Researchers discover novel method for detecting MIRCERA

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Austrian researchers have successfully developed a new electrophoretic method for detecting MIRCERA® and other erythropoietins (EPO) in the blood. The technique, using SARCOSYL-PAGE, has specifically enhanced sensitivity for MIRCERA, but does not alter the performance characteristics of SDS-PAGE for detecting other EPOs. Details of this study, funded by a grant from the World Anti-Doping Agency (WADA), appear in the latest issue of *Drug Testing and Analysis*.

MIRCERA (methoxy polyethylene glycol-epoetin beta), a PEGylated EPO, is a synthetic protein that helps the body produce red blood cells and used to treat anemia caused by kidney disease. The drug is manufactured by Hoffmann-La Roche and only available outside the U.S. due to an infringement upon Amgen's patents on recombinant EPO products and processes. In the past, EPOs have been used off-label as doping agents by athletes to enhance endurance in such sports as cycling, distance running, and rowing. WADA has banned the use of EPOs, such as MIRCERA, in all competitive sports.

"The detection of doping with recombinant EPOs is one of the most challenging topics in anti-doping control," said Dr. Christian Reichel from the Seibersdorf Laboratories at the Austrian Institute of Technology (AIT) and lead author of the study. Dr. Reichel and colleagues obtained blood samples from four healthy volunteers as part of the study. Each participant received a single dose of each EPO drug (NeoRecormon (66 IU/kg), Dynepo (35 IU/kg), and MIRCERA (50 @g)). Samples were collected for at least 14 days (NeoRecormon,

Dynepo) or up to 42 days post injection in the case of MIRCERA.

Researchers used a two-fold serial dilution of Dynepo (0.09 ng to 0.7 pg), NESP (0.07 ng to 0.5 pg), and MIRCERA (0.4 ng to 3.0 pg to compare the sensitivity of SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) for detecting MIRCERA by Western blotting. The intensity of the Dynepo and NESP bands gradually declined according to the decrease in their concentration, but a similar behavior was not observed for the MIRCERA bands. "We presumed from the experiments that the altered Western blotting performance of MIRCERA was due to its PEGylation (treatment with polyethylene glycol to obtain a long-acting effect of the protein)," noted Dr. Reichel.

PEGs of different average molecular masses (PEG 1500, 2000, 8000, 20000, 35000) were then separated on SDS-PAGE and visualized with a PEG-specific staining method. Due to a limited solubilizing power of SDS, PEGs - regardless of molecular size - were found to migrate as broad and smeared bands on SDS-PAGE. Given the results researchers hypothesized that the decreased sensitivity of MIRCERA on Western blots was due to an SDS-based solubility problem of MIRCERA's PEG-part.

To resolve the issue researches first used a detergent with higher solubilizing power for PEGs than SDS which did not improve the band shape of MIRCERA. Researchers then used SARCOSYL or (SAR)-PAGE with excellent results on Western blots by resolving MIRCERA in a sharp band and simultaneously keeping the resolution of rhEPOs and uhEPO on the performance level of SDS-PAGE. The authors found that "SARCOSYL was not binding to polyethylene glycol and was thus leaving PEGs in their native uncharged state." Redesigning SDS-PAGE by exchanging the SDS for SARCOSYL in the sample and running buffers solved the problem.

"In the past, there needed to be four methods performed to detect banned substances. Our method allows investigators to detect MIRCERA and other EPOs using just a single method," Dr. Reichel confirmed.

More information: "SARCOSYL-PAGE: A new method for the detection of MIRCERA and EPO-doping in blood." Christian Reichel, Friedrich Abzieher and Thomas Geisendorfer. Drug Testing and Analysis; Published Online: December 16, 2009 (DOI: DTA.97).

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