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To whom it may concern

Bratislava, 13th August 2017

Expert opinion report:

I hereby declare and confirm that I received a detailed written and verbal explanation of the matter regarding the investigation of possible anti-doping rule violations of Mr. Matej TOTH, born on 10.02.1983, a Slovak elite athlete, ABP P111K34. I have received advice and help from assoc. prof. Peter Celec MD, Dipl Ing., Dr. Rer. Nat., DSc., MPH, head of Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University in Bratislava, Slovakia and Dr. Michal Raab, data scientist, HighChem, s.r.o., Bratislava, Slovakia. I confirm that I have not entered into any arrangement where the amount or payment of my fees is in any way dependent on the outcome of the case.
Here, I would like to express my personal opinion.

Sample 7

Even though sample 7 was not considered as the main suspicious feature of the ABP P111K34 in the Joint Expert Opinion dated 8.6.2017, for the sake of clarity some arguments will be given.

The determined concentration of HB for sample 7 (16.9 g/dl) cannot be viewed as a deviation of the individual's haematological profile since it is equal to the upper limit of the expected pattern (16.9 g/dl) and does not, therefore, exceed the range of variation. In addition, the upper limit of the statistically estimated range of variation (the expected pattern) in the Athlete Haematological Passport (ABP) at the time point of sample 7 is the lowest value (16.9 g/dl) in more than a 5-year period (13.8.2009 - 16.5.2015), and can be considered a statistical outlier. A second value determined by a different laboratory could provide more clarification, however, this is not available. In addition, a single HB concentration value prohibits an analytical error estimate or an inter-laboratory interval estimation, such as a confidence interval.

Since it is not known what analytical error has been used in the ABP software to predict the adaptive model, one can only speculate about it. The Laboratory documentation package from the Swiss Laboratory for Doping Analyses (sample 529326) dated from 01.02.2017, exhibits the instrument's calibration variation between 0-0.2 g/dl (Annex N° 8 Check assay sheets - Level 2, e-CHECK XE ASSAY SHEET, Lot No. 11990811). It is standard practice in quantitative analysis to take into account the individual measurement variance for every data point. Utilising the observed instrument's calibration variation of 0.2 g/dl, the HB concentration of sample 7 is well within the expected concentration range.

In addition, sample 7 was collected in the morning, and this fact may contribute to its high HB level based on the findings of the diurnal pattern of Matej Toth presented later in this report.

Sample 20

The experimentally determined concentration of HB for sample 20 in ABP P111K34 was flagged as atypical. The interpretation of values resulting from a statistical model incorporating haematological laboratory results requires careful evaluation and the close scrutiny of several important factors relating to both a single HB concentration and to an observed long term pattern.

Pre-analytical and analytical errors cannot be ruled out

In relation to the range of variation (the expected pattern), the HB concentration of sample 20 is the only outlier in the entire ABP time frame, exhibiting 5% difference between the reported value of 13.5 g/dl and the lower threshold of 14.0 g/dl. In terms of quantitative analysis, a single concentration outlier emerging from a 7-year non-equidistant sampling can be classified as a suspect measurement, and it is very unusual to draw serious conclusions based on a single value without conducting a confirmatory test at an independent analytical facility. Since no other haematological value examined according to WADA ABP Operating Guidelines lies outside the expected range, except a single off-score that is derived from the HB value, from an analytical chemistry standpoint a single HB outlier must be viewed as a suspect measurement that may be attributed to human or instrument error. The official files from IAAF Results Management as sent to the athlete demonstrate that minor human errors are unavoidable. Based on the Lab documentation reports, the file names of samples 17 and 19 that encode the sample number, sample code and date of test (17_ABP_LDP_181465_02022016.pdf and 19_ABP_LDP_181765_27042016.pdf) have been mixed up.

A single concentration value in a haematological profile is inadequate evidence of blood doping

About 20 years ago, new scientific fields emerged that have revolutionised our understanding of the biochemical process in the human body: proteomics and metabolomics. With advancing analytical techniques, proteomic and metabolomic analysis has enabled us to study differences or changes in protein and small molecule concentration patterns of various compartments, including blood. Under several circumstances, a single atypical haematological value resulting from multi-year time-course measurements of biological samples, even if obtained within a subject, is an insignificant predictor of an abnormality or causality regardless of the type of study, especially for an endogenous substance such as haemoglobin which is involved in complex biochemical cascades[1]. In proteomics and metabolomics, biological data is inherently characterised by high variance from both biological variation and analytical errors[2]. Consideration of the expected variation is essential to ensure that experiments will have sufficient power to address the biological questions being addressed[3]. To define the biological variation accurately and validly, large sample sizes are required[4]. It is, therefore, of

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[1] Sankaran, Vijay G., and Mitchell J. Weiss. "Anemia: progress in molecular mechanisms and therapies." *Nature medicine* 21, no. 3 (2015): 221-230.

[2] Sriyudthsak, Kansuporn, Fumihide Shiraishi, and Masami Yokota Hirai. "Mathematical Modeling and Dynamic Simulation of Metabolic Reaction Systems Using Metabolome Time Series Data." *Frontiers in molecular biosciences* 3 (2016).

[3] Karp, Natasha A., Paul S. McCormick, Matthew R. Russell, and Kathryn S. Lilley. "Experimental and statistical considerations to avoid false conclusions in proteomics studies using differential in-gel electrophoresis." *Molecular & Cellular Proteomics* 6, no. 8 (2007): 1354-1364.

[4] Dunn, W.B., Broadhurst, D., Begley, P., Zelena, E., Francis-McIntyre, S., Anderson, N., Brown, M., Knowles, J.D., Halsall, A., Haselden, J.N. and Nicholls, A.W., 2011. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nature protocols*, 6(7), p.1060.

paramount importance that the method of examination is based on sufficient data points that support the declared outcomes to ensure valid conclusions.

In line with accepted proteomics and metabolomics standards, Sottas et al. (2008)[5] in their central publication upon which the Athlete Biological Passport is based, demonstrate in Figure 2. the longitudinal effects of a prohibited substance on an ABPS profile where a test is considered as positive if a measured value is higher than the corresponding threshold which "happened seven times out of eight" in the given example. In another study[6], a male endurance athlete tested positive to a homologous blood transfusion (BT) based on 13 tests carried out during a four-year period, while indirect markers of blood doping exceeded individual upper and lower limits of the ABP a number of times (2x HB, 5x off-score, 4x RET%, 5x ABPS).

In both cases, a distinct trend of multiple threshold outliers was conclusive evidence for blood manipulation. In contrast to the above, a single HB concentration along with a single off score, derived from the same HB concentration, during the entire ABP P111K34 history is used as the main argument for a blood doping scenario, while all other values, including those of RET%, remain in the expected range. Such a strict measure seems to be not only inconsistent with scientific literature, it goes far beyond the reference publication of "fathers" of ABP.

Applying objective scientific criteria adopted from peer-reviewed literature leads to the conclusion that a single concentration value in a haematological profile is not indicative of an abnormal situation in regard to blood doping.

Training cessation may decrease HB concentration

Plasma volume contributes significantly to variation in haemoglobin concentration in healthy volunteers, and across a variety of disease states[7]. According to the seminal paper of Costill and Fink (1974)[8], cited several thousand times, the mean relative change in plasma volume exceeded 10% after 3 h exercise, leading to HB concentration changes between 0.8-2.2 d/dl, while the highest difference of 2.2 g/dl was observed in two out of six runners.

According to the Joint Expert Opinion, "training suspension is usually associated with plasma volume contraction, mild haemoconcentration and relative HB increase, while HB can decrease as a consequence of the haemodilution which is caused by intense training or prolonged endurance competition". However, with regard to training suspension, Shaskey and Green argue in their extensive review article *"Sports Haematology"*[9] the opposite case. The stress of exhaustive exercise causes an initial volume contraction due to fluid loss, which is then followed by plasma volume expansion. Expansion may be 6 to 25% greater than baseline and the greatest plasma volume increase occurs in elite, endurance athletes following the cessation of training. This observation correlates with Matej Toth's training and HB profile. At the end of the well-documented training cessation period caused by

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[5] Sottas, Pierre-Edouard, Neil Robinson, Martial Saugy, and Olivier Niggli. "A forensic approach to the interpretation of blood doping markers." *Law, Probability & Risk* 7, no. 3 (2008): 191-210.

[6] Giraud, Sylvain, Pierre-Edouard Sottas, Neil Robinson, and Martial Saugy. "Blood transfusion in sports." In *Doping in Sports: Biochemical Principles, Effects and Analysis*, pp. 295-304. Springer Berlin Heidelberg, 2010.

[7] James M. Otto, James O. M. Plumb, Eleri Clissold, Shriya Kumar, Denis J.Wakeham, Walter Schmidt, Michael P.W. Grocott, Toby Richards, Hugh Montgomery "Hemoglobin concentration, total hemoglobin mass and plasma volume in patients: implications for anemia." *Haematologica* Jun (2017)

[8] Dill, D.B. and Costill, D.L., 1974. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of applied physiology*, 37(2), pp.247-248.

[9] Shaskey, David J., and Gary A. Green. "Sports haematology." Sports Medicine 29, no. 1 (2000): 27-38