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Case Report

Fatal Mephedrone Intoxication—A Case Report

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A death caused by a new designer drug, 4-methylmethcathinone (mephedrone), is reported. Eight small plastic bags containing white powder were found in the jacket of a young dead male. Spot tests conducted by the police officer indicated the presence of 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in the powders. Laboratory routine screening analyses of blood and vitreous humor did not reveal any positive results; therefore, 2C-B was excluded.

Analysis of powders was conducted using gas chromatography–mass spectrometry and high-pressure liquid chromatography with diode array detection. The purity of mephedrone found in all powder samples was in the range of 80.4–87.3%. In connection with these findings, blood and vitreous humor samples were analyzed for mephedrone. Analyses were conducted using liquid chromatography–tandem mass spectrometry. Mephedrone was found in blood and vitreous humor at the concentrations of 5.5 and 7.1 μg/mL, respectively, revealing that this was a fatal mephedrone intoxication.

Introduction

Mephedrone [4-methylmethcathinone; 4-methylephedrone; 4-MMC; UPAC: 2-(methylamino) -1-(4-methylphenyl)propan-1-one] is a designer drug derived from cathinone (Figure 1), which was marketed as a legal high or an afterburner in head shops throughout Poland within 2008–2010. Until now, mephedrone seemed to be the most popular of the synthesized cathinone stimulants. It appeared to be trendy among young clubbers, but was also used by a wider population of older adolescents and young adults (1, 2). Another concern is that mephedrone may be taken by young people with little previous experience of drug use. This compound was reported in the past two years to be linked with some fatalities and severe intoxications in users of legal highs (3). The rapid rise in the use of mephedrone and other synthetic designer drugs, together with a growing number of intoxications with legal highs have led to closure of 1,300 stationary legal high shops. Mephedrone, as a psychotropic substance of the I-P group, is controlled in Poland under the Drug Addiction Counteraction Act of July 29, 2005, as amended. Few data are available on the pharmacokinetics or pharmacodynamics, human or animal toxicity, addiction or acute overdose potential and long-term effects of the cathinones, including mephedrone. Most data regarding harm due to mephedrone use are self-reported and there are very few clinical data available. The effects of ring-substituted cathinones include feelings of empathy (openness, love, closeness, sociability and well-being), stimulation (alertness, rushing), euphoria, appreciation of music and awareness of senses (4). Self-reported dosages reach 200 mg or more for mephedrone, with some users reporting re-dosing to prolong the euphoric experience, leading to 1–2 g consumed in a session (3). The cathinones are sometimes used in conjunction with alcohol or other controlled substances. Mephedrone powder may be snorted (insufflated), swallowed, often after wrapping in tissue paper (bombing or dabbing) or, more rarely, injected (5). Reports from users at hospital toxicology units show that mephedrone is taken in staggered doses (6). Mephedrone users may develop tolerance quickly, and as a consequence, tend to consume higher doses more frequently (4).

Most of the preceding mephedrone effects seem similar to those already documented for amphetamine, methamphetamine and 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) (7), implicitly supporting a sympathomimetic activity of mephedrone. Conversely, symptoms of depression and anhedonia may tentatively be associated with a putative depletion of serotonin and dopamine (4), similarly to what may occur with other stimulants. A wide range of side effects have been observed in individuals presenting at hospitals, and these include tachycardia and palpitation, hypertension, dilated pupils, severe vasoconstriction in the peripheries, agitation, seizure, nausea and vomiting, headache, change in body temperature (sweating chills), insomnia, minor amnesia and in higher doses, hallucination and psychosis. Wood *et al.* (8) reported the first case of sympathomimetic toxicity related to mephedrone confirmed by toxicological screening, in which no other drugs or alcohol were detected. It has drawn wide attention from the media because it has been allegedly linked to a number of fatalities. Only a few formal papers and experimental/clinical data have been published (9–11).

This paper describes the circumstances of death, autopsy and toxicological findings in a fatal case in which mephedrone was detected and quantitated in blood and vitreous humor collected from a young male corpse.

Case History

A young male, approximately 30 years old, was found in a critical state in a staircase. Despite resuscitation attempts conducted by two ambulance teams, the man died at the scene. Eight small plastic bags, each containing 1 g of white powder, were found in the pocket of his jacket. Spot tests conducted by the police officer indicated the presence of 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in the powders. An autopsy showed brain stem failure and lung injury. The result of blood analysis showed that the blood was negative for alcohol. The histopathological examination was also negative. Blood and vitreous humor specimens were submitted to the Institute of Forensic Research (IFR) for confirmatory toxicological analysis for 2C-B. Routine screening analyses for common drugs of abuse (including 2C-B) and medicines conducted by gas

Cathinone
2-amino-1-phenylpropan-1-one
Molecular formula = $C_9H_{11}NO$
Average mass = 149.1897

Mephedrone
2-(methylamino)-1-(4-methylphenyl)propan-1-one
Molecular formula = $C_{11}H_{15}NO$
Average mass = 177.2429

Figure 1. Chemical structures of cathinone and mephedrone.

chromatography with flame ionization detection (GC–FID), gas chromatography–mass spectrometry (GC-MS), high-pressure liquid chromatography with diode array detection (HPLC–DAD) and liquid chromatography–mass spectrometry (LC–MS) did not reveal positive results; therefore, 2C-B in biological material was excluded. Laboratory requests for the secured powders were not fulfilled at that point. Five months later, the powders were sent to the IFR for identification.

Experimental

Reagents and materials

Mephedrone and mephedrone-d_3 were purchased from LGC Standards (Dziekanow Lesny, Poland). HPLC-grade acetonitrile (MeCN), methanol and formic acid, 98–100%, were bought from Merck (Warsaw, Poland). Orthophosphoric acid, 85%, was purchased from Poch S.A. (Gliwice, Poland). Blank blood samples used for the development and validation of the method and for preparing controls were obtained from a regional blood donation center. Vitreous humor drug-free samples were taken from persons with no history of drug abuse. These samples were collected from cadavers and sent to IFR for alcohol determination. Blank blood and vitreous humor screened for common drugs of abuse (including mephedrone) and alcohol were negative.

Biological material was stored at $+4^{\circ}$C before the analysis.

Standard, calibrators and control preparation

A stock solution of mephedrone (1 mg/mL in methanol) was stored at -22°C. Calibrators were prepared in 0.2 mL of drug-free blood samples by spiking them with mephedrone to obtain the concentrations of 1, 2, 5, 10, 20, 50 and 100 ng/mL. Control samples at 10 and 100 ng/mL in addition to negative blood and vitreous humor controls were prepared before each analysis series. Mephedrone-d_3 spiking solution was prepared for use as the internal standard (IS) at the concentration of 100 ng/mL. Postmortem blood and vitreous humor samples were diluted (100 times in two steps) with drug-free blood and drug-free vitreous humor, respectively, to fit into the linear range to allow quantification.

Analysis of white powders

Qualitative analysis of mephedrone by GC–MS in powders

Ten milligram samples of white powders collected from eight plastic bags were separately dissolved in methanol and the solutions were analyzed by GC–MS for semi-volatile organic compounds. The analysis was performed using a gas chromatograph (HP 6890 GC System) coupled to a mass spectrometer (Agilent 5973 Network Mass Selective Detector), equipped with a quadruple mass analyzer (Agilent Technologies, Palo Alto, CA). The injector was maintained at 280°C. Sample injection (1 μL) was in splitless mode. Sample components separation was conducted on an HP-5MS capillary column (Agilent Technologies; 30 m length, 0.25 nm inner diameter, 0.25 μm film thickness). Helium was used as a carrier gas at the flow rate of 1.0 mL/min. The temperature program consisted of three segments: the initial column temperature (75°C) was maintained for 1 min, then increased linearly by 20°C/min up to 275°C and maintained for 9 min. The mass detector was set to positive electron impact mode (EI) and the electron beam energy was 70 eV. The mass detector was operating in a full scan mode in range of 29–600 amu. The target compound was identified by matching its retention time (RT = 6.32 min) and spectra against a reference library (primary ions: m/z 58, 91 and 119). The acquisition and results analysis were conducted with MSD Chemstation (version E.02.01).

Quantitative analysis of mephedrone by HPLC–DAD in powders

To quantify mephedrone in the white powders, 10 mg samples were weighted out and dissolved in a mixture of 10 mL methanol–water (1:1, v/v), and diluted 50 times with water containing 100 μL 85% orthophosphoric acid per liter. The sample solution was analyzed by HPLC–DAD using a Merck-Hitachi HPLC LaChrom D-7000 System (Germany). Separation of sample components was performed on a LiChrospher 60 (125 × 4 mm, 5 μm) RP-Select B column maintained at 30°C. The mobile phase was made with MeCN and water containing 100 μL 85% orthophosphoric acid per 1 L. The flow rate was 1 mL/min, and the following elution gradient was applied: 0 min, 5% MeCN; 12 min, 60% MeCN; 13 min, 5% MeCN; 18 min 5% MeCN. The injection volume was 20 μL. The detector was set to monitor the 200–400 nm wavelength range. Identification was based on comparison with the reference spectrum and the RT of the target compound (8.85 min). The acquisition and data analysis were performed using D-7000 HPLC System Manager (version 4.1).

Validation of HPLC method

The HPLC mephedrone quantification method was validated. The validation covered determination of method linearity

limits, limit of detection (LOD), limit of quantification (LOQ) and precision and accuracy. The validation parameters were calculated using Validation Manager software by Merck. The results are shown in Table I. The HPLC method was linear over the entire range of tested concentrations from LOQ to 100% with $R^2 = 0.996$. The LOD was determined at 0.5 % and the LOQ was 1.5%. The intra-day and inter-day precision were determined at three concentration levels: 12.5, 50 and 100%. The measurements were conducted for five consecutive days, and five determinations were conducted for each concentration.

Analysis of biological material

Extraction

Twenty microliters of 1 μg/mL methanolic solution of mephedrone-d_3 (IS) was added to the blood and vitreous humor samples (0.2 mL) and placed in an Eppendorf vial to obtain a final concentration of 100 ng/mL. The analytes were isolated by acetonitrile precipitation. MeCN (600 μL) was added in 50 μL portions, and after each addition, the sample was vortexed for 10 s. The samples were mixed for 5 min, followed by centrifugation at 13,000 rpm for 3 min, and the MeCN was transferred to a 2 mL glass vial. Then, the organic solvent was evaporated under a stream of nitrogen at 37°C. The dry residue was reconstituted in 100 μL of 0.1% formic acid in water (v/v) and transferred to a polypropylene insert.

Chromatographic and spectrometric conditions

Blood and vitreous humor samples were analyzed using an Agilent Technologies 1200 series liquid chromatograph connected to a 6460 Triple Quad mass spectrometer. Separation was performed on a Zorbax SB-C18 column (2.1 × 50 mm, 1.8 μm) (Agilent Technologies), thermostated at 25°C. The mobile phase was composed of a mixture of 0.1% formic acid in MeCN (v/v) and 0.1% formic acid in water (v/v). The flow rate was 0.3 mL/min. All analyses were conducted in gradient mode (shown in relation to MeCN content): 0 min, 10%; 6 min, 100%; 7 min, 10%; 14 min, 10%. The injection volume was 10 μL. The total analysis time was 14 min. The retention time of mephedrone was 3.32 min.

Multiple reaction monitoring (MRM) with positive ion detection was used. The precursor ions and three fragment ions for each compound, selected as quantifiers (shown in bold) and qualifiers for mephedrone MRM, were **178.1 → 160.1**, 178.1 → 145.1, 178.1 → 77.1, and for mephedrone-d_3: **181.1 → 163.1**, 181.1 → 148.1, 181.1 → 91.1. The mass detector parameters were as follows: capillary voltage, 4,000 V; fragmentor voltage, 87 V; gas flow (nitrogen), 10 L/min; gas temperature, 325°C; sheath gas flow, 10 L/min; sheath gas temperature, 400°C; nebulizer pressure, 40 psi; dwell times, 25 ms. Collision energies (V) for mephedrone and mephedrone-d_3 transitions were 8, 20, 56 and 8, 20, 36, respectively. The apparatus maintenance and results analysis were conducted using MassHunter software by Agilent Technologies (version B.02.01).

Table I
Validation Data for the Quantification of Mephedrone in Powder by the HPLC–DAD Method*

	Mean (%)	Intra-day precision ($n = 5$)		Inter-day precision ($n = 5$)		Accuracy (%)
		SD (%)	RSD (%)	SD (%)	RSD (%)	
Low (12.5%)	15.4	0.9	6,1	2.1	13.7	122.9
Medium (50%)	51.4	1.7	3.4	3.9	7.6	102.9
High (100%)	99.0	2.7	6.7	6.3	6.4	99.0

*Note: Standard deviation (SD); relative standard deviation (RSD).

Method validation

The LC–MS method for mephedrone quantification was validated. The data are summarized in Table II. A seven-point mephedrone blood calibration curve (number of replicates for

Table II
Validation Data for the Quantification of Mephedrone in Blood by the LC–MS Method

	Mean concentration (± SD) (ng/mL)	RSD (%)	Accuracy (%)
Intra-day ($n = 5$)			
10 ng/mL	10.8 ± 1.5	14.2	107.8
100 ng/mL	104.0 ± 14.8	14.2	104.0
Inter-day ($n = 10$)			
10 ng/mL	11.1 ± 1.2	10.4	111.4
100 ng/mL	95.0 ± 13.8	14.5	95.0
LOD	0.08		
LOQ	1.00		

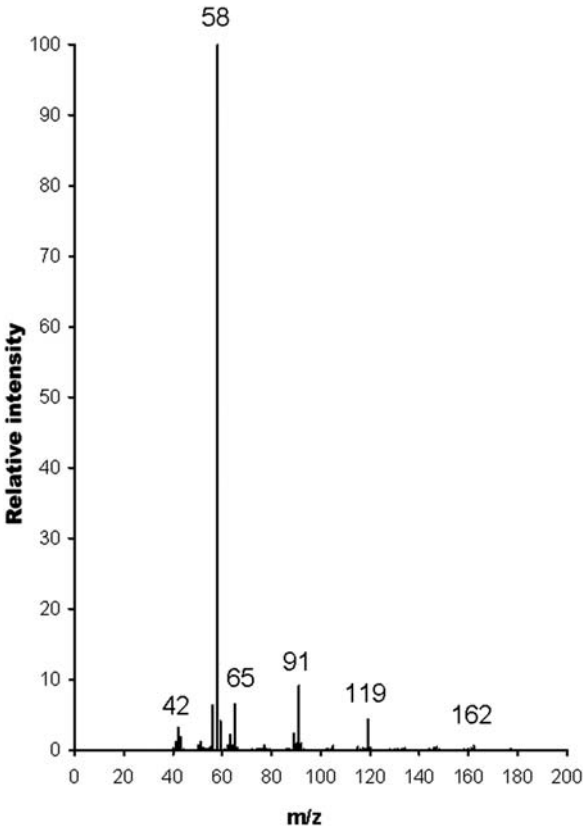

Figure 2. EI spectrum (GC-MS) of mephedrone detected in the powders.

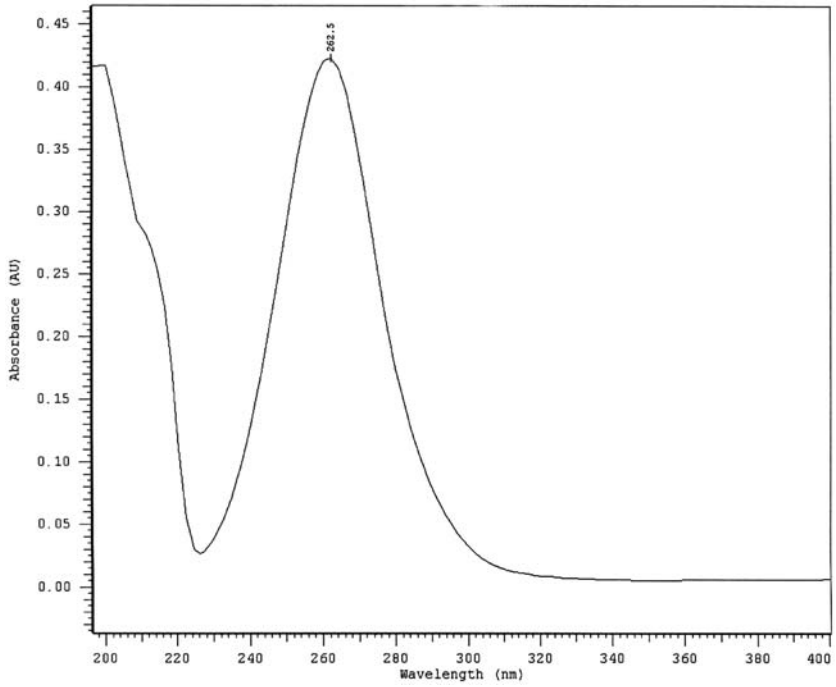

Figure 3. UV/Vis spectrum (HPLC-DAD) of mephedrone detected in the powders.

each level, $n = 5$) was linear in the range of 1–100 ng/mL with $R^2 = 0.994$. The LOD was 0.08 ng/mL and LOQ was assumed to be the lowest point from the calibration curve (1 ng/mL). The specificity of the assay was determined by analyzing mephedrone-free blood samples taken from eight people. Total extraction recovery calculated at concentration of 100 ng/mL ($n = 5$) was 84.4%, which was determined by comparing the responses (analyte area/IS area) of mephedrone extracted from blood with blank blood spiked with mephedrone after extraction. LC–MS-MS matrix effect was calculated by comparing the responses of known amounts of unextracted mephedrone ($n = 5$) with those measured in blank blood spiked with the same analyte amount (100 ng/mL) after extraction ($n = 5$). Absolute matrix effect for mephedrone was 132.3% and showed significant signal enhancement. Two levels of controls (10 and 100 ng/mL, three replicates each) were prepared for vitreous humor to test the usefulness of the blood calibration curve for the quantification of mephedrone in this matrix. Matrix matching experiments conducted between blood and vitreous humor showed a good correlation between the two matrices (measured values did not deviate more than 12% from the theoretical concentrations).

Enzyme-linked immunosorbent assay method

The ability of the enzyme-linked immunosorbent assays (ELISA) using Neogen Corporation kits for amphetamine and methamphetamine (purchased from STI, Warsaw, Poland) to cross-react with mephedrone and 2C-B was also investigated. Negative blood specimens were spiked at the concentrations of 0.1 and 1 μg/mL. Samples were prepared according to procedures

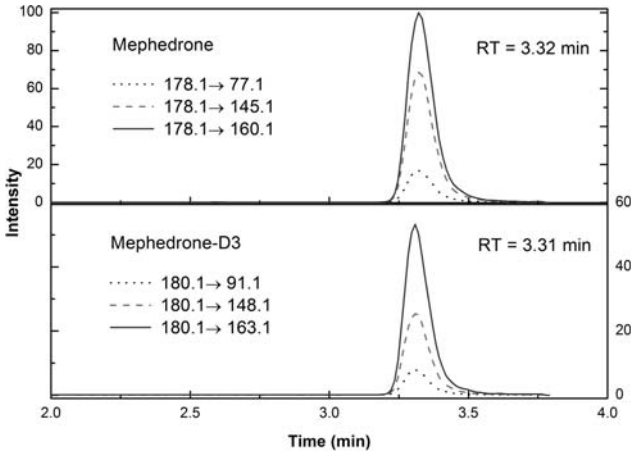

Figure 4. MRM chromatogram of the decedent's blood sample (LC–MS-MS).

provided by the manufacturer. Blood samples were analyzed on an Asys Expert Plus instrument (Asys Hitech GmbH).

Results

The purity of mephedrone base found in the 1 g (average) powders contained in eight plastic bags was in the range of 80.4–87.3 % (weight). The electron impact MS spectrum for mephedrone found in powders is shown in Figure 2, and the mephedrone UV spectrum is presented in Figure 3.

Mephedrone was found in the blood and vitreous humor at concentrations of 5.5 and 7.1 μg/mL, respectively. Concen-

Table III
Concentrations of Mephedrone in Fatal Poisonings*

Mephedrone (μg/mL)			Other detected substances	References
Blood	Urine	Vitreous humour		
5.5	NA	7.1	—	This case
5.1	186	NA	Blood: cocaine, MDMA, benzoylecgonine, methylecgonine, oxazepam, midazolam	(7)
0.98	NA	NA	Blood: atropine and naloxone (from resuscitation attempts)	(8)
2.24	+	NA	Blood: atropine, 3-TFMPP Urine: ethanol, acetone	(8)
0.13	+	NA	Blood: diazepam, nordiazepam, olanzapine, chlorpromazine metabolite Urine: methadone, EDDP, procyclidine	(8)
0.23	+	NA	—	(8)
0.5	198	NA	Blood: morphine Urine: 6-acetylmorphine, morphine, codeine, doxylamine	(9)
22	+	NA	Blood: amphetamine, diazepam, nordiazepam	(10)
3.3	>0.5	NA	Urine: benzoylecgonine, THCCOOH	(10)
5.7	+	NA	—	(10)
1.2	NA	NA	—	(10)

*Note: Not analyzed (NA); substance present but not quantified (+); 3-trifluoromethylphenylpiperazine (3-TFMPP); 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), the primary metabolite of methadone; 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCCOOH).

trations of mephedrone in vitreous humor samples were calculated from the standard curve prepared for blood. Comparison of the analysis results for blood controls and vitreous humor controls proved that the blood calibration curve can be applied to vitreous humor samples. MRM chromatograms of mephedrone and mephedrone-d_3 isolated from decedent's blood are presented in Figure 4.

No other drugs covered by routine screening analysis were detected in the powders and the biological specimens.

The results obtained by ELISA (with amphetamine and methamphetamine tests) for the samples with addition of mephedrone and 2C-B were classified as negative. The absorbance values for these compounds were not only higher than the absorbance of the blood samples spiked with amphetamine and MDMA at concentration of 50 ng/mL (established as a threshold for screening methods), but were also similar to the absorbance of the sample without any standards.

Discussion

The analysis of blood and vitreous humor samples showed the presence of mephedrone. No traces of initially targeted 2C-B were found. This was consistent with the results obtained for the powders. The concentration of mephedrone in biological samples appears to be very high, but reference concentrations are not readily available. The mephedrone concentration in blood in this case is in the range of the fatal concentrations reported to date (9–11). Mephedrone was not the primary cause of death in all described fatalities. Mephedrone was cited as a cause of death in some cases, but in others, death was attributed to multiple-drug toxicity associated with mephedrone and/or other drug use. In one case, mephedrone may have affected the ability to drive, leading to a fatal vehicular collision. In a recent study, two cases were presented in which death was attributed to mephedrone intoxication, the blood concentrations were found to be 22 and 3.3 μg/mL (12). Mephedrone concentrations determined in fatal poisonings are summarized in Table III. This study's results indicate that a high, fatal dose of mephedrone was taken. Oral dosages of 15–250 mg and intranasal dosages of 5–125 mg have been most frequently mentioned on the Internet; the corresponding concentrations in blood are unknown. In a non-fatal intoxication, mephedrone concentration in plasma was 0.15 μg/mL (6). Mephedrone intoxications that required hospitalization were reported after doses of 0.1–7.0 g (13). It is impossible to determine a safe dose for mephedrone, because negative side effects may be present in association with any dosage taken. Furthermore, similar dosages may have dramatically different consequences in different individuals (14).

Cross-reactivity ELISA with mephedrone and 2C-B was also tested. In this case, the amphetamine and methamphetamine assays were not triggered, although mephedrone has a similar structure to amine compounds. Daily routine screening procedures may not detect mephedrone, which may explain the lack of reference spectra in libraries.

Several fatalities were reported in the media across Europe, initially implicating mephedrone misuse, but a few have actually resulted in mephedrone cited as the cause of death (3). This paper presents the first documented fatal methedrone intoxication case in Poland.

This case also confirms the importance of analyses of non-biological materials by specific methods, and submitting the analyses together with biological material to one analytical laboratory. Negative or false positive results of spot tests conducted by the police (as in the described case) can lead to the wrong course of analysis for the biological material and new synthetic drugs may not be identified.

Conclusions

Mephedrone was found in blood and vitreous humor samples at concentrations of 5.5 and 7.1 μg/mL, respectively. The blood concentration of mephedrone was similar to those found in fatalities reported in literature.

It is important that even for non-biological materials, specific methods should be used, and both biological and non-biological materials from one case should be analyzed in the same institution.

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