The FEBS Journal

FEBS PRESS science publishing by scientists

VIEWPOINT

Improving scientific practice in sports-associated drug testing

Jon Nissen-Meyer[1], Tore Skotland[2], Bjarne Østerud[3] and Erik Boye[1,2]

1 Department of Biosciences, University of Oslo, Norway
2 Institute for Cancer Research, Oslo University Hospital, Norway
3 Institute for Medical Biology, UiT, The Norwegian Arctic University, Tromsø, Norway

Keywords
doping; EPO; PAGE; scientific analyses; WADA laboratories

Correspondence
E. Boye, Department of Radiation Biology, Institute for Cancer Research, Oslo University Hospital, Montebello, 0310 Oslo, Norway
Tel: +47-45475204
E-mail: ebo@rr-research.no

(Received 12 October 2018, revised 10 April 2019, accepted 1 May 2019)

Correction added on 30 May 2019, after first online publication: the original publication incorrectly included a superfluous paragraph from an earlier version of the manuscript in the section "Lack of accountability and transparency".

doi:10.1111/febs.14920

Antidoping work is heavily based on scientific analyses of biological material, such as urine and blood. Because of the high stakes both for sports and for the athletes involved it is important that analyses are performed and interpreted in agreement with established scientific standards and professional norms. This is not always the case, as we document here. It is our experience that the antidoping movement does not appear willing to consider that errors can occur and should be corrected. The consequences of the lack of transparency and responsibility are carried by unlucky athletes. Scientific, ethical and legal considerations urge the antidoping movement to reform some of their rules and regulations and to include the possibility that the World Anti-Doping Agency position could, in some cases, be incorrect.

Introduction

It is well known that there has been extensive and systematic use of performance-enhancing substances and methods by Russian elite athletes and that this use was covered up by the World Anti-Doping Agency (WADA)-accredited Moscow and Sochi laboratories, as evidenced by the McLaren report II (https://www.wada-ama.org/en/resources/doping-control-process/mclaren-independent-investigation-report-part-ii). The systematic doping was recently confirmed by a decision in Court of Arbitration for Sport (CAS) (https://www.wada-ama.org/en/media/news/2019-02/wada-welcomes-latest-court-of-arbitration-for-sport-decisions). The Moscow and Sochi laboratories are, however, not the only WADA-accredited laboratories that have breached good scientific practice and thereby jeopardized the rights of athletes. We have previously described the low quality and serious mistakes in the work performed at some of these laboratories and we have discussed in detail two cases where contradictory, irreproducible and questionable test results led to the

Abbreviations
CAS, Court of Arbitration for Sport; COC, Czech Olympic Committee; eEPO, endogenous EPO; EPO, erythropoietin; rEPO, recombinant EPO; SAR-PAGE, sarcosyl PAGE; WAADS, World Association of Anti-Doping Scientists; WADA, World Anti-Doping Agency.

sanctioning of athletes for using recombinant erythropoietin (rEPO) [1–4]. The two cases described below are additional examples where the shortfalls in good scientific practice has resulted in the unreasonable suspension of athletes.

Found guilty without evidence

In May 2016, the WADA-accredited laboratory in Dresden, Germany, reported that rEPO was present in the urine of the then 19-year-old Czech triathlon athlete and economics student Vojtěch Sommer. Czech experts in biochemistry and molecular biology reviewed the test results and stated that the laboratory's data did not prove the presence of rEPO in Sommer's urine [later documented in an award by the Arbitration Committee of the Czech Olympic Committee (COC), see below]. Late in 2016, the Czech Triathlon Association, with support of the Czech Anti-Doping Committee, nevertheless found Vojtěch Sommer guilty and he was suspended from competitions and organized training for 4 years.

Vojtěch 's father, Vladmir Sommer, contacted us in October 2016, after reading our article about the doping case against the Irish sprinter Steven Colvert [3]. Vladmir Sommer thought the two cases looked quite similar and asked if we could evaluate the results the laboratory obtained when testing Vojtěch 's urine. After carefully reviewing all the data we concluded that there was no scientific evidence that indicated the presence of rEPO in his urine (see our statement as Data S1).

We will now present and discuss the Dresden WADA-laboratory's test results of Vojtěch Sommer's urine, results that were made public in January 2017 at Vojtěch Sommer's home page (https://www.vojtechsommer.cz) and more easily accessible at the site of Loring Films, which made a documentary including the Sommer case (http://www.loringfilms.com/wp-content/uploads/2017/08/Sommer-results-Lab-B-sample.pdf)

The laboratory used sarcosyl PAGE (SAR-PAGE) to analyse the athlete's urine sample. The method is, for most practical purposes, identical to the more commonly used SDS/PAGE method, except that sarcosyl replaces SDS as the detergent. After the proteins in the urine samples had been separated by SAR-PAGE, erythropoietin (EPO)-like proteins were visualized by a double-immunoblotting technique, using a monoclonal antibody that recognizes the protein moiety of EPO [5]. In these tests, each protein has a characteristic migration rate. Proteins that move at different rates during electrophoresis and therefore end up in different places can, in general, be presumed to be different proteins.

Figure 1 shows the results after analysis of Sommer's urine A-sample (the two lanes indicated by red arrows) and B-sample (blue arrows), together with some other relevant samples. It is not at all apparent that the athlete's sample lanes are different from the negative control lanes (black arrows) that do not contain rEPO.

A more quantitative analysis of lanes 7, 8 and 15 of Fig. 1 was performed by the WADA-accredited

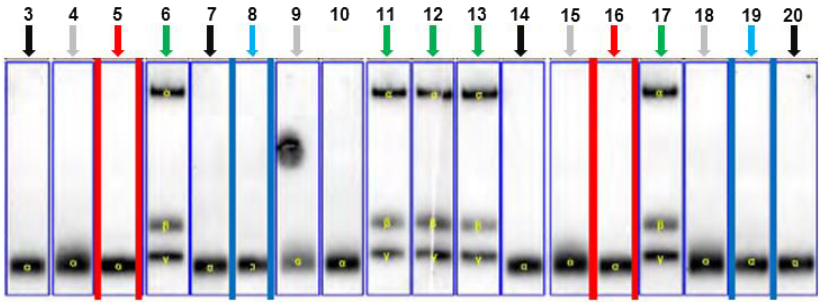

Fig. 1. GASepo-processed image of SAR-PAGE results. Shown is part of the gel where Vojtěch Sommer's urine A-sample (indicated by red arrows) and B-sample (blue arrows) were analysed. Positive control lanes that contain different rEPO-variants used in doping (from the top: CERA, NESP and rEPO) are marked by green arrows. Negative control lanes that contain normal eEPO, marked by black arrows, and positive control lanes that contain both rEPO and normal eEPO (grey arrows) are also shown. The figure is from page 14 of the 'Laboratory Documentation Sample June 2016' (http://www.loringfilms.com/wp-content/uploads/2017/08/Sommer-results-Lab-B-sample.pdf), but modified by us by including the arrows and lane numbers for presentation purposes. The first two lanes (1 and 2) and last two (21 and 22) of the original GASepo-processed image are not included in the present figure, since they were control lanes identical to those marked by green arrows. The details of the methods used by the WADA laboratory for these particular experiments are not available. The WADA Technical Documents contain some general information about the methods used and how the data should be interpreted: https://www.wada-ama.org/sites/default/files/resources/files/WADA-TD2014EPO-v1-Harmonization-of-Analysis-and-Reporting-of-ESAs-by-Electrophoretic-Techniques-EN.pdf.

Dresden laboratory, as shown in Fig. 2. The densitometric scan of the athlete's lane (panel B) appears to be identical to the scan of the negative control lane (A) that only contains the normal endogenous EPO (eEPO), which we all have in our blood and urine. In contrast, the two are clearly different from the scan of the positive control lane (C) where both normal eEPO and rEPO have been applied. Therefore, Fig. 2 does not provide evidence that there is rEPO in Sommer's urine. The WADA-accredited laboratory made an additional presentation of the gel shown in Fig. 1, this time with intensified staining in order to make visible a band in the athlete's sample lanes appearing above the band representing normal eEPO. Figure 3 shows lanes 6–9 of the gel in Fig. 1, with the intensified staining. The band appearing above normal EPO (eEPO) in the athlete's lane is indicated by a red arrow (lane 8, Fig. 3). We argue that this additional band cannot represent rEPO, since it has a different migration rate than all of the different rEPO controls (lane 6) and it migrates significantly more slowly than the 'mixed band' in lane 9, the positive control lane. If it is claimed that this additional band represents rEPO additional experiments and solid arguments must be added to support this unusual claim. Such information has never been supplied. We conclude that these data do not support the presence of rEPO in Sommer's urine. Nonspecific bands, such as that marked by a red arrow in lane 8, are quite common when the staining is intensified.

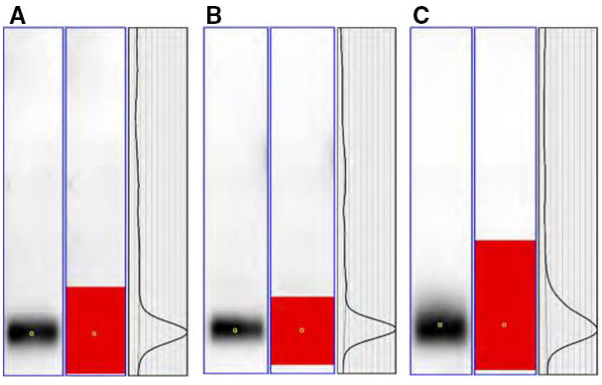

Fig. 2. Densitometric scans of the SAR-PAGE results. The lanes and densitometric scans of (A) a negative control (corresponds to lane 7 in Fig. 1) which contains only normal eEPO; (B) the athlete's B-sample (corresponds to lane 8 in Fig. 1); and (C) a positive control (corresponds to lane 15 Fig. 1) which contains both normal eEPO and rEPO. Results from the 'Laboratory Documentation Sample June 2016' (http://www.loringfilms.com/wp-content/uploads/2017/08/Sommer-results-Lab-B-sample.pdf). For methods, see Fig. 1 legend.

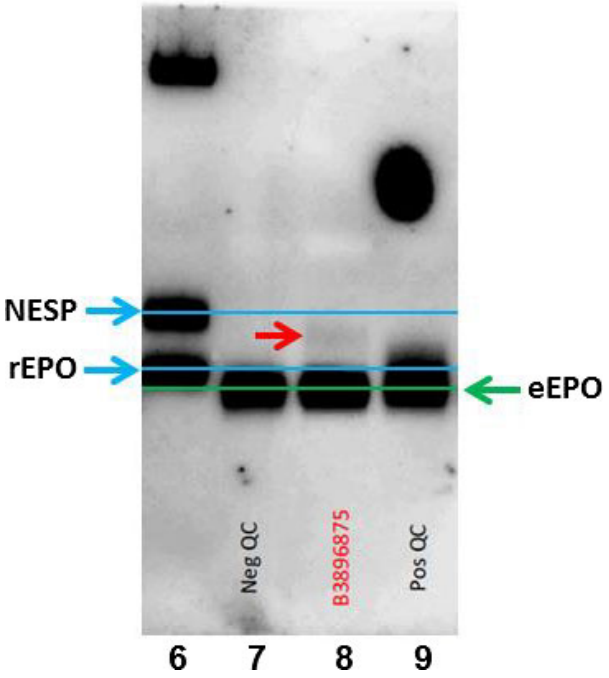

Fig. 3. SAR-PAGE data with intensified staining. Lanes 6 to 9 of the gel presented in Figure 1, but with intensified staining. Lane 6 contains three rEPO standards (from top: CERA, NESP and rEPO); lane 7 shows analysis of a negative control with normal, endogenous eEPO; lane 8 is the athlete's lane; and lane 9 shows a positive control containing rEPO and normal endogenous eEPO. The data in the figure are from page 10 of 'Laboratory Documentation Additional Explanation Sample B3896875 October 2016', conveniently found as fig. 6 in our evaluation presented to the Czech Olympic Arbitration Committee (https://www.olympic.cz/upload/files/arbitration-award-SOMMER.pdf). For methods, see Fig. 1 legend.

In April 2017, a first-instance civil court in Czechia cancelled Vojtěch Sommers 4-year suspension, but this decision was in July 2017 reversed by a higher level civil court. The Czech Olympic Arbitration Committee then decided to review the whole case upon the appeal filed by Vojtěch Sommer. In August 2017, at a hearing where evaluations by us and by the Czech scientists were presented, the Czech Olympic Arbitration Committee declared Vojtech Sommer innocent of doping and annulled his 4-year suspension (https://www.olympic.cz/upload/files/arbitration-award-SOMMER.pdf). Importantly, our interpretations of the data presented by the Dresden laboratory and also in this paper, was formally discussed and accepted at these hearings (see Data S1 for details of our evaluation). Interestingly, this award also referred to a statement from the Czech Anti-Doping Committee, which argued that '..in the interest of maintaining the reputation and effectiveness

of the antidoping system and WADA testing, the COC Arbitration Committee should uphold the previous decision. . ..'. Thus, the antidoping committee argued that the prestige of the antidoping movement was a factor that should influence the outcome of the arbitration.

In February 2018, WADA appealed the lifting of Vojtěch's suspension to CAS. In April 2018, however, before the case came to a hearing in CAS, WADA signed a confidential agreement that ended Vojtěch's 4-year suspension and allowed him to participate in competitions and organized training, and thereby essentially cleared Vojtěch of drug abuse. CAS approved the agreement in September 2018 and the 2 years that remained of his 4-year suspension were thereby annulled. This unfortunate and unnecessary affair has been damaging for Vojtěch 's athletic career and well-being and for his family, friends, club and team, as evidenced from Vojtěch's own account of the matter (https://www.vojtechsommer.cz/post/alone). The Sommer family has had to cover expenses for lawyers and for taking the case to civil court and sports arbitration. Remarkably, they even had to pay about 2000 euro to obtain the test results from the WADA-laboratory in Dresden, that is, the very same data that formed the basis for the accusations raised against him. When reviewing all the personal and financial burdens that have been laid on Vojtěch Sommer it is obvious that fighting for one's reputation and athletic career requires a strong personality and a healthy bank account. For athletes that are the victims of laboratory errors, the consequences for their athletic careers are catastrophic and their right of law is almost absent.

Serious consequences when detection limits are pushed too far

The prevalence of doping in elite sports is high – it may be 30% or higher according to a recent study [6], and only a small fraction of doped athletes are caught. Based on our experiences, some laboratories push the detection limit too far – beyond the documented state-of-the-art detection limits – and thereby greatly increase the risk of detecting irrelevant proteins and/or contaminants. This is particularly worrying because the WADA laboratories do not present and discuss the scientific evaluations of their results, not even when their results are clearly uncertain and/or inconsistent. We believe that both the Colvert [3] and Sommer cases are examples where the limits of sensitivity have been exceeded.

The Karus case

Another example of laboratory errors resulting in a questionable sanctioning of an athlete is the 2015 doping case against the German runner Benedikt Karus. Several years ago he published the relevant documents on his home page https://www.benedikt-karus.de/wissenschaftliche-stellungnahmen/. The WADA-accredited laboratory in Cologne claimed that the SAR-PAGE test revealed synthetic EPO in Karus' urine. But the WADA-accredited laboratory in Tokyo could not detect any synthetic EPO when independently testing the same sample using another technique, namely mass spectrometry (https://www.benedikt-karus.de/wissenschaftliche-stellungnahmen/; see document 'Certificate of Analysis – Confidential – Report ID BAG151221-09-R1 – LSI Medience Corporation – Anti-Doping Laboratory Tokyo Japan 24.3.2016'). The Cologne laboratory explained at the hearing – without referring to any scientific documentation – that the SAR-PAGE technique was, in their hands, more sensitive than mass spectrometry. The athlete was sanctioned for 4 years. What the Cologne laboratory did not mention, but must have known, was that the athlete's lane in the A-sample screening test was contaminated with synthetic EPOs from neighbouring lanes when the laboratory performed the analyses (see below). Karus was first made aware of this by us after the court hearing.

In Fig. 4 we present a part of the gel image of the A-sample screening test performed by the WADA-accredited laboratory in Cologne. Karus' sample is in lane 9, indicated by a black, vertical arrow. The position of normal eEPO is marked by the strongest bands in lanes 6–8 and 11–12. The amount of normal eEPO in the athlete's sample was very low and the staining of his lane was, presumably for this reason, greatly intensified compared to the staining of the neighbouring lanes. Staining was intensified to such an extent that bands resulting from diffusion of EPOs from the neighbouring lanes were detected in the athlete's lane. First, the asymmetric black-to-grey 'triangular band' (red arrow in Fig. 4) in the eEPO region of the athlete's lane is most likely due to diffusion of eEPO from the neighbouring lane 8 (see below). Second, the two even fainter black-to-grey 'triangular bands' (blue arrows in Fig. 4) in the rEPO regions of the athlete's lane seem to be due to diffusion into lane 9 of the two different rEPOs present in the neighbouring lane 10. Note the dramatic asymmetry in the bands marked with red and blue arrows, arguing that these bands do not reflect proteins that were present in the lane when the gel was loaded for electrophoresis, but appear as a result of leakage from neighbouring lanes. We do not

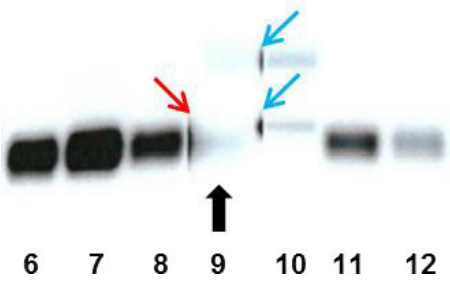

Fig. 4. SAR-PAGE analysis of Benedikt Karus' urine sample. Lanes 6 to 12 of the of the gel image of the A-sample screening test by the WADA-accredited laboratory in Cologne, presented in the laboratory's Documentation Package sample A142584 found at https://www.benedikt-karus.de/wissenschaftliche-stellungnahmen (page 19 in pdf named 'Documentation Package sample A 142584 – Institut für Biochemie DSHS Köln 8.4.2015'). Karus' sample was applied in lane 9 (indicated by a black arrow). Samples from other athletes were applied to lanes 6–8 and 11and 12, and the bands in these lanes represent normal eEPO. The two bands seen in lane 10 represent two different variants of synthetic EPOs. We have modified the image by adding blue, red and black arrows and the lane numbering. For methods, see Fig. 1 legend.

know whether Karus has used rEPO, but the data shown in Fig. 4 do not support such a hypothesis. Furthermore, the laboratory's interpretation of their results is certainly inconsistent with the negative mass spectrometry results from Tokyo. It seems to us that the laboratory in Cologne has shown suboptimal competence both in running and interpreting the gels, and it is disturbing that they did not give information about the contamination problem during the hearing.

The same WADA-accredited laboratory, in Cologne, previously presented questionable and inconsistent test results in the case against the Irish sprinter Steven Colvert in 2014 [3] http://www.newsweek.com/steven-colvert-epo-world-anti-doping-agency-sport-509189. Due to an unfortunate lack of critical expertise in the Colvert case, the defence and judges did not recognize the inconsistencies and the laboratory did not inform about it, so Steven Colvert was found guilty. In both the Karus and Colvert cases, it should have been the laboratory's duty to inform about the inconsistent results during the hearing. Judges are usually not qualified to interpret the test results and rely entirely on WADA's interpretation of WADA's own results. WADA's experts are closely involved in these cases and have a vested interest in defending their own position and prestige. Moreover, it seems that they hesitate to testify against each other and instead refer to a WADA code which states that a 'laboratory should not engage in analytical activities or expert testimony that would intentionally question the integrity of the individual or the scientific validity of work performed in the antidoping program' (International Standard for Laboratories, June 2016, Annex B, section 4).

Lack of accountability and transparency

One should expect WADA, WADA-accredited laboratories and sports organizations to respond in a constructive manner when informed about abuse and breach of good scientific practice, but that does not happen. WADA, the IOC and the International Association of Athletics Federations (IAAF) were informed about extensive doping abuse in Russia several years before the Olympics in Sochi in 2014 and the World Championships in Athletics 2013 in Moscow, but did little. From 2010, journalists and Russian whistleblowers repeatedly informed these sports organizations about systematic doping in Russia and the involvement of Russian sports and antidoping authorities(https://www.nytimes.com/2016/06/16/sports/olympics/world-anti-doping-agency-russia-cheating.html, http://www.sportingintelligence.com/2016/07/25/exposed-the-story-behind-the-story-of-russia-doping-and-the-ioc-250701/00000). Moreover, in 2011, a scientific paper by IAAF officials and analysts at the WADA-accredited laboratory in Lausanne reported that the prevalence of suspicious blood profiles was in the range 50–99% for Russian male elite track and field athletes compared to 0–3% for athletes from some other countries [7]. Nonetheless, WADA suspended the accreditation of the Moscow laboratory only in November 2015, long after the laboratory's deviations from good scientific practice had become evident for everyone.

Vladimir Sommer was met with silence when he contacted the WADA-accredited laboratory in Seibersdorf, which was involved in evaluating the test results. After several repeated request for additional information about the case against his son, Vladimir Sommer finally received the following reply: 'The WADA rules clearly state that laboratory employees are not allowed to provide information about testing to athletes or others than, for example the results management authority or the antidoping agency. This is part of the code of ethics that we have to follow. So may I ask you to understand that I cannot provide a statement to any analyses in the context of antidoping'. It seems that this 'code of ethics' does not function as a code of ethics. It does, however, function quite effectively to prevent transparency and accountability, which makes it all the more important that independent scientists react and become involved when they perceive that adherence to good scientific practice is breached by antidoping laboratories and organizations.

What should be improved?

The main shortcoming of the current testing regime is its lack of transparency. The unprocessed data should be made available to the athlete. It is a basic requirement of the right of law to have full insight into the details of an allegation. Sports organizations should adhere to generally accepted principles in science, ethics and law in order to maintain their position as objects of public sponsoring and to enjoy public confidence. Second, controversial analyses or interpretations should be reinvestigated by an external, independent party – and particularly if there is a mismatch between samples A and B. Such an instrument could act as a safety valve, in the few cases where it may apply. It does not help to send it to another WADA-accredited laboratory and particularly not as long as they have regulations telling them never to contradict one another. Third, the antidoping movement should be concerned about the fate of an athlete charged with and sanctioned for doping. Tragedies can be caused by an incorrect exclusion of an athlete and this should be taken seriously, both by the sport movement in general and especially by the antidoping organizations. We do not agree that a lack of time and funds should be used as an argument to ignore this huge social problem. Lastly, current regulations allow the WADA-accredited laboratories to dispose of samples taken for sanctioned athletes, whereas samples from other athletes shall always be stored. In the Colvert case, the remaining sample was destroyed in spite of the athlete's request that it should be stored. It appears obvious that all samples should be saved, pending further analyses.

Acknowledgements

We would like to thank Vojtěch and Vladimir Sommer and Benedikt Karus for communicating data and information. Data from the WADA-accredited laboratories have been reproduced in this article with the permission of both athletes.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

All authors have participated in evaluating the data and in writing the manuscript.

References

1 Nissen-Meyer J, Boye E, Østerud B & Skotland T (2013) WADA-accredited doping analyses cannot always be trusted. *Lab Times* **2013**, 18–23.
2 Nissen-Meyer J, Boye E, Østerud B & Skotland T (2015) Problems at a WADA-accredited antidoping lab: puzzling Discrepancy. *Lab Times* **2015**, 18–23.
3 Nissen-Meyer J, Boye E, Østerud B & Skotland T (2016) Another troubling doping case is questioning WADA's credibility again: borderline Analysis. *Lab Times* **2016**, 16–19.
4 Boye E, Skotland T, Østerud B & Nissen-Meyer J (2017) Doping and drug testing. *EMBO Rep* **18**, 351–354.
5 Lasne F, Martin L, Matin JA & de Ceaurriz J (2009) Detection of continuous erythropoietin receptor activator in blood and urine in anti-doping control. *Haematologica* **94**, 888–890.
6 Ulrich R, Pope HG, Cléret L, Petróczi A, Nepusz T, Schaffer J, Kanayama G, Comstock RD & Simon P (2018) Doping in two elite athletics competitions assessed by randomized-response surveys. *Sports Med* **48**, 211–219.
7 Sottas P-E, Robinson N, Fischetto G, Dollé G, Alonso JM & Saugy M (2011) Prevalence of blood doping in samples collected from elite track and field athletes. *Clin Chem* **57**, 762–769.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.
Data S1. Evaluation of Results of rEPO Tests Performed on the A and B Sample of Vojtěch Sommer's Urine.