UiO : Department of Biosciences
The Faculty of Mathematics and Natural Sciences

Executive Summary

The Australian athlete Peter Bol delivered on the 11th of October, 2022, a urine sample that was tested for recombinant EPO (rEPO) at the WADA-accredited Australian Sports Drug Testing Laboratory. The four undersigned scientists have evaluated the laboratory's documentation packages that report the tests performed on Peter Bol's A- and B-sample. 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 Peter Bol and have no relationship with him.

Notably, in all of Bol's EPO-tests, the amount of natural/endogenous EPO in the athlete's lane was found to be dramatically higher than that in the negative control lanes. That is problematic because the negative control test is thus less sensitive for detection of proteins than the athlete's test. The more sample added to a gel lane, the more protein bands may be detected and the bands become broader and blacker and the tailing edge of bands (that are generated upon electrophoresis) becomes more apparent. Moreover, the migration of proteins in PAGE gels may be influenced by the concentration/amount of protein put on the gel.

A reliable comparison of the athlete's lane and the control lanes thus necessitates that similar amounts are applied in all lanes; a negative control lane which does not have the same amount as the athlete's lane is definitely not a proper negative control.

The laboratory tries to some extent to correct for differences in the amounts applied to the various lanes by use of GASepo processing. By GASepo processing of the raw gel-images, the staining intensity in the lanes are adjusted so that the intensity becomes similar in all lanes and it thus appears as if similar amounts have been applied to all lanes. This enables to some extent lane comparison and makes interpretation of the results more reliable.

There is no obvious staining in athlete's lanes (or in the negative control lanes) in the "rEPO region" of the gels in any of the GASepo processed gel-images. Moreover, the athlete's band in all processed gel images is symmetric, indicating that it is not a mixed band that contains both natural EPO and synthetic EPO.

Curiously, in the second opinion of the A-sample test provided by the WADA-accredited laboratory in Cologne, it is concluded

> "*that the data from the Initial Testing Procedure …show a band indicating the presence of recombinant EPO. The confirmatory analysis…corroborates the presence of recombinant EPO in the sample*".

Remarkably, the data from the Initial Testing Procedure is not provided in the documentation package and there is no band in any of the confirmatory gels (nor does the laboratory specify such a band) that indicates the presence rEPO or confirms the alleged Initial Testing Procedure. In their "Adverse and Atypical Analytical Review Report" the laboratory refers only to "*small smear in rEPO region*". A small smear is not a band! Moreover, we have not been

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able to identify a significant small smear in the rEPO region of any of the GASepo processed gel images provided by the laboratory. Nor does the laboratory specify where it is. We can see a little faint staining in the “rEPO region” in **both** the athlete’s lanes and the negative control lanes in the **raw unprocessed** gel images, and there is a little more of it in the athlete’s lanes than in the negative control lanes. But that is evidently because of the larger amount of natural EPO - and thus a broad natural EPO band - in the athlete’s lane compared to the negative control lanes.

The second opinion of the B-sample test was provided by the WADA-accredited laboratory in Oslo, and it states that

> “*The “analyses show the presence of a very weak, diffuse signal (“smear”) above the strong endogenous EPO signal. Though this could be indicative of the presence of a low amount of recombinant EPO, a reliable identification of recombinant EPO in the sample is not possible in my opinion.*”

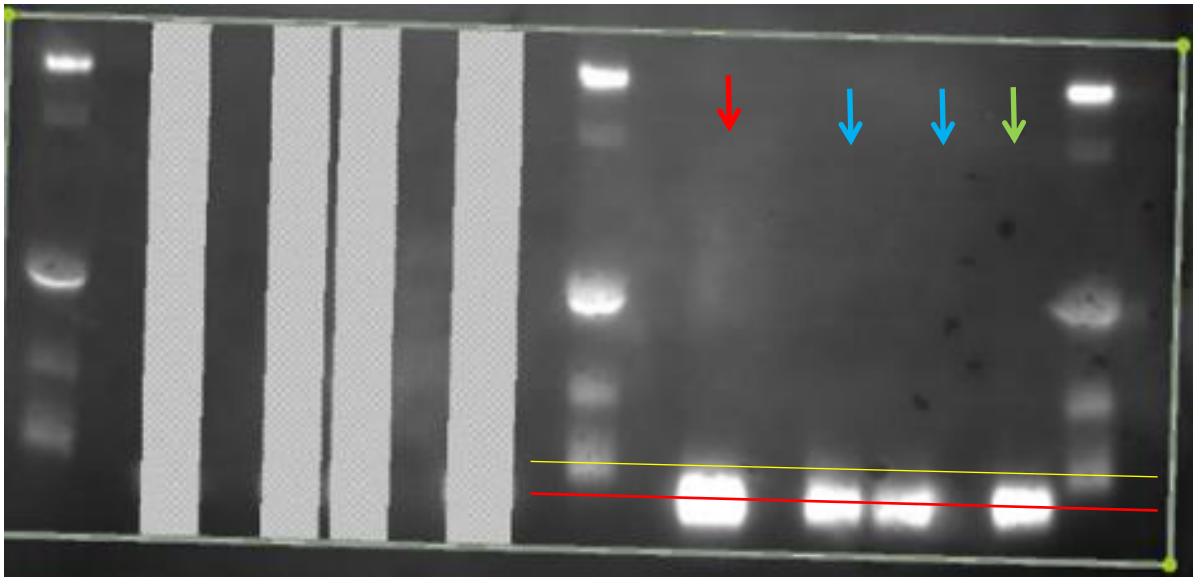
We conclude that there is **no scientific evidence provided by the laboratory which proves the presence of recombinant EPO in Bol's urine**. We refer to our evaluation more details.

Tore Skotland Bjarne Østerud Erik Boye

Jon Nissen-Meyer

Oslo, 14.03.23

Evaluation of Results of the EPO Tests Performed on Peter Bol's A-Sample



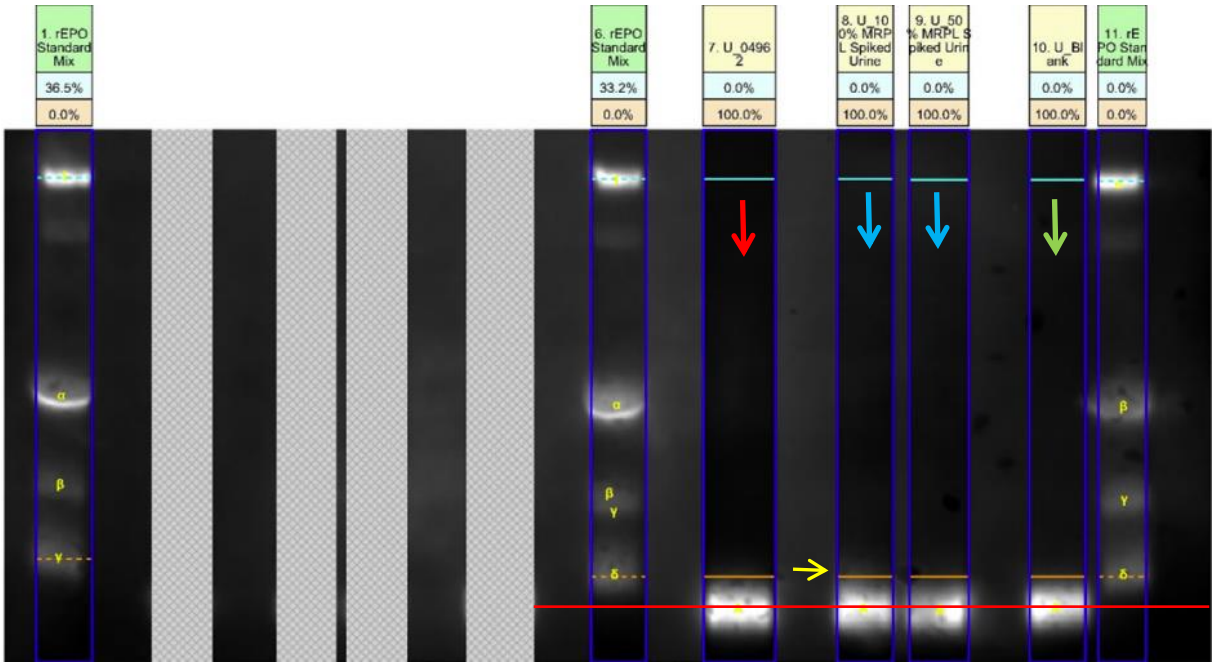
The first confirmation test: The unprocessed gel image from page 27 of the A-sample Documentation Package. It is a re-probed version of the gel presented on page 20 of the A-sample Documentation Package.

We have inserted the red arrow marking the athlete's (Bol's) lane, the green arrow marking the negative control lane (i.e. contains only natural [endogenous] EPO), and the blue arrows marking the positive control lanes (i.e. contain natural [endogenous] EPO and synthetic EPO). We have also inserted the yellow demarcation line that passed through the middle of the dynepo bands and the red line that passes through the middle of the negative control band. According to WADA's guidelines, staining above the yellow demarcation line may indicate the presence of synthetic EPO. And a band that is asymmetric with respect to the red line (which passes through the middle of the natural EPO band) may indicate a "mixed band" that contains both natural EPO and synthetic EPO.

There is some uncertainty as to where to position these two lines on this gel image because of the "skewed" migration of bands (the proteins on the right side of the gel migrated faster down than the ones on the left side). Moreover, the dynepo bands which specify the position of the yellow demarcation line are not "well defined" since they are rather blurred.

The amount of natural EPO in the athlete's lane is very much greater than it is in the negative and positive control lanes. This is problematic and makes it difficult to evaluate the results. The migration of proteins in PAGE gels may be influenced by the concentration/amount of protein put on the gel. More importantly for this case, with large amounts of protein, the bands become broader and trailing/tailing above the bands that occur during electrophoresis becomes more apparent (see the first explanatory note that was sent together with the evaluation of the B-sample test). For a good comparison, the amount of natural EPO must be similar in the athlete's lane, the positive control lane and the negative control lane.

There is a little staining above the yellow demarcation line (marked with yellow arrow) in the athlete's lane, more so than in the negative control lane, but that is likely due to the large amounts of natural EPO in the athlete's lane that resulted in a very broad natural EPO band. The two positive control lanes are not optimal; they should have been more distinct. The athlete's band seems to be symmetric with respect to the red line (i.e. there is as much staining above the red line as below), indicating that it is not a mixed band that contains both natural EPO and synthetic EPO.



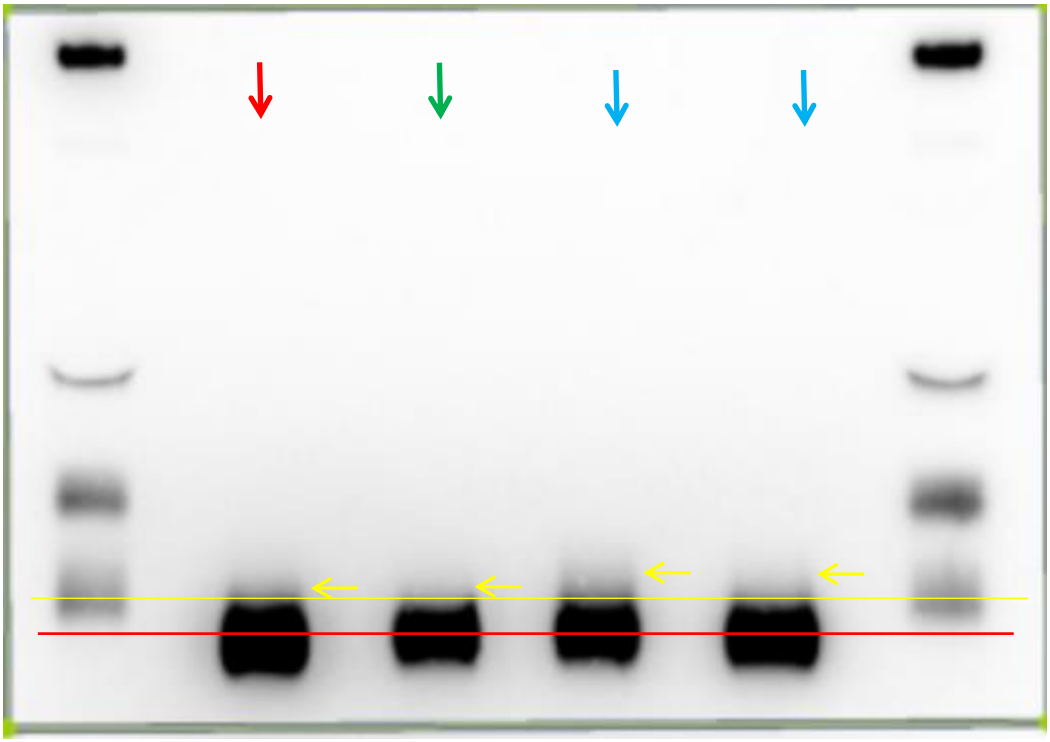
GASepo processed gel image of the unprocessed gel image shown on the previous page; from page 27 of the A-sample Documentation Package

We have inserted the red arrow marking the athlete's (Bol's) lane, the green arrow marking the negative control lane, and the blue arrows marking the positive control lanes. The laboratory inserted the yellow-orange demarcation line and we have inserted the red line that passes through the middle of the negative control band and the yellow arrow that marks very faint staining above the demarcation line in a positive control lane.

In this processed image the staining intensity in the lanes has been adjusted so that the intensity is similar in all lanes. With this adjustment, it appears as if similar amounts have been applied to each lane and this enables lane comparison and makes interpretation of the results more reliable. However, it would have been more correct to initially add nearly identical amounts to each lane so that only small adjustments were needed with the GASepo processing procedure. This because the migration of proteins in PAGE gels may be influenced by the concentration/amount of protein put on the gel. Moreover, if much smaller amounts are applied in the negative control lane than in the athlete's lane, the amount of relevant proteins in negative control lane may be so small that they are not visualized even with intensified staining. And finally, GASepo processing of a band/lane and the generation of band-profiles will not be accurate if the amount of protein in the band/lane is too large (as also pointed out on page 9; see the second explanatory note that is attached together with this evaluation).

There was no obvious staining above the orange demarcation line in the athlete's lane or in the negative control lane, indicating the absence of synthetic EPO in the athlete's sample. Moreover, the athlete's band is symmetric with respect to the red line, indicating that it is not a mixed band that contains both natural EPO and synthetic EPO. There was, however, only some very faint staining above the yellow demarcation line in one of the positive control lanes (yellow arrow).

As requested by expert, the rEPO analysis employing the SAR-PAG method was repeated by the laboratory. This test was annotated with a black background and white bands (on pages 38 -43 in the A-sample Documentation Package) and with a white background and black bands (on pages 44 -49 in the A-sample Documentation Package). We will present and discuss the results presented with a white background and black bands (pages 44-49 in the A-sample Documentation Package).

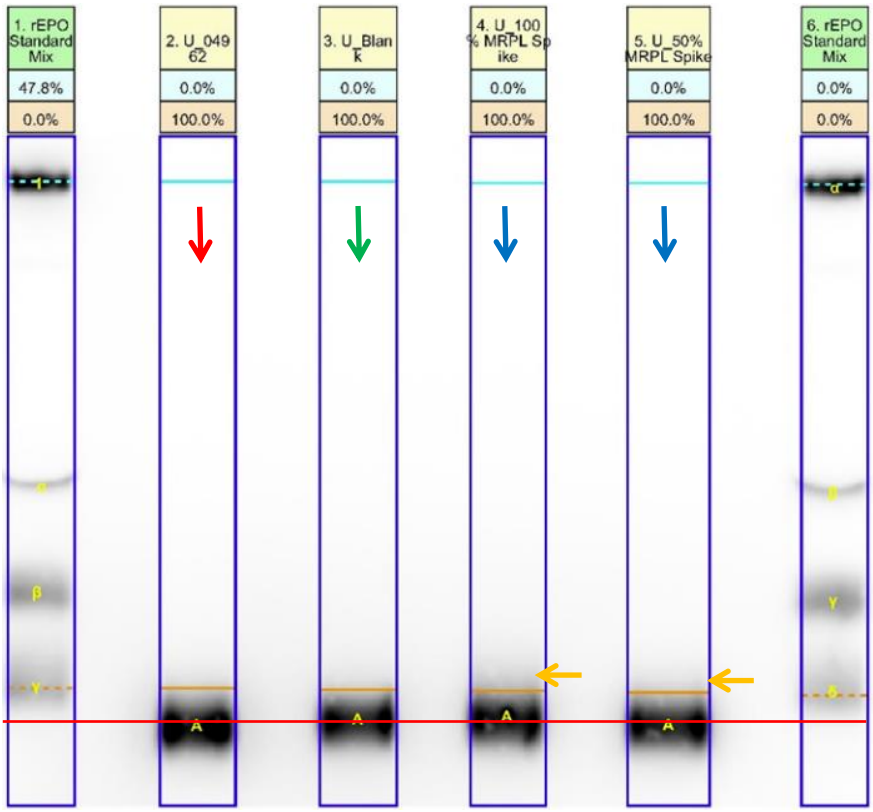


The second confirmation test: The unprocessed gel image from page 44 of the A-sample Documentation Package.

We have inserted the red arrow marking the athlete's (Bol's) lane, the green arrow marking the negative control lane (i.e. contains only natural [endogenous] EPO), and the blue arrows marking the positive control lanes (i.e. contain natural [endogenous] EPO and synthetic EPO). We have also inserted the yellow demarcation line and the red line that passes through the middle of the negative control band.

The amount of natural EPO is very large in athlete's lane, and greater than in the control lanes.

There is a little staining above the yellow demarcation line (marked with yellow arrows) in both the athlete's lane and negative control, although a little more in the athletes lane simply because of the larger amount of natural EPO - and thus a broad natural EPO band - in the athlete's lane. The athlete's band is symmetric with respect to the red line (i.e. there is as much staining above the red line as below), indicating that it is not a mixed band that contains both natural EPO and synthetic EPO. There is pronounced staining above the yellow demarcation line in both the positive control lanes (yellow arrows).



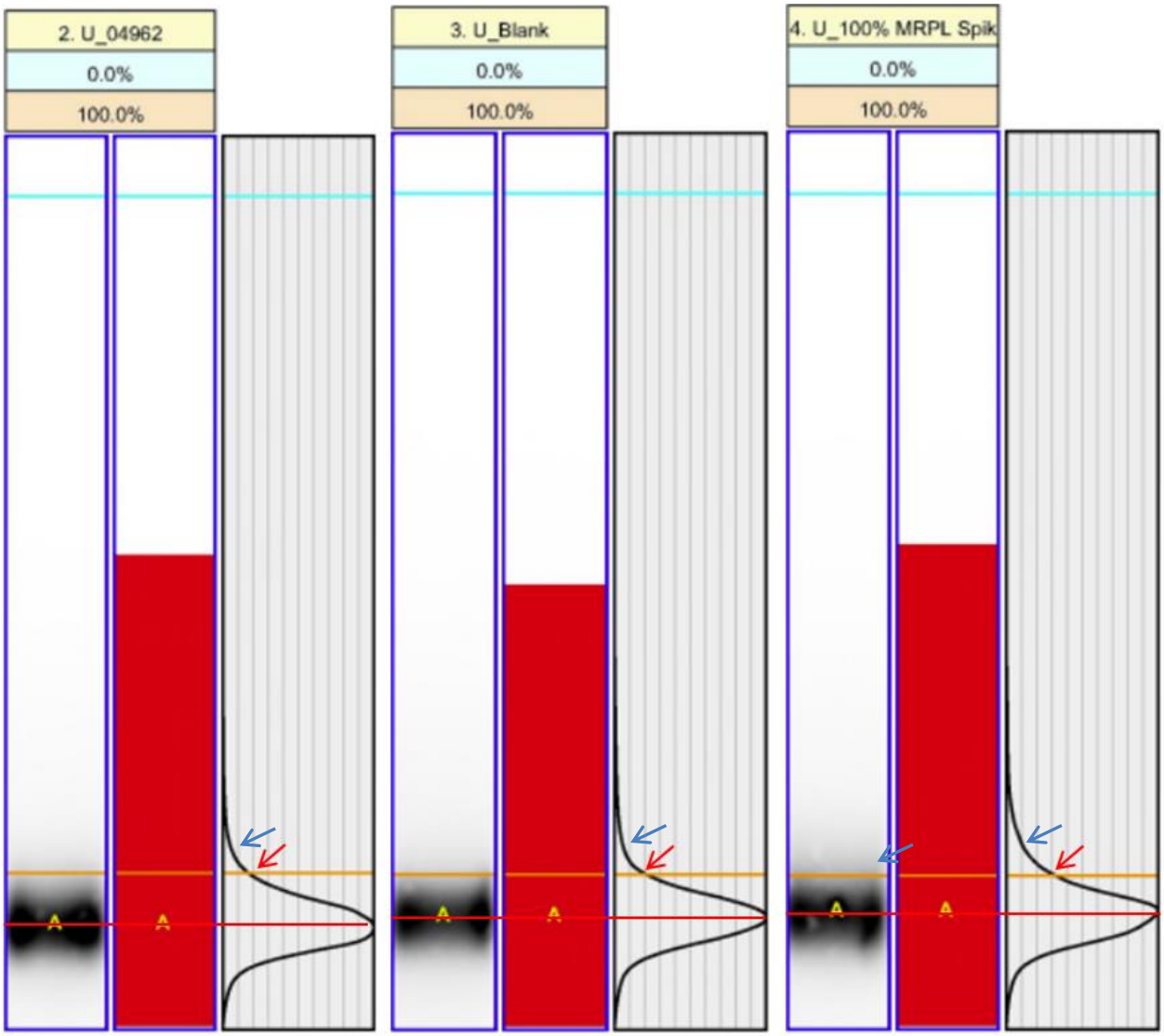
A GASepo processed version of the unprocessed gel image shown on the previous page; from page 45 of the A-sample Documentation Package

We have inserted the red arrow marking the athlete's (Bol's) lane, the green arrow marking the negative control lane, and the blue arrows marking the positive control lanes. The laboratory inserted the orange demarcation line and we have inserted the orange arrows that mark staining above the demarcation line, and the red line that passes through the middle of the negative control band.

In this processed image the staining intensity in the lanes has been adjusted so that the intensity is similar in all lanes, but there is nevertheless more in athlete's lane (the band is broader) than in negative control lane. With this adjustment, it appears as if similar amounts have been applied to each lane and this enables lane comparison and makes interpretation of the results more reliable. However, it would have been more correct to initially add nearly identical amounts to each lane so that only small adjustments were needed with the GASepo processing procedure. This because the migration of proteins in PAGE gels may be influenced by the concentration/amount of protein put on the gel. Moreover, if much smaller amounts are applied in the negative control lane than in the athlete's lane, the amount of relevant proteins in negative control lane may be so small that they are not visualized even with intensified staining.

There is some staining above the yellow demarcation line in both positive control lanes (orange arrows), but no distinct staining in the athlete's lane nor in the negative control lane, indicating the absence of synthetic EPO in the athlete's sample. Moreover, the athlete's band is symmetric with

respect to the red line, indicating that it is not a mixed band that contains both natural EPO and synthetic EPO.



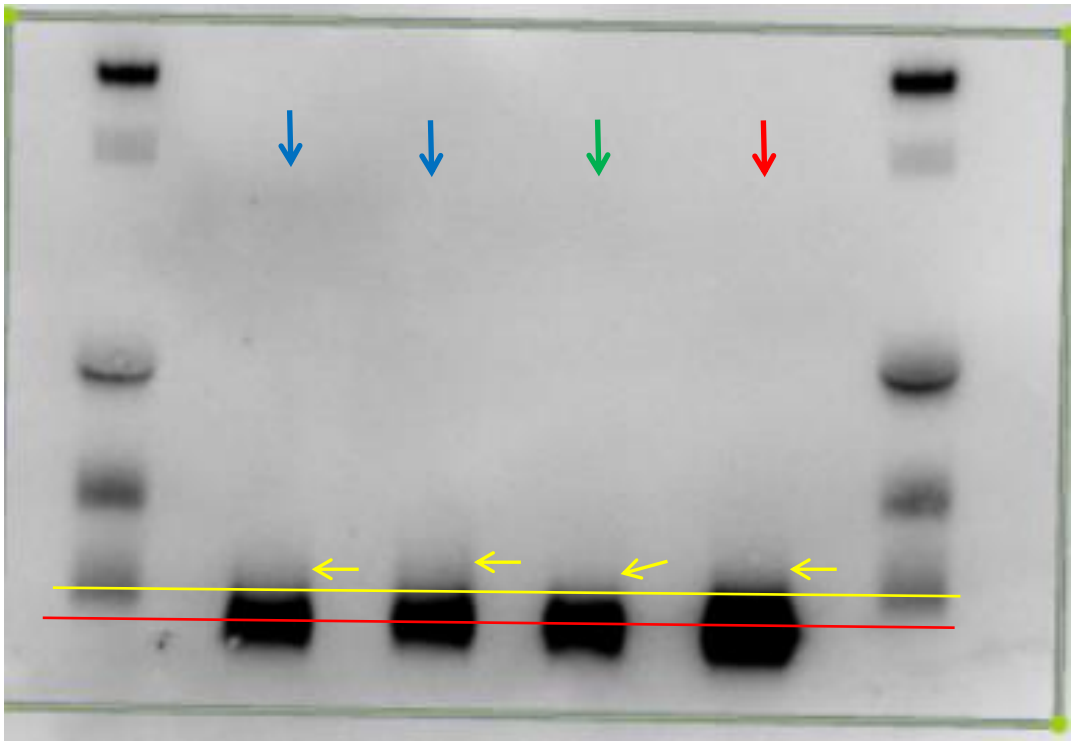
The band profiles that the laboratory presents on pages 46-47 of the A-sample Documentation Package. The profiles were obtained upon densitometric scanning the band in respectively (from left to right) the athlete's lane, the negative control lane, and a positive control lane.

These bands and profiles provide a more quantitative, rigorous and objective evaluation of the results than a mere visual inspection of the gel-bands. Importantly, these profiles have been "normalized" so that the amount of EPO is similar (i.e. the peak-height is the same) in all the profiles and this corrects to some extent the problem mentioned above – i.e. that different amounts were applied in the athlete's and in the control gel lanes.

The athlete's band and profile are clearly much more similar to the negative control band and profile than to the positive control band and profile. There is staining above the orange demarcation line in the positive control lane (blue arrow), but no distinct staining above this line in the athlete's lane or in the negative control lane. Note also that the small part of the profile (marked with blue arrow) that extends above the orange demarcation line in the athlete's lane is nearly identical to the

corresponding part (marked with blue arrow) of the negative control profile and very different from the corresponding part (marked with blue arrow) of the positive control profile. Moreover, the athlete's profile and the negative control profile intersect the orange demarcation line at the same point (marked with red arrows) which is very different from the positive control profile's intersection point (marked with red arrow). Finally, the negative control band and profile and the athlete's band and profile are symmetric with respect to the red line (inserted by us) indicating that the bands are not mixed bands that contain both natural EPO and synthetic EPO. This is in contrast to the positive control band and profile which are asymmetric with respect to the red line because the band is a "mixed band" consisting of both natural EPO and synthetic EPO.

As requested by expert, the rEPO analysis employing the SAR-PAGE/double blot method was repeated by laboratory. This test was annotated with a black background and white bands (on pages 56 -61 in the A-sample Documentation Package) and with a white background and black bands (on pages 62-67 in the A-sample Documentation Package). We will present and discuss the results presented with a white background and black bands (pages 62-67 in the A-sample Documentation Package).

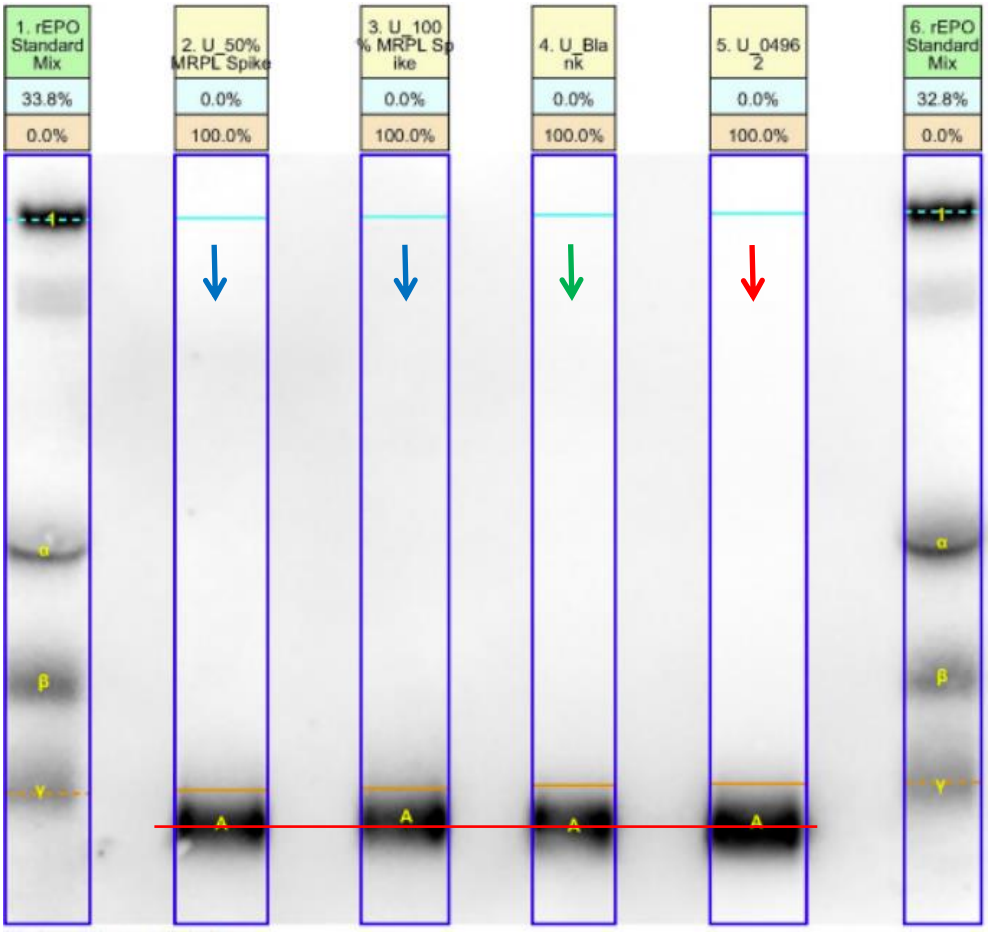


The third confirmation test: The unprocessed gel image from page 62 of the A-sample Documentation Package.

We have inserted the red arrow marking the athlete's (Bol's) lane, the green arrow marking the negative control lane (i.e. contains only natural [endogenous] EPO), and the blue arrows marking the positive control lanes (i.e. contain natural [endogenous] EPO and synthetic EPO). We have also inserted the yellow demarcation line and the red line that passes through the middle of the negative control band.

The amount of natural EPO is again much greater in the athlete's lane than in the control lanes. There is staining above the yellow demarcation line (marked with yellow arrows) in both the athlete's lane and negative control (yellow arrows), but more so in the athlete's lane which is likely due to the larger amount of natural EPO - and thus a broad natural EPO band - in the athlete's lane. The athlete's band is symmetric with respect to the red line, indicating that it is not a mixed band that

contains both natural EPO and synthetic EPO. There is marked staining above the yellow demarcation line in both the positive control lanes (yellow arrows).

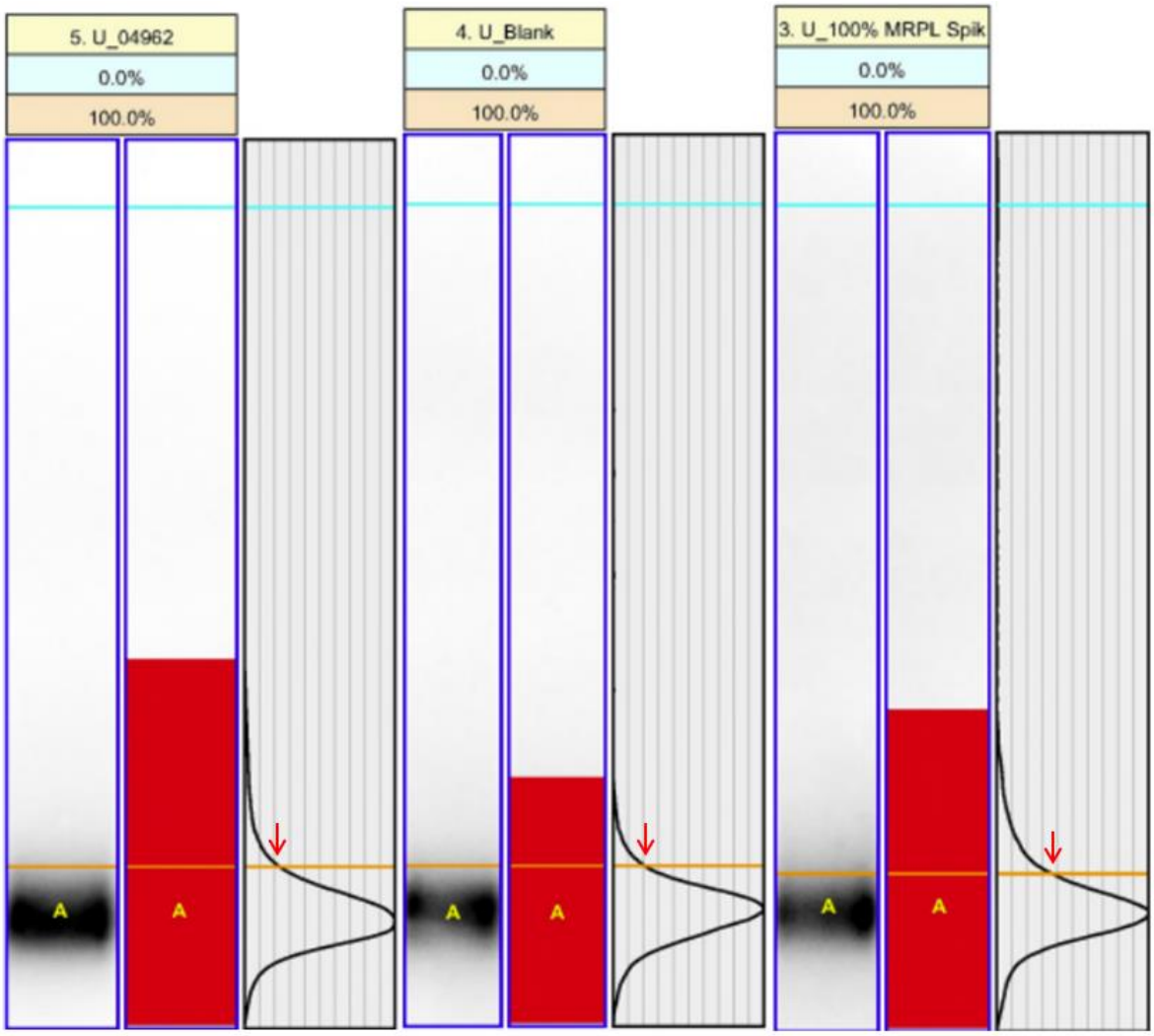


A GASepo processed version of the unprocessed gel image shown on the previous page; from page 63 of the A-sample Documentation Package

We have inserted the red arrow marking the athlete's (Bol's) lane, the green arrow marking the negative control lane, and the blue arrows marking the positive control lanes. The laboratory inserted the orange demarcation line and we have inserted the red line that passes through the middle of the negative control band.

In this processed image the staining intensity in the lanes should have been adjusted so that the intensity is similar in all lanes, but there is nevertheless more in athlete's lane (the band is broader) than in the other lanes.

There is no marked staining above the yellow demarcation line in any of these lanes. And there is in this respect not much difference between the athlete's lane and the negative control lane, even though the athlete's band is more intensely stained (the band is broader). The athlete's band is symmetric with respect to the red line, indicating that it is not a mixed band that contains both natural EPO and synthetic EPO. Both of the positive control bands are clearly asymmetric (i.e. "mixed bands") as expected, since they contain both natural EPO and synthetic EPO.



The band profiles that the laboratory presents on pages 64-65 of the A-sample Documentation Package. The profiles were obtained upon densitometric scanning the band in respectively (from left to right) the athlete's lane, the negative control lane, and a positive control lane.

These profiles have been "normalized" so that the amount of EPO is similar (i.e. the peak-height is the same) in all the profiles and this corrects to some extent the problem mentioned above – i.e. that different amounts were applied in the athlete's and in the control gel lanes – but there is still markedly more intense staining in the athlete's lane than in the other lanes in this image.

Note that the athlete's profile has a less "pointed/sharp" top and is a little broader than the negative and positive control profiles. And that is the case with all athlete-profiles shown in the A- and B-sample documentation packages. The reason for the less "pointed/sharp" top and a broader profile is that an accurate generation of a band-profile is not possible if the amount of protein in the band is too large (as also pointed out on page 2; see the second explanatory note that is attached together with this evaluation).

The athlete's band and profile are clearly more similar to the negative control band and profile than to the positive control band and profile. The athlete's profile and the negative control profile intersect

the orange demarcation line at nearly the same point (marked with red arrows) which is very different from the positive control profile's intersection point (marked with red arrow).

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 Peter Bol and have no relationship with him.

We have carefully evaluated the documents that report the tests performed on Peter Bol's A-sample and find **no scientific evidence in this document which proves the presence of synthetic EPO in Bol's urine.**

Jon Nissen-Meyer

Professor emeritus, Department of Biosciences, University of Oslo

Erik Boye

Professor emeritus, Department of Biosciences, University of Oslo

Tore Skotland

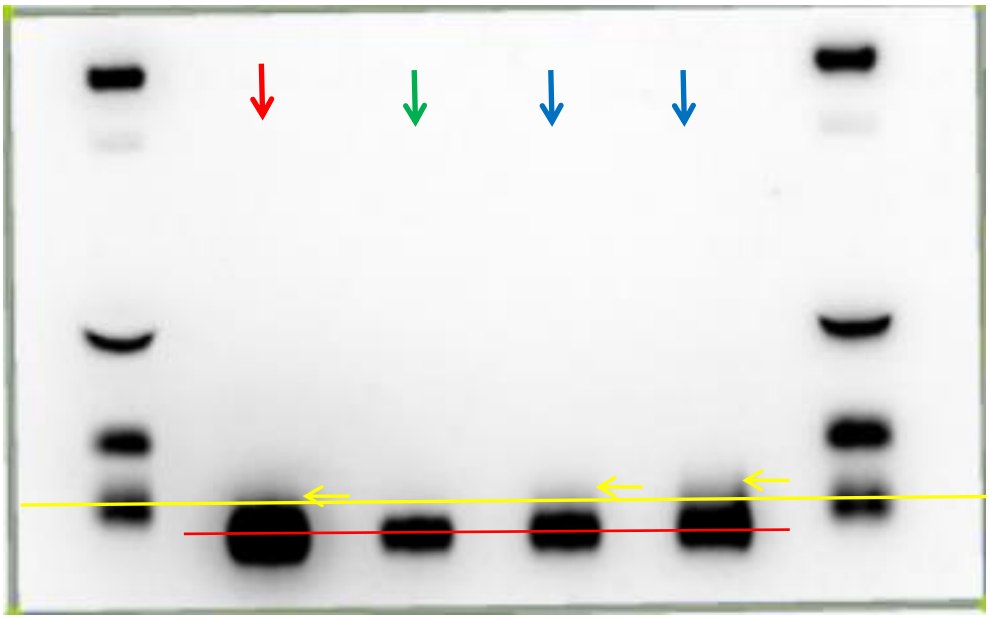
Senior scientist, Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital

Bjarne Østerud

Professor emeritus, Department of Medical Biology, UiT The Arctic University of Norway

Oslo, February 28th 2023

Evaluation of Results of the EPO Tests Performed on Peter Bol's B-Sample



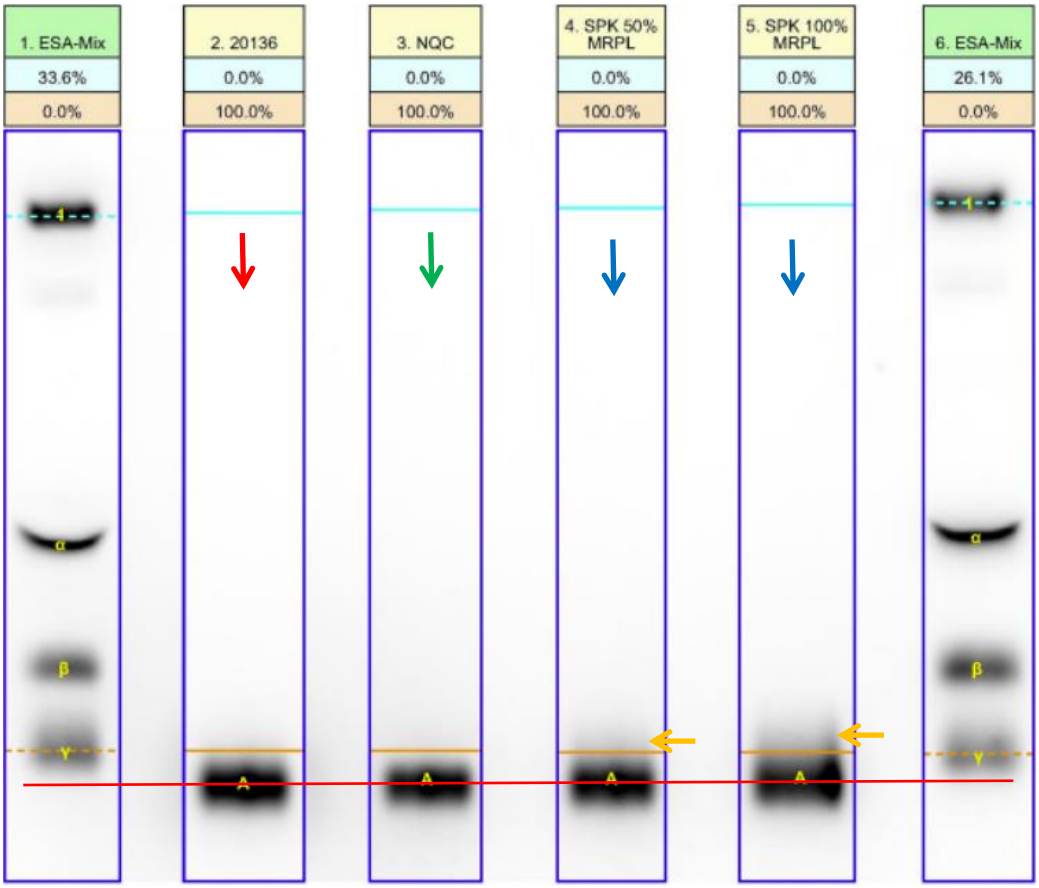
The first confirmation test: The unprocessed gel image, from page 26 of the B-sample Documentation Package.

We have inserted the red arrow marking the athlete's (Bol's) lane, the green arrow marking the negative control lane (i.e. contains only natural [endogenous] EPO), and the blue arrows marking the positive control lanes (i.e. contain natural [endogenous] EPO and synthetic EPO). We have also inserted the yellow demarcation line and the red line that passes through the middle of the negative control band.

Note that the amount of natural EPO in the athlete's lane is very much greater than it is in the negative control lane and also greater than in the two positive control lanes. This is problematic since the migration of proteins in PAGE gels may be influenced by the concentration/amount of protein put on the gel. With large amounts of protein, the bands become broader and trailing/tailing above the bands that occur during electrophoresis becomes more apparent (see the attached explanatory note). For a good comparison, the amount of natural EPO should be similar in the athlete's lane, the positive control lane and the negative control lane.

According to WADA's guidelines, staining above the yellow demarcation line may indicate the presence of synthetic EPO. And a band that is asymmetric with respect to the red line (which passes through the middle of the natural EPO band) may indicate a "mixed band" that contains both natural EPO and synthetic EPO.

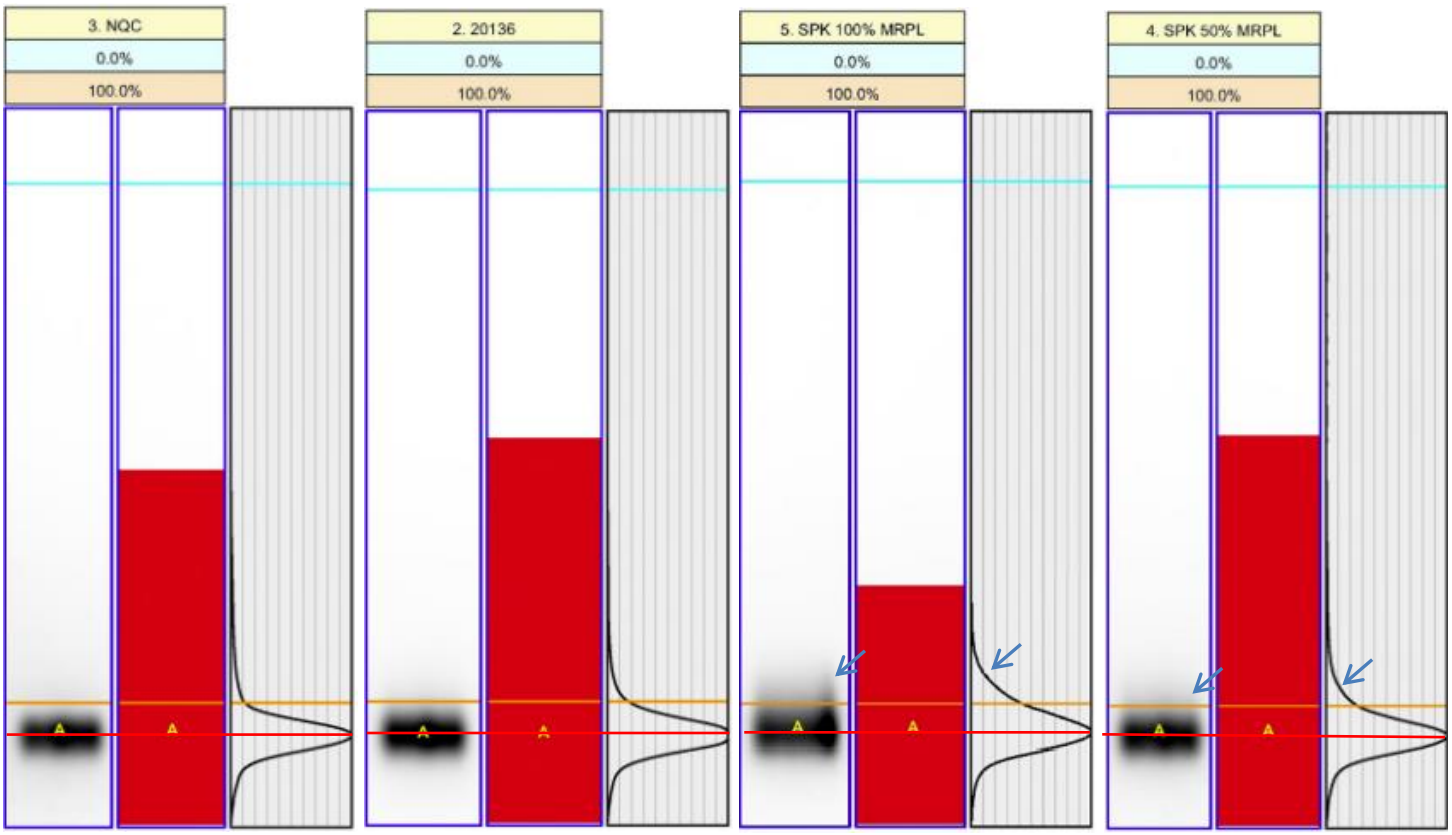
There is a little staining above the yellow demarcation line (marked with yellow arrow) in the athlete's lane, more so than in the negative control lane, but that is simply due the large amounts of natural EPO that result in a very broad natural EPO band. The staining above the yellow demarcation line is stronger in the positive control lanes, even though they contain less EPO than the athlete's lane. As is the case with the negative control band (and in contrast to the positive control bands), the athlete's band is symmetric with respect to the red line, indicating that it is not a mixed band that contains both natural EPO and synthetic EPO.



The GASepo processed gel image of the unprocessed gel image shown on the previous page; from page 26 of the B-sample Documentation Package. In this processed image the staining intensity in the lanes has been adjusted so that the intensity is about the same in all lanes. With this adjustment, it appears as if similar amounts have been applied to each lane and this enables lane comparison and makes interpretation of the results more reliable. However, it would have been more correct to initially add nearly identical amounts to each lane so that only small adjustments were needed with the GASepo processing procedure. This because the migration of proteins in PAGE gels may be influenced by the concentration/amount of protein put on the gel. Moreover, if much smaller amounts are applied in the negative control lane than in the athlete's lane, the amount of relevant proteins in negative control lane may be so small that they are not visualized even with intensified staining.

We have inserted the red arrow marking the athlete's (Bol's) lane, the green arrow marking the negative control lane, and the blue arrows marking the positive control lanes. The laboratory inserted the yellow-orange demarcation line and we have inserted the red line that passes through the middle of the negative control band and the orange arrows that mark staining above the demarcation line.

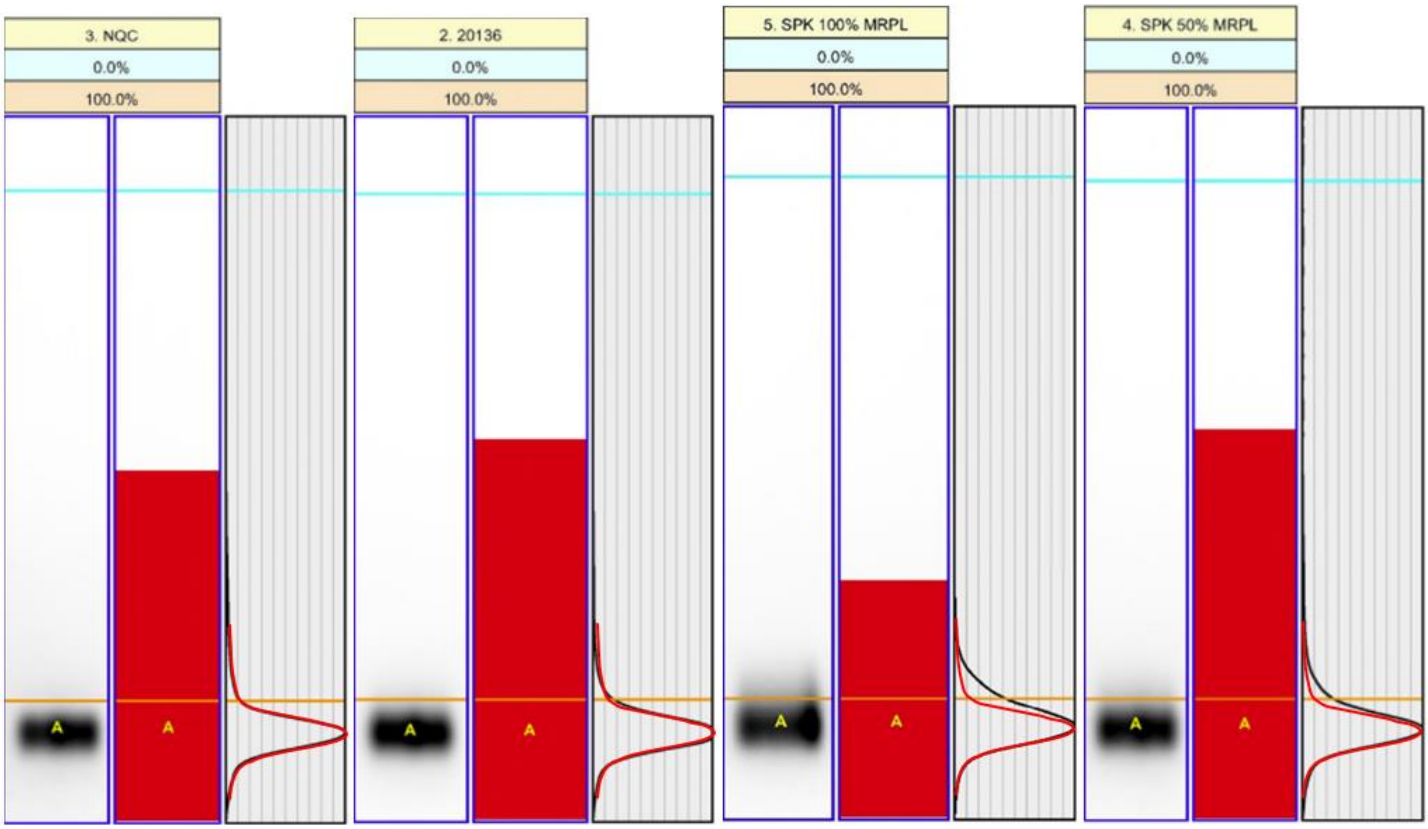
There is staining above the yellow demarcation line in both positive control lanes (orange arrows), but no staining in the athlete's lane nor in the negative control lane, indicating the absence of synthetic EPO in the athlete's sample. As is the case with the negative control band (and in contrast to the positive control bands), the athlete's band is symmetric with respect to the red line, indicating that it is not a mixed band that contains both natural EPO and synthetic EPO.



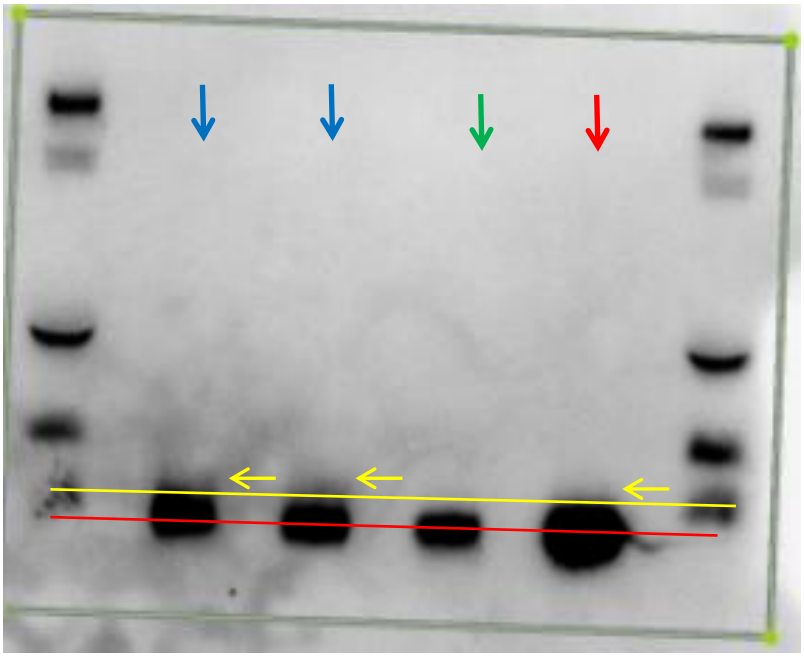
The band profiles that the laboratory presents on pages 27-29 of the B-sample Documentation Package. The profiles were obtained upon densitometric scanning the band in respectively (from left to right) the negative control lane, the athlete's lane, and two positive control lanes.

These bands and profiles provide a much more quantitative, rigorous and objective evaluation of the results than a mere visual inspection of the gel-bands. Importantly, these profiles have been "normalized" so that the amount of EPO is similar (i.e. the peak-height is the same) in all the profiles and this corrects to some extent the problem mentioned above – i.e. that different amounts were applied in the athlete's and in the control gel lanes.

The athlete's band and profile are clearly much more similar to the negative control band and profile than to the two positive control profiles. There is staining above the yellow demarcation line in both positive control lanes (blue arrows), but no staining in the athlete's lane nor in the negative control lane. Moreover, the negative control band and profile and the athlete's band and profile are symmetric with respect to the red line (inserted by us) indicating that the bands are not mixed bands that contain both natural EPO and synthetic EPO. The positive control bands and profiles are asymmetric with respect to the red line since the bands are "mixed bands" consisting of both natural EPO and synthetic EPO.



The profiles in black underneath the red profiles are the same as shown on the previous page and are, respectively, (from left to right) the negative control lane, the athlete's lane, and the two positive control lanes. We made a red copy of the negative control profile (left lane) and then superimposed this red copy on the athlete's profile (in black) and the two positive control profiles (also in black). The athlete's profile is clearly much more similar to the negative control profile than to the two positive control profiles, again indicating that the test of the athlete's B-sample is negative for synthetic EPO.



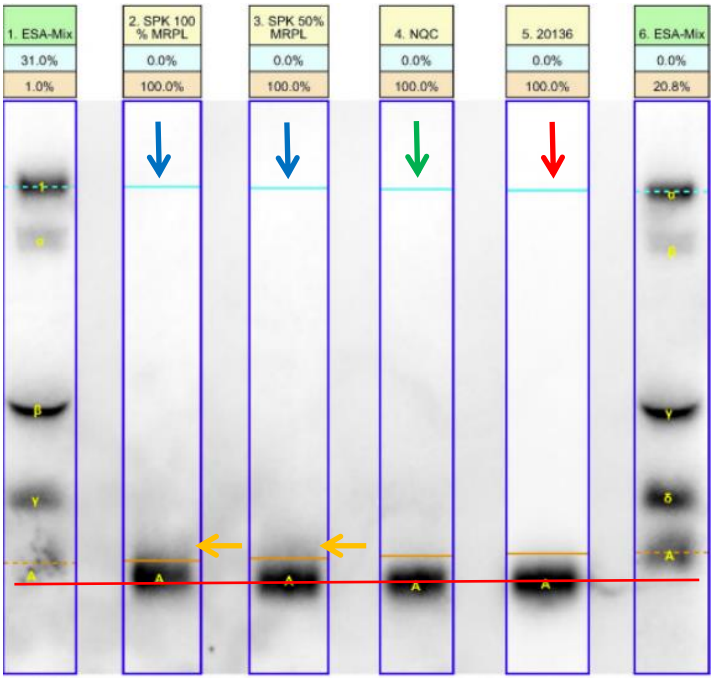
The second confirmation test: The unprocessed gel image from page 43 (same as on page 38) of the B-sample Documentation Package.

We have inserted the red arrow marking the athlete's (Bol's) lane, the green arrow marking the negative control lane (i.e. contains only natural [endogenous] EPO), and the blue arrows marking the positive control lanes (i.e. contain natural [endogenous] EPO and synthetic EPO). We have also inserted the yellow demarcation line and the red line that passes through the middle of the negative control band. There is some uncertainty where to position these lines because of the "skewed" migration of bands (the proteins on the right side of the gel migrated faster down than the ones in the left side) and because the dynepo band in the left lane is blurred.

Note that the amount of natural EPO in the athlete's lane is again very much greater than it is in the negative control lane and also greater than in the two positive control lanes. This is problematic since the migration of proteins in PAGE gels may be influenced by the concentration/amount of protein put on the gel. With large amounts of protein, the bands become broader and trailing/tailing above the bands that occur during electrophoresis becomes more apparent (see the attached explanatory note). For a good comparison, the amount of natural EPO should be similar in the athlete's lane, the positive control lane and the negative control lane.

According to WADA's guidelines, staining above the yellow demarcation line may indicate the presence of synthetic EPO. And a band that is asymmetric with respect to the red line (which passes through the middle of the natural EPO band) may indicate a "mixed band" that contains both natural EPO and synthetic EPO.

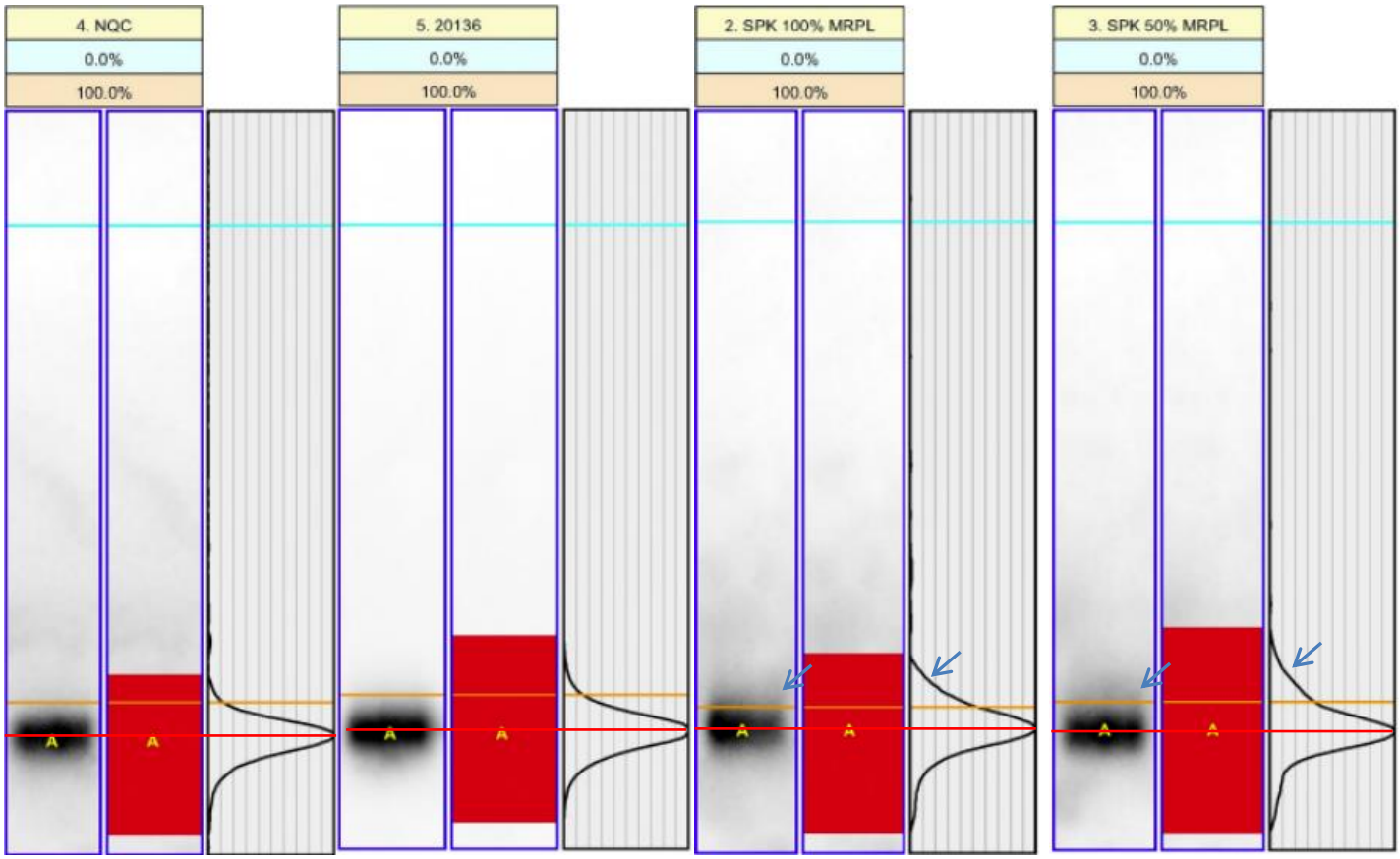
There is again a little staining above the yellow demarcation line (marked with yellow arrow) in the athlete's lane, more so than in the negative control lane, but that is simply due the large amounts of natural EPO that result in a very broad natural EPO band. The staining above the yellow demarcation line is stronger in the positive control lanes, even though they contain less EPO than the athlete's lane. As is the case with the negative control band (and in contrast to the positive control bands), the athlete's band is symmetric with respect to the red line, indicating that it is not a mixed band that contains both natural EPO and synthetic EPO.



The GASepo processed gel image of the unprocessed gel image shown on the previous page; from page 43 (same as on page 38, but now with white background) of the B-sample Documentation Package. In this processed image the staining intensity in the lanes has been adjusted so that the intensity is about the same in all lanes. With this adjustment, it appears as if similar amounts have been applied to each lane and this enables lane comparison and makes interpretation of the results more reliable. However, it would have been more correct to initially add nearly identical amounts to each lane so that only small adjustments were needed using the GASepo processing procedure. This because the migration of proteins in PAGE gels may be influenced by the concentration/amount of protein put on the gel. Moreover, if much smaller amounts are applied in the negative control lane than in the athlete's lane, the amount of relevant proteins in the negative control lane may be so small that they are not visualized even with intensified staining.

We have inserted the red arrow marking the athlete's lane, the green arrow marking the negative control lane, and the blue arrows marking the positive control lanes. The laboratory inserted the yellow-orange demarcation line and we have inserted the red line that passes through the middle of the negative control band and the orange arrows that mark staining above the demarcation line.

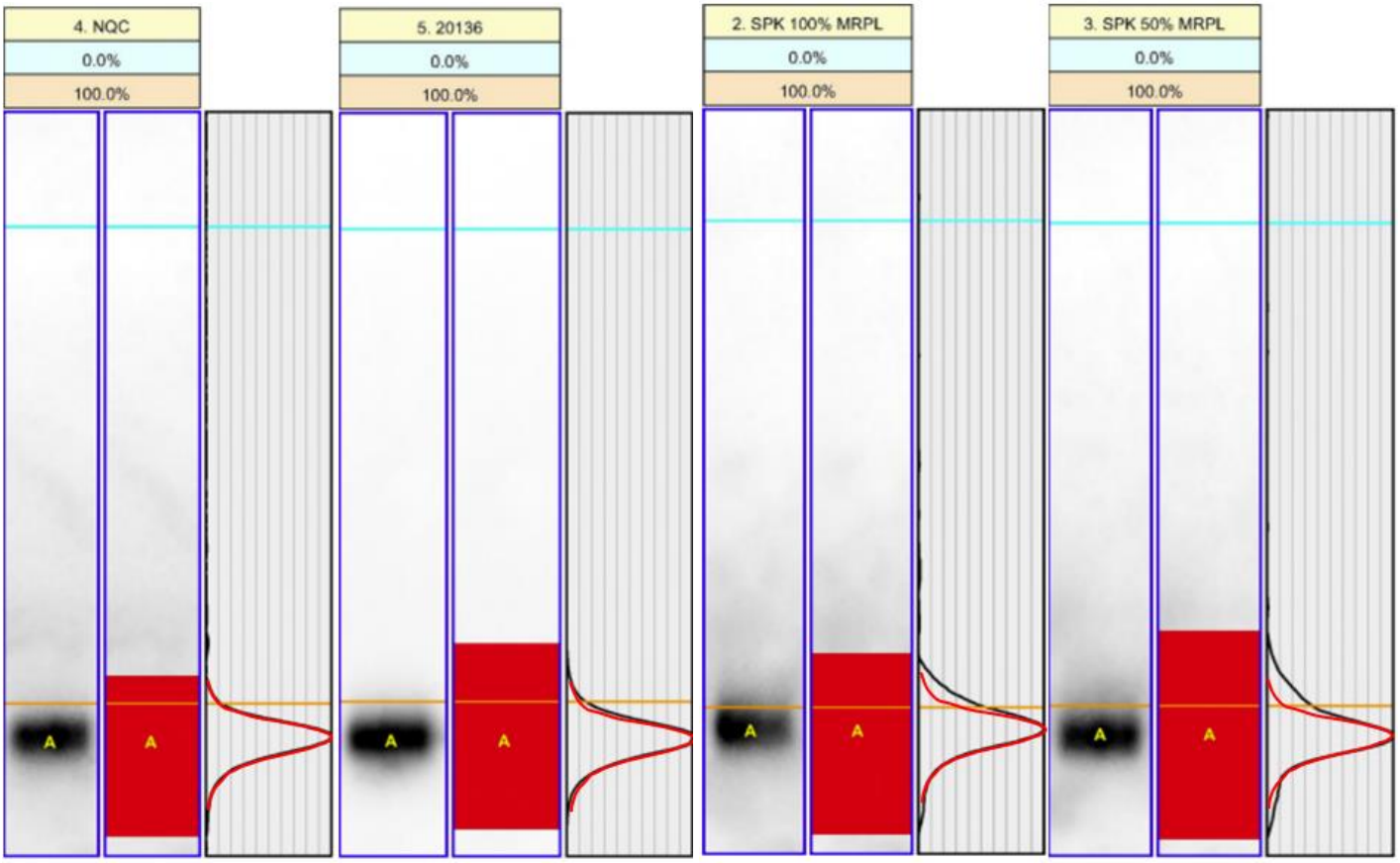
There is staining above the yellow demarcation line in both positive control lanes (orange arrows), but no staining in the athlete's lane nor in the negative control lane, indicating the absence of synthetic EPO in the athlete's sample. As is the case with the negative control band (and in contrast to the positive control bands), the athlete's band is symmetric with respect to the red line, indicating that it is not a mixed band that contains both natural EPO and synthetic EPO.



The band profiles that the laboratory presents on pages 44-46 of the B-sample Documentation Package. The profiles were obtained upon densitometric scanning the band in respectively (from left to right) the negative control lane, the athlete's lane, and two positive control lanes.

These bands and profiles provide a much more quantitative, rigorous and objective evaluation of the results than a mere visual inspection of the gel-bands. Importantly, these profiles have been "normalized" so that the amount of EPO is similar (i.e. the peak-height is the same) in all the profiles and this corrects to some extent the problem mentioned above – i.e. that different amounts were applied in the athlete's and in the control gel-lanes.

The athlete's band and profile are clearly much more similar to the negative control band and profile than to the two positive control profiles. There is staining above the yellow demarcation line in both positive control lanes (blue arrows), but no staining in the athlete's lane nor in the negative control lane. Moreover, the negative control band and profile and the athlete's band and profile are symmetric with respect to the red line (inserted by us) indicating that the bands are not mixed bands that contain both natural EPO and synthetic EPO. The positive control bands and profiles are asymmetric with respect to the red line since the bands are "mixed bands" consisting of both natural EPO and synthetic EPO.



The profiles in black underneath the red profiles are the same as shown on the previous page and are, respectively, (from left to right) the negative control lane, the athlete's lane, and the two positive control lanes. We made a red copy of the negative control profile (left lane) and then superimposed this red copy on the athlete's profile (in black) and the two positive control profiles (also in black). The athlete's profile is clearly much more similar to the negative control profile than to the two positive control profiles, again indicating that the test of the athlete's B-sample is negative for synthetic EPO.

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 Peter Bol and have no relationship with him.

We have carefully evaluated the documents that report the tests performed on Peter Bol‘s B-sample and find **no scientific evidence in this document which proves the presence of synthetic EPO in Bol's urine.**

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Professor emeritus, Department of Biosciences, University of Oslo

Erik Boye

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Tore Skotland

Senior scientist, Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital

Bjarne Østerud

Professor emeritus, Department of Medical Biology, UiT The Arctic University of Norway

Oslo, February 26th 2023

An explanatory note

As we state in our evaluation, in all of the EPO-tests of Bol's sample, there is very much more natural (endogenous) EPO applied to Bol's lanes than to the negative control lanes. And that is a major problem with the tests.

The more sample added to a gel lane, the more protein bands are detected and the bands become broader and blacker and the tailing edge of bands (that are generated upon electrophoresis) becomes more apparent. That is a (trivial and obvious) fact that should be evident to everyone that perform PAGE analysis. The "Merck gel" example shown below illustrates this point.

Effect of increasing the amount of protein sample added to gel lanes.

Different amounts (1, 5 and 10 microliters) of the exact same protein sample have been applied on the three lanes.

With increasing amounts added to lanes, bands become broader and a tailing edge towards higher molecular weights (above the bands) becomes more apparent, **simply because the system becomes more sensitive**. And new protein bands that were not detected with less amounts added to the gel, may appear upon increasing the amount added to the gel.

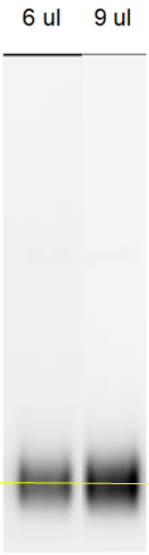
One could have obtained the same result as seen in the 5 ul and 10 ul lanes if one (instead of adding more amounts of the sample) had strongly increased the staining intensity of the 1 ul lane. I.e. one can increase the "sensitivity" both by adding more sample to a lane and by increasing the staining intensity.

A red box around one of the bands was inserted to illustrate the point that adding more of the sample to a lane generates broader bands and that tailing/trailing that may occur (to lesser or greater extent) during electrophoresis becomes more apparent. The yellow line marks the approximate middle of the intense part of the band. The red arrows mark some of the new protein bands that were not detected with less amount added to the gel.

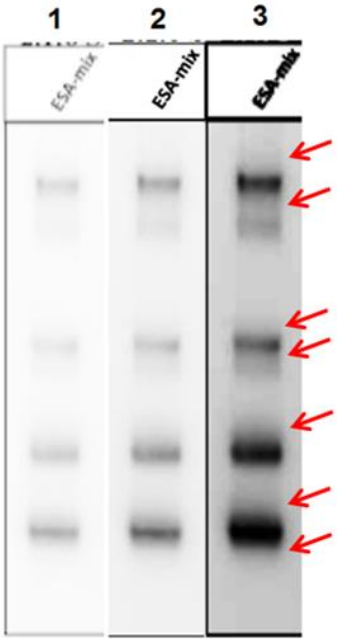
The figure is from *Merck* and can be found on the link:

https://www.sigmaaldrich.com/NO/en/technical-documents/technical-article/protein-biology/gel-electrophoresis/sample-preparation-gel-electrophoresis#BandsAreSmearedVertically

Even small differences (6 and 9 microliters, see figure below) in the amount of one and the same protein sample (in this case a sample that contains natural EPO) broadens and darkens protein bands and makes the diffuse grey staining around the dark part of the bands more apparent (yellow line marks the approximate middle of the bands).



As mentioned above, increasing the staining intensity increases the sensitivity and thus more protein is detected/“visualized”. Increasing the staining intensity has thus about the same effect as increasing the amount of protein sample added to gel lanes: the protein bands become broader, darker and protein tailing/trailing above protein bands that may occur during electrophoresis becomes more apparent. This is illustrated in the two figures below.



Lane 1 and 3 are the same as lane 2, except with reduced and increased staining intensity, respectively. Note how the appearance of the tailing above and below the bands increases markedly with the staining intensity (red arrows).

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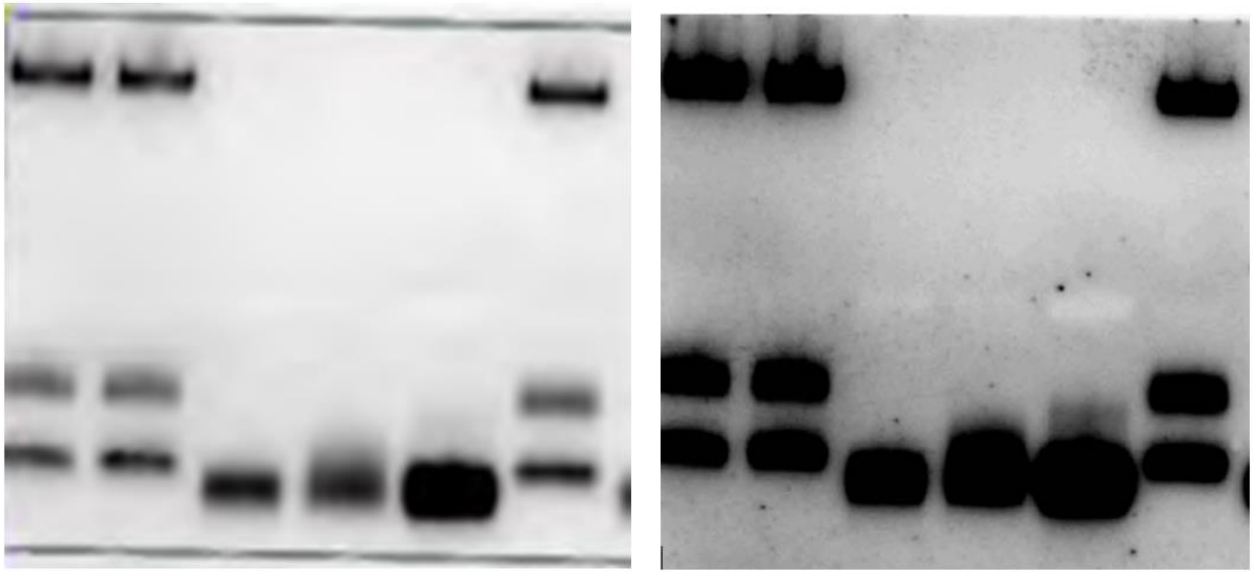
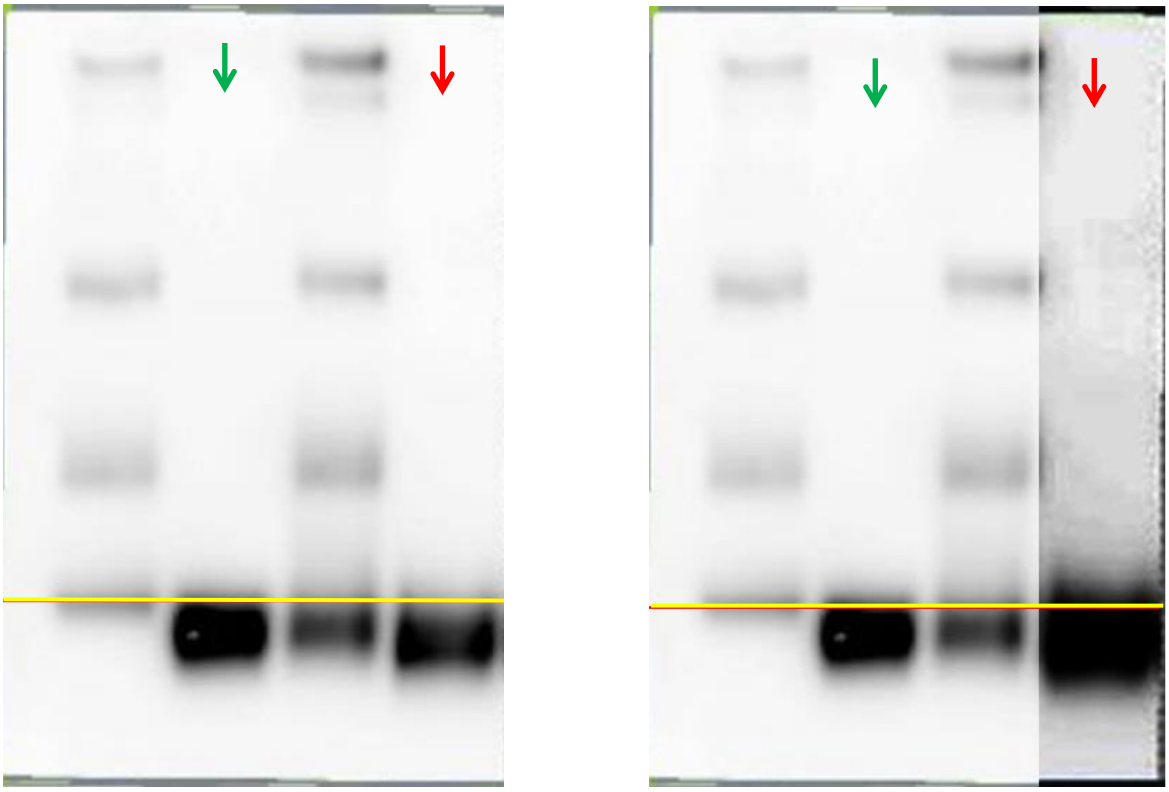
Had the left lane above been a negative control for a test and the right lane an athlete’s sample, then the conclusion would have been - I guess - that this must an adverse finding. Or perhaps just an atypical/suspicious finding, but two atypical/suspicious findings (one in in ITP and one in the CP) is equivalent an adverse finding and proves with 100% certainty that athlete is guilty, is’nt that how it works??.

The two gel images below are from one and the same gel, the only difference is that the right image has a higher staining intensity than the left image. Note how the bands in the right image are much broader and darker, almost as if the amount of protein in the right image is much greater than in the left image.



The (i) amount added to sample lanes and (ii) staining intensity of lanes **does** indeed matter when testing for EPO; alterations in these parameters can change a negative test to a positive test and a positive test to a negative test.

To illustrate this, I have intensified the staining of a test which was (correctly) judged by a WADA laboratory to be negative for synthetic EPO.



The left gel image is part of the unprocessed screening gel image which the laboratory correctly interpreted as negative for synthetic EPO. Note that the amount applied in negative control lane (green arrow) in this test is greater than the amount applied in the athlete's lane (red arrow). The opposite was very very much the case in all of the test performed on Bol's sample.

The right image is the same as the left image, except that I have intensified the "staining" of the athlete's lane (i.e. the right lane). The result would have been similar if (instead of intensified staining) more of the athlete's sample had been added to his lane. Now it looks more like the tests done on Bol's sample: the amount of natural EPO in the athlete's lane is now much greater than in the negative control lane. Many WADA laboratories would now (incorrectly) have judged this to be a positive test since there is marked staining above the yellow "demarcation line".

Thus: increasing the amount applied of athlete's sample and/or increasing the staining intensity of athlete's lane may change a negative test to a positive one!

This shows the importance of not "overloading" (or increasing the staining intensity of) the athlete's lane compared to the negative control lane. A negative control lane which does not have the same sensitivity as the athlete's lane is clearly not a correct negative control. Ideally, the negative control lane, the positive control lane and the athlete's lane should all contain the same amount of natural EPO. We see again and again that WADA laboratories disregard this important and obvious rule, especially when athletes are tested positive for synthetic EPO. Large amount in athlete's lane relative to the amount in the negative control was the problem when the Dresden laboratory incorrectly tested Vojtech Sommer positive for EPO and is the problem with Peter Bol's allegedly positive test.

Jon Nissen-Meyer

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26.02.23

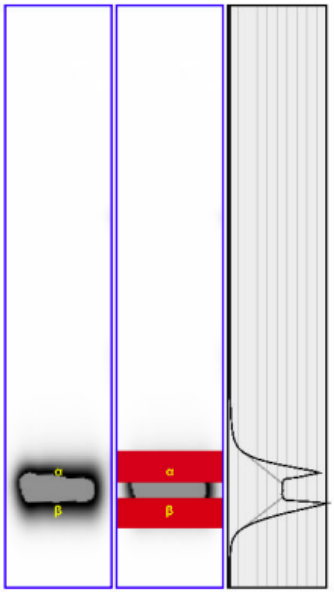
Explanatory note number two

I will in the following try to explain (somewhat simplified) why profiles of bands are not always an accurate presentation of the results, especially if the amount of protein in the band/lane is too large. And try to explain why such profiles may be broader (as is the case with Bol's profiles, they are a little broader) than they should be and why they have a flat or less pointed/sharp top/peak (as is the case with Bol's profiles, they do not have a sharp/pointed top/peak). The fact that Bol's profiles are broader (and may thus tend to traverse the demarcation line to a greater extent) than the negative control profiles may wrongly have been considered by the laboratory to be an atypical and suspicious finding, but is very likely the result of the large amount of natural EPO in his sample which in turn result in large and dark natural EPO bands.

The color of a protein-band in gel-images goes from white to light grey to darker grey and eventually to completely black (or from black to white if annotated with a black background) with increasing amounts of protein. The protein concentration is of course highest in the middle of a band and lowest along the edges of the band. If there is very much protein in the band, then the middle part of the band will be completely black and it will of course not become blacker upon increasing the amount of protein. Black is black, there are no different shades of black!

When performing band-scanning and thereby generating band profiles, one measures the intensity of the staining or color of the band – the more staining or darker the color, the more protein is measured and the height of the profile increases. But if there is very much protein in the middle part of the band, then that part of the band will be completely black and the scanner cannot detect any differences in "blackness" (or amount of protein) - even if the amount of protein is greatly increased. And that generates a flat top.

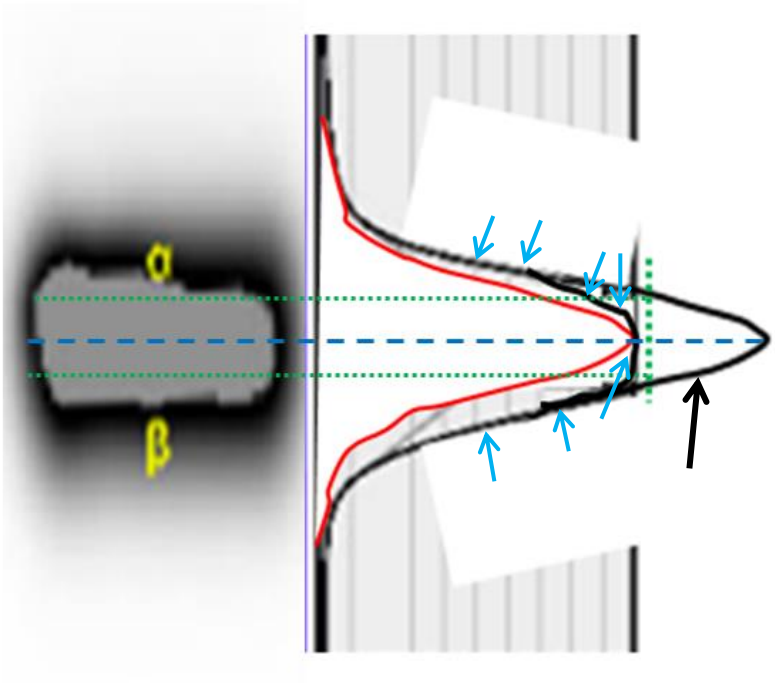
Some examples in order to illustrate this:



This is a scanned profile of a natural EPO band with so much EPO in the middle part of the band (indicated with grey color in this band) that the scanner measures the same amount

throughout the middle (grey) part of the band, although the amount of EPO in that part varies and it is highest in the very middle of the band.

Consider now the figure below:



The profile marked with black arrow indicates the distribution of EPO in this band and would have been the profile the scanner would have generated if it could have detected differences of “blackness” in the part of the band between the stippled horizontal green lines. But it cannot detect these difference and therefore measures the same amount (indicated by stippled vertical green line) of EPO throughout the middle part of the band. The top of the profile which the scanner generates will thus be the stippled vertical green line. It thus generates a profile with a flat top when the amount of protein in the band is extremely high. When it is not quite so extreme, but still too high, a less pointed top (as seen in Bol’s profile) is obtained. If the size of this profile is then reduced by data-processing (so that the profile has the same height as the other profiles) it will generate a profile (marked with blue arrows) that is broader/wider than it actually should have been. It should have been more like the red profile which reveals the actual of distribution of EPO in the band.

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01.03.23