Comments on Lundby et al.’s “Testing for recombinant human erythropoietin in urine: problems associated with current anti-doping testing”

ANTI-DOPING TESTING: FRIEND OR FOE?
TO THE EDITOR: Ideally, anti-doping testing is designed to ensure fair competition and prevent athletes’ harm from unfair practices. While the former issue is questionable, since fairness in sports is mostly a chimera, the latter is foremost and theoretically viable (1–3). The recent study of Lundby et al. (5) proved what everybody in the field of laboratory and sports medicine has supposed for years: a poor agreement in test results for detecting recombinant human erythropoietin (rHuEpo) comparing two World Anti-Doping Agency (WADA)-accredited laboratories. Laboratory tests are not always foolproof. For various reasons, false-positive and false-negative results can occur for any test in laboratory diagnostics (3). Traditionally, both sensitivity and specificity determine the diagnostic efficiency of a certain test in the clinical practice. However, the extension of this concept to the anti-doping context is challenging. Basically, anti-doping tests are not intended for diagnosing disorders or monitoring therapies. Therefore, sensitivity is only important to identify cheaters (rarely disorders), but specificity is foremost to prevent unfair sanctioning of clean athletes. Several lines of evidence attest that the current anti-doping strategy, based on repression rather than education or harm control, has returned negligible results, if none, in terms of preventing athletes from doping and modifying this upward trend. Probably it has only contributed to modify the pattern of drug consumption (4). The report of Lundby et al. must hence be regarded as an additional mainstay in the urgent process of reviewing the current anti-doping policy based on analytically questionable tests.

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TESTING FOR RECOMBINANT HUMAN ERYTHROPOIETIN—THE BOUQUET OF COMPOUNDS
TO THE EDITOR: Lundby and coworkers (4) show poor agreement in the results obtained in two WADA-accredited laboratories applying isoelectric focusing (IEF) for detection of recombinant human erythropoietin (rhEpo) in urines from male volunteers treated with Epoetin beta. This rhEpo and its analog, Epoetin alpha, are Epo transfected Chinese hamster ovary (CHO) cell-derived drugs clinically used for almost 20 years. Since process patents for the originator rhEpo have expired in the EU and elsewhere, biosimilar follow-on biologics have entered the market. Recently, two novel CHO cell-derived Epoetin biosimilars have been approved in the EU, which are marketed as Epoetin alpha and, respectively, Epoetin zeta (3). Moreover, several companies outside the US and EU are producing so-called Epoetin alpha. While the amino acid sequence of all Epoetins is identical, the structure of their glycans differs depending on the production procedures. The glycans determine the behavior on IEF. Some products exhibit more acidic and others more basic isoforms (1). Furthermore, Epoetin delta is available, which is homologously expressed in human cells, thus lacking N-glycosylation similar to endogenous Epo (3). No method is established yet to detect pegylated Epoetin beta in urine. As noted earlier, searching rhEpo in urine is “tilting at windmills” (2).

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PROBLEMS ASSOCIATED WITH CURRENT ANTI-DOPING TESTING: IS QUALITY ASSESSMENT A RELIABLE SOLUTION?
TO THE EDITOR: In the recent article of Lundy et al. (1), a rather poor agreement of results was demonstrated between two World Anti-Doping Agency (WADA)-accredited laboratories. This is not really surprising, in that laboratory test results have not always been, nor are they, as reliable as they should be due to several incidental variables, which variably affect the preanalytical, analytical, and post-analytical phases (2). Laboratory professionals have struggled for decades with this issue in an attempt to make test results reliable and, especially, comparable between different laboratories. One of the best solutions to standardize or harmonize (when standardization is unreachable) test results is to establish a broad concept of quality control (QC) for the whole testing process (3). Basically, QC refers to procedures for monitoring the work processes, detecting problems, and making corrections prior to delivery of products or services. Statistical process control, or statistical quality control, is the major procedure for monitoring the analytical performance of laboratory methods. Quality assessment (QA) refers to the broader monitoring of other...
dimensions or characteristics of quality, such as patient preparation, specimen acquisition, and handling. Then, proficiency testing provides an external or outside measure of analytical performance (3, 4). Regardless of inherent limits of the current technique to detect recombinant human erythropoietin in urine, the implementation of rigorous external quality assessment schemes (EQAS), based on urine samples shipped to all the accredited WADA laboratories worldwide, would probably be helpful to increase consistency in results between different facilities.

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TO THE EDITOR: The Lundby paper (2) and Delanghe and Joyner editorial (1) describe numerous technical issues related to testing blood and urine for the presence of recombinant human erythropoietin (rhEpo). The blood level is affected by plasma volume and the urine level is affected by the renal tubular handling of Epo, which is poorly understood. My major concern with this report is that erythropoietin treatment elevates hemoglobin concentration by increasing red cell volume (RCV) and decreasing plasma volume (PV). RCV was measured directly using carbon monoxide. PV was estimated indirectly from the carbon monoxide RCV and the total body hemocrit, using the f-factor of 1.0 to correct the peripheral venous hemocrit value, at baseline, 5, 11, and 13 wk after Epo treatment. To assert that Epo decreases PV, a direct measurement of PV is needed and it should be done 2 wk after treatment with Epo to assess the renin-angiotensin-alosterone activity.

Our experience has shown the factor to correct the peripheral venous hemocrit to estimate the total volume hemocrit depends on the method used to measure PV (3). Our data in chronic hypovolemic anemic patients and acute hypovolemic anemic dogs demonstrated that transfusion of viable washed red cells increased RCV, PV, and total blood volume (4–7). The information reported that erythropoietin treatment elevated hemoglobin concentration by increasing the RCV and decreasing PV is not supported by our data. A direct measurement of PV is needed to document that erythropoietin decreases PV, which will affect the measurement of erythropoietin treatment.

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A NEGATIVE TEST CANNOT EXCLUDE DOPING

TO THE EDITOR: Lundby et al. (3) illustrate an Achilles heel of anti-doping policy. The war on doping aims at eradication using a strong repressive system, based, among other strategies, on testing for forbidden substances and metabolites in bodily specimens, mainly urine but also blood. For surprise controls outside competition athletes have to report where they are 365 days/yr to be compelled to provide urine samples, being nude from nipple to knee while a doping officer looks on, making sure the sample is genuine. But assuming that testing protocols accurately expose the use of drugs is scotomizing the fact that testing is condemned to remain one step behind the advances in biomedicine. False-negatives and false-positives are inherent possibilities with testing technology as clearly shown by Lundby et al. (3). This uncertainty of laboratory testing is acceptable in the field of therapeutic medicine but problematic in sport. Athletes can never be declared “truly” clean, whereas false accusations should be avoided by all means. Marion Jones never tested positive throughout her career. On the other hand, many athletes are treated as cheaters even if there is good reason to doubt their intention to dope (1). Instead of pursuing an increasingly repressive strategy at rising cost and with limited efficacy, there are good reasons to question current anti-doping policies and to plea for a more pragmatic approach aiming at reducing harm to the individual and society at large (2).

REFERENCES


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TO THE EDITOR: As the developer of the test validated for anti-doping control of recombinant erythropoietin (rHuEPO), I
have been asked to comment on the article of Lundby et al. “Testing for recombinant human erythropoietin in urine: problems associated with current anti-doping testing” (2).

The French anti-doping laboratory did not participate in this study and the two laboratories involved certainly will give detailed technical explanations about the real conditions and the conclusions of this study. I am fully confident in their ability to set the record straight.

However, I would like to address just one question to the authors of this article.

The article aims to demonstrate the failure of the test validated for anti-doping control [isoelectric focusing followed by double-blotting (1, 3, 4)] based on the assertion of a discrepancy in the results obtained by the two laboratories. For this, the authors state that both laboratories used the validated method. In fact, this method was used by laboratory A, while laboratory B used another method (SDS electrophoresis), currently under investigation as a possible complementary method. My question is this: What is the value of a scientific procedure that uses the results of method B to prove the failure of method A?

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NEW STRATEGIES IN THE FIGHT AGAINST DOPING ARE NECESSARY

TO THE EDITOR: Although several issues concerning the EPO urine test remain controversial, the article by Lundby et al. (1) emphasizes the fact that the fight against doping might need a significant modification in the future. Since the first in-competition doping tests in the 1960s and the introduction of out-of-competition urine tests 20 years later, anti-doping strategies have evolved considerably. Subsequent to the development of recombinant doping substances, which are difficult if not impossible to detect in urine such as EPO, blood data are used to screen for suspicious athletes. Not an abused substance itself, but its effects on the organism are analyzed. Through these measures, anti-doping developed from the purely biochemical detection of a forbidden substances to a more biological approach. The study of Lundby et al. strengthens this direction and suggests new options: Despite the fact that only a limited number of samples was found positive according to the WADA criterion, a larger number was defined as “suspicious.” This important information should be included in the so-called “biological passport,” which aims at a longitudinal monitoring of variables indicative of doping: It is likely that the entity of increased Hb mass, increased hematocrit (as shown in Table 1 of the article), and suspicious EPO urine test would result in a high prospect of doping in the examined athletes in any probabilistic calculation (2). With more innovative doping methods looming, the anti-doping fight should develop from its pure biochemical/biological horizon into a probabilistic framework, considering all available information.

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EXPANDING THE ANALYSIS REPORTING WILL LEAD TO MORE EFFICIENT TESTING

TO THE EDITOR: Lundby et al. (3) show marked differences between results obtained from two WADA-accredited laboratories using isoelectric focusing (IEF) to detect recombinant human erythropoietin (rhEpo) in urine samples. As proved by Laboratory A, there is no doubt that the implemented rhEpo test (2) can detect rhEPO, but the test also has certain limitations, i.e., a short window of detection (1) due to the strict criteria used to deem a test result as an “adverse analytical finding.” Today, sport federations that receive test results from the rhEpo test are only provided with either one of the following “results”: “negative,” “adverse analytical finding,” or “atypical analytical finding” from the laboratory. Therefore, highly suspicious sample results are likely to be deemed “negative,” with no further reporting to the federation. Large amounts of important analysis data are simply wasted, because it never reaches the right body—the federations. Information that can be used by federations not only to compare previous data with new on a individual level as is currently being done for various blood variables (4) and anabolic steroids (5) but also used in combination with blood screening results to give a better overall picture of the individual athlete. This will not only increase the sensitivity of testing for rhEpo but also make the targeting of urine analysis much more efficient. Therefore, in the future, federations must be provided with a full report of the specific analysis, including images and quantitative data from the analysis. The data are already there, why don’t we use it???

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TO THE EDITOR: The report by Lundby et al. (1) has deficiencies regarding information about the design and performance of the current study, comparing results of EPO analyses from two WADA-accredited laboratories. The obvious weakness of this relatively simple study is the lack of a description of how the samples from the EPO-treated subjects were submitted for analysis and also which procedures were utilized by the laboratories. Did Lundby et al. follow a specific protocol describing how the contact with the two labs should be handled for the laboratories. Did Lundby et al. follow a specific protocol describing how the contact with the two labs should be handled for the results to be comparable? In Methods, the current paper briefly presents the transport of the samples from Copenhagen to the chosen doping laboratories and refers only to the official WADA web page concerning the performance of the analyses. However, the section does not contain any information of how similar conditions in the labs were pursued. This confers insecurity to the evaluation of the results. The authors state that the two labs were blinded toward the treatment. What does this blinding imply? Did the authors ensure that the same procedure(s) were applied in both labs; how were the samples introduced to the labs, as research or ordinary doping samples? Lundby et al. are stating “In ‘real life,’ WADA requires,” implying that the samples to be tested in fact were presented as test samples not ordinary ones.

A prerequisite for a test model like this is a detailed protocol approved in advance to ensure proper quality assurance of every step of the study.

REFERENCE


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TO THE EDITOR: CONTEXT: Since spring 2006, the laboratory of Lausanne has been collaborating with the group of Lundby et al. Among others, we accepted that, during the operational phase, the principle of double-blind study would be preserved. We also accepted to discuss the results to avoid any misunderstanding. This discussion never happened and we were never informed about any publication release from this study.

Laboratory A: By reading the data presented in the publication and by carefully reviewing those we originally sent to the group, we can assume that our laboratory is the so-called Laboratory A and will produce our comments in that perspective. To be fair in our response to the Editor regarding this publication, we recently asked the first author to confirm in writing that it was actually the case and we did not get any formal answer.

Material and Methods: Four deficient points at least must be highlighted in the description of the methods.

1) Treatment: When the dosage is described, the type of administration is not. It is, however, known that subcutaneous and intravenous administration of EPO yield very different pharmacokinetic results (1). As the authors aim to provide a good picture of the detection power of a method to the scientific community, they should have known that it is directly related to the application mode of the substance. No clear description exists concerning the treatment schedule during the maintenance period (“one single injection was given weekly”). This leaves to the authors the possibility of playing with the detection power of the laboratories by occulting to the reader the precise information about how far from the last injection time the urine collection took place.

2) Parameters of interest: There is no mention of the type of technology used to perform the hematocrit (Hct) measurement. Not any other blood parameter was apparently measured. As the authors state in their conclusions, the longitudinal monitoring of athletes is one solution for the detection of blood manipulation. From then on, it is really surprising to see that the authors limit themselves to using Hct, which is nowadays claimed to be highly insufficient for providing a good picture of blood doping. No reticulocyte (Ret%) count, no hemoglobin (Hb) concentration, as well as no off-score have been provided to the reader, even if these parameters are currently considered as the state of the art and the minimum requirement for this monitoring.

3) Samples: On the contrary to what is asserted by the authors, several shipments were necessary to receive the biological material in acceptable conditions for an optimal application of the EPO analysis method. Multiple analyses were performed in duplicate to ensure undiscussable results to be delivered.

4) EPO detection method: There is simply no mention of description of the method(s) that was(were) used by the laboratories to screen the presence of rhEPO in the urine samples.

Discussion: Because of a lack of common and usual definition on the reporting of the results from the two laboratories to the authors, as well as because of no mention that one of the laboratories did not use the official method of EPO detection, the argumentation is basically not based on the same rationale through the entire discussion. In our particular case, we analyzed all samples using the official accredited EPO detection method and returned all the results according to the WADA positivity criteria (TD2007EPO), allowing us, in case of necessity, to defend all of them in front of a court.

Negative Day 30, Positive Day 35: Every scientist involved in the field perfectly knows how the urinary matrix can be difficult to handle. Even for other doping substances, it has been observed that the detection can strongly depend on the matrix. In this particular case, day 30 sample, which is presented as negative by the authors, was in fact an undetectable sample that was easily explainable by a low urinary EPO concentration (3) as well as by a low specific gravity of the sample. The main investigators were obviously informed about that but did not take it into account. Moreover, the authors claim that this pseudo-negativity is a proof of false positivity for the day 35 sample. Indeed, they consider that, as the detection window for rhEPO has been reported to be of about 3 days, no positive case should be declared after a longer
period of time. However, our results are completely in accordance with Breidbach et al.’s paper (2), who reported that, on the 7th day after the last of 9 rhEPO doses, this last was detected in approximately one-half of the participants to his study.

**Conclusion:** In conclusion, for all the previously mentioned reasons, the published results cannot be credited with any scientific credibility and demonstrate of a great lack of knowledge of the authors regarding the EPO detection method and, more generally, of the whole anti-doping procedures. This publication has to be considered as a major offense to the scientists working years to help the sports authorities to efficiently deal with doping in their disciplines.

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**COMMENT OF LABORATORY B ON THE PUBLICATION BY LUNDBY ET AL.**

TO THE EDITOR: In the article “Testing for recombinant human erythropoietin in urine: problems associated with current anti-doping testing,” Lundby and coworkers (3) reported that the analyses of urine samples of rhHuEpo-treated volunteers with the World Anti-Doping Agency (WADA)-approved isoelectric focusing (IEF) test (2) in two different laboratories (laboratories A and B) led to entirely different results. This statement of the study is wrong for the following reasons:

The two laboratories did not use the same methods. Laboratory B reported only results obtained with a newly established sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method (1) and not with the WADA-approved IEF procedure (2) as employed by Laboratory A.

The two laboratories used different reporting categories. The study design did not include a scheme about how to report results. Laboratory A used three reporting categories termed “negative,” “suspicious,” and “positive,” while Laboratory B used only the expression “suspicious.” Lundby and coworkers interpreted the “suspicious” results of Laboratory B as “no misuse” of rhHuEpo. This is a misinterpretation. All 15 “suspicious” results reported from Laboratory B fulfilled the screening criteria for the presence of rhHuEpo (1). As no confirmation analyses were performed and a new non/WADA-approved method was used, the term “positive” was not used.

The two laboratories analyzed different numbers of samples. In Table 1 of the publication it is mentioned that 52 samples were provided to the laboratories. Laboratory B received only 48 samples, and only 48 samples were reported.

The correct comparison of the results of the two laboratories would have led to the following conclusion: Laboratory B obtained the same results compared with Laboratory A, even with a different method.

In general, the performance of inter-laboratory studies is very difficult and has to be performed by professional institutions. In the field of doping control this is done by WADA. Regarding the study of Lundby et al., it is questionable if a group, which is neither experienced nor accredited for inter-laboratory studies, performs such studies with non-certified test material.

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