WORLD ANTI-DOPING AGENCY
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TD2021EPO

Summary of Major Modifications

The *Technical Document* on Harmonization of Analysis and Reporting of Erythropoietin (EPO) and other Erythropoietin-Receptor Agonists (ERAs) by Polyacrylamide Gel Electrophoretic (PAGE) Analytical Methods, TD2021EPO, has been aligned with the 2021 World Anti-Doping Code (*Code*) and the recently approved 2021 *International Standard* for Laboratories (ISL); and, the other *International Standards*, which are set to come into force on 1 January 2021.

The main changes in the TD2021EPO include:

- The adjustment of the title to reflect the current classification of these erythropoiesis stimulating agents in the 2021 *Prohibited List*.

Article 2.1 Pre-Analytical Procedure

- This is a new article providing guidance on the pre-analytical procedures to be followed for urine and blood *Samples* for ERA analysis, aimed at reducing the risks of ERA degradation and increasing the efficiency of the analysis;

Article 2.2.1 PAGE Analytical Methods

- Several requirements for the performance of PAGE methods have been revised or new ones added, including, among others:
  - The requirement that immunopurification be performed prior to the electrophoretic separation of ERAs, both during the Initial *Testing* Procedure (ITP) and the Confirmation Procedure (CP);
  - the requirement to add an appropriate carrier protein to the immunopurified eluate and use an appropriate buffer system ensuring the effective transfer of large biomolecules (*e.g.* CERA, EPO-Fc) when applying SDS-PAGE;
  - the recommendation that, after electrophoretic separation, Laboratories apply a single blotting procedure using a cross-reactivity minimized protocol;
  - the recommendation to use test sensitivity controls to verify that the electrophoretic separation method is working as expected in accordance with validation results;
  - the definition of Minimum Required Performance Levels (MRPL) for ERAs in urine and blood, with the requirement that the Limit of Detection (LOD) of the ITP, as estimated in the matrix of analysis during method validation, shall not be higher than 50% of the corresponding MRPL;
  - Better guidance on the selection of positive QCs, and recommendation for use of test sensitivity controls, during the CP;

- An updated Table 2 on the PAGE methods to be used for detection of ERAs during the ITP and CP.

- An extensive comment is provided to explain why PAGE methods, which include immunopurification and immunoblotting procedures in addition to electrophoretic separation, comply with the Selectivity requirements established in the ISL for affinity-binding assays.

Article 2.3 Description of PAGE Analytical Methods

- Better description and revised requirements for the four different steps (Sample preparation; electrophoretic separation; immunoblotting; and detection) of the PAGE methods (IEF-PAGE and SAR- or SDS-PAGE). For example, it has been clarified that for both the ITP and the CP, immunopurification shall be performed prior to the electrophoretic separation; vertical electrophoresis shall be applied for SAR-/SDS-PAGE; immunoblotting shall be performed by electroblotting to optimize the transfer of the ERA(s); single-blotting using a cross-reactivity minimized protocol (*e.g.* use of the biotinylated monoclonal mouse anti-human EPO clone AE7A5) or double-blotting shall be performed.

Article 2.4 Evaluation and Interpretation of Results

- In this Article, the acceptance and identification criteria applicable to the analysis of ERAs by PAGE methods have been updated, including

  - Interpretation of results when test sensitivity controls are used;

  - Updates of figures to illustrate the PAGE acceptance and identification criteria, including a new Figure 1b showing an IEF-PAGE immunoblot image after the analysis of ERAs at pH = 2 – 8; an updated figure 2 of a SAR-PAGE analysis of different commercially available Epoetin-α, Epoetin–β and EPO-Fc preparations; a new Figure 3 with the SDS-PAGE analysis of a Dynepo excretion urine; a new Figure 4 of a SAR-PAGE image obtained for samples collected at different timepoints after Retacrit (epoetin-ζ) administration; and a new Figure 5 showing the SAR-PAGE separation of urine samples collected at different timepoints after subcutaneous application of Biopoin, including images obtained without contrast processing (A) and after contrast optimization with GASepo software v2.1 (B);

  - A comment as been included to clarify that the electrophoretic behaviour of ERAs may be different from that of ERA standards or PQC samples depending on the source of the particular preparation analyzed.

Article 3.0 Documentation and Reporting

- Clear guidance is provided on the conditions for reporting an ERA finding as an *AAF*, *ATF* or Negative Finding. This Article also includes the recommendation to apply an additional CP method to obtain conclusive scientific evidence when initial CP results for NESP, CERA or EPO-Fc are inconclusive, and guidance on how to report the results of the analyses.

- A comment is included to provide guidance to the Laboratories on conditions that should trigger a recommendation to *Testing* Authorities to conduct additional ERA analysis on urine and/or blood *Sample(s)* from the *Athlete.*

Article 4.0 References

The list of scientific publications and *WADA* laboratory standards references has been updated.

Annex A: This new Annex include templates for second opinion requests on ERA analysis

In addition:

- Analytical requirements for Peginesatide have been removed from this *TD*, since this EPO mimetic is no longer available (shown to induce serious side effects, including death), and Laboratories do not receive requests for analysis from *Testing* Authorities;
- Terms and definitions have been updated where relevant;
- Footnotes have been inserted as Comments where relevant in the main text.

The TD2021EPO replaces the former TD2014EPO and becomes effective on 1 May 2021.