Sample 3959325, collected 01 January 2016, AAF for testosterone

Opinion on the manipulation scenario of the investigating judge of the Court of Bolzano (as set out in his decision of 18 February 2021)

A. Summary of the manipulation scenario:

The investigating judge alleged that both samples A and B 3959325 had been manipulated by a third person, possibly by a member of the staff of the laboratory in charge of the analysis, in order to artificially create an Adverse Analytical Finding (AAF) for testosterone for both A and B samples.
More precisely, the judge put forward a manipulation scenario as follows:

- A third person opened the A and the sealed B samples of the athlete and mixed the athlete's urine with urine that was "positive for testosterone" (supposedly, from another person who had ingested testosterone).
- Before mixing the positive urine with the athlete's urine, the DNA in the positive urine was allegedly destroyed by exposure to UV rays, in order to have only one DNA signature in the final sample.
- Then, to resolve the problem of dilution of the steroids resulting from mixing the urines and in order to bring the final urine "*above the threshold of positivity*", the manipulator allegedly concentrated the doping substance present in the urine (e.g. by heating).

B. Opinion as to the plausibility of the alleged manipulation scenario:

I have been asked to evaluate the plausibility of the judge's manipulation scenario from a scientific and analytical perspective. For the reasons set out below, I consider that the manipulation scenario is flawed and implausible as (i) it is based on a fundamental misconception as to how the relevant analytical method works and (ii) it would be extremely difficult (if not to say) impossible to achieve without leaving analytical traces.

(i) The manipulation scenario is based on a fundamental misconception

The athlete tested positive for metabolites of testosterone. These metabolites are also produced 'endogenously' which means that they are naturally produced by the body. Therefore, in order to record an AAF against an athlete for endogenous steroids such as testosterone and its metabolites, it is not sufficient to merely detect those substances. Rather, it is necessary to demonstrate that the testosterone and/or its metabolites were administered 'exogenously' rather than being produced in the body.
A specific analytical method known as the Gas Chromatography-Combustion-Isotopic Ratio Mass Spectrometry (GC/C/IRMS) is used to differentiate steroids of endogenous and exogenous origin. This analysis is based on the carbon isotopic signature of the relevant compounds, which itself is calculated based on the ratio of carbon 13 (^{13}C) and carbon 12 (^{12}C) isotopes (the "carbon isotope ratio" or "CIR"). Synthetic steroids administered exogenously will typically have a different CIR to endogenous steroids. More particularly, synthetic steroids will contain a greater proportion of ^{12}C, which will result in a more negative (or more depleted) CIR value (expressed as $\delta^{13}C$ values). Conversely, whereas the CIR of endogenous steroids will vary between different populations (depending, in particular, on diet), the values will typically be more positive (or more enriched) as a result of a higher proportion of ^{13}C. To put that in numbers for the purposes of illustration: Endogenous steroids in European populations will typically have a CIR value in the region of minus 22 to minus 25 whereas synthetic steroids will typically have a more negative value in the region of minus 27 to minus 32.
As all the endogenous steroids of a given individual should present similar CIR values, a positive IRMS result is triggered, in simple terms, when the CIR value of a given target compound (such as testosterone or its metabolites) varies by more than a prescribed amount from the CIR value of an endogenous

reference compound (ERC), which is selected because its CIR value would not be influenced by the use of the prohibited substance, since it is metabolically unrelated to it. For example, where the CIR value of the two testosterone metabolites known as 5αAdiol and 5βAdiol vary by more than 3 points (per mil, or ‰) from the CIR value of the ERC, the testosterone metabolites are considered as being exogenous in origin. This was precisely the case with the athlete's sample, where the GC/C/IRMS values were as follows:

ERC: δ (‰) = [redacted]
5αAdiol: : δ (‰) = [redacted]
5βAdiol: : δ (‰) = [redacted]

As the two testosterone metabolites present CIR values that are > 3 ‰ (viz. [redacted] respectively) lower than the ERC (which was pregnanediol in this case), the GC/C/IRMS positivity criteria were met.
Whereas it is true that mixing a negative sample with a positive sample will result in testosterone metabolites with more enriched (i.e., less negative) CIR values than the positive urine would have (making it less likely to trigger a positive result), it is a misconception that heating (or otherwise concentrating) the resulting mixture would then increase the likelihood of a positive. This might be the case if the positive were triggered by the simple detection of a prohibited substance (above the laboratory method's detection capacity) or by the detection of a substance above a certain threshold concentration. But as explained above, that is not how the GC/C/IRMS analysis works. Concentrating the mixture might increase the overall concentration of the analyte (from both exogenous and endogenous origin) but it would not affect the CIR value of the relevant compounds. It would therefore have no effect on the likelihood of the mixture to test positive.
Therefore, in my opinion, the notion of concentrating a sample to increase the likelihood of a positive GC/C/IRMS result makes no sense from an analytical perspective. The premise of the manipulation scenario is therefore flawed.

(ii) It would be extremely difficult (if not to say impossible) to perform the manipulation without leaving traces

When doping control samples (urine) are analyzed, the laboratory routinely measures the concentrations and ratios of certain steroids. For example, laboratories will measure the ratio of testosterone to epitestosterone (the so-called T/E value) as well as the ratio of the two testosterone metabolites mentioned above (5aAdiol and 5bAdiol). These steroid ratios are (absent doping) quite stable within a given individual over time, but they vary between individuals. The various steroid ratios and concentrations therefore give a specific steroid profile for each individual athlete.
The manipulation scenario postulates that the athlete's sample was mixed with the sample of a third person and then subject to heating (or other concentrating procedure). If these processes of mixing and concentration occurred, they would, with a very high degree of probability, have upset the athlete's individual steroid profile (see below). However, this is not the case. In fact, the ratios of the steroids in sample A-3959325 are consistent with the results of other samples collected from the athlete shortly afterwards on 24.01.2016 and 02.02.2016. For example, the ratio of Androsterone/Etiocholanolone (A/Etio), which is generally not materially affected by the ingestion of testosterone, was [redacted], respectively, for the samples collected on 01.01.2016, 24.01.2016 and 02.02.2016. The ratio 5aAdiol/5bAdiol is also very stable for an individual and, in this case, for the same three samples described above, the ratios were respectively [redacted].
Other steroid profile ratios of sample 3959325 were of course affected by the use of testosterone. The T/E ratio was [redacted] on 1 January 2016 (the date of the sample collection); the subsequent results from 24.01 and 02.02 were, respectively, [redacted], which are consistent with a return to the athlete's normal steroid profile. The same is true for the A/T ratio, which is also used as a diagnostic ratio for the use of exogenous testosterone (like the T/E ratio). From a value of [redacted] on 01.01, the ratio increases to [redacted] (24.01), then to [redacted] (02.02). This is exactly what one would expect from an individual in the recovery phase after administering testosterone; as the exogenous testosterone is cleared (metabolized and excreted) by the body, the A/T ratio increases.

For the following reasons, it would be extremely difficult to maintain the athlete's particular steroid profile if the manipulations postulated by the judge had occurred:

The premise of the steroid passport is based on the low variability of the relevant steroid ratios within an individual. It is well known that those ratios may vary significantly between individuals. It would therefore be extremely difficult to maintain a consistent steroid profile of an athlete after mixing his/her urine with that of another athlete. In particular, it would require access to the athlete's steroid profile (which the

Cologne laboratory would not have as it was not the APMU for World Athletics) and involve a series of highly complex calculations for each of the endogenous steroids to keep the ratios consistent with the athlete's reference values.
In my opinion, therefore, the sample does not present any analytical evidence that it was manipulated (whether in the manner described by the judge or otherwise). Rather, the results are typical for a positive urine from a single individual that has used testosterone and fit well with the expected alteration of the steroidal profile of the athlete in question. That would have been extremely difficult, if not to say impossible, to achieve by way of manipulation.

(iii) Conclusion

In conclusion, I consider the manipulation scenario of the judge to be extremely implausible and extremely unlikely to have occurred. First, the very premise of the manipulation scenario (i.e. concentration to increase detectability) is flawed. Second, the lack of any indication of manipulation and compatibility of the positive sample with the athlete's steroid profile are a very strong indication that no manipulation occurred. It strikes me as seems implausible in the extreme that a scientist with the sophistication to pull off the manipulation protocol without upsetting the athlete's steroid profile would not have realized that concentrating the sample was a futile exercise.

Lausanne, 30 March 2022

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Professor Martial Saugy

Note: Some details of this opinion have been redacted for legal reasons.