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Written by:	*WADA* Laboratory Expert Group	Approved by:	*WADA* Executive Committee
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MINIMUM REQUIRED PERFORMANCE LEVELS FOR DETECTION AND IDENTIFICATION OF NON-THRESHOLD SUBSTANCES

In order to ensure that all *WADA*-accredited Laboratories can report the presence of *Prohibited Substances*, their *Metabolite(s)* or their *Marker(s)* in a uniform way, a minimum routine detection and identification capability for testing methods has been established. It is recognized that some Laboratories will be able to identify lower concentrations of *Prohibited Substances* than other Laboratories. While such individual capabilities are encouraged in order to improve the overall system, it is also recognized that there are Minimum Required Performance Levels (MRPL) at which all Laboratories shall operate (Table 1).

1.0 Minimum Required Performance Levels (MRPL)

The MRPL is intended to harmonize the analytical performance of methods applied to the detection of Non-Threshold Substances. The MRPL is a mandatory analytical parameter of technical performance established by *WADA* with which the Laboratories shall comply when testing for the presence of a particular *Prohibited Substance*, its *Metabolite(s)* or *Marker(s).* The MRPL is the minimum concentration of a *Prohibited Substance* or *Metabolite* of a *Prohibited Substance* or *Marker* of a *Prohibited Substance* or *Method* that Laboratories shall be able to reliably detect and identify in routine daily operations.

- The MRPL is not a threshold (T) nor is it a Limit of Detection (LOD). *Adverse Analytical Findings* may result from concentrations below the established MRPL values;
- MRPL values are relevant for the detection and identification of Non-Threshold Substances; they do not apply to Threshold Substances, which are covered in other Technical Documents (*e.g.* TD DL[1], TD GH[2]);
- MRPL values are established taking into account the metabolism, stability, pharmacokinetics and pharmacodynamics of the *Prohibited Substance*. Thus, substances with a long-term doping effect which are prohibited at all times (*e.g.* anabolic steroids) will have lower MRPL values than substances which are taken for an immediate ergogenic effect and are prohibited *In-Competition* only (*e.g.* stimulants);

[1] *WADA* Technical Document TD DL: Decision Limits for the Confirmatory Quantification of Threshold Substances.
https://www.wada-ama.org/en/what-we-do/science-medical/laboratories

[2] *WADA* Technical Document TD GH: human Growth Hormone (hGH) Isoform Differential Immunoassays for *Doping Control* Analyses.
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- The MRPL is established for the *Prohibited Substance* itself and/or its *Metabolite(s)*, *Marker (s)* or degradation product(s) depending on the extent of their metabolism and/or stability in the *Sample* matrix;
- Since the metabolic and excretion patterns of *Prohibited Substances* may vary substantially with time after administration, it is important that Laboratories include in their analytical procedures relevant target analytes to ensure the detection of the *Prohibited Substance* as extensively as possible.

Table 1. MRPLs for detection of Non-Threshold Substances in human urine

Prohibited Class	Specific Examples / Exceptions	MRPL[a]
S1.1a Exogenous Anabolic Androgenic Steroids (AAS)		5 ng/mL
	Dehydrochlormethyltestosterone	2 ng/mL
	Metandienone	2 ng/mL
	17α-Methyltestosterone	2 ng/mL
	Stanozolol	2 ng/mL
S1.1b Endogenous AAS administered exogenously	19-norandrosterone (19-NA)[b] 19-noretiocholanolone (19-NE)[b]	2 ng/mL
	Boldenone[c]	5 ng/mL
S1.2 Other Anabolic Agents		2 ng/mL
	Clenbuterol	0.2 ng/mL
S2.1.2 HIF Activating Agents	Molidustat Roxadustat (FG-4592)	2 ng/mL
S2.2.1 Gonadotropin (CG/LH) Releasing Factors [d]	Buserelin, Deslorelin, Gonadorelin, Goselerin, Leuprorelin, Narfarelin, Triptorelin,	2 ng/mL
S2.2.3 Growth Hormone (GH), its fragments and Releasing Factors:		
• GH fragments [d]	AOD9604, hGH 176-191	2 ng/mL
• GH-Releasing Hormone (GHRH) and its analogues	CJC-1295, CJC-1293, Sermorelin, Tesamorelin	1 ng/mL
• GH-Secretagogues (GHS) [d]	Anamorelin, Ipamorelin, Tabimorelin	2 ng/mL
• GH-Releasing Peptides (GHRPs)[d]	Alexamorelin, GHRP-1, -2, -3, -4, -5 and -6; Hexarelin	2 ng/mL

S2.3 Growth Factors and Growth Factor Modulators	TB-500 (N-Ac LKKTETQ) [d]	2 ng/mL
S3. Beta-2 Agonists [e]		20 ng/mL
S4. Hormone and Metabolic Modulators	Aromatase inhibitors, SERMs and other anti-estrogenic substances	20 ng/mL
	Formestane [f]	50 ng/mL
	Meldonium	200 ng/mL
	Insulins	50 pg/mL
S5. Diuretics and Masking Agents		200 ng/mL
	Desmopressin and analogs[d]	2 ng/mL
S6. Stimulants [g]		100 ng/mL
	Octopamine	1000 ng/mL
S7. Narcotics [h]		50 ng/mL
	Buprenorphine	5 ng/mL
	Fentanyl (and derivatives)	2 ng/mL
S8. Cannabimimetics [i]		1 ng/mL
S9. Glucocorticoids		30 ng/mL
	Budesonide (6β-hydroxy-budesonide) [j]	30 ng/mL
P1. Beta-Blockers		100 ng/mL

[a] In each case, the MRPL applies to the parent compound or appropriate *Metabolite(s)* or *Marker(s)* depending on each substance's biotransformation pathways, excretion profile and/or stability in the *Sample* matrix.

[b] The detection and reporting of findings for 19-norsteroids is covered in the Technical Document on 19-NA (TD NA)[3].

[c] GC/C/IRMS analysis shall be conducted before reporting an *Adverse Analytical Finding* for *Samples* containing boldenone and/or its *Metabolites* between 5 ng/mL and 30 ng/mL (after adjustment for the specific gravity of the *Sample* when SG > 1.020). Refer to the Technical Document on GC/C/IRMS [4].

[3] *WADA* Technical Document TD NA. Harmonization of Analysis and Reporting of 19-norsteroids related to Nandrolone. https://www.wada-ama.org/en/what-we-do/science-medical/laboratories

[4] *WADA* Technical Document TD IRMS: Detection of synthetic forms of Endogenous Anabolic Androgenic Steroids by GC/C/IRMS.

(d) All Laboratories shall have analytical capacity to test for small peptides, including GHSs, GHRPs, gonadotropin-releasing factors, TB-500, AOD9604, desmopressin, etc. However, Testing Authorities should be aware that *Testing* for these substances may not be part of the Laboratory routine Analytical Testing menu, and therefore their analysis, if required, should be requested either in the *Sample* Chain of Custody Form, through a direct communication with the Laboratory or by prior agreement between the Laboratory and the Testing Authority. However, Laboratories shall apply the analysis for gonadotropin-releasing factors as a Confirmation Procedure for elevated LH findings (refer to the TD CG/LH [5]).

(e) Salbutamol and Formoterol are considered Threshold Substances; therefore their determination and reporting is covered in the Technical Document on Decision Limits (TD DL)[1]. When detected in conjunction with a prohibited diuretic or other masking agent, these substances shall be reported as an *Adverse Analytical Finding* at any concentration.

Reporting of salmeterol and higenamine is described in section 4.0 of this Technical Document.

(f) GC/C/IRMS analysis shall be conducted before reporting an *Adverse Analytical Finding* for *Samples* containing formestane between 50 ng/mL and 150 ng/mL (after adjustment for the specific gravity of the *Sample* when SG > 1.020). Refer to the Technical Document on GC/C/IRMS [4].

(g) Cathine, Ephedrine, Methylephedrine and Pseudoephedrine are considered Threshold Substances; therefore their determination and reporting is covered in the Technical Document on Decision Limits (TD DL)[1]. When detected in conjunction with a prohibited diuretic or other masking agent, the reporting limit established for stimulants (*i.e.* 50 ng/mL – refer to section 4.0 of this Technical Document) should be applied.

(h) Morphine is considered a Threshold Substance; therefore its determination and reporting is covered in the Technical Document on Decision Limits (TD DL)[1].

(i) 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (carboxy-THC) is considered a Threshold Substance; therefore its determination and reporting is covered in the Technical Document on Decision Limits (TD DL)[1].

(j) For detection of budesonide administration *via* systemic routes, Laboratories shall target the detection of the 6β-hydroxylated *Metabolite* [6].

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[5] *WADA* Technical Document TD CG/LH. Reporting and Management of Urinary Human Chorionic Gonadotrophin (hCG) and Luteinizing Hormone (LH) Findings in Male *Athletes*.
https://www.wada-ama.org/en/what-we-do/science-medical/laboratories

[6] X. Matabosch, O.J. Pozo, C. Pérez-Mña, M. Farré, J. Marcos, J. Segura, R. Ventura. Discrimination of prohibited oral use from authorized inhaled treatment of budesonide in sports. *Therapeutic Drug Monitoring* **35**(1):118-128, 2013.

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2.0 Limit of Detection (LOD) of the Initial Testing Procedure

The Laboratory's method validation of the Initial Testing Procedure shall include the estimation of the LOD for each Non-Threshold Substance or its representative, target *Metabolite(s)* or *Marker(s)* using the relevant reference material, when available. It is not necessary to estimate the LOD for all potential *Metabolites* of a given Non-Threshold Substance. The estimated LOD shall be not higher than 50 % of the MRPL. In the absence of a suitable reference material for a specific Non-Threshold Substance or its representative *Metabolite(s)* or *Marker(s)*, the LOD will be assumed to be similar to that of a related *Prohibited Substance* of the same class.

When detecting Non-Threshold Substances using chromatography and mass spectrometry methods, the LOD is expressed as the minimum concentration of the analyte that can be detected with reasonable certainty in urine. The estimation of the LOD may be based on the Signal-to-Noise (S/N) ratio, which may be obtained by *c*omparing measured signals from samples with known low concentrations of analyte with those of blank samples. A S/N ratio of 3 is generally considered acceptable. However, other widely recognised procedures may be applied (*e.g.* signal repeatability data for HRMS applications).

3.0 Confirmation Procedure

The Laboratory shall document that the Confirmation Procedures for Non-Threshold Substances allow the identification of every Non-Threshold Substance or its representative, target *Metabolite(s)* or *Marker(s)* (in compliance with the Technical Document on Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the Identity of Analytes, TD IDCR[7]) at the MRPL or less.

[7] *WADA* Technical Document TD IDCR: Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the Identity of Analytes for Doping Control Purposes. https://www.wada-ama.org/en/what-we-do/science-medical/laboratories

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4.0 Reporting of Non-Threshold Substances

A confirmed identification of a Non-Threshold Substance at any concentration shall be reported as an *Adverse Analytical Finding*, with the following exceptions:

- Non-Threshold Substances in classes S6, S7, S8, and P1, which are prohibited *In-Competition* only, should not be reported below 50 % of the MRPL[8];
- Glucocorticoids (S9) should not be reported at levels below the MRPL of 30 ng/mL;
- Salmeterol and higenamine should not be reported at levels below 10 ng/mL (*i.e.* 50 % of the MRPL for beta-2 agonists)[9];
- Meldonium should not be reported at levels below 100 ng/mL (*i.e.* 50 % of the MRPL);
- Octopamine should not be reported at levels below the MRPL of 1000 ng/mL[10].

[8] The reporting limits specified for Non-Threshold Substances in classes S6, S7, S8, S9 and P1 apply to either the parent compound or a specific *Metabolite*, depending on the substance metabolism and excretion pattern (or unless otherwise specified in this Technical Document). These reporting limits shall not be applied to the sum of concentrations of different molecular species [*e.g.* parent compound and phase-I *Metabolite*(s) or different phase-I *Metabolite*(s)]. However, when the method used includes the determination of phase-II *Metabolites* of the specific target substance (*e.g.* glucuronides, sulfates), the reporting limit applies to the total content of the free and conjugated substance (*i.e.*, parent compound and its phase-II *Metabolites*). This is obtained either by separate determination of the molecular species (*e.g.* by LC-MS analysis) or after hydrolysis of the phase-II *Metabolite*(s) (*e.g.* for GC-MS analysis), and shall be expressed as equivalent concentration of the parent compound.

[9] The reporting limits specified for salmeterol and higenamine apply to the determination of the free parent compound only.

[10] The reporting limit specified for octopamine applies to the sum of the parent compound and its phase-II sulfate *Metabolite* (expressed as equivalent concentration of the parent compound).