TD2014EPO

Summary of Major Modifications

The Technical Document on Harmonization of Analysis and Reporting of Recombinant Erythropoietins and Analogues by Electrophoretic Techniques has undergone a revision process by WADA's EPO Working Group. The modified document, TD2014EPO, renamed "Harmonization of Analysis and Reporting of Erythropoiesis-Stimulating Agents (ESAs) by Electrophoretic Techniques", has been updated to reflect recent developments in the detection of ESAs, including Recombinant Erythropoietins and their Analogues.

1.0 Introduction

- The name of the Omontys®, Affymax Inc. product has been changed from hematide to its international non-proprietary name, peginesatide.
- Aranesp® has been identified as an example of darbepoetin-α under the definition of NESP, since there are other analogues of darbepoetin-α, such as Cresp.

2.0 Analysis

2.1 Electrophoretic Methods

2.1.1 Initial Testing Procedure

- The same methods are applied for detection of ESAs in urine and blood.
- A differentiation has been established for the electrophoretic analysis of ESAs with a chemical structure related to EPO (*e.g.* rEPO, NESP, CERA, EPO-Fc) and peginesatide, a pegylated peptide with no structural relationship to EPO. For the former, IEF and/or SAR-PAGE methods shall be applied, whereas for the latter the laboratories shall apply SDS-PAGE or SAR-PAGE.
- For the initial testing procedure of recombinant EPOs (rEPO), laboratories may apply IEF or SAR-PAGE, or a combination of these methods (at laboratory's discretion).
- It has been clarified that the methodology employed for EPO enrichment prior to the application of electrophoretic methods shall be demonstrated not to change the IEF isoform profiles or the SDS/SAR-PAGE behaviour of the endogenous EPO and the ESAs being analyzed.

2.1.2 Confirmation Procedure

- The same methods are applied for detection of ESAs in urine and blood.
- Specific subparagraphs have been included specifying the confirmation methods for different ESAs (rEPOs, NESP, CERA, EPO-Fc and peginesatide). In each case,

it is clarified that specific methods (*e.g.* IEF or SAR-PAGE) may be applied for both the Initial Testing Procedure and the Confirmation Procedure. This is in compliance with the ISL provision 5.2.4.3.1.3 for the application of two different affinity-binding assays, because the specificity of the substance detected is guaranteed through the application of electrophoretic methods which are based on the detection of ESAs through two different principles: i) the electrophoretic behaviour of the substances (isoelectric point for IEF, different molecular mass for SDS-/SAR-PAGE) and ii) the targeted binding of the ESA with specific antibodies (immunoblotting). In addition, all samples subject to confirmation analyses shall be immunopurified prior to confirmation using antibodies different from the one used for the immunoblotting.

- It has been established that for the confirmation of rEPO, laboratories shall not apply the IEF method anymore. The presence of rEPO shall be confirmed by SDS-PAGE or SAR-PAGE, which allow for a longer time window of detection of rEPO administration. In addition, with the application of SDS-PAGE or SAR-PAGE, the stability test is no longer necessary for the identification of "active" urines, because in these urines the EPO band shifts to or below the molecular mass range of endogenous EPO resulting in a sample "negative" for rEPO.
- Due to its lower sensitivity, the application of SDS-PAGE is not recommended for the confirmation of CERA (instead, IEF or SAR-PAGE shall be used).

2.2 Other (non-electrophoretic) methodologies

- The application of substance-specific detection methods during the Initial Testing Procedure is only allowed for the analysis of ESAs with a structure unrelated to EPO (*e.g.* peginesatide). For all other ESAs, the electrophoretic methods specified in Table 1 shall be applied. Thus, for example, the specific screening for CERA using ELISA will not be applied anymore (since CERA could be detected, for example, with SAR-PAGE, which is a more sensitive and specific method and allows the detection of other ESAs, if present in the sample).
- For confirmation procedures, substance-specific detection methods may be applied at the laboratory's discretion as further scientific evidence in addition to the one provided by the electrophoretic methods.
- Also, where a mass spectrometric method is available, no independent second method is required for the Confirmation Procedure (e.g. LC-MS for detection of peginesatide).

3.0 Description of the Methods

- IEF and SDS-/SAR-PAGE methods are described in 4 subsections, including: i) sample preparation, ii) electrophoretic separation, iii) immunoblotting and iv) detection.
- For sample preparation, it is specified that immunopurification of the sample is required prior to the application of the electrophoretic method during confirmation analyses.
- For immunoblotting, it is specified that a double blotting procedure is mandatory after IEF separation of urine or serum/plasma samples as well as for the confirmation of serum/plasma samples by SDS- or SAR-PAGE. For the analysis of urine samples by SDS- or SAR-PAGE, single- or double-blotting may be applied. In addition, anti-human EPO antibodies with specificity and sensitivity characteristics similar to the primarily recommended AE7A5 clone

may now be used for the immunoblotting. For peginesatide, the antibody must target the peptidic moiety of the drug.

4.0 Evaluation and Interpretation of Results

The acceptance and identification criteria applicable to the Confirmation Procedures when using electrophoretic techniques are defined.

Since now only one method (*i.e.* IEF or SDS-PAGE or SAR-PAGE, as applicable) is established for confirmation procedures of ESAs, the reference to the co-application of two different confirmation methods or to the reporting of the sample as an *Atypical Finding* (which was applicable when more than one method was applied and the method's acceptance criteria were met for only one of them) has been removed.

IEF method:

- There are no identification criteria for the confirmation of rEPOs since this method is not to be used for that purpose anymore. However, criteria are given as a recommendation for evaluation of the results of the Initial Testing Procedure, with minor modifications in comparison with the previous TD2013EPO.
- The second criterion for NESP has been changed to: "At least one band in the "acidic area" must be more intense than the last band of the endogenous area" (in place of the two most intense bands being in the acidic area), to account for the slightly different IEF pattern of other darbepoetin-α preparations.

SDS-PAGE and SAR-PAGE methods:

- The third criterion in the TD2013EAAS (2 or more bands) has been transferred into 4.2.2.1. Single band(s) as multiple single bands.

5.0 Documentation and Reporting

- Explanatory footnotes have been included to define what is meant by Control Samples and Positive Control Samples to be used for Confirmation Procedures.
- Two new members of the EPO WG have been named as providers of second opinion:
  - Philipp Reihlen (Cologne)
  - Jean Francois Naud (Montreal)

6.0 References

The list of references has been updated to include relevant papers which have been recently published.