.2, the Laboratory may contact the *Testing* Authority (or *Results Management* Authority, if different) to enquire whether an approved *TUE* exists before confirming a PAAF for formoterol or salbutamol. In cases where a diuretic or masking agent is co-detected in the *Sample* and there is no approved *TUE* for the beta-2 agonist (irrespective of whether there is an approved *TUE* for the diuretic or not), the Laboratory shall perform the (qualitative) CP and report the result as an *AAF* for the beta-2 agonist if identified **at any concentration level** in compliance with the TD IDCR [5].~~

3.0 ~~2.2 Stimulants – Cathine~~Target Analytes

3.1 Cobalt

a) The CP for cobalt shall be able to separate the inorganic (Co^{2+}) and organic forms (vitamin B12, cobalamin) of cobalt, and shall target specifically the quantification (and identification, where applicable) of Co^{2+} for the reporting of an *AAF*. Suitable CPs could be based, for example, on:

i. Solid Phase Extraction (SPE) to remove organic cobalt followed by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS).

ii. High Performance Liquid Chromatography (HPLC) combined with ICP-MS [7].

b) It is not necessary for the Initial *Testing* Procedure (ITP) to separate the inorganic and organic forms of cobalt (*e.g.*, ICP-MS). However, the Laboratory shall establish, during the ITP validation, a concentration cut-off for total cobalt (inorganic + organic), which would trigger a CP for Co^{2+}.

[Comment to Article 3.1: The Analytical Testing Procedure (ATP) for the analysis of cobalt is not a mandatory ATP (see ISL TD ATP [8]) and, therefore, it is not applied to all urine Samples. A Laboratory with appropriate analytical capacity shall perform the analysis upon request by the responsible TA (or RMA, if different) or WADA.]

Cathine~~, Ephedrine, Methylephedrine and Pseudoephedrine~~

3.2 ~~The concentration level is based on the parent compound of each target~~

a) In addition to targeting cathine, the CP for cathine shall ensure the adequate chromatographic resolution of cathine and its non-prohibited diastereoisomer phenylpropanolamine (also known as norephedrine).

~~Threshold Substance in the free fraction.~~

~~• If either of these exogenous Threshold Substances is identified in a *Sample* in conjunction with diuretic subject to an *MRL* (at an estimated concentration higher than (>) the corresponding *MRL*, as defined in the TD MRPL [6]), or in the presence of any other diuretic or a masking agent (at any concentration), the confirmation of the stimulant requires only the identification of the compound and the estimation of its concentration, not its quantification. In such cases, the Laboratory shall:~~

~~- As per ISL 2021 Article 5.3.6.2.2, when there is a PAAF for a diuretic, the Laboratory may contact the *Testing* Authority (or *Results Management* Authority, if different) to enquire whether an approved *TUE* exists for the diuretic detected. If there is no approved *TUE* for the diuretic, the Laboratory shall perform the CP and report the result as an *AAF* for the diuretic in compliance with the TD MRPL [6] and the TD IDCR [5];~~

~~- Irrespective of the existence or not of an approved *TUE* for the diuretic, the Laboratory shall perform the (qualitative) CP for the stimulant and report the results as an *AAF* if identified, in compliance with the TD IDCR [5], at an estimated concentration level greater than (>) the applicable *MRL* for stimulants (as defined in the TD MRPL [6]). Whether the *AAF* for the stimulant is associated with an approved *TUE* shall be determined during the *Results Management* process.~~

~~c)~~b) The Laboratory shall report cathine as an *AAF* when found at a urinary concentration level greater than (>) the *DL* of 6 µg/mL. However, since cathine is a *Metabolite* of pseudoephedrine, if pseudoephedrine is also detected in the *Sample*, but at a concentration ~~levels~~ below (<) the *DL*, ~~the concentration level of pseudoephedrine shall also be reported, and~~ of 170 µg/mL, a comment shall be made in the Test Report that the cathine finding may have resulted from the administration of pseudoephedrine. In addition, the concentration of pseudoephedrine shall also be reported.

~~• The Laboratory shall refer to TL05 (Oxilofrine) [7] or any other relevant Technical Letter providing guidance on findings related to Threshold Substances classified as stimulants in the *Prohibited List* [8].~~

3.3 ~~2.3~~ Morphine

~~The concentration level is based on content of morphine, which is defined as the combination of free substance (free morphine) and its glucuronide conjugated forms (morphine-3-glucuronide and morphine-6-glucuronide), expressed as morphine equivalent.~~

Occasionally, a morphine (M) finding may result from the administration of a permitted substance such as codeine (C) [9,10] or ethylmorphine~~:~~ (EtM) [11-14]:

~~• The Laboratories shall refer to the Technical Letter TL22 (Ethylmorphine) [9], which provides details on morphine findings that may be related to the administration of ethylmorphine;~~

~~b)~~a) ~~When codeine~~Where M is detected in a *Sample*~~, Laboratories~~ together with C, the Laboratory shall report an *AAF* for ~~morphine in cases~~M when both of the following conditions are met:

i. [M] > *DL*:

– The ~~morphine~~total M concentration level (free M + M3G + M6G, expressed as M equivalent) in urine is higher than (>) the *DL* or the adjusted *DL* (if SG > 1.018), and

ii. M/C ≥ 2.00, except if C > 5.00 µg/mL:

– The ratio M/C of ~~morphine (M) to codeine (C, defined as the combination of free codeine + codeine-6-glucuronide~~total M (free M + M3G + M6G, expressed as ~~codeine~~M equivalent) to total C (free C + codeine-6-glucuronide (C6G), expressed as C equivalent) is equal to or higher than ~~(≥)~~(≥) 2.00 (expressed truncated to three (3) significant figures), except when C > 5.00 µg/mL, which is indicative of only codeine intake ~~(in~~. In this case, the quantification of ~~morphine~~M is not necessary, and the finding shall be reported as a Negative Finding~~).~~.

[Comment~~:~~ to Article 3.3 a): The total concentration ~~level~~ of C is expressed truncated to three (3) significant figures.]

~~2.4 Carboxy-THC (11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid)~~

b) ~~The concentration level~~Where M is ~~based on~~ detected in a *Sample* together with EtM, the ~~content~~Laboratory shall report an *AAF* for M when both of ~~carboxy-THC, which is defined as~~ the ~~combination of~~ following conditions are met:

i. [M] > *DL*:

– The total M concentration level (free ~~substance and its~~ M + M3G + M6G, expressed as M equivalent) in urine is higher than (>) the *DL* or the adjusted *DL* (if SG > 1.018), and

ii. M/EtM > 1.00 and M/nor-EtM (norethylmorpine) > 20.0 (free nor-EtM + nor-EtM-6-glucuronide ~~conjugated forms~~)

– The M/EtM ratio of total M (free M + M3G + M6G, expressed as ~~substance equivalent~~ M equivalent) to total EtM (free EtM + ethylmorphine-6-glucuronide (EtM6G), expressed as EtM equivalent) is higher than (>) 1.00 (expressed truncated to three (3) significant figures); and

– The M/nor-EtM ratio of total M (free M + M3G + M6G, expressed as M equivalent) to total nor-EtM (free nor-EtM + norethylmorphine-6-glucuronide (nor-EtM6G), expressed as nor-EtM equivalent) is higher than (>) 20.0 (expressed truncated to three (3) significant figures).

[Comment 1 to Article 3.3 b): The total concentration of EtM and/or nor-EtM is expressed truncated to three (3) significant figures.]

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[Comment 2 to Article 3.3 b): When reporting an AAF for M in the presence of EtM and nor-EtM, a comment shall be included in the Test Report indicating that "Morphine was detected at a concentration greater than the DL, which was also higher than the concentration of total ethylmorphine detected in the Sample. In addition, the ratio of total morphine to total norethylmorphine was higher than 20. This is consistent with the mixed intake of morphine and ethylmorphine."]

[Comment 3 to Article 3.3 b): The Laboratory should evaluate the rate of hydrolysis of EtM-glucuronide and nor-EtM-6-glucuronide during their CP method validation, if applicable. The evaluation should also confirm the lack of artifact(s) formation. In the absence of nor-EtM-6-glucuronide Reference Material, the evaluation should consider a similar conjugate such as norcodeine-6-glucuronide.]

4.0 Detection of Exogenous Threshold Substances in the Co-Presence of Diuretics or Masking Agents

Where an exogenous Threshold Substance is detected in a *Sample* at concentrations equal to or lower than (≤) the corresponding *DL* (see Table 1) and in the co-presence of an identified diuretic or masking agent, the Laboratory shall report the finding as an *AAF* for the Threshold Substance when the three (3) conditions listed below are met.

[Comment to Article 4.0: As per the ISL Article 5.3.4.1.3 [1], when there is a Presumptive Adverse Analytical Finding (PAAF) for a diuretic, the Laboratory may contact the Testing Authority (TA) (or Results Management Authority (RMA), if different) to enquire whether an approved Therapeutic Use Exemption exists for the diuretic. However, where a diuretic is detected in a Sample together with a Threshold Substance, the Laboratory shall proceed with the CP of both substances and report the confirmed findings according to the ISL TD MRL [6] (for the diuretic, where applicable) and this ISL TD DL (for the Threshold Substance). Whether there is an approved Therapeutic Use Exemption for the diuretic and/or the Threshold Substance shall be determined during the Results Management process.]

i. $SG_{Sample} \leq 1.018$: The SG of the *Sample* (as measured by the Laboratory during the CP and expressed rounded to three (3) decimal places) is not higher than (≤) 1.018.

The measured concentration of the Threshold Substance shall be adjusted to SG = 1.020 as per Eq. 3. If the $SG_{Sample} < 1.003$, the measured concentration shall be adjusted to SG = 1.020 based on $SG_{Sample} = 1.003$.

(Eq. 3) $$\mathbf{Conc_{adj}} = \frac{(\mathbf{1.020-1})}{\mathbf{SG_{Sample\_Max}-1}} \times \mathbf{Conc_{measured}}$$

where SG_{Sample_Max} is calculated as:

(Eq. 4) $$\mathbf{SG_{Sample\_Max} = SG_{Sample} + U_{max\_SG} = SG_{Sample} + 0.002}$$

[Comment to Article 4.0-i: This adjustment of the concentration shall not be performed when $SG_{Sample} > 1.018$.

Instead, in those situations where SG_{Sample} > 1.018, only the DL shall be adjusted (as per Article 7.0, and irrespective of whether a diuretic or masking agent is present in the Sample) and the result for the Threshold Substance shall be reported as AAF if the concentration exceeds the adjusted DL (DL_{adj}).]

ii. After SG adjustment to 1.020, the adjusted concentration of the Analyte(s) of the Threshold Substance is higher than (>) the corresponding *DL*, and

iii. The diuretic or masking agent whose presence has been confirmed (as per ISL *TD* IDCR [5]) in the *Sample* is either not subject to a *Minimum Reporting Level* (*MRL*) or, where applicable, its estimated concentration is higher than (>) the corresponding *MRL*, in accordance with the ISL *TD MRL* [6]. In those cases, since $SG_{Sample} \leq 1.018$, the concentration of the diuretic or masking agent subject to an *MRL* shall not be adjusted.

~~4.0~~5.0 Threshold (T) and *Decision Limit* (*DL*)

Where a T has been established for a *Prohibited Substance*, the *DL* represents the value for that ~~*Prohibited*~~Threshold Substance above which it can be decided that the result in a given *Sample*, obtained using a validated measurement procedure, has exceeded the T with a statistical confidence of at least 95 %, and hence that an *AAF* is justified. This is illustrated in **Figure 1**.

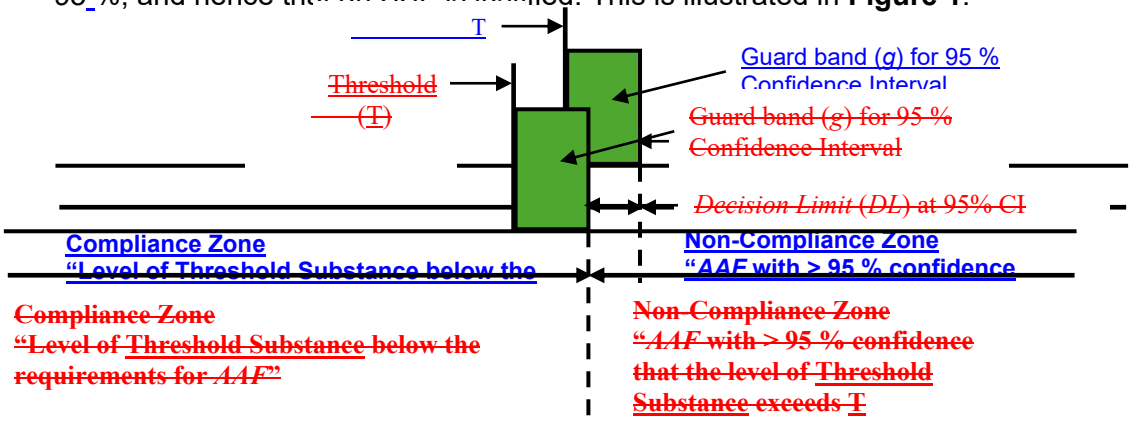

Figure 1: Use of a guard band (*g*) to establish a *DL* relative to a T and to differentiate between compliance and non-compliance zones.

The *DL* value shall be calculated as the sum of the T value and the guard band (*g*), where *g* is calculated based *on* the relevant *WADA* maximum acceptable value (unit/mL) of the combined standard uncertainty

(u_{c_Max}) given in Table 1, using a coverage factor *k* of 1.645 (95 % coverage range, one-tailed normal distribution). The resulting value of the *DL* is then rounded up to the second significant figure.

~~(Eq. 1) $DL = T + g$~~

~~(Eq. 2) $g = k \cdot u_{c\_Max}$~~ (Eq. 5) $\boldsymbol{DL = T + g}$

(Eq. 6) $\boldsymbol{g = k \times u_{c\_Max}}$, with *k* = 1.645

~~(Eq. 3) $u_{c\_Max} = T \cdot u_{c_{Max}}(\%)$~~

~~(Eq. 4) $AAF > DL$~~

~~When a value found in a *Sample* exceeds the T value but is less than or equal to (≤) the *DL*, the Laboratory shall report this result as a Negative Finding and include a recommendation (*e.g.*, in the opinion section of the Test Report) for the *Results Management* Authority to consider this result within its future "target and intelligence" test planning. This result shall not constitute an *AAF* regardless of the value of MU the Laboratory reports for the result.~~

(Eq. 7) $\boldsymbol{u_{c\_Max} = T \times u_{c_{Max}}(\%)}$

(Eq. 8) $\boldsymbol{AAF > DL}$

~~5.0~~6.0 Maximum Levels of Measurement Uncertainty

The maximum acceptable relative combined standard uncertainty (u_{c_Max}, %) represents the minimum requirement to be met by a Laboratory for the uncertainty of the measurement, estimated at levels close to the T value, when reporting a result for the determination of a Threshold Substance. The u_{c_Max} (%) values are set such that a Laboratory can reasonably expect to work within them when applying ~~quantitative CPs~~Quantitative Procedures for the ~~determination~~confirmation of Threshold Substances.

In most cases, the u_{c_Max} (%) is assigned using robust estimates of method Reproducibility (S_R) obtained from the combined participant Laboratory results from relevant rounds of the External Quality Assessment Scheme (EQAS~~).~~) – see ISL *TD* EQAS [15]. In cases where a new Threshold Substance is introduced into this ISL *TD* before EQAS performance data are available, alternative approaches will be used to assign the relevant u_{c_Max} (%). In this case the assignment of u_{c_Max} (%) must be reviewed and approved by the *WADA* Laboratory Expert Advisory Group (~~LabEG~~Lab EAG). When data obtained from

subsequent EQAS rounds becomes available, the u_{c_Max} (%) may be revised to reflect the actual analytical performance of the Laboratories.

The results obtained from the *WADA* EQAS indicate that these minimum requirements are conservative. When setting the target values, the degrees of freedom associated with the MU data are assumed to be large.

a) Laboratories shall estimate the relative combined standard uncertainty (u_c, %) for a result at levels close to the T value for each ~~quantitative CP~~Quantitative Procedure for Threshold Substances~~;~~.

b) The estimated u_c (%) shall be not greater than (≤) the u_{c_Max} (%) value given in Table 1.

[Comment~~:~~ to Article 6.0 b): As mentioned above, these u_{c_Max} (%) values are considered to be conservative; therefore, smaller u_c (%) values may be reported by Laboratories.]

Various approaches to obtain Fit-for-Purpose estimates of u_c (%) associated with the results from a given measurement procedure are given in Annex A.

~~9.0~~7.0 Adjustment of the *DL* for ~~the Urine~~a High Specific Gravity ~~(SG)~~of the *Sample*

a) For any of the Threshold Substances ~~treated in~~subject of this ~~document~~ISL *TD DL*, when the ~~SG of the urine *Sample* (~~SG_{Sample} (as measured by the Laboratory during the CP and expressed rounded to three (3) decimal places) is greater than (>) 1.018~~,~~ (including in the presence of diuretics/masking agents), an adjusted *DL* for an individual test result (DL_{adj}) shall be calculated as per Eq. ~~5~~9 below~~;~~ to determine whether the finding constitutes an *AAF*.

(Eq. 9) $$\boldsymbol{DL_{adj} = \frac{(SG_{Sample\ Max} - 1)}{(1.020 - 1)} . DL}$$ where $SG_{Sample\ Max}$ is calculated as per Eq.4.

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[Comment~~:~~ to Article 7.0 a): The SG_{Sample} cut-off value for adjustment of the DL has been set at 1.018 to account for the lower limit of the 95~~% coverage interval~~ % CI, based on a two-tailed normal distribution, of a reference value of SG at 1.020 for normally hydrated individuals (calculated as 1.020 – U_{Max_SG}~~)]~~., where U_{Max_SG} = 0.002, k = 2)].

- ~~The SG value (SG_{Sample}) to be used in applying Eq. 6 for the calculation of SG_{Sample_Max} is that measured in the Laboratory.~~

~~*[Comment: The Laboratory shall measure the SG_{Sample} in a single Aliquot during the Initial Testing Procedure (ITP) and the CP, using a method that is included within the Laboratory's ISO/IEC 17025 Scope of Accreditation, as follows:*~~

- ~~*ITP: In all Samples, using either a digital refractometer or a densitometer;*~~
- ~~*CP: A digital refractometer shall be used in all "A" and "B" Samples.*~~ The adjustment of the *DL* for the SG is not needed for~~:~~

~~*(i) "A" and "B" Sample confirmations for those exogenous Threshold Substances that shall not be quantified if detected in the presence of a prohibited diuretic or other masking agent, and*~~

~~g)~~b) ~~*(ii)*~~ "B" *Sample* confirmations of exogenous Threshold Substances, since in those cases, in accordance with the ISL [~~4~~1], "B" *Sample* results shall only confirm the ~~*"A" Sample*~~ identification ~~*(in compliance with*~~of the ~~*TD IDCR [5])*~~target Analyte(s) of the Threshold Substance for the *AAF* to be valid.

~~*If the SG_{Sample}, as measured by the instrument, reads to ≥ 4 decimal places, the SG_{Sample} is the value obtained after rounding the instrumental value and expressing it to three (3) decimal places (e.g., 1.0223 should be expressed as 1.022; 1.0227 as 1.023. When the measured value finishes in 5, it should be expressed to the nearest higher 3-decimal place value, e.g., 1.0225 should be expressed as 1.023).]*~~

- ~~The SG adjustment to the *DL* shall be made using the following formula:~~

~~$$(\text{Eq. 5}) \quad DL_{adj} = \frac{(SG_{Sample\ Max} - 1)}{(1.020 - 1)} . DL$$~~

~~Where SG_{Sample_Max} is calculated as:~~

~~$$(\text{Eq. 6}) \quad SG_{Sample\_Max} = SG_{Sample} + U_{Max\_SG} = SG_{Sample} + 0.002$$~~

~~U_{Max_SG} = 0.002 is the maximum allowed expanded uncertainty ($U_{95\%}$, k = 2) for SG.~~

~~n)~~c) The determined DL_{adj} shall be expressed truncated to three (3) significant figures (trailing zeros (0) shall be considered as significant figures, *e.g.*, 1.50; 100) (see Annex B).

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~~12.0~~8.0 Reporting Quantitative Results

The minimum ~~requirements for~~information required when reporting an *AAF* for a Threshold Substance ~~are~~includes:

a) The quantitative result (reported as the mean value from triplicate determinations, truncated to three (3) significant figures; trailing zeros (0) shall be considered as significant figures, *e.g.*, 13.0; 190~~);~~).

b) A statement that the quantitative result exceeds (>) the relevant *DL* (or DL_{adj}, if SG > 1.018~~);~~), and

c) The u_c (%) associated with a result at levels close to the T value, as determined during the ~~quantitative CP method~~Quantitative Procedure validation (which shall not be higher than (≤) the corresponding u_{c_Max} (%) specified in Table 1).

d) Where the concentration of the Threshold Substance exceeds the Threshold value but does not exceed the (adjusted, if applicable) *DL*, the Laboratory shall report this result as a Negative Finding and include a recommendation (*e.g.*, in the opinion section of the Test Report) for the RMA to consider this result for *Target Testing* purposes.

Reporting Example for the Test Report:

The concentration level of '*Prohibited Substance* A' in the *Sample* is X.XX (units). This exceeds the *DL* (after adjustment for the SG, if applicable) for ~~A of Y (units). The relative combined standard uncertainty (u_c %) estimated by the Laboratory for a result at the Threshold Z is '*b*' (%). This result constitutes an *Adverse Analytical Finding* for the presence of A in the *Sample*.~~

~~13.0~~1.0 *Prohibited Substance* A of Y.YY (units). ~~Interpretation Examples~~

~~Ephedrine is detected in a *Sample* with an SG of 1.018 at a concentration level of 11.208 µg/mL using a quantitative Analytical Method where the u_c (%) is 3.6% for a result at the T of 10.0 µg/mL.~~

~~In accordance with the reporting rules established in this *TD* (see Article 6.0), this result constitutes an *AAF* since the concentration level of ephedrine in the *Sample*, truncated to three (3) significant figures, is 11.2 µg/mL and exceeds the *DL* for ephedrine of 11.0 µg/mL. The u_c (%) of 3.6 % is lower than the corresponding u_{c_Max} (%) of 5.0. Such a finding shall be reported as follows:~~

~~Test Report: The concentration level of ephedrine in the *Sample* is 11.2 µg/mL. This exceeds the *DL* for ephedrine of 11.0 µg/mL.~~ The relative combined standard uncertainty (u_c %) estimated by the Laboratory for a result at the Threshold (~~10.0 µg/mL~~Z.ZZ) is ~~3.6%.~~*b*' (%). This result constitutes an ~~*AAF*~~*Adverse Analytical Finding* for the presence of ~~ephedrine~~ '*Prohibited Substance* A' in the *Sample*.

9.0 Interpretation Examples

a) ~~Carboxy THC~~The presence of ephedrine is ~~detected~~confirmed in a *Sample* with a ~~SG of~~SG_{Sample} = 1.~~022~~018 at a concentration ~~level~~ of ~~216.7 ng~~11.23 µg/mL using a ~~quantitative Analytical Method where the~~Quantitative Procedure with a u_c ~~is 9.0~~ = 3.6% for a result at the T of 10.0 µg/mL.

This result constitutes an *AAF* since the concentration of ephedrine in the *Sample*, truncated to three (3) significant figures, is 11.2 µg/mL and exceeds the *DL* for ephedrine of 11.0 µg/mL. The u_c of 3.6% is lower than (<) the corresponding u_{c_Max} of 5%. Such a finding shall be reported as follows:

[Test Report: The concentration of ephedrine in the *Sample* is 11.2 µg/mL. This exceeds the *DL* for ephedrine of 11.0 µg/mL. The relative combined standard uncertainty (u_c%) estimated by the Laboratory for a result at the Threshold (10.0 µg/mL) is 3.6%. This constitutes an *AAF* for the presence of ephedrine in the *Sample*.]

b) The presence of salbutamol is confirmed in a *Sample* with a SG_{Sample} = 1.012 at a concentration of 0.90 µg/mL using a Quantitative Procedure with a u_c = 7% for a result at the T of 1.00 µg/mL. In addition, furosemide, a prohibited diuretic subject to an *MRL* at 20 ng/mL, is detected and confirmed in the *Sample* at a concentration of 55 ng/mL.

After adjusting the concentration for a SG = 1.020 as per Eq.3, this result constitutes an *AAF* for salbutamol, since the adjusted concentration, truncated to three (3) significant figures, is 1.29 µg/mL and exceeds the *DL* for salbutamol of 1.20 µg/mL. The u_c of 7% is lower than (<) the corresponding u_{c_Max} of 10%. Such a finding shall be reported as follows:

Test Report:

The presence of furosemide was confirmed in the *Sample* at a concentration of 55 ng/mL, which is higher than the *MRL* of 20 ng/mL. This constitutes an *AAF* for the presence of furosemide in the *Sample*.

In addition, the presence of salbutamol was also confirmed in the *Sample* at a concentration of 0.90 µg/mL. The concentration of salbutamol adjusted for a SG = 1.020 is 1.29 µg/mL, which exceeds the *DL* of 1.20 µg/mL. The relative combined standard uncertainty (u_c%) estimated by the Laboratory for a result at the Threshold (1.00 µg/mL) is 7%. This constitutes an *AAF* for the presence of salbutamol in the co-presence of a diuretic in the *Sample*.

~~a)~~c) The presence of Carboxy-THC is confirmed in a *Sample* with a SG_{Sample} = 1.022 at a concentration of 216.7 ng/mL using a Quantitative Procedure with a u_c = 9% for a result at the T of 150 ng/mL. The DL_{adj} calculated according to Eq. ~~5~~9 and expressed to three (3) significant figures is 216 ng/mL (see Annex B).

~~In accordance with the reporting rules established in this *TD* (see Article 6.0), this~~This result does not constitute an *AAF*, since the concentration ~~level~~ of carboxy-THC in the *Sample*, truncated to three (3) significant figures, is 216 ng/mL and does not exceed the DL_{adj} for carboxy-THC of 216 ng/mL.

Test Report:

Since the concentration ~~level~~ of carboxy-THC exceeds the Threshold value but does not exceed the adjusted *DL*, the Laboratory shall report this result as a Negative Finding and include a recommendation (*e.g.,* in the opinion section of the Test Report) for the ~~*Results Management* Authority~~RMA to consider this result ~~within its Test Distribution Plan~~for *Target Testing* purposes.

[Comment~~:~~ to Article 9.0 c): When the result for a Threshold Substance in a Sample scantily exceeds the DL, the *95% confidence interval [mean ± expanded uncertainty $U_{95\%}$ (k = 2)] ~~for~~of the Laboratory result may extend below the DL. It is important to note that this shall not invalidate an AAF. For appropriate statistical comparison, the u_c with a single-tailed distribution coverage factor (k = 1.645) is taken into consideration when the Laboratory result is compared to the T to demonstrate that the result obtained for the Threshold Substance exceeds the T at greater than (>) 95 % confidence.]*

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~~ANNEX A~~

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ANNEX A – Estimation and Verification of Measurement Uncertainty

1. Estimating Measurement Uncertainty (MU)

The International Vocabulary of Metrology (~~ISO/IEC Guide 99:2007)~~ [~~10~~]JCGM-200:2012) [16] formally defines MU as a parameter characterizing the dispersion of quantity values attributed to a measurand.

More simply stated, the combined standard MU of a result [$u_c(y)$] is equivalent to an estimate of the standard deviation (SD) associated with the result (y) that would have been obtained ~~for~~if the measurement of the sample under analysis ~~if~~had been repeated several times with all influence quantities perturbed in line with their associated uncertainties. Multiplication of $u_c(y)$ by a coverage factor (k) gives the expanded MU (U) associated with result (y). For a given sample, the combination of the result (y) and its associated U ~~specifies~~determines a range ~~describing~~representing the dispersion of the values that can reasonably be attributed to the measurand at a stated level of statistical confidence. For *Doping Control* purposes, a value of U corresponding to a 95 % coverage ~~range~~interval is applied.

Accreditation to ISO/IEC 17025 [~~11~~17], as well as compliance with the ISL [~~4~~1], requires that Laboratories evaluate the MU associated with their results at levels close to the Threshold~~,~~ (T), and report the uncertainty where applicable. The ~~ISO/IEC~~JCGM Guide to the Expression of Uncertainty in Measurement (GUM) establishes general rules for evaluating and expressing uncertainty in measurement that are applicable to ISO/IEC 17025 accredited laboratories [~~12~~18].

~~The examples cited in~~To evaluate the MU of a measurement procedure, the Laboratory may use any approach consistent with the GUM ~~concentrate on one method,~~. Such approaches include "bottom-up" methods (referred to elsewhere as the "analytical~~",~~" or "modelling" ~~or "bottom-up" approach, for uncertainty evaluation. The basic GUM principles also allow for more global approaches for estimating the sources of MU, generally referred to~~method) as well as "top-down" or "empirical" ~~approaches, using~~methods that use data derived from intra- or inter-laboratory method validation studies, internal quality control procedures or ~~the results of EQAS. These approaches are all potentially compliant with the GUM principles provided the MU estimate obtained is suitable for the intended purpose of the measurement.~~from proficiency testing schemes (such as the *WADA* EQAS). Various references are

available which give worked examples of both the "bottom-up" and "top-down" approaches to MU estimation [~~13, 14, 15, 16, 17~~]19-25].

Different approaches may be applied for the estimation of the combined standard measurement uncertainty $u_c(y)$ associated with an individual result (y). They use:

A. A modelling approach based on the principles described in the GUM~~;~~.

B. Intra-laboratory approach: "In-house" method validation data combined with quality control data~~;~~.

C. Inter-laboratory approach: Data derived from inter-laboratory collaborative trials or from EQAS.

~~The strategy used for uncertainty estimation does not have to follow one exclusive model and in practice the combination of data obtained from two or more different approaches can be employed.~~

All of these approaches are considered acceptable. Any of these approaches may be employed by a Laboratory to estimate the MU associated with their measurement results, provided the Laboratory estimate does not exceed the maximum acceptable (target) MU associated with the determination of specific Threshold Substances that have been established by *WADA*. The strategy used for uncertainty estimation does not have to follow one exclusive model and in practice the combination of data obtained from two or more different approaches can be employed.~~These maximum acceptable MU are conservative estimates derived from EQAS performance data.~~

~~. Modeling Approach~~

~~In this case, the Laboratory develops a measurement equation or model in which result (y) is a function of independent input parameters $x_1, x_2, x_3 \ldots x_n$ that all influence the measurement result.~~

~~If the mathematical model is a combination of addition/subtraction and multiplication/addition operations, then an appropriate quadratic combination is used to calculate the $u_c(y)$. This approach is also referred to as the "bottom-up" or "GUM" approach.~~

~~The GUM approach is based on the propagation of uncertainties where the estimated standard deviation associated with the measurement result (y) is named $u_c(y)$ and is determined from the estimated standard deviations associated with each input estimate (x_i). These uncertainty components from the input quantities are then combined to give the combined standard uncertainty $u_c(y)$.~~

~~When the input quantities are independent, the $u_c(y)$ is given as:~~

~~(Eq. 7) $$u_c(y) = \sqrt{\sum_{i=1}^{N} \left(\frac{\partial f}{\partial x_i}\right)^2 u^2(x_i)}$$~~

~~Where f is the function that defines the measurand.~~

~~More details on the application of this method and the implications in cases where two or more of the input quantities are correlated can be found in the GUM and elsewhere in the literature [12, 15].~~

~~*[Comment: The uncertainty budget derived using this approach indicates the relative magnitude of the various sources of uncertainty but carries the risk of missing a contributing factor which may significantly affect the overall estimate of MU. Nonetheless, it is a valuable means of establishing where the major sources of uncertainty are found in a quantitative CP and for identifying where efforts should be focused if a reduction is desired in the overall MU of results obtained through use of the quantitative CP.]*~~

However, for *Doping Control* purposes, a **'top-down' approach**, based on the determination of the following method performance characteristics, is the recommended procedure:

a) Repeatability (s_r) and Intermediate Precision ($s_{w,}$ also referred to as the within-Laboratory reproducibility or imprecision) from within-Laboratory QC and/or validation data, and

b) Bias (*B*) from either QC/validation data, for example, when there is an available Certified Reference Material (CRM), or from *WADA* EQAS data.

A. Intra-Laboratory Data Approach

This approach assumes that the ~~quantitative CP~~Quantitative Procedure has undergone intra-Laboratory validation including an estimation of the ~~Intermediate Precision (also referred to as the within-Laboratory reproducibility or imprecision).~~ s_w and *B*. It is based on a three~~-~~ (3)-component measurement model:

~~(Eq. 8)~~ $y = m + B + e$

(Eq. 10) $\boldsymbol{y = m + B + e}$

The result (y) is the sum, under Intermediate Precision conditions, of the measurement method mean (m), ~~an estimate of~~ a systematic error contribution (method bias ~~(~~, B) and a random error contribution (e~~) and the~~). The $u_c(y)$ associated with the result is given by:

(Eq. 11) ~~(Eq. 9)~~ $u_c(y) = \sqrt{u(m)^2 + u(B)^2 + u(e)^2}$

$$\boldsymbol{u_c(y) = \sqrt{u_m^2 + u_B^2 + u_e^2}}$$

The estimate of within-Laboratory Intermediate Precision of results, usually obtained from intra-Laboratory QC and method validation data, can be expressed as a standard deviation (s_w). It provides a Fit-for-Purpose estimate of the uncertainty ~~contribution from the $u(m)$ and $u(e)$ terms and the "internally visible" bias component (B_{Int}).~~contributions related to random errors (u_e) and the mean values (u_m). Consequently, Eq. 10 can be simplified by including this s_w component and the uncertainty associated with the estimate of *B* (u_B), as determined during method validation:

~~(Eq. 10)~~ $S_w \sim \sqrt{u(m)^2 + u(e)^2 + u(B_{Int})^2}$

~~If (y) is the result of a single analysis, the equation for calculating the standard uncertainty associated with the result simplifies to:~~

(Eq. 12) $s_w = \sqrt{u_m^2 + u_e^2}$

(Eq. 13) $u_c(y) = \sqrt{{s_w}^2 + {u_B}^2}$

~~(Eq. 11) $u_c(y) = \sqrt{{s_w}^2 + u(B_{Ext})^2}$~~

When (y) is the average of n replicate analyses:

~~(Eq. 12) $u_c(y) = \sqrt{\frac{{s_w}^2}{n} + u(B_{Ext})^2}$~~

~~In both cases, B_{Ext} is an estimate for bias not accounted for by intra-Laboratory studies and the~~ (Eq. 14) $u_c(y) = \sqrt{\frac{{s_w}^2}{n} + {u_B}^2}$

The uncertainty ~~due to bias [u_{bias} or $u(B_{ext})$]~~ associated with B (u_B) can be estimated by using the following equations~~[13]~~:

~~(Eq. 13) $u_{Bias} = \sqrt{\Delta_i^2 + \frac{s^2}{n} + u_{ref}^2}$~~

(Eq. 15) $u_B = \sqrt{{\Delta_{lab}}^2 + \frac{s_{ref}^2}{n} + u_{ref}^2}$

where:

Δ_{lab} - Difference between the Laboratory's measurement result (y_{lab}) and the quantity value of the reference sample.

n - ~~number~~Number of replicate measurements of the ~~sample used as~~ reference ~~(~~sample (*e.g.*, CRM, QC or EQAS sample~~) prepared at a specified dilution level;~~).

~~s – standard deviation~~s_{ref} - Standard Deviation (SD) under Repeatability conditions of the results obtained for the replicate measurements of the reference sample ~~at a specified dilution level;~~.

u_{ref} ~~–~~ – Combined standard uncertainty associated with the quantity value of the reference sample~~, and;~~.

~~$\Delta_t = C_{lab,t} - C_{ref,t}$~~

(Eq. 16) $$\Delta_{lab} = y_{lab} - C_{ref}$$

where:

y_{lab} - Laboratory measurement result

C_{ref} – Concentration of the reference sample

Where information is available from ~~n_{bias}~~n_B separate ~~bias~~ determinations of *B*, then the ~~u_{bias}~~u_B shall be expressed as the root mean square of the ~~bias~~ (~~RMS_{bias}~~Bias (RMS_B).

~~(Eq. 17)~~ $$u_B = RMS_B = \sqrt{\frac{\sum u_B^2}{n_B}}$$ where: n_B

~~(Eq. 14) $u_{bias} = RMS_{bias} = \sqrt{\frac{\sum u_{bias,i}^2}{n_{bias}}}$~~

~~where:~~

~~n_{bias}~~ - number of independent ~~bias~~*B* determinations~~.~~

~~*[Comment: When appropriately applied, this approach, as with the other empirical approaches, is as valid as the modeling approach, and should provide a conservative but pragmatic estimation of MU.]*~~

~~C.~~B. Inter-Laboratory Method Performance or EQAS Approach

Where a Laboratory has participated in an inter-Laboratory comparison or EQAS to evaluate a ~~quantitative CP, or has demonstrated appropriate implementation of a literature method validated using such an approach~~Quantitative Procedure, the inter-Laboratory Reproducibility of the method (s_R), *i.e.*, the SD of the Participants' results calculated after exclusion of outliers or from ~~the results of the comparison and expressed as *SD*~~robust statistics, can be used as an estimate of the u_c of an individual result obtained using the method:

~~(Eq. 15) $u_c(y) = \frac{s_R}{\sqrt{n}}$~~ (Eq. 18) $$u_c(y) = \frac{s_R}{\sqrt{n}}$$ where *y* is the average of *n* replicate analyses~~)~~

~~This approach is applicable, in practice, only when the validation study includes a multi-centre, inter-~~Laboratory ~~trial conducted to a pre-defined experimental protocol.~~

*[*Comment *1 to Article A-1B: The major sources of variability can be assessed by inter-Laboratory studies and provide estimates of Repeatability ~~standard deviation~~ (s_r), Reproducibility (s_R) and Bias (B) of the ~~method~~Test Method (with respect to a known reference value). The ~~Reproducibility (~~s_R~~)~~ can be used as an estimate of the u_c associated with an individual measurement result obtained using this ~~quantitative CP~~Quantitative Procedure.]*

~~Data obtained from ongoing participation in an EQAS also allows, in some cases, for the calculation of a performance characteristic of the ensemble of methods used by participants that can serve, in the absence of a properly constituted inter-Laboratory study, as a conservative estimate of the Reproducibility (s_R) of the quantitative CP used by an individual Laboratory. It is mostly in the latter sense that the term s_R is used in the current draft. This estimate is only valid when:~~

- ~~The values reported by participants in the EQAS round (after exclusion of outliers) fall into a normal Gaussian distribution;~~

This estimate is only valid when:

a) The intra-Laboratory ~~Repeatability (s_r) for the method~~ is smaller than (<) the variation of the ~~participants'~~Participants' results ($s_r < s_R$~~);~~).

- ~~Uncertainty contributions from instability or heterogeneity of the EQAS sample are negligible;~~
- ~~The matrices utilized correspond closely to those encountered in routine analytical conditions (*i.e.*, "representative" matrices are used to prepare the EQAS materials);~~

~~d)~~b) The target values of the study ~~fall~~ (including any sample dilution needed to fit the instrument measurement capacity) fall within the range of application of the ~~method;~~Quantitative Procedure.

~~e)~~c) The Laboratory obtains satisfactory results in a minimum number (2) of consecutive EQAS rounds.

~~In this case the SD of the participants' results after exclusion of outliers or as calculated from robust statistics can be used as an estimate of the u_c associated with a result obtained by the method. This value can then be applied as described for the s_R estimate above.~~

[Comment 2 to Article A-1B: As noted before, the ~~Reproducibility (s_R)~~s_R estimate can be used as a conservative estimate of the u_c associated with a result. Moreover, a Laboratory can, by its participation in the WADA EQAS, verify and demonstrate the validity of its chosen approach to estimate the MU.]

The tools currently used to assess the quantitative EQAS results provide the estimate of u_B for each participating Laboratory along with the consensus values. This offers an estimate of u_B that can be used in the estimation of the u_c. The u_B values estimated for each Laboratory in each EQAS round can be combined with s_w using Eq. 13. If multiple values of u_B are used, regardless of whether they arise from EQAS results and/or internal estimates, they can be combined using Eq. 17 before being included in Eq. 13.

2. Verification of Measurement Uncertainty

Regardless of the approach employed by a Laboratory to estimate the MU for the results it obtains using a particular ~~quantitative CP~~Quantitative Procedure, it is important that this MU estimate be validated, and its veracity continuously monitored. This can be accomplished by regular comparison with an appropriate QC sample, preferably a ~~Certified Reference Material (~~CRM~~),~~ (if available~~,~~) and/or through evaluation of method performance using EQAS data.

The tools currently used to assess the quantitative EQAS results provide an estimate of MU for each participating Laboratory, $u(y_{lab})$, along with its expanded uncertainty, $U_{95}(y_{lab})$. The estimate $u(y_{lab})$ is realistic when $u(y_{lab})$ is between $u(x_{PT})$ and S_R (robust reproducibility standard deviation of all Participant's results) and is non-compliant when $u(y_{lab}) - U_{95\%}(u(y_{lab}) > u_{c\ Max}$.

The MU for a particular ~~quantitative CP~~Quantitative Procedure, estimated by a Laboratory can also be checked by comparison to data generated from an appropriate EQAS by employing the ~~E_n number.~~normalized error (E_n).

~~(Eq. 16) $E_n = \frac{x - x_a}{\sqrt{U(x)^2 + U(x_a)^2}}$~~

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~~Where x_{α} is the~~ (Eq. 19) $$E_n = \frac{\Delta_{lab}}{U(\Delta_{lab})} = \frac{y_{lab} - x_{PT}}{2\sqrt{u(y_{lab})^2 + u(x_{PT})^2 - \frac{2}{N}u(y_{lab})^2}}$$

where:

Δ_{lab} - Difference between the Laboratory's measurement result and the consensus value (assigned value for ~~the EQAS study, x is~~ proficiency testing).

$U(\Delta_{lab})$ - Expanded uncertainty (95%, k = 2) of the difference.

y_{lab} - Laboratory ~~result, and $U(x_{\alpha})$ and $U(x)$ are respectively the expanded uncertainties associated with each~~measurement result.

x_{PT} - Assigned proficiency testing value for the EQAS study.

$u(y_{Lab})$ – Combined standard uncertainty of the Laboratory's measurement result (y_{lab}).

$u(x_{PT})$ - Combined standard uncertainty of the consensus value.

N – Number of Participants.

Monitoring the ~~|~~ABS(E_n~~|~~) values over time provides the Laboratory ~~an important~~with a tool to evaluate the agreement between its ~~MU~~u_c estimation ~~for a quantitative procedure~~ and the actual performance of ~~that procedure.~~the Quantitative Procedure. Provided that the estimated ~~MU~~u_c is less than or equal to (≤) the u_{c_Max} required by *WADA*, it is considered that when ~~|~~ABS(E_n~~|~~) is distributed:

a) Around one (1): ~~then~~ the estimated ~~MU~~u_c is in good agreement with the Laboratory's EQAS performance~~;~~.

b) Repeatedly at levels considerably smaller than (<<) one (1): ~~then~~ the ~~MU~~u_c could be overestimated. This shows that the historical Laboratory performance in the EQAS compared to the inter-Laboratory consensus values is better than its estimated ~~MU~~u_c. The Laboratory should evaluate the need for re-assessing the ~~MU~~u_c for this ~~particular quantitative CP;~~Quantitative Procedure.

c) Repeatedly greater than (>) one (1): the ~~MU~~u_c could be underestimated as the Laboratory's performance in the EQAS is worse than its estimated ~~MU~~u_c. In this case the reason for the high E_n value should be re-assessed. If necessary, steps should be taken to re-evaluate the ~~MU~~u_c.

It is important to highlight that individual ~~|~~ABS(E_n~~|~~) values greater than (>) or lower than (<) one (1) may not necessarily justify actions to be taken by the Laboratory. Rather, the history of values and their trends should be monitored.

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Whenever there is a change in the ~~quantitative CP~~Quantitative Procedure (extraction step, derivatization conditions, internal standard, etc~~.~~).), a re-validation of the procedure and a re-assessment of the MU of results obtained using the altered procedure is required. It is necessary to check that the ~~quantitative CP~~Quantitative Procedure is still Fit-for-Purpose (*e.g.*, the ~~MU~~u_c estimated by the Laboratory for a particular ~~quantitative CP~~Quantitative Procedure is below the acceptable u_{c_Max} given in Table 1 above).

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ANNEX B — Adjusted *Decision Limits*

Table 2. Adjusted *DL*s calculated for SG > 1.018 as per Eq. ~~5~~9 and expressed truncated to three (3) significant figures.

		Salbutamol	Formoterol	Cathine	Ephedrine	MethylE	PSE	Morphine	C-THC
SG	SG_{Max}	**1.20**	**50.0**	**6.00**	**11.0**	**11.0**	**170**	**1.30**	**180**
1.019	1.021	1.26	52.5	6.30	11.5	11.5	178	1.36	189
1.020	1.022	1.32	55.0	6.60	12.1	12.1	187	1.43	198
1.021	1.023	1.38	57.5	6.90	12.6	12.6	195	1.49	207
1.022	1.024	1.44	60.0	7.20	13.2	13.2	204	1.56	216
1.023	1.025	1.50	62.5	7.50	13.7	13.7	212	1.62	225
1.024	1.026	1.56	65.0	7.80	14.3	14.3	221	1.69	234
1.025	1.027	1.62	67.5	8.10	14.8	14.8	229	1.75	243
1.026	1.028	1.68	70.0	8.40	15.4	15.4	238	1.82	252
1.027	1.029	1.74	72.5	8.70	15.9	15.9	246	1.88	261
1.028	1.030	1.80	75.0	9.00	16.5	16.5	255	1.95	270
1.029	1.031	1.86	77.5	9.30	17.0	17.0	263	2.01	279
1.030	1.032	1.92	80.0	9.60	17.6	17.6	272	2.08	288
1.031	1.033	1.98	82.5	9.90	18.1	18.1	280	2.14	297
1.032	1.034	2.04	85.0	10.2	18.7	18.7	289	2.21	306
1.033	1.035	2.10	87.5	10.5	19.2	19.2	297	2.27	315
1.034	1.036	2.16	90.0	10.8	19.8	19.8	306	2.34	324
1.035	1.037	2.22	92.5	11.1	20.3	20.3	314	2.40	333
1.036	1.038	2.28	95.0	11.4	20.9	20.9	323	2.47	342
1.037	1.039	2.34	97.5	11.7	21.4	21.4	331	2.53	351
1.038	1.04	2.40	100	12.0	22.0	22.0	340	2.60	360
1.039	1.041	2.46	102	12.3	22.5	22.5	348	2.66	369
1.040	1.042	2.52	105	12.6	23.1	23.1	357	2.73	378

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SG	SG_Max	Cobalt	Formoterol	Salbutamol	Cathine	Ephedrine	Methylephedrine	PSE	M	C-THC
1.018	1.020	80.0	50.0	1.20	6.00	11.0	11.0	170	1.30	180
1.019	1.021	84.0	52.5	1.26	6.30	11.5	11.5	178	1.36	189
1.020	1.022	88.0	55.0	1.32	6.60	12.1	12.1	187	1.43	198
1.021	1.023	92.0	57.5	1.38	6.90	12.6	12.6	195	1.49	207
1.022	1.024	96.0	60.0	1.44	7.20	13.2	13.2	204	1.56	216
1.023	1.025	100	62.5	1.50	7.50	13.7	13.7	212	1.62	225
1.024	1.026	104	65.0	1.56	7.80	14.3	14.3	221	1.69	234
1.025	1.027	108	67.5	1.62	8.10	14.8	14.8	229	1.75	243
1.026	1.028	112	70.0	1.68	8.40	15.4	15.4	238	1.82	252
1.027	1.029	116	72.5	1.74	8.70	15.9	15.9	246	1.88	261
1.028	1.03	120	75.0	1.80	9.00	16.5	16.5	255	1.95	270
1.029	1.031	124	77.5	1.86	9.30	17.0	17.0	263	2.01	279
1.030	1.032	128	80.0	1.92	9.60	17.6	17.6	272	2.08	288
1.031	1.033	132	82.5	1.98	9.90	18.1	18.1	280	2.14	297
1.032	1.034	136	85.0	2.04	10.2	18.7	18.7	289	2.21	306
1.033	1.035	140	87.5	2.10	10.5	19.2	19.2	297	2.27	315
1.034	1.036	144	90.0	2.16	10.8	19.8	19.8	306	2.34	324
1.035	1.037	148	92.5	2.22	11.1	20.3	20.3	314	2.40	333
1.036	1.038	152	95.0	2.28	11.4	20.9	20.9	323	2.47	342
1.037	1.039	156	97.5	2.34	11.7	21.4	21.4	331	2.53	351
1.038	1.04	160	100	2.40	12.0	22.0	22.0	340	2.60	360
1.039	1.041	164	102	2.46	12.3	22.5	22.5	348	2.66	369
1.040	1.042	168	105	2.52	12.6	23.1	23.1	357	2.73	378

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World Anti-Doping *Code International Standard* for Laboratories (ISL). ~~*[Current versions of WADA ISL, Technical Documents and Laboratory Guidelines may be found at https://www.wada-ama.org/en/what-we-do/science-medical/laboratories]*~~

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