TD2024EPO

Summary of Major Modifications

This document summarizes the modifications in the Technical Document (TD) on Harmonization of Analysis and Reporting of Erythropoietin (EPO) and Other EPO Receptor Agonists (ERAs) by Polyacrylamide Gel Electrophoretic (PAGE) Analytical Methods. The TD2024EPO has been revised to improve the formatting, structure, and clarity of pre-analytical, analytical and reporting requirements for ERA findings, including the following main modifications:

Title

The title has been modified to reflect that this TD now also includes guidance on the analysis of Transforming Growth Factor-Beta Signalling Inhibitors (e.g., Luspatercept, Sotatercept). The modified TD2024EPO is now titled: Harmonization of Analysis and Reporting of Erythropoietin (EPO) Receptor Agonists (ERAs) and Transforming Growth Factor-Beta (TGF-b) Signalling Inhibitors by Polyacrylamide Gel Electrophoretic (PAGE) Analytical Methods.

1.0 Introduction

- Nesbell, a new example of darbepoietin preparations, has been added to the definition of the dEPO acronym.
- The TGF-b acronym has been defined.

2.0 Analytical Testing for ERAs

2.1 Pre-Analytical Procedure

This section has been, on the one hand, simplified by referring to the initial sample storage and aliquoting requirements described in the International Standard for Laboratories (ISL) whereas, on the other hand, further guidance is provided to the Laboratories on the procedures to follow to minimize ERA degradation, and to the Testing/Results Management Authorities to expedite the performance of ERA “B” Confirmation Procedures (CP) within one month of being notified by the Laboratory of the “A” sample Adverse Analytical Finding (AAF).

In addition, a new article 2.1.3 has been introduced to include pre-analytical requirements for the analysis of ERAs in dried blood spot (DBS) samples.

2.2 Description of the PAGE Analytical Methods

The order of the articles describing the PAGE analytical methods and the analytical testing strategy for their application has been inverted for ease of reading and understanding.

In this new Article 2.2, some requirements on the application of PAGE Analytical Methods have been clarified including, for example, the possibility to use a preparation of recombinant Epoetin-α (*e.g.*, Eprex) as an alternative to Epoetin-d (Dynepo) for placing the rEPO migration cut-off line, as well as the need to produce 16-bit grayscale TIFF-files for proper evaluation of results.

2.3 Analytical Testing Strategy

- The Minimum Required Performance Levels (MRPL) defined for ERA analysis in serum/plasma are now applicable also to the analysis of DBS samples.
- For the Initial Testing Procedure (ITP), it is clarified that the Laboratory may add an internal standard to the sample aliquot, the negative control sample (NQC) and the Test Sensitivity Control (if used) to verify the sample preparation procedure (for example, when neither endogenous EPO nor other ERA signals could be initially detected in the sample).
- It is now emphasized that the ERA CP shall differ from the ITP, and examples are provided (for example, it is now clarified that the application of different immunopurification procedures shall be based on the use of different capture antibodies).
- It is now required that, to avoid signal interferences from adjacent lanes, all lanes in the CP gels ("A" or "B" Aliquots, NQC, PQC, reference standards, TSC) shall be flanked by empty lanes.
- The use of IEF-PAGE as an additional Analytical Method for confirmation of rEPO: It is now clarified that IEF-PAGE may be applied as an additional Analytical Method to obtain complementary evidence of the presence/absence of rEPO if requested by a second opinion provider. This is not mandatory and is not necessary in all cases.
- The adjustment of ERA signal intensity for rEPO confirmations: When needed and based on the results from the ITP, the intensity signals of the confirmation Aliquot and/or the NQC and/or the PQC shall be adjusted to ensure appropriate ERA intensity signals (endogenous EPO ± rEPO) and facilitate the interpretation of results.

3.0 Review and Interpretation of ERA Results

This article has been revised to clarify the initial steps in the review of ERA results by the Laboratory before suspicious results are subject to a second opinion review process by Experts of the WADA EPO Working Group (WG).

- In addition, it has been clarified that when a second, complementary confirmation Analytical Method is applied to obtain additional scientific evidence and it does not confirm the presence of ERA(s) in the sample, the result shall be reported as an Atypical Finding (ATF).
- Further, a result shall be interpreted as a Negative Finding when no electrophoretic band is detected in the sample's gel lane [*i.e.,* no band signal for endogenous EPO and any of the exogenous ERA(s)].

4.0 Reporting of ERA Results

In this Article, the procedural requirements for reporting ERA results by the Laboratory are outlined, including the obtaining of second opinions before reporting an AAF or an ATF, the consideration of WADA instructions before reporting an AAF for rEPO, the reporting of any signs of microbial and/or proteolytic activity in the sample that may affect the stability of the ERA(s) detected, and the recommendations to Testing Authorities to perform ERA analysis on collected blood samples, or to collect further urine and blood sample(s) from the athlete for ERA analysis if a urine sample is associated with either a non-confirmed Presumptive Adverse Analytical Finding (PAAF), an "A" sample AAF with low-intensity signals, or an ATF for any ERA.

5.0 Analytical Testing for TGF-ß Signalling Inhibitors

This is a new article describing the requirements for the analysis of TGFβ signaling inhibitors, with reference to published studies on the application of PAGE and other Test Methods (capillary immunoassays, LC-MS).

6.0 References

The list of published studies and documents referenced in the TD has been updated accordingly.

ANNEX A – ERA Second Opinion Procedure

This is a new section describing a new, comprehensive procedure for provision of second opinions for ERA findings by Experts of the WADA EPO WG. The new procedure includes the results review and provision of independent second opinions by two Experts.

The procedure is directly managed by WADA through an assigned and access-restricted data management system. Conditions for concluding a result as an AAF, ATF or a Negative Finding based on the second opinions provided are also described in the Annex A. In particular, a finding would be reported as an AAF only when both Experts conclude that an ERA(s) is present in the sample. However, while considering the second opinions received, the laboratory will be ultimately responsible for reporting the result. A flow chart has been added representing the second opinion procedure.

ANNEX C – *EPO* c.577del Variant

This Annex, now renamed Annex C, deals with the management of VAR-EPO cases. This revised version of the TD EPO has been updated to provide further clarity and guidance on the investigations to be carried out to determine whether a presumptive rEPO finding may be related to the expression of VAR-EPO, including the possible application of two recent scientific developments: i) Test Methods allowing to determine rEPO use by athletes expressing the VAR-EPO, and ii) the possibility to perform DNA analysis targeting the EPO gene on sample matrices other than whole blood (urine, DBS).

In addition, the flowchart describing the investigation process for VAR-EPO has been updated to provide further clarity and better reflect the text description in the Annex C.