Evaluation of Results of rEPO Tests Performed on the A and B Sample of Vojtěch Sommer's Urine

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Mr. Vojtěch Sommer, born 8. 6. 1996, residing at Sokolovská 34/5, Prague 8 – Karlín

Mr. Vojtěch Sommer is a successful Czech triathlete who was found to have committed an anti-doping rule violation. It is asserted by the WADA accredited laboratory in Dresden that the recombinant erythropoietin (rEPO) was found in his A and B urine samples.

Documents provided

Laboratory Documentation Sample 3896875

Laboratory Documentation Sample B3896875

Laboratory Documentation Additional Explanation Sample B3896875

Test Report

WADA Technical Document – TD2014EPO

Analyses from Czech experts?

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Declaration of Experts

All four of us are experienced molecular biologists and biochemists and well versed in the techniques in question here. We have not and will not receive any compensation for this evaluation, and we have never met Vojtěch Sommer and have no relationship with him.

Executive Summary

We have carefully evaluated the documents that report the tests performed on Vojtěch Sommer's A- and B-samples and also additional explanation from the Dreden laboratory. We find **no scientific evidence in these documents which proves the presence of rEPO in Sommer's urine.**

In our opinion, the differences between results obtained with the athlete's sample and the **negative-control samples are due to a protein that can not be claimed to be rEPO (since its size differs from rEPOs) and to spreading and or tailing of the athlete's endogenous EPO (eEPO).**

Note for instance the clear staining below the athlete's eEPO band in the A-sample screening test (see page 15 of the A-sample Documentation package dated December, 2016). The staining below the band is much stronger than that above the eEPO band and is definitety not due to any known EPO variants used for doping. So why can the laboratory be certain that the (less intense) staining above the eEPO band is due to rEPO? The fact is that spreading of bands is not uncommon upon PAGE analyses of proteins. As an illustrative example, note the marked tailing seen in all the beta-bands on pages 19-23 of the B-sample Documentation package dated June 2016. Moreover, the athlete's EPO profiles (for instance that shown on page 24 of the A-sample; Documentation package dated December) is almost identical to the negative-control profile. It can not be concluded without further investigations that the small shoulder on the athlete's profile is due to rEPO. The conclusion that the shoulder is due to rEPO presumes that the alleged rEPO in the athlete's sample migrates more slowly than the rEPO in the positive-control sample, which may or may not be true. More importantly, it is not at all evident from the positive- and negative-control profiles that it is possible to obtain a profile similar to that obtained from the athlete's sample by combining, in different proportions, the profile with no sign of rEPO with that of only rEPO added. The reason being that the protein the laboratory apparently claims is rEPO in the athlete's urine has a different migration speed and positions differently in the gels than rEPOs and can therefore not claimed to be rEPO.

We note that the laboratory seems to present the fluorescent gel image on page 13 of the B-sample Documentation package dated June 2016 in an incorrect way. It seems that this image is inverted (it reads from right to left) compared to the corresponding gel images on page 14 (which reads from left to right). Note also the variations in the mobility and splitting of bands (the top band in lanes 5 and 8 [from right to left], which seem to be lanes on which the athlete's sample was tested. This also illustrates anomalies that often are observed upon PAGE-analyses of proteins and illustrates the uncertainty of assigning significance to minor differences. Note that there is no staining on the top of the fluorescent image for four lanes (lanes **4,7,15** and 18 from left), which seem to be the four lanes in which the athlete's sample was tested (and note that the top band in these four lanes migrate somewhat more slowly than the top band in the other lanes), indicating that the athlete's sample behaves somewhat differently than the control samples. This difference is exactly what one could expect when samples with a very different protein composition and concentration are directly compared on a gel.

We also note that the laboratory has masked most of the gel-images shown on pages 15 and 19 of the A-sample Documentation package (December 2016). This is unacceptable, as it prevents the evaluation of the general quality of the PAGE-test run.

PAGE analysis of proteins is a method fraught with problems when trying to measure small differences in protein composition or migration. With large differences, there may be small problems, but with small differences, like here, the chances of incorrectly reporting an adverse

finding are significant. Therefore, this athlete should be considered innocent in the absence of further and clearer data.

Evaluation of Results of rEPO Tests Performed on the A-sample of Vojtech Sommer

Figure 1A

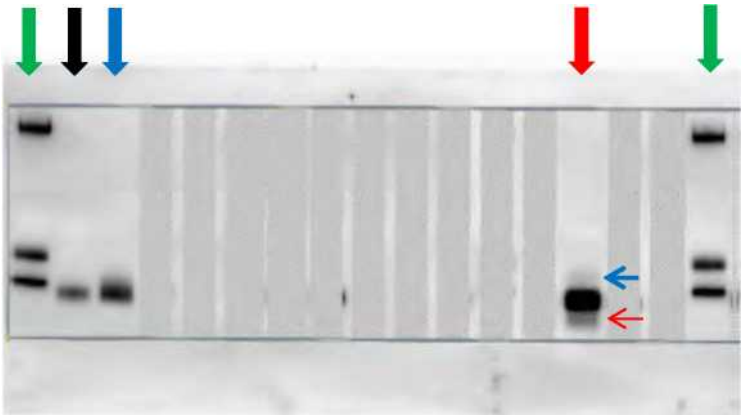
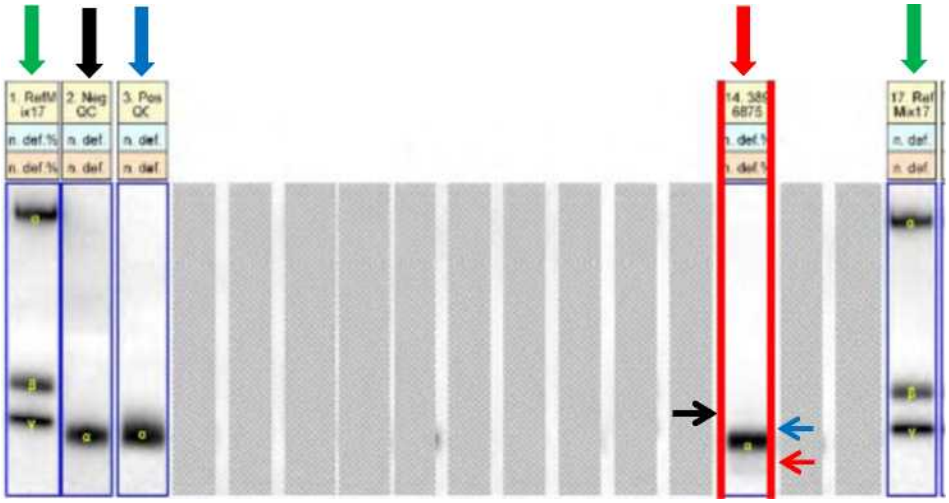


Figure 1B



Figures 1A and 1B are both from page 15 of the «Laboratory Documentation Sample» dated December 2016.

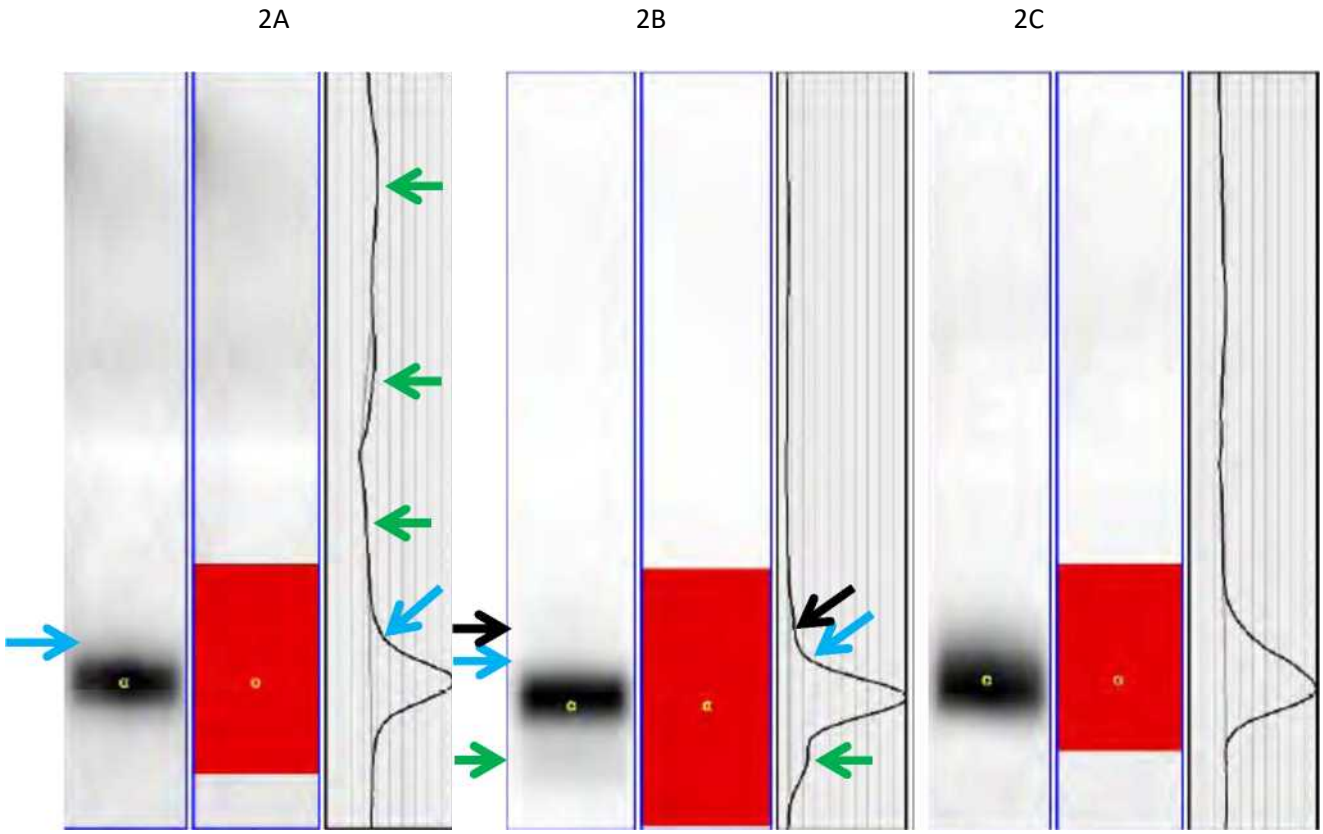
Fig. 1A is a part of the (unprocessed) gel image of the first (i.e.screening) test of the A-sample whereas Fig. 1B is a «GASepo processed» image of the image shown in Fig 1A. The vertical green arrows indicate control lanes that contain different «synthetic» EPO-variants used in doping (from top: CERA, NESP and rhEPO). The vertical black arrows indicate negative control lanes that contain normal endogenous EPO (eEPO). The vertical blue arrows indicate positive control lanes that contain normal endogenous EPO (eEPO) and rEPO(s). The vertical red arrows indicate athlete's lanes.

In Fig. 1A there is staining both below (red horizontal arrow) and above (blue horizontal arrow) the athletes normal

endogenous EPO (eEPO) band. The staining below and above the athlete's normal eEPO band could be due to spreading, tailing and/or heterogeneity of the athlete's eEPO, or to other proteins. Note that the athlete's eEPO band in Fig.1A is strongly "exposed" (i.e.the athlete's eEPO band is much more strongly stained than the bands in neighbouring control lanes) presumably because of high eEPO concentrations in his sample. The staining above and below his band thus becomes more apparent than in the bands in neighbouring lanes.

The staining below (red horizontal arrow) the athlete's eEPO-band can obviously not be attributed to any known rEPO variants used for doping, since rEPOs do not migrate below (i.e. faster than) eEPO. So why can the laboratory be so certain that the (less intense) staining above (blue and black horizontal arrows) the eEPO-band is due to rEPO? It is acceptable that the laboratory might want to analyse the staining above the eEPO-band further, but the test results they obtain are not at all convincing and definitely do not prove that the staining above the athlete's eEPO is due to rEPO.

In the in the «GASepo processed» image shown in Fig. 1B the staining of the athlete's lane has been reduced (more normalized relative to the other bands) and the staining below and (especially) above is reduced and more similar to that in the neighbouring control bands. And it seems that the staining above the eEPO band splits into (i) a faint (not very convincing) band marked with black horizontal arrow (Fig. 1B) and (ii) a region marked with a blue horizontal arrow (Fig. 1B). The latter region is not much different from the "normal" spreading/tailing one sees above the control bands in neighbouring lanes in Figure 1, as is perhaps more evident in Figure 2 A-C below (pages 16 & 18 in the «Laboratory Documentation Sample» December 2016).



Figures 2A, B and C show the lanes and corresponding profiles of, respectively, (A) the negative control which contains just normal endogenous EPO (eEPO); (B) the athlete's lane and profile; and (C) the positive control which contains both normal eEPO and rEPO. It is not at all obvious that the staining marked with blue arrow in the athlete's lane differs from that (marked with blue arrows) in the negative control. There is some very faint staining (perhaps not convincing) marked with black

arrow in the athlete's lane, but (as will be more evident later) it is not clear that this migrates as rEPO. Note that there are also other regions (marked with green arrows; most are in the negative control) that stain more strongly (than that marked with black arrow in athlete's lane) and which cannot be attributed to rEPO.

The fact is that spreading/tailing of bands (as seen in Fig. 1 and 2 above - and especially evident in athlete's lane in Fig. 1 because of strong staining) is not uncommon upon PAGE analyses of proteins. As an illustrative example, note the marked tailing seen in all the beta-and CERA-bands (blue arrows) in Fig. 3 below.

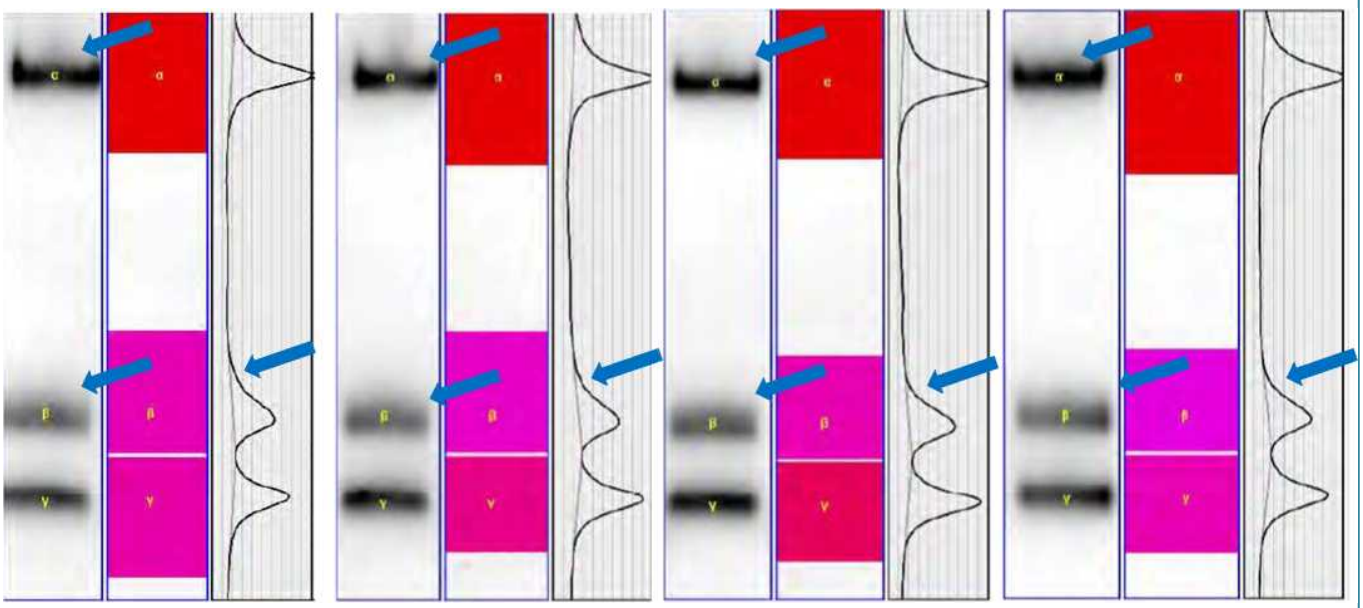


Figure 3. Some of the «GASepo images» of standard mixtures (CERA, NESP rEPO) on pages 19-23 of the B- sample Documentation package dated June 2016.

Another example which reveals variability in the migration and/or spreading/tailing of normal endogenous EPO (eEPO) is shown in Figure 4 below.

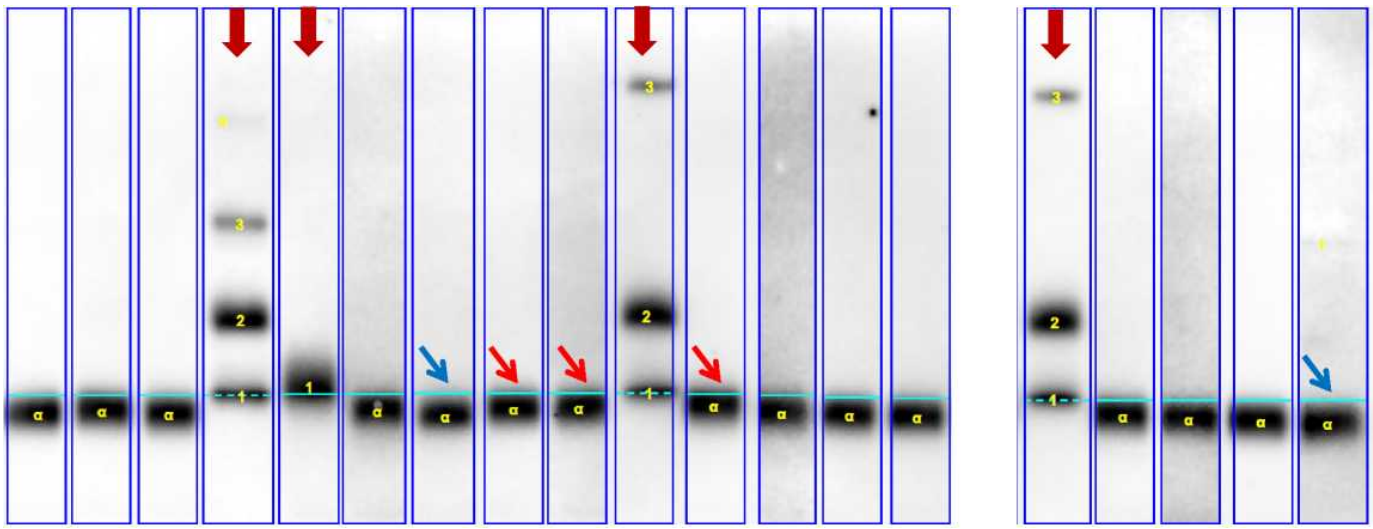
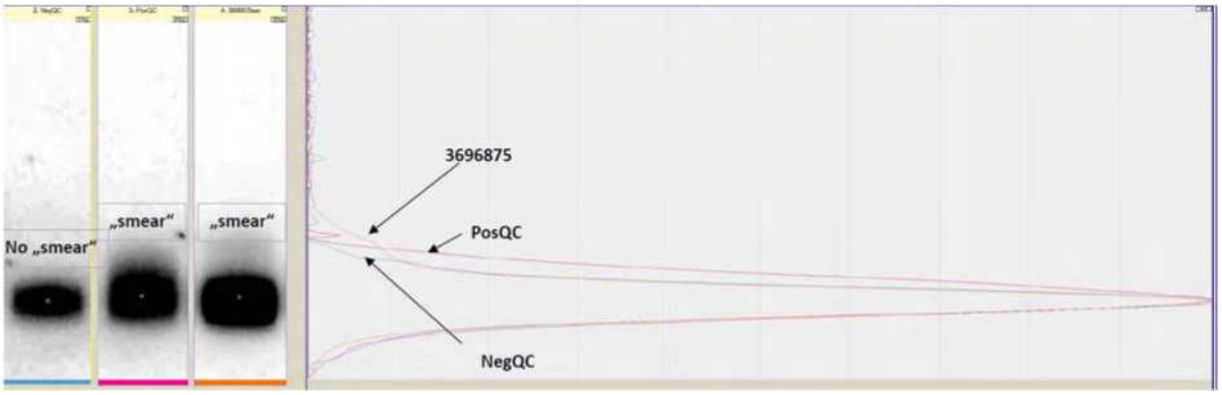


Figure 4 shows variability in the migration of endogenous EPO (eEPO) when analysed by SAR-PAGE. The lanes marked with vertical brown arrow above the lanes indicate control lanes on which different rEPO variants have been applied. On all the other lanes, urine samples from different individuals (that presumably are "non-dopers") have been applied, and the alpha-band in these lanes is their eEPO. Note the differences (variability) in how far these "normal" eEPOs migrates. The blue arrow indicates some of the eEPOs that migrate farthest down and all/most staining/tailing is below the blue horizontal line. The red arrow indicates some of the eEPOs that have migrated more slowly and some of the band staining/tailing is above the blue horizontal line. The blue horizontal line represents where rEPO (band 1) migrates, and staining above this line may raise suspicion of rEPO-doping. Lane 15 is not included, since that was a test of an athlete accused of rEPO-doping, and some might consider that the variation in his alpha-band is not that of a non-doper. The figure is from Lab Times 2016-5: 16-19, but modified by addition of arrows and removal of lane 15 and lane numbering. The test was performed by the WADA laboratory in Cologne (we probably could have used Figure 1A to illustrate this point, had the laboratory not masked all the other lanes in the gel image).

Now we turn to the results the laboratory obtained when they retested the urine A-sample; i.e. when they performed the so-called confirmation test of the A-sample (pages 19-24 of the «Laboratory Documentation Sample» dated December 2016). The results were similar to the screening test discussed above (but the staining below the eEPO band (marked with red horizontal arrow in Figure 1 above) was not evident).

The following figure from page 24 in the «Laboratory Documentation Sample» dated December 2016 summarizes the results obtained when the laboratory retested the urine A-sample.



The athlete's EPO profile in the above figure (labelled 3696875; note the laboratory labelled it incorrectly, it should be 3896875) is almost identical to the negative-control profile (labelled NegQC). I.e. the athlete's profile coincides perfectly with the negative control profile, except for the small shoulder. And it cannot be concluded that the small shoulder on the athlete's profile is due to rEPO since it is not at all evident from the positive- and negative-control profiles that it is possible to obtain a profile similar to that obtained from the athlete's sample by combining, in different proportions, the profile with no sign of rEPO with that of only rEPO added. The fact is that the small shoulder in the athlete's profile does not migrate similarly to any known rEPOs and can thus not be attributed to rEPO. This is more easily seen in the profiles presented upon testing the B-sample, which we describe later. But it is also evident in this figure and Figure 5 below.

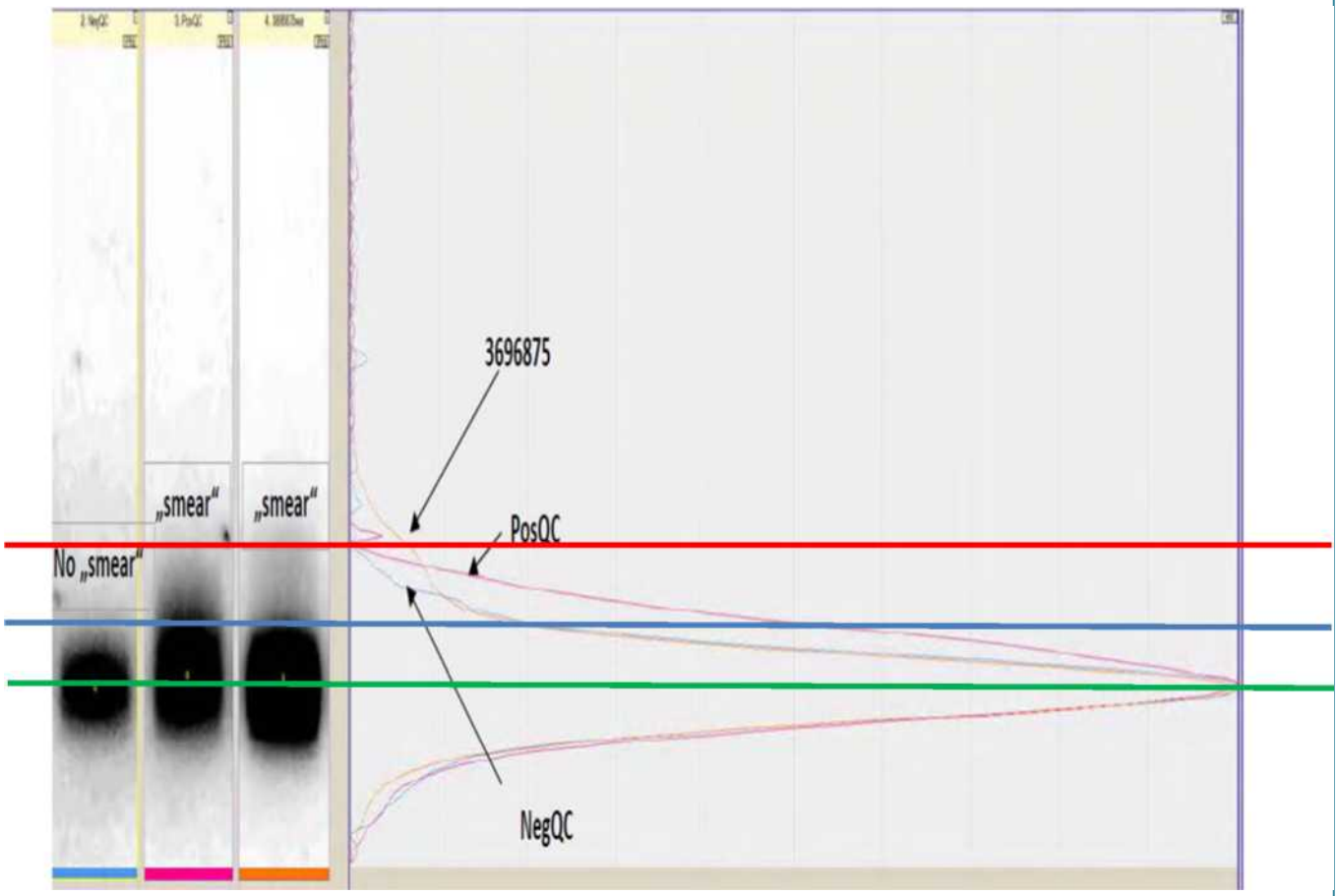


Figure 5: The same as the figure on previous page but with expanded vertical axis in order to more clearly see the differeces between the three profiles. Moreover, the figure includes horizontal lines that indicate where the peak due to eEPO appears (green horizontal line), where the peak due to rEPO is expected (blue horizontal line) and where the peak that contributes to the «shoulder» in the athlete's lane is expected (red horizontal line). Where these peaks are predicted to come (if the positive control and the athletes sample did not contain eEPO) is estimated by subtracting the negative control profile (NegQC) from, respectively, the positive control profile (PosQC) and the athlete's profile. It is assumed that the three samples contain similar amounts of eEPO, which should not be a "way-off" assumption since these profiles have been normalised/standardised so that their maximum heights/intensity (which is mainly due to eEPO) is identical. The conclusion that the athlete's "shoulder peak" migrates more slowly than rEPO and thus does not coincide with rEPO in the positive control is evident in all subsequent profiles the laboratory presents upon testing the B-sample.

Evaluation of Results of rEPO Tests Performed on the B-sample of Vojtěch Sommer's Urine

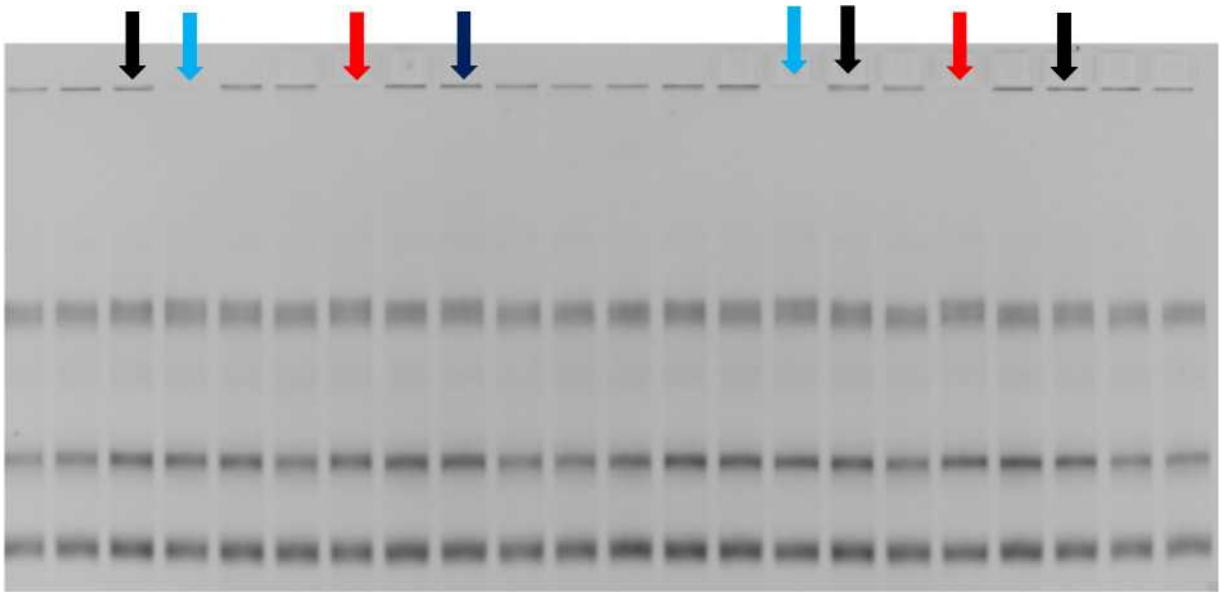


Figure 1. The fluorescence gel image on page 13 of the B-sample Documentation package dated June 2016. The vertical red arrows indicate lanes where the athlete's A-sample was applied, the vertical blue arrows indicate lanes where the athlete's B-sample was applied, and the vertical black arrows indicate lanes where negative controls (i.e. eEPO) was applied.

The laboratory presents this fluorescence gel image (Figure 1) in an incorrect way; the image is inverted (it reads from right to left) compared to the corresponding gel images (Figure 2 on page 2 below) which reads from left to right.

Note the variations in the mobility of the top band: the top band in athletes lanes (marked with red and blue vertical arrows) migrates a little more slowly (i.e. appears slightly higher in the gel) than in most of the neighbouring lanes; this is also the case (but to a lesser extent) for the top band in the negative control lanes (marked with black vertical arrows). Note also the splitting of the top band in lanes 5 and 8 [from right to left; two of the athlete's lanes], which are two lanes on which the athlete's sample was tested. This illustrates anomalies that often are observed upon PAGE-analyses of proteins and illustrates the uncertainty of assigning significance to minor differences. Note also that there is no staining on the top of the fluorescent image for four lanes (lanes 4, 7, 15 and 18 from left to right), which are the four lanes in which the athlete's sample was tested, indicating that the athlete's sample behaves somewhat differently than the control samples. Such differences are not uncommon when samples with a very different protein composition and concentration are directly compared on a gel. PAGE analysis of proteins is a method fraught with problems when trying to measure small differences in protein composition or migration. With large differences, there may be small problems, but with small differences, the chances of incorrectly reporting an adverse finding are significant.

We note that the laboratory does not disagree that the fluorescent gel image is presented incorrectly but states that the image has no influence on data evaluation.

Figure 2A

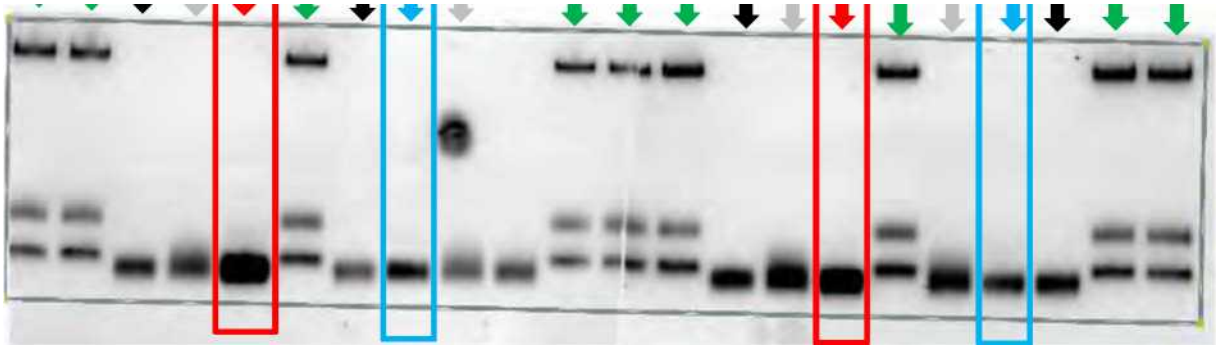
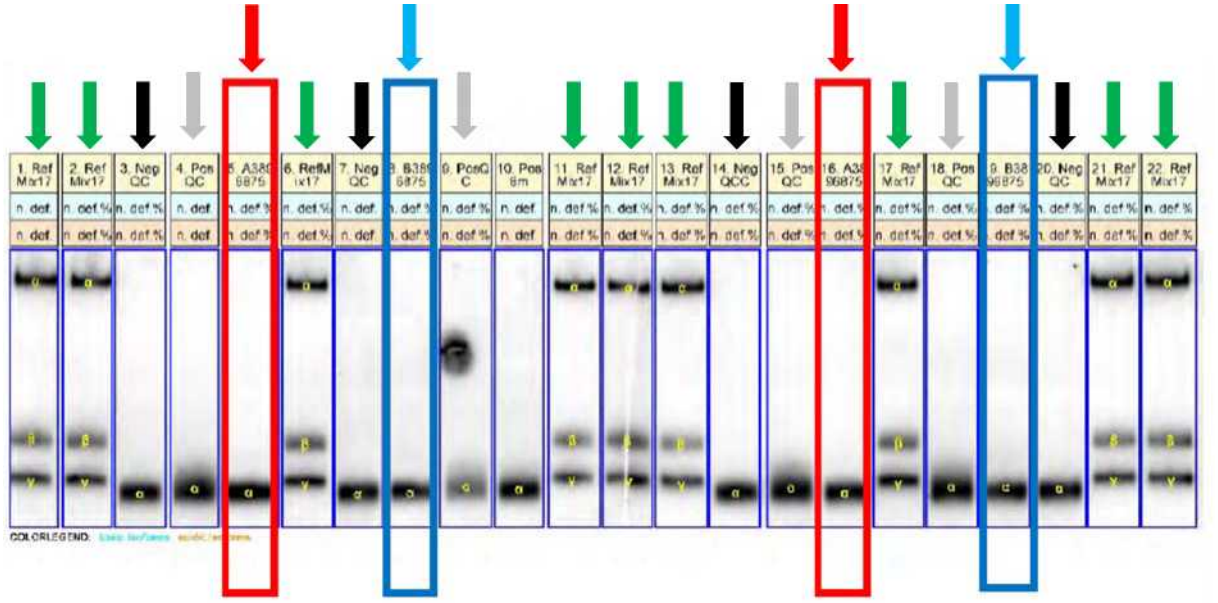


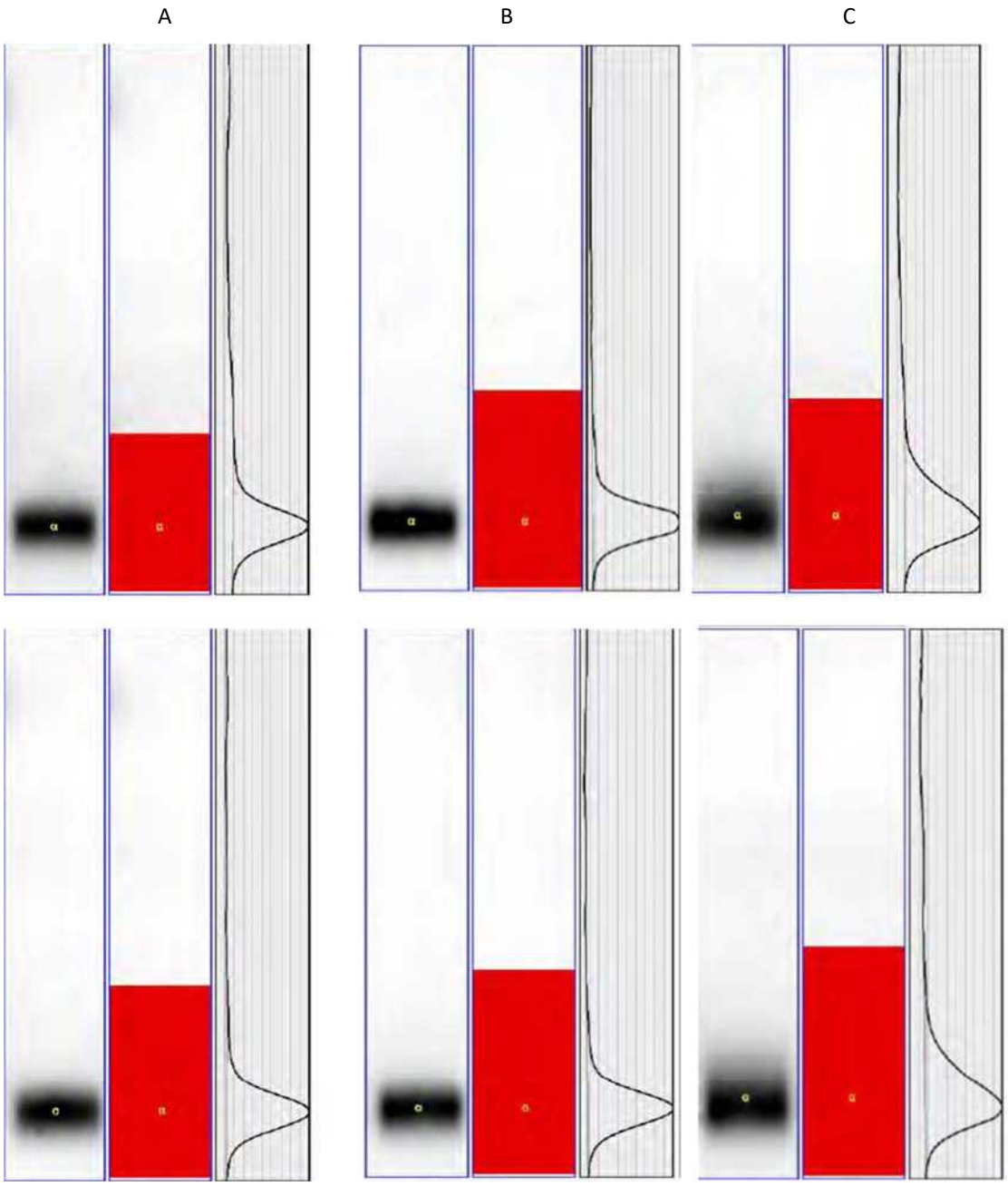
Figure 2B



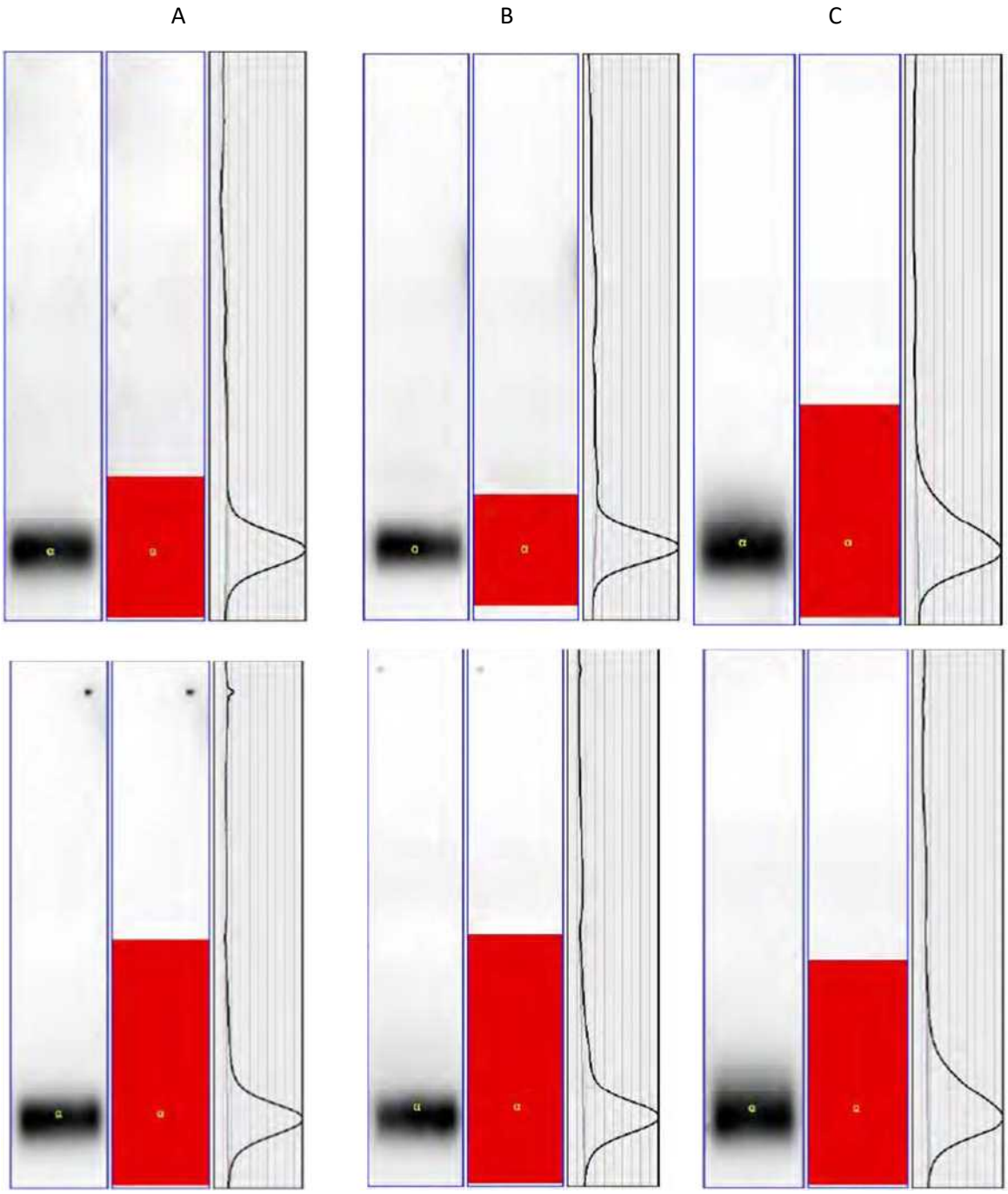
Figures 2A and 2B are both from page 14 of the «Laboratory Documentation Sample» dated June 2016.

Fig. 2A is a part of the (unprocessed) gel image of the B-sample (although test of the A-sample was also included) whereas Fig. 2B is a «GASepo processed» image of the image shown in Fig. 2A. The vertical green arrows indicate control lanes that contain different «synthetic» EPO-variants used in doping (from top: CERA, NESP and rhEPO). The vertical black arrows indicate negative control lanes that contain normal endogenous EPO (eEPO). The vertical grey arrows indicate positive control lanes that contain normal endogenous EPO (eEPO) and rEPO(s). The vertical red and blue arrows indicate athlete's lanes where the, respectively, A-sample and B-sample are tested.

Note that the athlete's A-sample eEPO band in Fig. 2A is strongly "exposed" (i.e. the athlete's eEPO band is much more strongly stained than the bands in neighbouring control lanes) presumably because of high eEPO concentrations in his A-sample. The staining above and below his band thus becomes more apparent than in the bands in neighbouring lanes. In the in the «GASepo processed» image shown in Fig. 2B the staining of the athlete's lane has been reduced (more normalized relative to the other bands) and the staining below and above is reduced and more similar to that in the neighbouring negative control bands, as is more evident in Figure 3 A-C and Figure 4 A-C below (from pages 16 & 18 in the «Laboratory Documentation Sample» December 2016)



Figures 3 A, B and C show the lanes and corresponding profiles of, respectively, (A) negative Controls (correspond to lanes 3 & 14 in Fig. 2) which contains just normal endogenous EPO (eEPO); (B) the athlete's A-sample lane and profile (correspond to lanes 5 & 16 in Fig. 2); and (C) the positive control (correspond to lanes 4 & 18 in Fig. 2) which contains both normal eEPO and rEPO. The athlete's band and profile (in B, in the middle) is similar to negative controls (in A, on the left), but clearly different from positive controls (in C, on the right). Results from pages 15-25 of the «Laboratory Documentation Sample» dated June 2016.



Figures 4 A, B and C show the lanes and corresponding profiles of, respectively, (A) negative Controls (correspond to lanes 7 & 20 in Fig. 2) which contains just normal endogenous EPO (eEPO); (B) the athlete's B-sample lane and profile (correspond to lanes 8 & 19 in Fig. 2); and (C) the positive control (correspond to lanes 15 & 18 in Fig. 2) which contains both normal eEPO and rEPO. The athlete's band and profile (in B, in the middle) is similar to negative controls (in A, on the left), but clearly different from positive controls (in C, on the right). Results from pages 15-25 of the «Laboratory Documentation Sample» dated June 2016.

Explanation to the Laboratory Documentation *Aditional Explanation Sample B3896875*

Now we turn to the results the laboratory presents in the *Laboratory Documentation Additional Explanation Sample B3896875* dated October 2016. The following figures (Figure 5) is from page 7 in that rapport.

Negative control Athletes B-sample Positive control

Negative kontrol Athlete B-sample Positive control

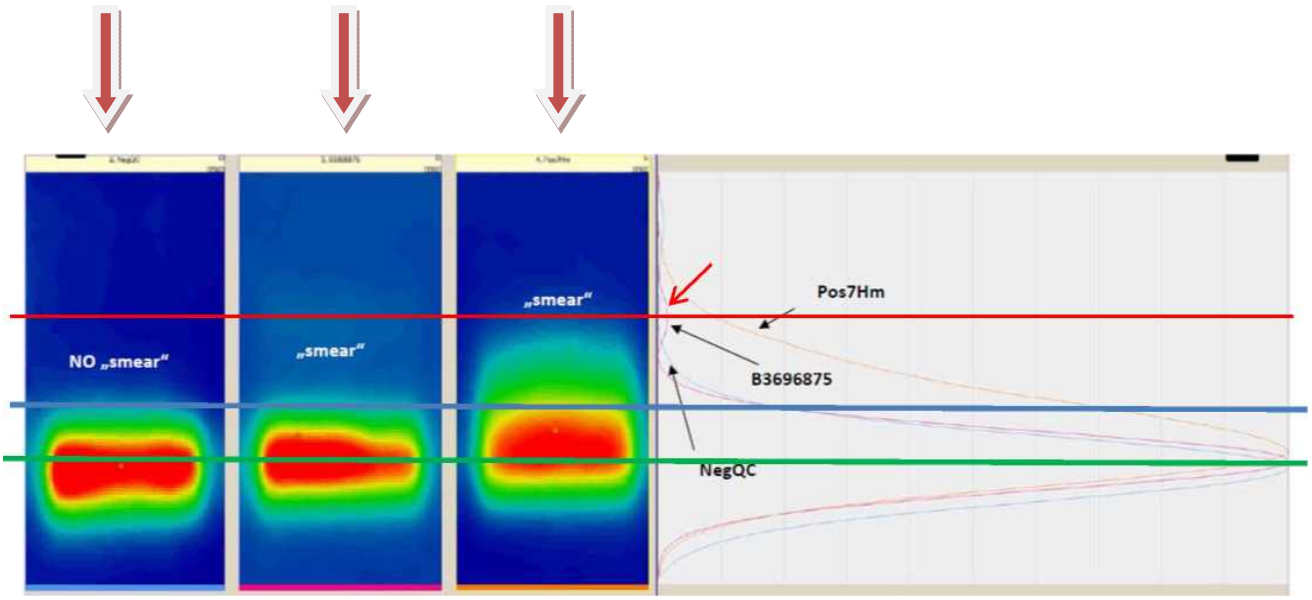


Figure 5. The athlete's EPO profile in the above figure (labelled 3696875; note the laboratory labelled it incorrectly, it should be 3896875) is almost identical to the negative-control profile (labelled NegQC). I.e. the athlete's profile coincides with the negative control profile, except for the small peak (marked with red arrow). And it cannot be concluded that the small peak on the athlete's profile is due to rEPO since it is not at all evident from the positive- and negative-control profiles that it is possible to obtain a profile similar to that obtained from the athlete's sample by combining, in different proportions, the profile with no sign of rEPO with that of only rEPO added. The horizontal green line indicates where the peak due to eEPO appears; the blue horizontal line indicates where the peak due to rEPO is expected and the red horizontal line indicates where the top of the small peak in the athlete's lane is. Where these peaks are predicted to come (if the positive control and the athletes sample did not contain eEPO) is estimated by subtracting the negative control profile (NegQC) from, respectively, the positive control profile (Pos7Hm) and the athlete's profile. It is assumed that the three samples contain similar amounts of eEPO, which should not be a "way-off" assumption since these profiles have been normalised/standardised so that their maximum heights/intensity (which is mainly due to eEPO) is identical.

The small peak (marked with red arrow) in the athlete's profile does not migrate similarly to any known rEPOs and can thus not be attributed to rEPO. This conclusion is also seen if one looks at the corresponding gel image shown on the next page (Figure 6)

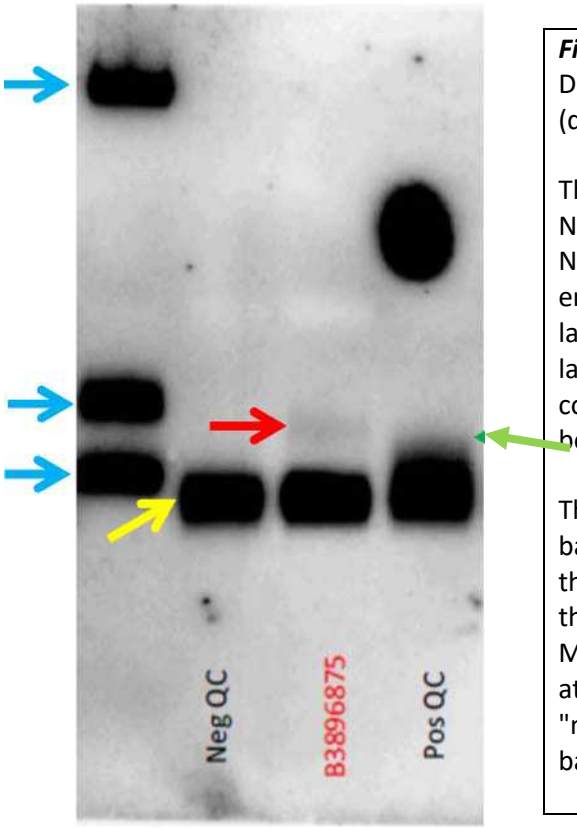


Figure 6. The figure on the left is from page 10 of Laboratory Documentation Additional Explanation Sample B3896875 (dated October 2016).

The left lane contains 3 "rEPO standards" (From top: CERA, NESP and rhEPO) marked with blue arrows; the lane labelled NegQC is a negative control showing where normal endogenous EPO (marked with yellow arrow) migrates; the lane labelled B3896875 is the athletes lane; and the lane labelled PosQC is a positive control showing a mixed band containing normal endogenous EPO and rEPO- alpha and beta.

The band labelled with the red arrow in the atletes lane (this band corresponds to the small peak labelled with red arrow in the profile in Fig. 5 above) DOES NOT correspond to any of the rEPOs in the left lane (labelled with blue arrows). Moreover, the band labelled with the red arrow in the athletes lane migrates significantly more slowly than the "mixed band in the positive control (the top of this mixed band is labelled with a green arrow).

The figure below (Figure 7) is from page 9 in the *Laboratory Documentation Additional Explanation Sample B3896875* dated October 2016. It shows basically the same as Figure 5 above, but is derived from another set of negative, positive and B-sample lanes than those shown in Figure 5.

Positive control Athletes B-sample Negative control

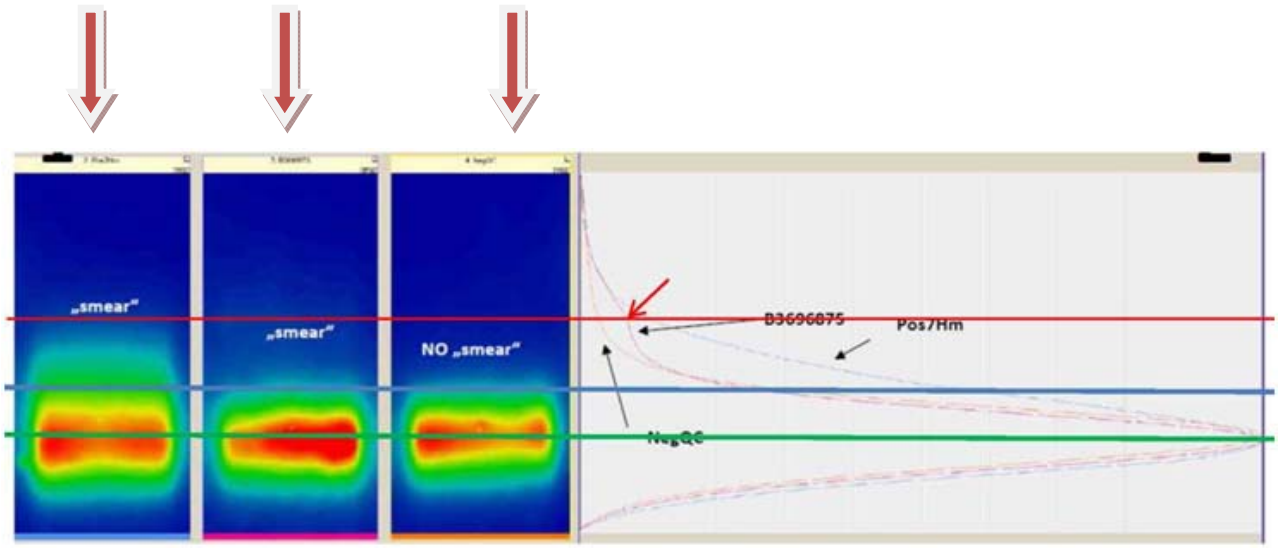


Figure 7. The athlete's EPO profile in the above figure (labelled 3696875; note the laboratory labelled it incorrectly, it should be 3896875) is almost identical to the negative-control profile (labelled NegQC). I.e.the athlete's profile coincides perfectly with the negative control profile, except for the small shoulder (marked with red arrow). And it cannot be concluded that the small shoulder on the

athlete's profile is due to rEPO since it is not at all evident from the positive- and negative-control profiles that it is possible to obtain a profile similar to that obtained from the athlete's sample by combining, in different proportions, the profile with no sign of rEPO with that of only rEPO added.

The horizontal green line indicates where the peak due to eEPO appears; the blue horizontal line indicates where the peak due to rEPO is expected and the red horizontal line indicates where the top of the small peak in the athlete's lane is. Where these peaks are predicted to come (if the positive control and the athletes sample did not contain eEPO) is estimated by subtracting the negative control profile (NegQC) from, respectively, the positive control profile (Pos7Hm) and the athlete's profile. It is assumed that the three samples contain similar amounts of eEPO, which should not be a "way-off" assumption since these profiles have been normalised/standardised so that their maximum heights/intensity (which is mainly due to eEPO) is identical.

The small peak (marked with red arrow) in the athlete's profile does not migrate similarly to any known rEPOs and can thus not be attributed to rEPO.

CONCLUSION

The differences between results obtained with the athlete's sample and the negative-control samples are due to a protein that can not be claimed to be rEPO (since its size differs from rEPOs) and to spreading and or tailing of the athlete's endogenous EPO (eEPO).

In PAGE-tests, two proteins are not identical if they do not migrate with the same speed and thus become positioned at different positions. In all the PAGE-tests performed on Vojtech Sommer's urine, it is clear that the protein that the laboratory apparently claims is rEPO has a different migration speed and positions differently in the gels than rEPOs and can therefore not claimed to be rEPO. Consequently, there is no evidence that Vojtêch Sommer used rEPO.

The laboratory's treatment of the analysis results is superficial, and illustrates again that all too many WADA accredited laboratories produce sub-optimal work that fall short of quality standards expected of analytical laboratories (see refrences 1-8). Such behaviour clearly jeopardizes the rights of athletes and can in some cases best be characterized as abusive (see for instance reference 8 and the "WAADS-letter to which it refers).

Tore Skotland Bjarne Østerud Erik Boye

Jon Nissen-Meyer

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