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O/ref. : 21-T02499-02503 and 21-T02540-2544-vic

Report on the forensic genetics analysis of biological specimens

Dear Dr Kuuranne,

We have been asked to determine the DNA concentration of 100 urine samples numbered DN001 to DN100 using the same method which was used for the analysis of the sample 12966171DN (17-T05676a) in 2017.

You also asked our opinion regarding the following statements from the report issued by the Judge, Mr. Walter Pelino, dated 18.02.2021:

p.15 "*This involves, therefore, not only the concentration of exogenous testosterone, desired by the hypothetical manipulator, but also of everything else, including DNA, and what the expert has definitely found is just a completely abnormal, unnatural, DNA concentration.*"

p.16 "*In other words, not only does the manipulation hypothesis allow us to explain how and why that abnormal DNA concentration occurred, but this is also, at present, the only convincing explanation.*"

p.31 "*Actually, the experimentation carried out by the expert, Col. PhD Giampietro Lago, has demonstrated that already after 6 months the samples show on average (weighted average) a decay of approximately 70% of the DNA present thereto, a decay that becomes higher, approximately 87%, after 12 months (see supplementary report filed on 3.09.2019, p. 43).*

The subsequent supplement to the expert report, filed on 5.09.2020, demonstrated the asymptotic trend of this progressive decay, where the curve that graphically expresses the decrease, very pronounced in the first 6 months, continues over time becoming progressively milder the closer it gets to zero (p. 30)."

p.51 "*Moreover, the figure concerning the DNA concentration (14,013 pg/µl in urine frozen for a year and a half) was manifestly implausible, given that (as a result of the decay due to freezing, the passage of time and the thermal stress suffered) it would presuppose a concentration at the time of taking the sample of more than 100,000 pg/µl, comparable with that of blood or saliva, as repeatedly stated by the expert who was not contested on the point.*"

p.51-52 "*Instead, if we consider that the sample of 27.06.2016, at the time of the analysis (October 2017) had been frozen for a year and four months and we assume, with a conservative estimate, that due to the long time elapsed it had undergone a decay of 80% (the examination showed a median decay after 12 months of 87%), we can estimate the original concentration at 112,104 pg/µl on 4 ml of urine, i.e. 28,026 pg/µl on 1 ml, a concentration significantly higher (approximately +9%) compared to the (already implausible) concentration of 25,780 pg/µl that WADA's Director of Legal Affairs, Sieveking, asserted to be the maximum quantity found at the Lausanne laboratory (see p. 2 of the note of 10.12.2019), without however documenting this assertion in any way.*"

p.70 "*Is it not the case that this phantom study is completely bogus and has been referred to solely in an attempt to make the figure of 14,013 pg/µl that emerged from the Lausanne analysis of the 27.06.2016 sample appear true, a figure that is completely implausible, especially one year and four months after freezing?*"

Our findings and comments are reported thereafter.

METHODS

The samples were analyzed using the following methods:

SAMPLING
The requesting authorities forward specimens to the Forensic Genetics Unit (FGU). FGU staff cannot be held responsible for any damage to these samples prior to their reception. The part of the specimen that is submitted for analysis is called a sample. When the transmitted specimen is used in its entirety for analysis, for example in the case of a swab or a piece of clothing, FGU is not responsible for the sampling plan and method. When this is not the case, the FGU is responsible for the sample taken. Where appropriate, these are described in the "Analyzed material" section of this report.

DNA EXTRACTION
The QIAamp Circulating Nucleic Acid Kit (Qiagen) was used to extract and purify the DNA.

DNA QUANTIFICATION
The concentrations of male and total DNA (i.e. male and female) present in the samples are determined prior to amplification by means of real-time quantitative PCR with the Quantifiler Trio kit (Applied Biosystems) on an Applied Biosystems QuantStudio 5 Real-Time PCR System.

Complementary information on the methods can be obtained upon request. In accordance with the Ordinance of the Federal Department of Justice and Police on the DNA analysis laboratories (RO 363.11), the results were confirmed with at least two independent analyses.

MATERIAL AND RESULTS

LAD nb : DN001 to DN100
Material received : about 6 ml urine for each sample
Reception date : 11.05.21
Lab nb : 21-T02499a - 21-T02503j and 21-T02540a - 21-T02544j

ANALYZED MATERIAL
4 ml of each urine sample

DNA CONCENTRATION [ng/uL] from 50 uL extract
Range : 0.001 - 20.183 ; Mean : 1.845 ; SD: 3.670
See Fig. 1 and the appendix

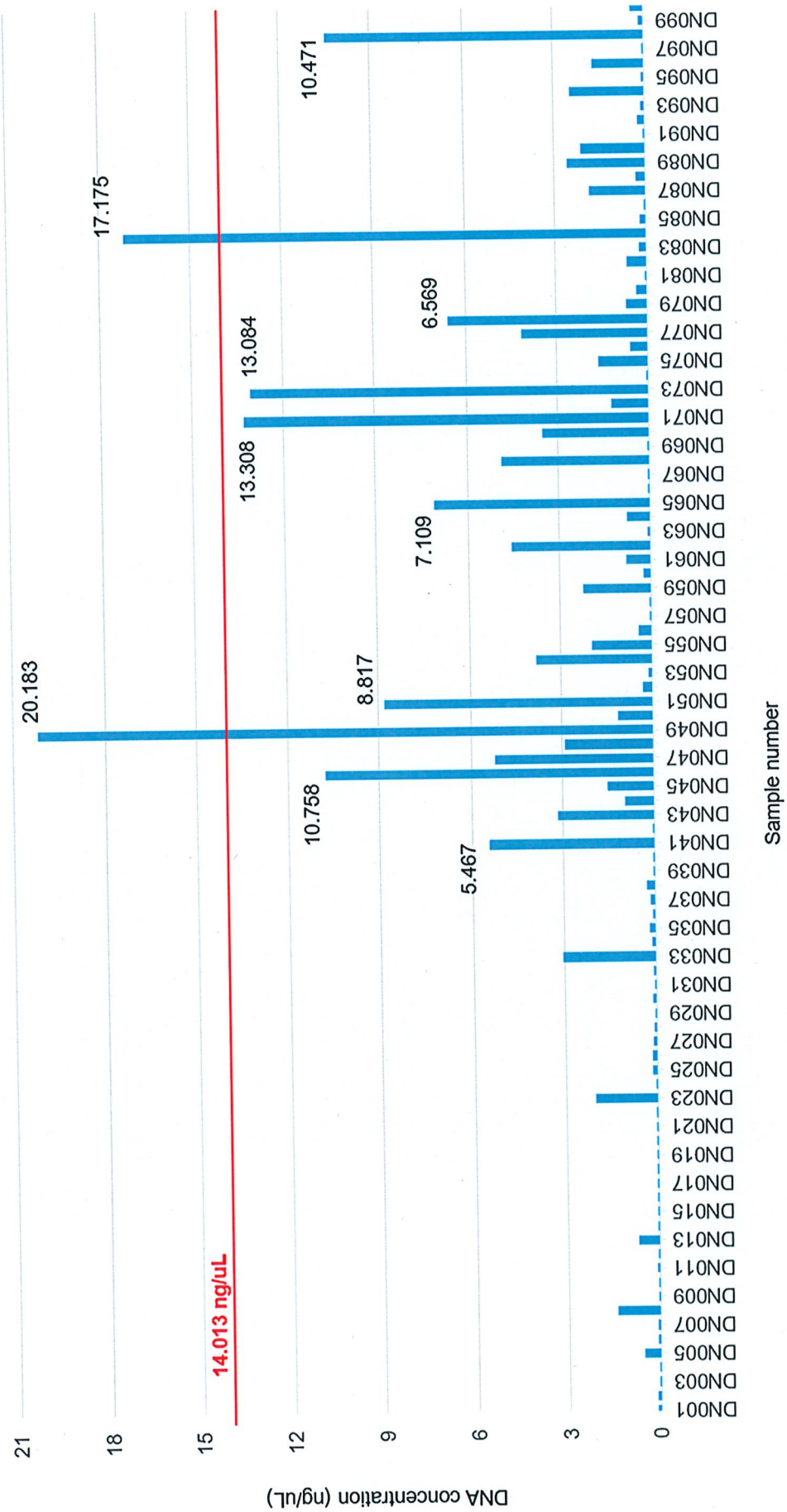

Figure 1: DNA concentration of the urine samples DN001 to DN100. DNA was extracted from 4mL urine.

COMMENTS AND INTERPRETATION

The individual DNA concentrations measured from 4mL of each of the 100 urine samples provided (named DN001 to DN100) ranged from 0.001 to 20.183 ng/ul (Fig. 1). The corresponding mean DNA concentration is 1.845±3.670 ng/uL. With DNA extracts of 50 uL, these DNA concentrations correspond to a range of 0.013 to 252.288 ng of DNA per mL of urine and a mean DNA concentration of 23.062±45.880 ng of DNA per mL of urine. This corresponds to the typical amounts of DNA expected to be extracted from urine samples (from 1 to 20 ng/mL according to [1]).

Among the 100 urine samples analyzed, 6 of them had DNA concentrations exceeding 10 ng/uL among which 2 even exceeded 17 ng/uL. This definitely proves that the DNA concentration of 14.013 ng/uL (or 14'013 pg/uL) observed for the urine sample 17-T05676a analyzed in Lausanne with the same method is neither "manifestly implausible" nor "abnormal" or "unnatural" as stated by the Judge, Mr. Walter Pelino, in his report.

During storage, the DNA present within a biological sample may suffer degradation. When we focus on a sample of interest, it is better to consider the characteristics of this particular sample rather than an average value obtained from samples that may not have been exposed to the same environmental conditions.

DNA profiles obtained from good quality DNA samples (*i.e.* not significantly degraded) show peaks with about similar heights between the shortest (on the left side) and the longest loci (on the right side) within the different color channels (Fig. 2). DNA degradation will statistically affect the largest DNA fragments first. At the level of the DNA profile, this will result in the so-called "ski-slope effect", the height of the largest loci being smaller than the one of the shortest loci. Extreme degradation results in partial DNA profiles with alleles missing for the largest loci.

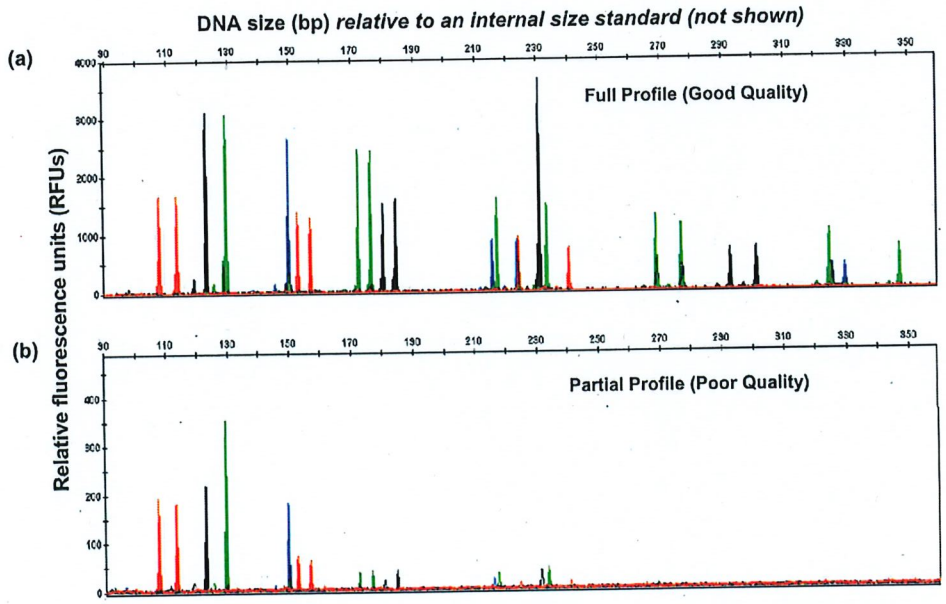

Figure 2 (from Ref [1]): A comparison of DNA profiles from the same person but of different qualities. (a) Intact, good-quality DNA yields a full DNA profile and (b) Degraded, poor-quality DNA yields a partial DNA profile with alleles missing at the larger-sized loci.

Hence, when considering a DNA profile, it is possible to determine whether the corresponding DNA is severely, slightly or not degraded.

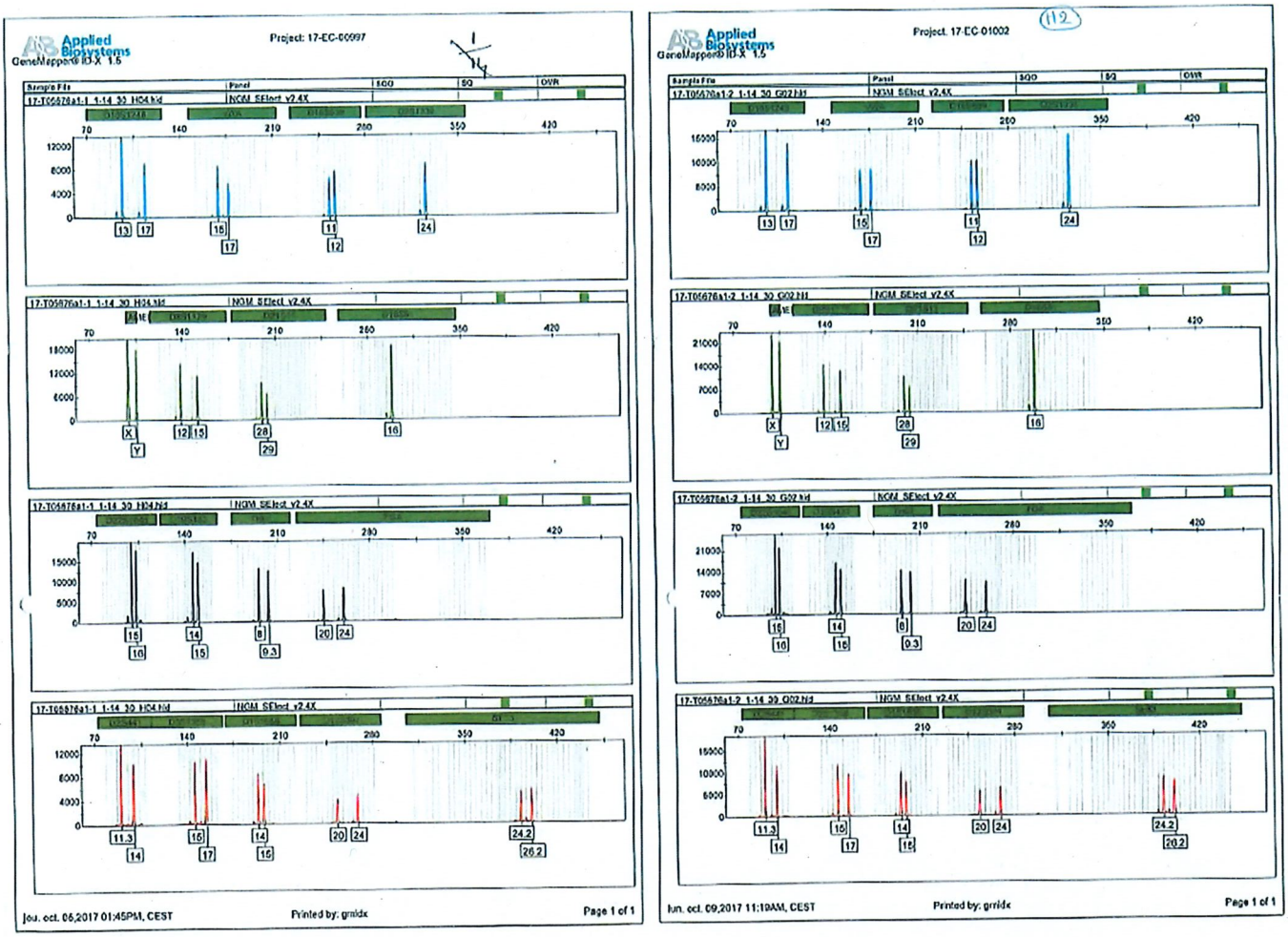

Figure 3: DNA profiles (replicates) obtained for the urine sample 17-T05676a. These are full profiles with peaks present at every locus. Furthermore, the height of the peaks corresponding to the largest loci is similar to the one of the shortest loci. These characteristics are diagnostics of good-quality DNA.

As illustrated in Fig. 3, the DNA recovered from the urine sample 17-T05676a can be qualified as a good-quality DNA. Using the method described in [2], we estimated that the integrity index, which is a measure of non-degraded DNA, is equal to 0.86 for this urine sample. This implies that the degradation is about 14%. This represents a slight degradation and certainly not a decay of 80% (or even more) as stated by the Judge, Mr. Walter Pelino, in his report.

The Quantifiler Trio kit used for the DNA quantification provides a degradation index (DI). DI is calculated as the ratio of the DNA concentration of a short autosomal DNA target of 80 bases and the DNA concentration of a large DNA target of 214 bases. The DI of the urine sample 17-T05676a provided with this method is 1.0. This means that the concentration of the short DNA fragments is equal to the concentration of the large DNA fragments. According to the manufacturer user guide [3], a DI <1 typically indicates the absence of DNA degradation, whereas a DI between 1 and 10 indicates that the DNA is slightly to moderately degraded. The DNA is significantly degraded when the DI is reaches values >10.

All these considerations indicate that the urine sample 17-T05676a contains good-quality DNA. This sample has been stored frozen for a year and four months but it clearly does not show signs of at least 80% decay as written by the Judge, Mr. Walter Pelino, in his report. Consequently, the calculation made by the expert, taking into account such an exaggerated decay, in order to estimate an "original concentration of 112,104 pg/uL" is totally erroneous and fallacious.

As a summary, we measured the DNA concentrations of 100 individual urine samples and examined the DNA profiles obtained for the urine sample 17-T05676a. Contrary to what the Judge, Mr. Walter Pelino, has written in his report, the scientific elements presented here prove that the DNA concentration of urine measured for the sample 17-T05676a is high but not "manifestly implausible" nor "abnormal" or "unnatural" and also proves the absence of severe degradation of DNA in this urine sample.

Should you need any further information, please feel free to contact us.

With our best regards,

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DrSc. V. CASTELLA, PD, MER
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Bibliography:

[1] Butler, JM. Advanced topics in forensic DNA typing: methodology. Waltham, MA: Elsevier, 2012, 704 pp

[2] Comte J, Baechler S, Gervaix L, Lock E, Milon M-P, Delemont O and Castella V (2019) Touch DNA collection - Performance of four different swabs. Forensic Science International: Genetics 43: 102113 (open access)

[3] Applied Biosystems (2018) Quantifiler™ HP and Trio DNA Quantification Kits User Guide Rev. H. P/N 4485354.

Appendix 1 : DNA concertation of 100 urine samples

Appendix to the report 21-T02499-02503 and 21-T02540-02544 28.06.2021
DNA concentration of 100 urine samples

N° LAD	DNA conc. ng/uL	N° LAD	DNA conc. ng/uL
DN001	0.122	DN051	8.817
DN002	0.119	DN052	0.33
DN003	0.017	DN053	0.113
DN004	0.003	DN054	3.813
DN005	0.549	DN055	1.987
DN006	0.103	DN056	0.425
DN007	0.083	DN057	0.023
DN008	1.401	DN058	0.009
DN009	0.071	DN059	2.243
DN010	0.011	DN060	0.248
DN011	0.094	DN061	0.795
DN012	0.002	DN062	4.566
DN013	0.704	DN063	0.089
DN014	0.033	DN064	0.786
DN015	0.015	DN065	7.109
DN016	0.054	DN066	0.045
DN017	0.021	DN067	0.025
DN018	0.041	DN068	4.878
DN019	0.019	DN069	0.04
DN020	0.023	DN070	3.529
DN021	0.012	DN071	13.308
DN022	0.044	DN072	1.231
DN023	2.048	DN073	13.084
DN024	0.065	DN074	0.061
DN025	0.167	DN075	1.622
DN026	0.168	DN076	0.571
DN027	0.123	DN077	4.181
DN028	0.084	DN078	6.569
DN029	0.021	DN079	0.713
DN030	0.139	DN080	0.363
DN031	0.044	DN081	0.019
DN032	0.099	DN082	0.645
DN033	3.055	DN083	0.24
DN034	0.141	DN084	17.175
DN035	0.2	DN085	0.199
DN036	0.094	DN086	0.049
DN037	0.152	DN087	1.849
DN038	0.277	DN088	0.323
DN039	0.001	DN089	2.58
DN040	0.054	DN090	2.112
DN041	5.467	DN091	0.03
DN042	0.067	DN092	0.239
DN043	3.204	DN093	0.133
DN044	0.951	DN094	2.467
DN045	1.543	DN095	0.101
DN046	10.758	DN096	1.723
DN047	5.231	DN097	0.009
DN048	2.921	DN098	10.471
DN049	20.183	DN099	0.169
DN050	1.146	DN100	0.446