WADA ISL *Technical Letter* TL27– *Sample Tampering*

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Written by: Reviewed by:	*WADA* Science / EAAS Working Group *WADA* Laboratory Expert Advisory Group	Approved by:	*WADA* Executive Committee
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Urine *Sample Tampering* or *Attempted Tampering*

1.0 Introduction

WADA wishes to draw the attention of the Laboratories, *Anti-Doping Organizations* (*ADOs*) and *Delegated Third Parties* (*DTP*) in charge of *Doping Control* activities on behalf of *ADOs* to the following observations and instructions regarding circumstances where a urine *Sample* is suspected to have been subjected to a possible *Tampering* or *Attempted Tampering*.

Whenever a urine *Sample* is suspected of *Tampering* or *Attempted Tampering*, the Laboratory shall perform a series of analytical assessments to determine the authenticity and validity of the *Sample*. These evaluations are intended to identify the absence of typical urinary constituents, the presence of atypical compounds, and/or indicators of *Sample* adulteration [1].

2.0 *Sample Tampering* or *Attempted Tampering* Analytical Assessment

2.1 Initial Assessment

a) Atypical *Sample* Appearance

Upon inspection of the *Sample* at the time of "A" *Sample* aliquoting, the Laboratory shall document as part of the *Sample*'s records any significant *Sample* differences from physiological urinary characteristics *(e.g.*, color, odor, turbidity, presence of particulates not typically found in human urine) that may indicate a possible *Sample Tampering* or *Attempted Tampering*. If necessary, the Laboratory shall inform the *Testing* Authority (TA) and seek instructions on the performance of Analytical *Testing* on the *Sample*, in writing, as soon as possible.

[Comment to Article 2.1 a): As per the International Standard for Laboratories (ISL) Article 5.3.2.1 [2], the TA shall inform the Laboratory, in writing within seven (7) days, whether a urine Sample with the noted Sample appearance irregularity(-ies) shall be analyzed or not, and/or of any further measures to be taken (e.g., forensic analysis, DNA analysis), or that the Sample should be stored for Further Analysis. The communication between the Laboratory and the TA shall be recorded as part of the Sample's documentation. In the absence of a timely reply (within seven (7) days) by the TA, the Laboratory should report the Sample as "Not Analyzed" in ADAMS or, at the Laboratory's discretion, analyze the Sample for other indicators of Sample Tampering or Attempted Tampering as described below.]

Differences between the "A" and "B" *Samples* shall be handled as per ISL *TL*14 [3]. It is recommended that pictures of both the "A" and "B" S*amples* be taken wherever relevant.

b) Specific Gravity and pH

The Laboratory's initial assessment of the Specific Gravity (SG) and pH values outside the physiological range, i.e.,

- SG < 1.001 [4,5] and pH < 4.0 or > 9.0 [6].

[Comment to Article 2.1 b): Differences between SG and pH values obtained from Sample collection and Laboratory measurements may be observed and not necessarily indicate possible Tampering or Attempted Tampering]

c) Evaluation of the Urinary Steroid *Markers*

The absence or suppression of the urinary steroid *Markers* [7] may suggest non-urine matrices or a diluted *Sample* and, therefore, may further support the possibility of *Sample Tampering* or *Attempted Tampering*.

Wherever applicable, the Laboratory should inform the relevant TA or *Results Management* Authority (RMA, if different) or the Passport Custodian and recommend that the associated *Athlete* Passport Management Unit (APMU) investigate the *Athlete*'s steroidal Passport with particular attention to the suspected *Sample*.

[Comment to Article 2.1.c: the substitution of an Athlete's urine Sample with the urine of another individual (urine exchange) can also be uncovered using the steroidal Passport and confirmed by DNA analysis across multiple Samples, as described in the ISL TD APMU [8].]

d) Evaluation of Other Endogenous Substances

In addition, depending on the analytical capability of the Laboratory, the evaluation of other endogenous substances typically excreted in urine, such as 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), cortisol and cortisone, should be performed. Moreover, other relevant endogenous substances that may be evaluated include pregnanediol (PD), pregnanetriol (PT), 5α-androst-16-en-3α-ol (16-en), 11β-hydroxy-androsterone (11-OH-A), and 11-oxo-etiocholanolone (11-oxo-Etio).

The absence or relatively low concentrations of these additional endogenous substances should be assessed in conjunction with other physiological parameters to determine the consistency of the *Sample* with normal human urine. If GC/C/IRMS [9] analysis of any endogenous substances listed above has been performed, and the carbon isotope ratio values are incompatible with human reference ranges, the data may also be used to support the investigation of *Tampering* or *Attempted Tampering.*

e) Evaluation of Proteolytic Activity

The Laboratory may assess the presence and activity of proteolytic enzymes (proteases), which can degrade endogenous proteins normally present in urine *Samples*.

The Laboratory may analyze the integrity of specific endogenous proteins commonly found in urine, such as endogenous erythropoietin (EPO) [10], albumin, and others. The detection of abnormal fragmentation patterns or the absence of intact proteins, particularly when corresponding unexpected degradation products (peptides or fragments) are present, may provide evidence of protease activity.

In addition, proteolytic enzymes may be detected directly using enzyme activity assays or mass spectrometry-based proteomics targeting known protease *Markers*. The presence of active proteases in properly stored urine *Samples* is unusual and may indicate *Tampering* or *Attempted Tampering*.

2.2 Further Assessment

The Laboratory shall conduct the further assessments when the initial evaluations described in Article 2.1 produce abnormal results. If the Laboratory lacks the analytical capacity to perform the required additional analyses, the Laboratory may, in consultation with the relevant TA (or RMA, if different), subcontract the analysis to another Laboratory or *WADA*-authorized laboratory that has the procedure included in its ISO/IEC 17025 [11] Scope of Accreditation.

a) Concentrations of creatinine, electrical conductivity, and other physiological constituents of urine

The Laboratory is not required to have Analytical *Testing* Procedures (ATPs) [12] for the measurement of creatinine, urinary electrical conductivity, or any other physiological constituents of urine (*e.g.,* glucose/sugars, electrolytes, uric acid, etc.) that may be used as corroborating evidence to determine the origin or authenticity of the *Sample*; however, assessing these parameters may help identify a possible case of *Tampering* or *Attempted Tampering*. Therefore, the Laboratory may consider implementing or subcontracting [13] such analysis.

[Comment to Article 2.2.a): All procedures used in these evaluations shall be conducted using validated Test Methods. If the Laboratory subcontracts the analysis, the subcontracted laboratory shall be accredited to the ISO/IEC 17025 [11] (or ISO 15189 [14]) for the applied analytical procedures.]

Concentrations significantly different from normal ranges for [6]:

- Creatinine < 20 mg/dL (*i.e.*, < 200 mg/L).
- Urinary electrical conductivity < 1mS/cm [15].

These suggested values may be used to indicate that the *Sample*'s urinary parameters are significantly different from normal ranges and may therefore not be representative of authentic urine.

A complete record of all results, interpretations, and justifications shall be documented and maintained as part of the *Sample*'s records.

b) Detection of Atypical Compounds

The Laboratory may analyze and evaluate results for non-physiological compounds that are not typically found in urine *Samples* or that are found in concentrations inconsistent with normal excretion patterns [16, 17]. This includes substances commonly associated with commercial beverages (*e.g.,* high levels of caffeine, artificial sweeteners, or colorants), industrial products (protease reagents), or even related to the preparation of synthetic urine. While some of these compounds may be present in urine following dietary intake or supplementation, unusually high concentrations and/or unexpected combinations may indicate *Sample Tampering* or *Attempted Tampering*.

[Comment to Article 2.2.b: An ATP applied by the Laboratory for the evaluation of the presence of non-prohibited atypical compounds is not required to be ISO/IEC 17025 accredited [11], and such compounds may be detected in

Samples without the need for identification in compliance with the ISL TD IDCR [18]. Nevertheless, the Laboratory shall have established Quality Assurance requirements for the procedure applied.]

2.3 Urine Substitution: DNA Profiling

If deemed necessary, the Laboratory may perform DNA profiling to confirm whether the *Sample* contains human DNA and, if so, whether the DNA profile matches that of the *Athlete*. If necessary, a reference *Sample* of the *Athlete* shall be obtained in consultation with the TA (or RMA, if different) or *WADA*.

[Comment 1 to Article 2.3: For the avoidance of doubt, the absence of DNA is not conclusive evidence that the Sample is non-human or that it has been substituted, as DNA may be present in insufficient quantities in urine for profiling.]

[Comment 2 to Article 2.3: The Laboratory shall inform WADA when DNA profiling analysis needs to be performed. WADA will then verify whether another Sample belonging to the same Athlete has been previously collected and is available to be used as a reference Sample.]

If the Laboratory lacks the analytical capacity to perform the DNA analysis, the Laboratory may, in consultation with the relevant TA (or RMA, if different), subcontract the DNA analysis to another Laboratory or *WADA*-authorized laboratory that has the procedure included in its ISO/IEC 17025 [13] Scope of Accreditation.

3.0 Reporting of *Sample Tampering* or *Attempted Tampering*

3.1 *Adverse Analytical Finding*

The Laboratory shall report the finding as an *Adverse Analytical Finding* (*AAF*) for *Tampering* or *Attempted Tampering* if the Laboratory can determine that the general nature/type of the adulterated *Sample* is not consistent with human urine, *i.e.,* when:

a) The assessments described in Article 2.1 show irregular results together with anomalous results obtained with the assessments described in Article 2.2,

and/or

b) The DNA profiling performed according to Article 2.3 concludes that the *Athlete's* urine has been substituted.

3.2 *Atypical Finding*

The Laboratory shall report the finding as an *Atypical Finding* (*ATF*) for *Tampering* or *Attempted Tampering* if the assessments described in Article 2.1 show irregular results without the observation of anomalous results in the assessments described in Articles 2.2 and/or 2.3.

The Laboratory shall also include a comment in *ADAMS* advising the TA (or RMA, if different) to perform further investigations (*e.g.,* additional analyses on the *Sample*, *Target Testing* the *Athlete,* or placing the *Sample* in long-term storage for Further Analysis).

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4.0 References

[1] Piper, T., et al. "When is a sample a urine sample? Markers for urine sample authenticity assessment in sports drug testing." *Bioanalysis* (2025): 1-9.

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

[3] *WADA Technical Letter* ISL *TL*14 – Difference in "A" and "B" *Sample* Urine Characteristics.

[4] Edgell, Kenneth, et al. "The defined HHS/DOT substituted urine criteria validated through a controlled hydration study." *Journal of Analytical Toxicology* 26.7 (2002): 419-423.

[5] Athanasiadou, I., et al. "The effect of athletes hyperhydration on the urinary 'steroid profile' markers in doping control analysis." *Drug Testing and Analysis* 10.9 (2018): 1458-1468.

[6] Department of Health and Human Services. "Mandatory guidelines for federal workplace drug testing programs." Substance Abuse and Mental Health Services Administration, *Fed Reg* (2023): 70814-50.

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

[8] *WADA Technical Documen*t ISL *TD* APMU: Athlete Passport Management Unit – Requirements and Procedures.

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

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

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

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

[13] *WADA* Laboratory Guidelines: Conducting and Reporting Subcontracted Analysis and Further Analysis for *Doping Control.*

[14] ISO 15189:2022 - Medical Laboratories — Requirements for Quality and Competence.

[15] Fazil Marickar, Y.M. Electrical conductivity and total dissolved solids in urine. Urol Res 38, 233–235 (2010).

[16] Segura, Jordi, *et al.* "If you play with fire, you may get burned." *Drug Testing and Analysis* 12.5 (2020): 582-587.

[17] Alaejos, Ana Rubio, *et al.* "Evaluation of doping control samples to determine the prevalence of nicotine use by German elite athletes." *Manfred Donike Workshop on Dope Analysis: Cologne Workshop on Dope Analysis*. Sportverlag Strauß, 2021.

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

[Comment to Article 4.0: Current versions of WADA's ISL, Technical Documents, Technical Letters and Laboratory Guidelines may be found at https://www.wada-ama.org/en/what-we-do/science-medical/laboratories]