WADA Technical Document – ISL TD2027ATP

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Written by: Reviewed by:	*WADA* Science *WADA* Laboratory Expert Advisory Group	Approved by:	*WADA* Executive Committee
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Analytical *Testing* Procedures

1.0 Introduction

This *Technical Document* (*TD*), which constitutes an integral part of the *International Standard* for Laboratories (ISL [1]), establishes the framework for the application of Analytical *Testing* Procedures (ATPs) for *Doping Control*. The intent is to ensure consistency and transparency in the implementation of ATPs across all Laboratories, including *WADA*-accredited Laboratories (*i.e.,* Laboratories) and, where applicable, Laboratories approved to analyze whole blood *Samples* for the *Markers* of the Hematological Module of the *Athlete Biological Passport* (*ABP*) (*i.e*., *ABP* Laboratories). ATPs include mandatory and non-mandatory Analytical Methods.

2.0 Analytical *Testing* Procedures

ATPs are a fundamental part of *Doping Control*, enabling the Laboratories to reliably detect, identify, and/or quantify specific Analytes in *Samples*. ATPs, also referred to as Analytical Methods or Test Methods, are carried out in accordance with the requirements established in the *WADA International Standard* for Laboratories (ISL) [1] and its associated ISL *TDs* (*e.g*., see ISL *TD* VAL [2]) and ISL *Technical Letters* (*TL*s).

2.1 Fitness-for-Purpose: Validation and ISO Accreditation of ATPs

The Fitness-for-Purpose of ATPs shall be demonstrated through method validation in conformity with ISO/IEC 17025 [3] (or ISO 15189 [4], as applicable to *ABP* Laboratories) and ISL [1] requirements, including its applicable ISL *TD*s and ISL *TL*s.

Irrespective of whether an ATP is mandatory or not, it shall be validated and should be included within the Laboratory's Scope of ISO/IEC 17025 Accreditation [3] (or ISO 15189 [4], as applicable to *ABP* Laboratories) before it can be applied for the analysis and reporting of analytical results [either as Negative Findings, *Adverse Analytical Findings* (*AAFs*) or *Atypical Findings* (*ATFs*)] for *Samples*. Whenever possible, the ATP should be subjected to the evaluation of method performance through the Laboratory's participation in the *WADA* External Quality Assessment Scheme (EQAS) or other inter-Laboratory collaborative studies.

[Comment to Article 2.1: Laboratories may analyze Samples using open-profiling screening assays (untargeted screening strategies) without having completed the validation of the procedures according to the requirements of ISL TD VAL. However, results can only be reported as an AAF or ATF after being confirmed using a CP that has been validated in accordance with ISL TD VAL [2].]

As established in the ISL [1], under exceptional circumstances, and upon informing *WADA*, a Laboratory may apply a validated ATP to the analysis of *Samples* before its inclusion into the Laboratory's Scope of ISO/IEC 17025 Accreditation. However, in such cases, the Laboratory would not automatically benefit from the presumption that the Test Method is valid and, consequently, the Laboratory may be required to provide Test Method validation documentation or EQAS performance data in support of a reported *AAF* or *ATF*.

2.2 Classification of Analytical *Testing* Procedures

2.2.1 Mandatory ATPs (see Table 1)

All Laboratories shall have the mandatory ATPs (Initial *Testing* Procedures (ITPs) and Confirmation Procedures (CPs), where applicable) included within their Scope of ISO/IEC 17025 Accreditation [3] (or ISO 15189 [4], as applicable to *ABP* Laboratories).

Mandatory ATPs shall be applied to:

a) All *Samples* collected, both *In-Competition* (*IC*) and *Out-of-Competition* (O*OC*) (*i.e.*, for the analysis of *Prohibited Substance(s)* and/or *Prohibited Method(s)* that are prohibited at all times) (see Table 1A), or

b) All *Samples* collected *IC* only (for *Prohibited Substances* that are prohibited *IC* only) (see Table 1B), or

c) All *Samples* collected in specific sports (for *Prohibited Substances* that are banned only in those sports) (see Table 1C).

d) In cases of temporary disruption of Laboratory analytical capacity, these mandatory ATPs may be subcontracted to other Laboratories.

2.2.2 Additional Mandatory ATPs (see Table 2)

a) These ATPs shall be applied upon request by the *Testing* Authority (TA) or *Results Management* Authority (RMA, if different)/ *Athlete* Passport Management Unit (APMU) or *WADA*, or shall be applied as mandatory CPs, as required.

b) All Laboratories shall have the additional mandatory ATPs (ITPs and CPs, where applicable), included within their Scope of ISO/IEC 17025 Accreditation [3].

c) In cases of temporary disruption of Laboratory analytical capacity, these mandatory ATPs may be subcontracted to other Laboratories.

2.2.3 Non-mandatory ATPs (see Table 3).

a) The implementation of a non-mandatory ATP by a Laboratory is optional. Therefore, not all Laboratories would have the non-mandatory ATPs within their Scope of ISO/IEC 17025 Accreditation [3].

b) These Analytical Methods may be subcontracted to other Laboratories or to *WADA*-approved laboratories (except for the analysis of the *Markers* of the Hematological Module of the *ABP*, due to the limited time requirements for such analysis – see also ISL [1] and ISL *TD* HEM [5]).

The classification of an ATP as either mandatory or non-mandatory is not static. A non-mandatory ATP can become mandatory (or *vice versa*) depending on several factors, including but not limited to, the prevalence of *Use* of the *Prohibited Substance*(s) or *Prohibited Method*(s), numbers of *Samples* collected for analysis by *Anti-doping Organizations* (*ADO*s), ease of Laboratory access to instrumentation/technology or Reference Materials (RMs), technological and/or analytical complexity of the Test Method, availability of national/regional access to the Test Method, or analytical costs.

Therefore, considering the developments in the Laboratory's capacity to implement non-mandatory ATPs, the Laboratory shall report and maintain in *ADAMS* an up-to-date list of available ATPs and services [1]. In addition, upon request by an *ADO*, the Laboratory should cooperate by providing other relevant information (*e.g.,* Laboratory analytical capabilities or prices for analytical services) to assist the *ADO* with their *Testing* plans.

[Comment to Article 2.2: Given the dynamic nature of the classification of ATPs as either mandatory or non-mandatory, as well as the continuous development of new ATPs for Doping Control, this ISL TD ATP may require frequent updates. For avoidance of doubt, Laboratories are not constrained from implementing a new non-mandatory ATP or from applying an approved ATP in a new matrix of analysis not listed in this ISL TD ATP as long as that new ATP / matrix of analysis has been demonstrated to be Fit-for-Purpose through method validation (in conformity with ISO/IEC 17025 and ISL requirements, including its applicable ISL TDs and ISL TLs). Under exceptional circumstances, and upon informing WADA, the Laboratory may apply the validated new ATP / matrix to the analysis of Samples before its inclusion into the Laboratory's Scope of ISO/IEC 17025 Accreditation (see also Article 2.1 and ISL Article 4.1.4.2.4.]

2.3 Temporary Disruptions to Analytical Capacity

Where a Laboratory cannot apply an ATP to the analysis of *Samples* due to, for example, a temporary disruption of its analytical capacity (*e.g.,* instrumental or staffing issues) that may affect test results reporting timelines, the Laboratory shall inform its customers and *WADA*.

Where a Laboratory's inability to apply a mandatory ATP leads to the subcontracting of the analysis for *Samples* already received at the Laboratory, the Laboratory shall bear the costs of *Sample* transportation to the subcontracted Laboratory(-ies) as well as any additional analytical costs, unless otherwise agreed with the responsible TA.

3.0 *WADA*-specific Analytical *Testing* Procedures

WADA-specific ATPs (see Table 4), which are applied by Laboratories for the analysis of certain *Prohibited Substances* and *Prohibited Methods*, require specific implementation steps prior to their application to *Samples* (see Appendix 1):

a) Application of Flexible Scope of ISO/IEC 17025 Accreditation is not allowed for the *WADA*-specific ATPs, even if the addition of the proposed Test Method would fall within the boundaries of allowed flexibility [6].

b) Therefore, in such cases, the Laboratory shall validate the *WADA*-specific ATP and inform *WADA* before requesting an extension to their Scope of ISO/IEC 17025 Accreditation by the relevant

Accreditation Body (AB). The Laboratory may also be required to successfully participate in an inter-laboratory collaborative study or *WADA*-organized EQAS round (see ISL*TD* EQAS [6]).

c) An assessment of *WADA*-specific ATPs by an AB is required for an extension of the Scope of ISO/IEC 17025 [3] Accreditation before application of the Test Method to the analysis of *Samples*.

d) However, once included within the Scope of ISO/IEC 17025 Accreditation, limited changes to these Test Methods can be made within the allowed boundaries of flexibility.

For example:

i. A Laboratory that has an accredited method (*e.g.,* LC-MS^n) for the quantification of Threshold Substances may include a new Threshold Substance within the Flexible Scope of ISO/IEC 17025 Accreditation if this new Threshold Substance and its applicable *Decision Limit* (*DL*) is defined in the ISL *TD* DL [7] or another relevant ISL *TD* or ISL *TL*.

ii. A Laboratory that has an accredited *WADA*-specific Test Method for the analysis of a specific type of substances (*e.g.,* LC-MS^n for the analysis of insulins), may include additional analogs of the substance into the Test Method within the Flexible Scope of ISO/IEC 17025 Accreditation.

e) Nonetheless, this flexibility does not allow the Laboratory to introduce new Analytes into these Test Methods if specific compliance decision criteria (applicable to these new Analytes) are required but are not yet defined in an applicable ISL *TD* or ISL *TL*.

For example:

i. New Target Compounds (TCs) or Endogenous Reference Compounds (ERCs) for GC/C/IRMS analysis shall not be flexibly included within the accredited GC/C/IRMS Test Method if specific method performance characteristics [*e.g.,* Limit of Quantification (LOQ), Measurement Uncertainty (MU)] and compliance decision criteria (*e.g., DL*) for the new Analyte have not been established in the ISL *TD* IRMS [8] or another relevant ISL *TD* or ISL *TL*. In such cases, the Laboratory shall request the AB for an extension to the method's scope to include the new Analyte within the Scope of ISO/IEC 17025 Accreditation [3].

ii. In contrast, if analytical and compliance requirements for these Analytes have been defined in a ISL *TD* or ISL *TL*, then the Laboratory may include them into the accredited GC/C/IRMS Test Method within the allowed boundaries of flexibility.

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Table 1. List of Mandatory Analytical *Testing* Procedures

MANDATORY TEST METHODS					
A. APPLICABLE TO ALL *SAMPLES*: *IN-* and *OUT-OF-COMPETITION*					
***Prohibited Substance* Class**	***Prohibited Substance* Subclass / Specific Examples**	**Matrix**	**Test Method(s)** [1, 2, 3]		**Exceptions** (not mandatory)
			Qualitative Procedure	**Quantitative Procedure**	
S1. Anabolic Agents	S1.1 Anabolic Androgenic Steroids (AAS) • Exogenous AAS • 19-NA; 19-NE	Urine	ITP and CP: GC-MS^n or LC-MS^n	n/a	n/a [4]
	• Urinary *Markers* of the Steroidal Module of the *ABP* [5]		CP: GC-MS^n	ITP and CP: GC-MS^n	
	S1.2 Other Anabolic Agents	Urine	ITP and CP: GC-MS^n or LC-MS^n	n/a	n/a

[1] Where an "*n*" is indicated for mass spectrometric (MS) methods, $n \geq 1$.

[2] The listing of chromatographic-mass spectrometric Test Methods (*e.g.,* GC-MS^n, LC-MS^n) shall not be interpreted as a restriction to the use of gas- (GC) or liquid chromatography (LC) methods. Other chromatographic Test Methods, such as, for example, Supercritical Fluid Chromatography (SFC) may also be applied if they have been validated and included in the Laboratory's Scope of ISO/IEC 17025 Accreditation.

[3] If not specified, the Test Method applies to both ITP and CP.

[4] n/a: Not applicable

[5] This ATP is also considered a *WADA*-specific ATP. Refer to Table 4 - *WADA*-specific ATPs.

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***Prohibited Substance* Class**	***Prohibited Substance* Subclass / Specific Examples**	**Matrix**	**Test Method(s)** [1, 2, 3]		**Exceptions** (not mandatory)
			Qualitative Procedure	**Quantitative Procedure**	
S2. Peptide Hormones, Growth Factors, Related Substances, and Mimetics	S2.1.2 Hypoxia-inducible Factor (HIF) Activating Agents	Urine	ITP and CP: LC-MS^n or GC-MS^n	n/a	Xenon (GC-MS) Cobalt (ICP-MS)
	S2.2.1 Chorionic gonadotrophin (CG) [5, 6]	Urine	n/a	ITP for hCG: Immunoassay or LC-MS^n	CP for hCG (Quantitative Procedure): Immunoassay [7] or LC-MS^n
S3. Beta-2 Agonists		Urine	Non-Threshold Substances (ITP, CP) LC-MS^n, or GC-MS^n Threshold Substances (ITP, CP) LC-MS^n or GC-MS^n	n/a Threshold Substances (CP) LC-MS^n or GC-MS^n	n/a

[6] *Testing* for hCG is applicable only to *Samples* from male *Athletes* or when a test for pregnancy is needed on *Samples* from female *Athletes* in accordance with the ISL *TD* NA [9].

[7] If the Laboratory uses immunoassays specific for the α/β heterodimer of hCG for both the ITP and the CP, then the confirmation immunoassay shall be different from the immunoassay applied for the ITP [10].

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Prohibited Substance **Class**	***Prohibited Substance*** **Subclass / Specific Examples**	**Matrix**	**Test Method(s)** [1, 2, 3]		**Exceptions** (not mandatory)
			Qualitative Procedure	**Quantitative Procedure**	
S4. Hormone and Metabolic Modulators	S4.1 Aromatase Inhibitors	Urine	ITP and CP: LC-MS^n or GC-MS^n	n/a	n/a
	S4.2 Anti-estrogenic Substances	Urine	ITP and CP: LC-MS^n or GC-MS^n	n/a	n/a
	S4.4.1 Activators of the AMP-activated protein kinase (AMPK) and Peroxisome Proliferator Activated Receptor δ (PPARδ) agonists	Urine	ITP and CP: LC-MS^n or GC-MS^n	n/a	n/a
	S4.4.3 Meldonium	Urine	ITP and CP: LC-MS^n	n/a	n/a
	S4.4.4 Trimetazidine	Urine	ITP and CP: LC-MS^n or GC-MS^n	n/a	n/a
S5. Diuretics and Masking Agents	• Diuretics	Urine	ITP and CP: LC-MS^n or GC-MS^n	n/a	n/a
	• Masking Agents				Other plasma expanders
pH		Urine	Electrochemistry pH strips	n/a	n/a
Specific Gravity (SG)		Urine	n/a	ITP: Densitometry ITP and CP: Refractometry	n/a

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			Qualitative Procedure	**Quantitative Procedure**	
B. APPLICABLE TO *IN-COMPETITION SAMPLES*					
S6. Stimulants		Urine	ITP and CP: LC-MS^n or GC-MS^n Threshold Substances (ITP, CP) LC-MS^n, or GC-MS^n	Threshold Substances (CP) GC-NPD, or LC-MS^n, or GC-MS^n	n/a
S7. Narcotics		Urine	ITP and CP: LC-MS^n or GC-MS^n Threshold Substances (ITP, CP) LC-MS^n or GC-MS^n	Threshold Substances CP) LC-MS^n or GC-MS^n	n/a
S8. Cannabinoids		Urine	ITP and CP: LC-MS^n or GC-MS^n Threshold Substances (ITP, CP) LC-MS^n or GC-MS^n	Threshold Substances CP) LC-MS^n or GC-MS^n	n/a
S9. Glucocorticoids		Urine	ITP and CP: LC-MS^n or GC-MS^n	n/a	n/a

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			Qualitative Procedure	**Quantitative Procedure**	
C. APPLICABLE TO SPECIFIC SPORTS					
P1. Beta-blockers		Urine	ITP and CP: LC-MS^n or GC-MS^n	n/a	n/a
For some specific sports, beta-blockers are prohibited at all times (*In-* and *Out-of-Competition*), whereas for some other sports, they are prohibited only *In-Competition*. Refer to the *Prohibited List* [11].					

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Table 2. List of Additional Mandatory Analytical *Testing* Procedures

ADDITIONAL MANDATORY TEST METHODS **(WHEN REQUESTED BY *TESTING* AUTHORITIES / APMU or AS MANDATORY CONFIRMATION PROCEDURES)**					
***Prohibited Substance* Class**	***Prohibited Substance* Subclass / Specific Examples**	**Matrix**	**Test Method(s)** [1, 2, 3]		**Exceptions** (not mandatory) *
			Qualitative Procedure	**Quantitative Procedure**	
S1. Anabolic Agents	S1.1 Anabolic Androgenic Steroids (AAS) • Urinary *Markers* of the Steroidal Module of the *ABP*	Urine	n/a	CP: GC/C/IRMS [5]	n/a
S2. Peptide Hormones, Growth Factors, Related Substances, and Mimetics	S2.1.1 Erythropoietin Receptor Agonists (ERAs) [5] • Recombinant EPOs, NESP, CERA, EPO-Fc.	Urine Plasma Serum	ITP and CP: PAGE Method [12]	n/a	EPO-mimetic agents (*e.g.,* CNTO-530, Peginesatide)
	S2.2.1 GnRH *,[8] and its agonist analogues	Urine	ITP and CP: LC-MS^n	n/a	n/a
	S2.2.3 Growth Hormone • hGH Isoforms Test [5]	Serum	n/a	Immunoassay	n/a
	S2.2.3 Growth Hormone Fragments (AOD-9604, hGH 176-191) *	Urine	ITP and CP: LC-MS^n	n/a	n/a

[8] *Testing* for GnRHs is applicable only to *Samples* from male *Athletes*.

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***Prohibited Substance* Class**	***Prohibited Substance* Subclass / Specific Examples**	**Matrix**	**Test Method(s)** [1, 2, 3]		**Exceptions** (not mandatory) *
			Qualitative Procedure	**Quantitative Procedure**	
S2. Peptide Hormones, Growth Factors, Related Substances, and Mimetics (continued)	S2.2.4 Growth Hormone Releasing Factors Growth Hormone Secretagogues (GHS) and Growth Hormone Releasing Peptides (GHRPs) *	Urine	ITP and CP: LC-MS^n	n/a	n/a
	S2.3 Growth Factors and Growth Factor Modulators TB-500 *				
S5. Diuretics and Masking Agents	Masking Agent(s) • Desmopressin *, Felypressin *	Urine	ITP and CP: LC-MS^n or GC-MS^n	n/a	n/a
* These are often referred to as “small peptides”					

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Table 3. List of Non-Mandatory Analytical *Testing* Procedures

NON-MANDATORY (OPTIONAL) TEST METHODS				
***Prohibited Substance* Class**	***Prohibited Substance* Subclass / Specific Examples**	**Matrix**	**Test Method(s)** [1, 2, 3]	
			Qualitative Procedure	**Quantitative Procedure**
Relevant Target Analytes	• 6α-Hydroxy-androstenedione (6α-OH-AD) • 19-NA, 19-NE • Boldenone and Boldenone *Metabolite*(s) • Epiandrosterone sulfate • Formestane	Urine	n/a	CP: GC/C/IRMS [5]
S1. Anabolic Agents	S1.1 Anabolic Androgenic Steroids (AAS)			
	• Steroid Esters	Serum, Plasma DBS	ITP and CP: LC-MS^n or GC-MS^n	n/a
	• Blood *Markers* of the Steroidal Module of the *ABP* [5]	Serum DBS	CP: LC-MS^n	ITP/CP: LC-MS^n
	• Exogenous AAS	DBS [9]	ITP and CP: LC-MS^n or GC-MS^n	n/a

[9] DBS *Testing* can only be applied to Non-Threshold Substances without *MRL* [13]

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			Qualitative Procedure	**Quantitative Procedure**
S2. Peptide Hormones, Growth Factors, Related Substances, and Mimetics	S2.1.1 Erythropoietin Receptor Agonists (ERAs) [5]	Urine DBS	ITP and CP: PAGE Methods [12] or LC-MS^n	n/a
	S2.1.2 Hypoxia-inducible Factor (HIF) Activating Agents	DBS	ITP and CP: LC-MS^n	n/a
	S2.1.2 Hypoxia-inducible Factor (HIF) Activating Agents • Cobalt • Xenon	Urine	n/a	ITP and CP: ICP-MS or GC-MS
	S2.1.4 TGFβ Signaling Inhibitors	Urine Serum, Plasma DBS	ITP and CP: PAGE Methods [12] or LC-MS^n	n/a
	S2.1.5 Innate Repair Receptor Agonists • Asialo EPO • Carbamylated EPO	Urine	ITP and CP: PAGE Methods	n/a
	S2.2.1 • Intact hCG [5, 6, 7]	Urine	n/a	CP: Immunoassay or LC-MS^n
	• LH [5, 10]		n/a	ITP: Immunoassay
	S2.2.1 GnRH [*,8] and its agonist analogues	DBS	ITP and CP: LC-MS^n	

[10] *Testing* for LH is applicable only to *Samples* from male *Athletes*.

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			Qualitative Procedure	**Quantitative Procedure**
S2. Peptide Hormones, Growth Factors, Related Substances, and Mimetics (Continued)	S2.2.2 Corticorelin, Tetracosactide	Urine, DBS	ITP and CP: LC-MS^n	n/a
	S2.2.3 Growth Hormone, its Analogues and Fragments hGH Biomarkers Test [5]	Serum	IGF-I (CP): LC-MS^n	P-III-NP (ITP, CP): immunoassay IGF-I (ITP, CP): Immunoassay or LC-MS^n (bottom-up)
	hGH Analogues (Somatrogon)	Urine Serum, Plasma	CP: LC-MS^n	ITP: hGH isoforms test kit 2
	Blood *Markers* of the Endocrine Module of the *ABP* [5]	Serum	IGF-I (CP): LC-MS^n	P-III-NP (ITP, CP): Siemens Centaur Immunoassay IGF-I (ITP, CP): LC-MS^n (top down)
	S2.2.4 Growth Hormone-Releasing Factors Growth Hormone-Releasing Hormone (GHRH) and its analogues ** [5]	Urine Serum, Plasma	ITP and CP: LC-MS^n	n/a
	Growth Hormone Secretagogues (GHS) and Growth Hormone Releasing Peptides (GHRPs) *	Serum, Plasma DBS	ITP and CP: LC-MS^n	n/a
	S2.3 Growth Factors and Growth Factor Modulators [5] • IGF-I and its analogues ** • Mechano Growth Factors	Urine Serum, Plasma DBS	ITP and CP: LC-MS^n	n/a
	• TB-500 *	DBS	ITP and CP: LC-MS^n	n/a

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			Qualitative Procedure	**Quantitative Procedure**
S3. Beta-2 Agonists		DBS	ITP and CP: LC-MS^n or GC-MS^n	n/a
S4. Hormone and Metabolic Modulators	S4.1 Aromatase Inhibitors	DBS	ITP and CP: LC-MS^n or GC-MS^n	n/a
	S4.2 Anti-estrogenic Substances	DBS	ITP and CP: LC-MS^n or GC-MS^n	n/a
	S4.3 Agents Preventing Activin Receptor IIB Activation *e.g.,* Bimagrumab	Urine Serum, Plasma	ITP and CP: LC-MS^n or PAGE Methods	n/a
	S4.4.1 Activators of the AMP-activated Protein Kinase (AMPK) and Peroxisome Proliferator Activated Receptor δ (PPARδ) Agonists	DBS	ITP and CP: LC-MS^n or GC-MS^n	n/a
	S4.4.2 Metabolic Modulators Insulins and insulin-mimetics **, [5]	Urine Serum, Plasma DBS	ITP and CP: LC-MS^n	n/a
	S4.4.4 Trimetazidine	DBS	ITP and CP: LC-MS^n or GC-MS^n	n/a
S5. Diuretics and Masking Agents	Diuretics	DBS	ITP and CP: LC-MS^n or GC-MS^n	n/a
	Masking Agent(s) • Desmopressin *, Felypressin *	DBS		n/a
	Plasma Expanders • Dextran, HES, Mannitol	Urine		n/a

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			Qualitative Procedure	**Quantitative Procedure**
M1. Manipulation of Blood and Blood Components	M1.1 • Homologous Blood Transfusion (HBT) [5]	Whole blood	n/a	ITP and CP: Flow Cytometry
	• *Markers* of the Hematological Module of the *ABP* [5, 11]	Whole blood	n/a	ITP and CP: Flow Cytometry
	• Phthalates (DEHP *Metabolites*)	Urine	ITP and CP: LC-MS^n	n/a
	M1.2 • Efaproxiral (RSR 13)	Urine, DBS	ITP and CP: LC-MS^n	n/a
	• HBOCs [5]	Serum, Plasma Whole blood	ITP: Visual inspection Spectrophotometry (serum/plasma) ITP and CP: Native-PAGE; or Capillary Electrophoresis; or Flow Cytometry; or LC-MS^n	n/a
	• Myo-inositol Trispyrophosphate (ITPP)	Urine Serum, Plasma DBS	ITP and CP: LC-MS^n	n/a

[11] Due to the requirements to analyze *ABP Samples* within timelines that meet the Blood Stability Score (BSS) criteria, the analysis of the *Markers* of the Hematological Module of the *ABP* is an ATP that cannot be subcontracted.

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***Prohibited Substance* Class**	***Prohibited Substance* Subclass / Specific Examples**	**Matrix**	**Test Method(s)** [1, 2, 3]	
			Qualitative Procedure	**Quantitative Procedure**
M1. Manipulation of Blood and Blood Components (Continued)	• Voxelotor	Urine DBS	ITP and CP: LC-MS^n	n/a
M2. Chemical and Physical Manipulation	M2.1 *Tampering* or *Attempting to Tamper* • Proteases	Urine	ITP: SDS-PAGE, ITP and CP: LC-MS^n	n/a
	• *Sample* Substitution	Urine Serum, Plasma Whole blood DBS	ITP and CP: DNA sequencing, DNA Profiling, e.g., Short Tandem Repeat (STR)	n/a
	• *Sample* Adulteration		n/a	ITP and CP: Urinalysis Hemograms[12]
M3. Gene and Cell Doping	M3.1 Use of Nucleic Acids or Nucleic Acid Analogues • cDNA transfer (*e.g.,* cDNA-EPO)	Whole blood DBS	ITP and CP: PCR based methods [5]	n/a
	• siRNA (*e.g.,* myostatin siRNA)	Urine Whole blood DBS	ITP and CP: PAGE Methods or LC-MS^n	n/a
* These are often referred to as “small peptides”. ** These are often referred to as “large peptides”.				

[12] The urinalysis and hematograms may include the measurement of pH, creatinine, salt concentrations, glucose, ketones, endogenous hormones, red blood cells, etc.

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Table 4. *WADA*-specific Analytical *Testing* Procedures

Prohibited Substances and *Prohibited Methods* Class	Approved Matrix	Analytical Method [a]
S1.1 (Blood and Urine) *Markers* of the Steroidal Module of the *ABP*	Urine Serum	GC-MS^n LC-MS^n
S2.1.1 Erythropoietin Receptor Agonists (ERAs)	Urine Plasma Serum DBS [b]	PAGE methods (IEF-PAGE, SDS-PAGE, SAR-PAGE) or LC-MS^n
S2.2.1 Intact Human Chorionic Gonadotrophin (hCG)	Urine	LC-MS^n, Immunoassay
S2.2.1 Luteinizing Hormone (LH)	Urine	Immunoassay
S2.2.3 Human Growth Hormone (hGH) Isoforms	Serum	Isoforms Differential Immunoassays
S2.2.3 Blood *Markers* of the Endocrine Module of the *ABP* IGF-I & P-III-NP	Serum	LC-MS^n (top-down) for IGF-I Siemens Centaur Immunoassay for P-III-NP
Large peptides *e.g.,* S2.2.4 GHRH analogues, S2.3 IGF-I analogues S4.4.2 Insulins	Urine Plasma Serum DBS [b]	LC-MS^n
Relevant classes [c]: Determination of origin by GC/C/IRMS	Urine	GC/C/IRMS
Relevant classes [c]: Confirmation of Threshold Substances by Quantitative Procedure	Urine	GC-MS^n, LC-MS^n, GC-NPD
M1. Homologous Blood Transfusion (HBT)	Whole Blood	Flow cytometry
M1. *Markers* of the Hematological Module of the *ABP*	Whole Blood	Flow cytometry

M1. Haemoglobin-based Oxygen Carriers (HBOCs)	Plasma Serum	Native-PAGE
M3. Gene and Cell Doping	Whole Blood DBS [b]	PCR based methods

[a] Examples of Analytical Methods, which are currently applied to the analysis of these *Prohibited Substances* and *Prohibited Methods*. Any other analytical techniques not specifically mentioned in Table 4 also require *WADA* to be informed before the analysis of the listed *Prohibited Substances* and *Prohibited Methods*.

[b] DBS: Dried Blood Spots

[c] As determined in a relevant *WADA* ISL *TD* or ISL *TL*.

4.0 References

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

[2] *WADA Technical Document* ISL *TD* VAL: Minimum Requirements for Validation of Analytical *Testing* Procedures for *Doping Control*.

[3] ISO/IEC 17025:2017 - General Requirements for the Competence of Testing and Calibration Laboratories.

[4] ISO 15189:2022 - Medical laboratories — Requirements for Quality and Competence.

[5] *WADA Technical Document* ISL *TD* HEM: Analytical and Reporting Requirements for the *Markers* of the Hematological Module of the *Athlete Biological Passport*.

[6] *WADA Technical Document* ISL *TD* EQAS: *WADA* External Quality Assessment Scheme.

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

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

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

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

[11] The World Anti-Doping *Code Prohibited List*.

[12] *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.

[13] *WADA Technical Document* ISL *TD* DBS: Dried Blood Spots (DBS) for *Doping Control* - Requirements and Procedures for Analytical *Testing* and *Sample* Storage.

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

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Appendix 1. Summary of the steps for approval of *WADA*-specific Analytical *Testing* Procedures

Step	Action to be performed by the Laboratories and Accreditation Bodies (ABs)
1	The new *WADA*-specific ATP shall be validated and determined as Fit-for Purpose by the Laboratory;
2	The Laboratory shall inform *WADA* that a new *WADA*-specific ATP has been validated and determined as Fit-for-Purpose. *[Comment: It is not necessary to inform WADA of changes in procedures that are already included in the Laboratory's Scope of ISO/IEC 17025 Accreditation.]*
3	The Laboratory may be required to successfully participate in a *WADA* educational EQAS (specific to this Test Method) or another relevant inter-laboratory collaborative study – to be determined by *WADA.*
4	The Laboratory shall contact the Accreditation Body (AB) to request an ISO/IEC 17025 scope extension for the Test Method
5	The *WADA*-specific ATP shall be assessed by the relevant AB, and the Laboratory shall inform *WADA* of the outcome of the assessment.
6	The *WADA*-specific ATP may only be applied to *Samples* after it is included within the Laboratory's Scope of ISO/IEC 17025 Accreditation. Then limited changes can be made to the *WADA*-specific ATP within the allowed boundaries of flexibility.