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WORLD ANTI-DOPING AGENCY

TD2013 EPO v1.0

Summary of Major Modifications

The title of the Technical Document has been slightly modified to specify that it applies to the analysis and reporting of recombinant EPO and its analogues when electrophoretic techniques are employed.

1. Introduction

The list of abbreviations, acronyms and trademarks used throughout the document has been updated.

2.0 Analysis

- The analytical methodologies to be employed for the Initial Testing Procedures and Confirmation Procedures for recombinant EPO (rEPO) and the different EPO analogues in urine and blood (serum/plasma) are specified.

- Attention is drawn to the use of IEF and SDS-PAGE or SAR-PAGE methods as the two main methodologies, whereas the application of substance-specific methods for detection of EPO analogues is also described.

- It is specified that immunopurification is required prior to the performance of confirmation analyses by electrophoretic methods.

- A table is provided summarizing the testing strategy for detection of rEPO and analogues (NESP, CERA, Hematide, EPO-Fc):

    ° For the confirmation of rEPO in urine, in particular, both IEF and SAD-PAGE or SAR-PAGE methods shall be applied on newly, immunopurified Aliquots of *Sample* "A" (unless the Initial Testing Procedure was performed by IEF following immunopurification, in which case the confirmation by SDS-PAGE or SAR-PAGE is sufficient).

- For confirmation of rEPO in serum/plasma, however, only SDS-PAGE or SAR-PAGE shall be applied (IEF is not recommended due to the more basic profile of endogenous blood EPO in comparison to urinary EPO).

- 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).

- Due to their distinct and characteristic profiles, for the analysis of NESP and CERA the Laboratory may choose to use the same method for the Initial Testing and Confirmation Procedures (IEF or SDS-PAGE or SAR-PAGE).

- 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 Hematide).

- Several specific methods are listed for the detection of CERA, EPO-Fc and Hematide.

3.0 Description of the Methods

A brief description is provided on the different electrophoretic methods used for detection of rEPO and analogues, including IEF, SDS-PAGE and SAR-PAGE as well as a SDS-PAGE method specifically developed for the detection of Hematide.

4.0 Evaluation and Interpretation of Results

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

It is specified that when more than one method is used for the Confirmation Procedure, the acceptance and identification criteria must be fulfilled on both procedures employed before reporting an *Adverse Analytical Finding*. If the acceptance and identification criteria are met for only one of the methods employed for the Confirmation Procedure, the *Sample* shall be reported as an *Atypical Finding*.

IEF method:

- The identification criteria for the analysis of rEPO (Epoietins) in urine have been made less strict in comparison to the TD2009EPO. This results from the required mandatory application of SDS-PAGE or SAR-

PAGE for the confirmation analyses for rEPO, which brings additional evidence to the results of the analyses.

SDS-PAGE and SAR-PAGE methods:

- Identification criteria are provided in three (3) categories: i) a single band, ii) a mixed band and iii) two or more bands.
- Figures are included exemplifying the detection of rEPO, CERA, NESP and EPO-Fc by SDS-PAGE or SAR-PAGE techniques.

SDS-PAGE for Hematide

- Identification criteria and a figure are provided exemplifying the detection of Hematide by SDS-PAGE.

The section **3.3 Stability Criteria** of the TD2009EPO has been removed. Due to the mandatory application of SDS-PAGE or SAR-PAGE for confirmation of rEPOs, the performance of a stability test is considered not to be necessary.

5.0 Documentation and Reporting

The requirements for the Initial Testing Procedures and Confirmation Procedures are listed.

5.1 Provision of a Second Opinion

It is specified that one second opinion is required from one of the experts designated in the document before any *Adverse Analytical Finding* for rEPOs or the EPO analogues is reported.