

# Anti-Doping Fact Sheet

## WADA-Accredited Laboratories

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### Overview

Anti-doping laboratories are dedicated to the analysis of sport's doping control tests. Laboratories that wish to perform the analysis of doping controls for sports under the World Anti-Doping Code must achieve and maintain accreditation from the World Anti-Doping Agency (WADA). The International Standard for Laboratories (ISL) and its related technical documents specify the criteria that must be met for accreditation and re-accreditation, as well as standards that must be met for the production of valid test results and evidentiary data.

### Methodologies

Mass spectrometry (MS) coupled to either gas (GC) or liquid chromatography (LC) is the analytical technique of choice for confirmation of prohibited substances, metabolite(s) of a prohibited substance, or marker(s) of the use of a prohibited substance or prohibited method.

Affinity Binding Assays (e.g. Immunoassays) are also routinely used for detection of macromolecules in urine samples.

### Gas Chromatography – Mass Spectrometry (GCMS) and Liquid Chromatography – Mass Spectrometry (LCMS)

Using this method, compounds within the urine are separated and identified.

In gas chromatography, a mixture (the urine) is made into a gas and injected into a heated column filled with an inert liquid. A carrier gas pushes the mixture through the column and the different components of the mixture are separated as they move at different speeds through the column.

In liquid chromatography, the urine is separated on the bases of varying chemical and physical properties. A sample mixture is passed through a column packed with solid particles which may or may not be coated with another liquid. With the proper conditions some components in the sample will travel through the column more slowly than others, resulting in the desired separation.

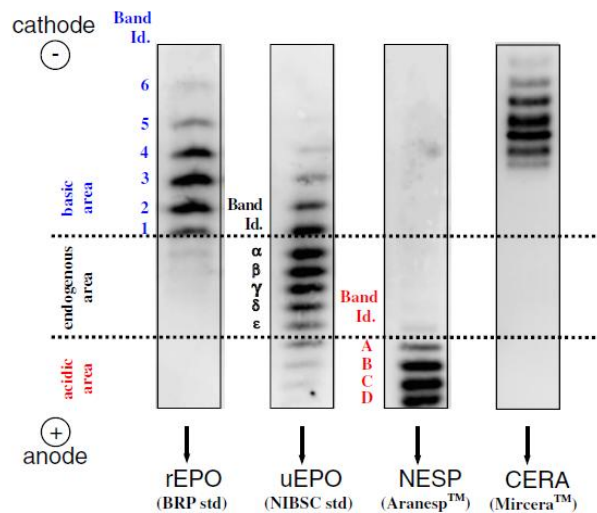
Mass spectrometry determines the composition of a sample by creating a chemical fingerprint of the compounds within it. The sample (already separated by chromatography) is bombarded with electrons to fragment the compounds into smaller ions whose abundance is recorded according to its mass. A given chemical will always fragment in the same reproducible way; therefore its mass spectrum (chemical fingerprint) is characteristic for that compound.

### Isotope Ratio Mass Spectrometry (IRMS)

This technique is used to distinguish between an endogenous and an exogenous source of testosterone. This analyses the ratio between two stable carbon isotopes (C13/C12). Exogenous testosterone contains less C13 than the endogenous counterpart and IRMS will demonstrate this difference.

## IEF-PAGE

IEF-Page stands for Isoelectric focusing (IEF) polyacrylamide gel electrophoresis (PAGE) and was the first method which enabled the detection of recombinant EPO for doping purposes. Due to its charge EPO can be separated into EPO isoforms, which together form an EPO isoform profile. These profiles normally differ between human urinary EPO and recombinant EPO.



**Fig. 1** Identification of Epo isoforms according to TD2009EPO (WADA). Whereas isoforms of uhEpo (National Institute for Biological Standards and Control (NIBSC); Hertfordshire, UK) are mostly found in the “endogenous area” of the IEF gel, isoforms of rhEpo (BRP-Epo, European Directorate for the Quality of Medicines; Strasbourg, France) and Mircera are primarily focussed in the “basic area”. Most of the NESP isoforms migrate into the “acidic area”. Effort and unstable urine samples cause shifts of the uhEpo profile toward the cathode. *Source:* TD2009EPO [7], World Anti-Doping Agency (WADA) (permission granted)

## Immunoassay

Traditional drug testing in sport has involved urinary sampling, however it is not viable for recombinant human growth hormone (rhGH) detection because neither hGH itself nor markers of hGH are secreted into the urine in sufficient and reliable quantities.

As a result, the two methods of detecting hGH widely discussed in the literature both rely on blood samples.

Both the GH Isoform and GH Markers approaches to detecting Growth Hormone use an immunoassay approach. This approach measures the concentration of a specific substance in a mixture using an antibody capable of binding to that specific substance.

## Further Information

- [www.wada-ama.org/en/Science-Medicine/Athlete-Biological-Passport](http://www.wada-ama.org/en/Science-Medicine/Athlete-Biological-Passport)
- [www.wada-ama.org/en/Science-Medicine/Anti-Doping-Laboratories](http://www.wada-ama.org/en/Science-Medicine/Anti-Doping-Laboratories)
- [www.youtube.com/watch?v=cpOJ2dDEWU4](https://www.youtube.com/watch?v=cpOJ2dDEWU4)