EPO Test

EPO (erythropoietin) is a natural hormone and a blood booster. It is also available as a pharmaceutical. The use of this artificial form, or recombinant EPO, to enhance performance by boosting endurance, is prohibited.

Historically, the EPO test at the Olympics (2000 to 2006) was done on a pair of urine and blood samples collected from the athlete at the same time. The urine test identifies the prohibited substance, therefore it is called a direct test. In contrast, the blood test is called an indirect test because it does not show the presence of recombinant EPO. Instead, it shows the "footprints" of drug use, in other words its impact on multiple lab readings on the blood. The readings are used to calculate a score which indicates whether the person is on a prohibited blood booster ("on score") or recently stopped taking it ("off score"). Since 2002, EPO tests done by U.S. sports authorities have consisted of only the urine test.

At first the only urine EPO test was the French test, also known as IEF (isoelectric focusing) test[1]. Soon researchers, in their quest for improvement, began exploring alternatives. One example is a different way of separating recombinant from natural EPO, using an SDS (sodium dodecyl sulfate) gel instead of an IEF gel [2].

The IEF test for EPO takes two and a half days. Although it consists of many steps, those can be grouped in four main steps:

1. sample preparation
2. IEF
3. double blotting
4. visualization

Step 1 of 4: sample preparation

The sample preparation consists in concentrating a little more than a tablespoon of urine to reduce it to roughly one drop. The EPO present in the urine ends up in the drop. So do other proteins.

Step 2 of 4: IEF

IEF stands for isoelectric focusing. The drop of urine concentrate containing EPO is put on a gel. Electricity is run through it. This spreads out the EPO forms, which all have essentially the same protein backbone but differ from one another by the sugars attached. The forms spread out on the gel like the rungs of a ladder. The good news is that the different EPOs—natural or artificial—spread out in different, characteristic patterns that allow for their identification. The bad news is that they are invisible. Steps 3 and 4 are done to make EPO visible.

Step 3 of 4: double blotting

Blotting is what you do when there are drops of water on the table and you press a napkin straight down onto them to pick them up. Doing this moves the water drops from the table to the napkin. If the water drops formed a pattern on the table, such as the rungs of a ladder, they still form that same pattern on the napkin. In the EPO test, the EPO is like the water drops, the gel is like the table, and instead of a napkin, what is used to blot is a special sheet called membrane. The bad news is that if there was anything else on the table (juice, milk), the napkin will blot it too; by analogy, there are other proteins in urine, and the membrane picks them all up. The EPO and the other proteins are still invisible, but if you could see them, the EPO ladder rungs would be lost in a giant smear due to the other, more abundant proteins. To solve this problem, chemicals-that-stick-to-EPO (antibodies) are put in contact with the membrane. Antibodies stick only to EPO because they fit each other perfectly like lock and key. Antibodies stick to EPO, natural or artificial, wherever it is—in the ladder rung pattern.

At this point, blotting is done again: this time the membrane is blotted with a second piece of membrane of the same kind. This second blotting moves only the EPO antibodies to the second membrane, in the ladder rung pattern, but it is still invisible.

Step 3 is called double blotting because blotting was done twice.

Step 4 of 4: visualization

Visualization means “making visible.” The second membrane is exposed to chemicals which react with the EPO antibodies to produce light. This phenomenon is called chemiluminescence and this step is also called chemiluminescent detection. The reaction happens only where the EPO antibodies are located, therefore light is emitted in the ladder rung pattern, and the pattern becomes visible. A special camera is used to capture the image or electropherogram.

To see an example of such an image, click on the link below to go to the relevant WADA (World Anti-Doping Agency) Technical Document and page down to the figure. Note that the four main steps described above are the same as the four points of the “Description of the method” in the WADA document.

http://www.wada-ama.org/rtecontent/document/td2007epo_en.pdf

References

[1] Lasne F, Martin L, Crepin N, de Ceaurriz J (2002) Detection of isoelectric profiles of erythropoietin in urine: differentiation of natural and administered recombinant hormones. *Anal. Biochem.* 311:119-26.

[2] Kohler M, Ayotte C, Desharnais P, Flenker U, Ludke S, Thevis M, Volker-Schanzer E, Schanzer W (2008) Discrimination of recombinant and endogenous urinary erythropoietin by calculating relative mobility values from SDS gels. *Int J Sports Med* 29:1-6.