

Another troubling doping case is questioning WADA's credibility again

Borderline Analysis



Some WADA-accredited laboratories and also sports judges base their conclusions and verdicts on uncertain, inconsistent results and interpretations. That's fatal for those individual athletes who are innocent and for the credibility of the entire anti-doping system.

In May 2014, the Irish sprinter and law student Steven Colvert was tested for performance-enhancing drugs. The WADA-accredited laboratory in Cologne reported that his urine contained traces of synthetic recombinant erythropoietin (rEPO), a drug that must be injected and cannot be unintentionally ingested. Colvert claimed he was innocent, but was found guilty and deprived of the right to participate in competitions and organised training for two years (see www.independent.ie/sport/other-sports/steven-colvert-the-damage-to-my-name-is-done-but-science-isnt-infallible-it-wasnt-clearcut-31388457.html). He is completely open about his case and presents all relevant information at <http://stevencolvert.ie>.

After reading our article in *Lab Times* in September 2015, Colvert contacted us and asked if we could help him evaluate the data that formed the basis for the conviction. We found that there are indeed troubling aspects to the data and our considerations concern the level of certainty that one can achieve when measuring very small differences with scientific methods.

We fear that Colvert's sentence for drug abuse might have been unjustified.

The PAGE test results

The results obtained in the first of two PAGE tests which the laboratory carried out on Colvert's A-sample, are shown in Figure 1 (from the A-Sample Analytical Report, made available on <http://stevencolvert.ie>). The PAGE test (SAR-PAGE, an analysis somewhat similar to SDS-PAGE) separates different EPO forms according to size: The smallest forms migrate farthest down the lane. Lanes 4, 5, 10, and 16 in Figure 1 are control lanes, on which different recombinant EPO (rEPO) forms have been applied.

The PAGE test is good for detecting some of these rEPO forms because their migration rates differ greatly from that of endogenous EPO, which our body naturally produces. However, the PAGE test is not optimal for detecting the rEPO form shown as band 1 in lanes 4, 10 and 16 in Figure 1, because it migrates only slightly more slowly than endogenous EPO. It is this or a similar variant of rEPO that the laboratory claims is present in Colvert's urine.

In the first PAGE test, urine samples from 15 athletes were applied on lanes 1-3, 6-9, 11-15, 17-19 (Figure 1); Colvert's sample is on lane 15. The bands marked alpha (α) are due to endogenous EPO and an alpha-band is found in the lanes of all 15 athletes.

The WADA laboratory claims that Colvert's alpha-band contains (in addition to endogenous EPO) small amounts of rEPO because some diffuse staining may be perceived above the horizontal line in lane 15, and similarly when they retested Colvert's sample in two subsequent PAGE tests.

Not much different from other lanes

In our opinion, however, Colvert's lane is not much different from lanes 2, 6, 8, 9, 11, 12, and 17 in Figure 1, all of which are considered negative for rEPO. Furthermore, Colvert's lane (lane 15) is not much different from some of the negative controls obtained in the second PAGE test (Figure 2B and C). Figure 2A shows a more quantitative representation of Colvert's lane in the first PAGE-test and Figure 2B

Information box 1: Not much different from the other lanes...

The results obtained in the first PAGE test of Colvert's urine A-sample:

Urine samples from 15 athletes were applied on lanes 1-3, 6-9, 11-15, 17-19; Colvert's sample is on lane 15.

Lanes 4, 5, 10, and 16 are control lanes, on which only different recombinant EPO (rEPO) forms have been applied (bands marked 1, 2 and 3).

The bands marked α (red arrows) in each lane are due to endogenous EPO. The light blue horizontal line indicates the position of the rEPO form, resulting in band number 1 in lanes 4, 10 and 16.

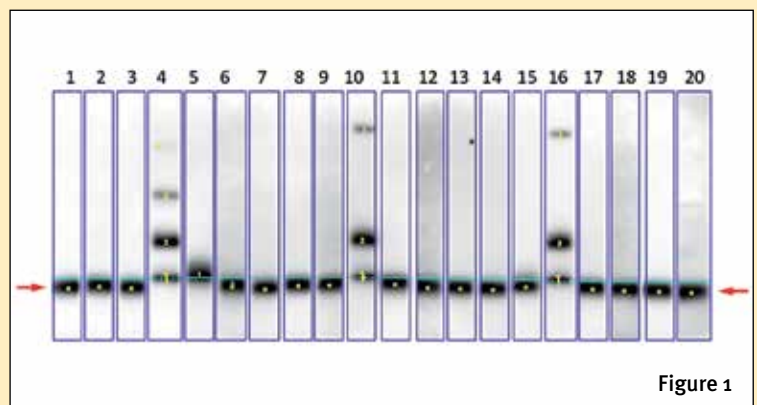


Figure 1

Source: Institute of Biochemistry, German Sport University Cologne, WADA-accredited Laboratory for Doping Analysis, Documentation Package sample A 6050816 (25.06.2014) Analytical report S20143705-1, Lab no. 7397 (page 16) (downloadable at <http://stevencolvert.ie> under "A Sample Analytical Data").

and C show similar representations of negative control lanes (i.e. negative for rEPO) from the second PAGE test.

Here due to rEPO, there not?

The laboratory claims that the "density profile" above the horizontal line in Figure 2A must be due to rEPO whereas that above the horizontal line of the nearly identical "density profile" of some of the negative control samples (Figure 2B and 2C) is simply due to "tailing/spreading" of endogenous EPO. In his witness statements (see the Hearing Transcript found on <http://stevencolvert.ie>), the scientific associate representing the WADA-accredited laboratory in Cologne stated that from his experience of seeing 21,000 samples, he can say that lane 15 (i.e. Colvert's lane) is clearly that of a positive sample whereas the others are not. And he also states,

"...that in finding a sample which has such low doses of rEPO, you need to be expert to clearly identify it".

He may have analysed 21,000 urine samples over the years, but it is most likely that only a few, if any, of these samples are relevant in the sense that they were athlete's samples proven to be positive for rEPO by independent methods and with a staining profile similar to that in lane 15 in Figure 1. It is worrisome that the outcome of an athlete's doping test can be determined inside the heads of a few people and without objective and robust criteria.

Influenced by multiple factors

The type of tailing or spreading seen in Colvert's lane – and in many of the other lanes in Figure 1 – is common when doing PAGE analyses; the extent of spreading is influenced by electrophoretic, sample and staining conditions. Note for instance that the vertical positions of the alpha-bands are different in the different lanes shown in Figure 1. Compare the alpha-band in lane 2 with that in lane 3, or the one in lane 7 with the ones in lane 8 and 11. Such variability will influence the amount of staining that occurs above the horizontal line.

Another problem is that the results presented in the A- and B-Sample Analytical Reports are not pictures of the original PAGE-gels, but pictures obtained after software analysis (GASepo) and after the lanes have been "cut out" from the original gel image and realigned. Even small alterations in the realignment of the lanes may drastically influence how much of the various alpha-bands come above or below the horizontal line.



Photo: private

Is Irish sprinter Steven Colvert a wrongly convicted victim or a lying sports fraudster? We don't know. But what we do know is that the underlying doping analyses are insufficient and cannot clarify this question.

To resolve whether or not Colvert's alpha-band differs significantly from other alpha-bands it is necessary to evaluate the original gel images.

Colvert has asked Anti-Doping Sport Ireland for the original images. Their reply was, incorrectly, that the GASepo images comprise the raw data and that the A- and B-Sample Analytical Reports are the entire documentation to which he is entitled.

Uncertain PAGE conclusions ...

As it is now, one can not conclude with any certainty that the small band tailing or spreading in Colvert's lane is due to rEPO.

But let us say – just for the sake of argument – that the weak signal that the laboratory refers to in the PAGE test is due to rEPO. The results would then imply that there is only a very small amount of rEPO compared to the amount of Colvert's endogenous EPO. This interpretation is ►►

indeed in agreement with the laboratory's own claim. In their witness statements (see the Hearing Transcript found on <http://stevencolvert.ie>), experts from the WADA-accredited laboratories in Cologne and Seibersdorf state that there is just a small amount of rEPO compared to the amount of endogenous EPO in Colvert's sample. Judging from the first PAGE test (lane 15 in Figure 1 and Figure 2A), the amount of endogenous EPO is at least ten times greater than the amount of alleged rEPO.

But this conclusion is in conflict with the results the laboratory obtained when they performed the so-called IEF test after the first PAGE-test, as outlined below.

... in conflict with IEF test results

The laboratory must, according to WADA's Technical Document that was valid in the relevant time period (<https://wada-main-prod.s3.amazonaws.com/resources/files/WADA-TD2013EPO-Harmonization-Analysis-of-Recombinant-Erythropoietins-EN.pdf>), perform at least one IEF test on the A-sample. In this test, the EPO variants are separated according to differences in charge instead of size.

The results of the IEF test are shown in Figure 3 on top of page 19 (from the A-Sample Analytical Report; <http://stevencolvert.ie>). Lane 1 shows a negative control sample, which contains endogenous EPO, but no rEPO; lane 3 is a positive control that contains both endogenous EPO and rEPO; and lane 2 shows Colvert's sample.

Most of the staining above the blue horizontal line between the bands labelled 1 and alpha in lane 2 is considered to be due

to rEPO, whereas the staining below is due to Colvert's endogenous EPO.

A requirement for judging the IEF-test positive for rEPO is, according to WADA's Technical Document, that the two most intense bands must be above the horizontal line, i.e. they must be in the rEPO region of the gel.



The main entrance of the German Sport University in Cologne. Here, at the WADA-accredited Manfred Donike Institut für Dopinganalytik, Steven Colvert's urine samples were analysed.

This requirement was not expected to be fulfilled, considering that the PAGE test of Colvert's sample indicated that the amount of rEPO, if present at all, was very small (about one-tenth) compared to the amount of endogenous EPO.

Implausible finding

Nevertheless, the laboratory obtained a clearly positive IEF test, which, surprisingly, indicated that there was nearly twice as much rEPO as endogenous EPO (estimated

from data on page 27 of the A-Sample Analytical Report).

How can one explain this large discrepancy, namely that the IEF-test indicated a twenty-fold higher ratio than the PAGE-test between what was claimed to be rEPO and endogenous EPO? It is obvious that something is wrong and the problem must be clarified before making any judgement on whether or not Steven Colvert did use rEPO. If not clarified, the only fair decision should have been to drop the case against Colvert, and give the WADA laboratories feedback that they must improve the rigour of their analyses.

Need to improve analyses

It is odd that the laboratory did not repeat the IEF test when they did the final confirmation test on the B-sample. Judging from the Hearing Transcript (see <http://stevencolvert.ie>) the

laboratory was asked why they did not choose this obvious alternative (i.e. to use the IEF test on the B-sample).

They suggested that there was not enough urine to perform a second IEF test. But that is difficult to understand, considering that there was initially 140 ml (to be divided between the A- and B-sample) and each PAGE and IEF test requires only 15-20 ml.

WADA tests their laboratories for false negatives – the Beijing laboratory was in

Information box 2: GASepo analyses and densitometric scans

GASepo analyses and densitometric scans of the first PAGE-test of Colvert's A-sample (panel A) and two negative samples (negative for rEPO; panels B and C) from the second PAGE-test.

The blue horizontal line in each lane indicates where the rEPO form migrates, producing band number 1 in lanes 4, 10 and 16 in Figure 1.

Source: Institute of Biochemistry, German Sport University Cologne, WADA-accredited Laboratory for Doping Analysis, Documentation Package sample A 6050816 (25.06.2014) Analytical report S20143705-1, Lab no. 7397 (pages 17, 36 and 40, respectively) (downloadable at <http://stevencolvert.ie> under "A Sample Analytical Data").

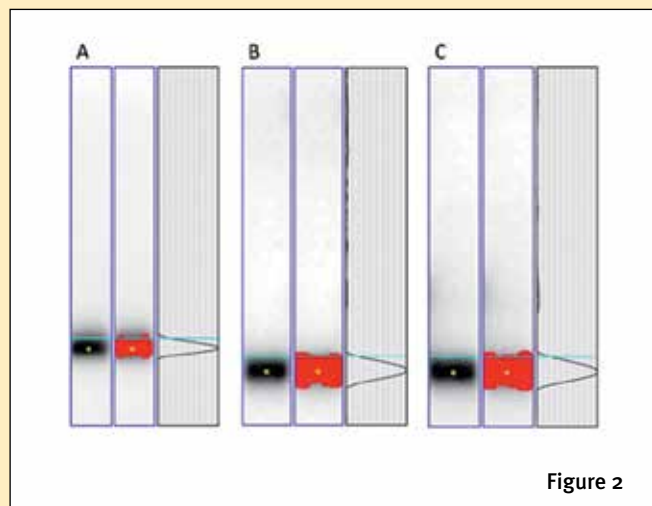


Figure 2

Information box 3: IEF test exhibits a completely different result

The results obtained with the IEF test of Colvert's urine A-sample:

Lane 1 shows a negative control sample that contains endogenous EPO, but no rEPO. Lane 2 shows Colvert's sample. Lane 3 shows a positive control sample that contains both rEPO and small amounts of endogenous EPO. Most of the staining above the blue horizontal line in each lane is due to rEPO, whereas the staining below is largely due to endogenous EPO.

The IEF test detected nearly twice as much rEPO as endogenous EPO, while the PAGE test (Figure 1, page 16) detected only very small amounts of rEPO, compared to large amounts of endogenous EPO. That discrepancy is implausible.

Source: Institute of Biochemistry, German Sport University Cologne, WADA-accredited Laboratory for Doping Analysis, Documentation Package sample A 6050816 (25.06.2014) Analytical report S20143705-1, Lab no. 7397 (page 23) (downloadable at <http://stevencolvert.ie> under "A Sample Analytical Data").

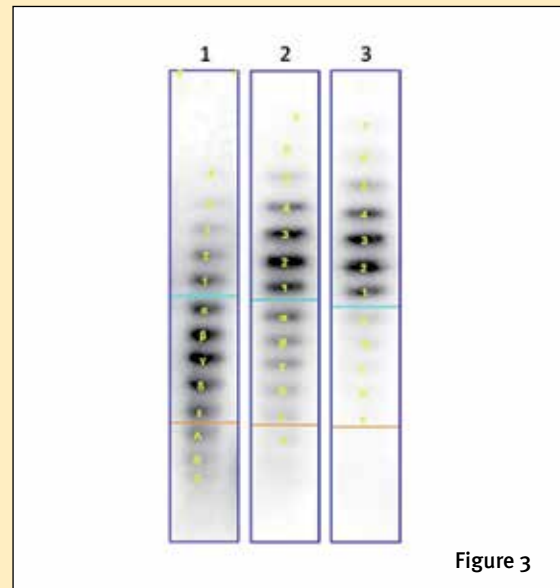


Figure 3

fact recently suspended because it reported two false negative tests – but does WADA have any tests for uncovering false positives? At the moment, clean athletes have reason to be concerned. It would be reassuring if the A- and B-samples were routinely tested independently at different laboratories.

Reasons to be concerned

As it is now, it is almost impossible to correct a laboratory mistake arising, for instance, from contamination or an exchange of two samples. Errors can occur in any laboratory. It is important that such errors are detected and that reanalyses are performed. In the present case, the large internal disagreements between different methods of analysis should have been sorted out before going to the dramatic step of charging an athlete with doping.

"I always thought science was infallible but I've seen now that it sometimes comes down to 'interpretations' of results. It's not always that clear-cut", Steven Colvert remarked on 20th July 2015 to an *Irish Independent* reporter.

Frightening résumé

The sprinter is certainly right; scientific results are not always clear-cut and thus do not always permit reliable interpretations. After examining the doping case against Erik Tysse (discussed in *Lab Times* 1/2013, page 18-23 and *Lab Times* 5/2015, page 18-23) and now the case against Colvert, it is our opinion that some WADA-accredited laboratories and also sports judges do not

recognise such ambiguities and base their conclusions and verdicts on uncertain and inconsistent results and interpretations.

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*"I always thought science was infallible but I've seen now that it sometimes comes down to 'interpretations' of results. It's not always that clear-cut".
 – Steven Colvert*



Photo: private

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