Recommendations from the Brazilian Society of Clinical Analysis – SBAC regarding the preservation of urine samples for forensic purposes from female individuals suspected of sexual abuse

Recomendações da Sociedade Brasileira de Análises Clínicas – SBAC quanto à preservação de amostras de urina, para fins forenses, de indivíduos do sexo feminino sob suspeita de abuso sexual

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INTRODUCTION

The presence of spermatozoa in urine samples from female children and adolescents under 14 years old must be described in the laboratory report[1,2] and notified to the Guardianship Council or to the Childhood and Youth Court or to the Public Prosecutor's Office, in compliance with Law number 8.069/90 (Brazilian Child and Adolescent's Statute - ECA)[3] and Decree-Law number 2,848/40 (Brazilian Criminal Code),[4] under the risk of criminal violation.

The urine sample may contain various elements, such as cells from the from the vagina, penile urethra, and spermatozoa,[5] which can be used for forensic human identification purposes. By comparing reference samples with sperm cells, it is possible to identify the perpetrator in cases of sexual violence. The detection of spermatozoa helps confirm the occurrence of sexual activity, and subsequent DNA analysis may lead to the identification of the alleged perpetrator.[6]

Urine collection is a non-invasive option in cases of suspected sexual abuse, as it is possible to detect traces of Y chromosome-linked DNA (male sex marker) and spermatozoa in the urine of female patients up to 24 hours after penile-vaginal penetration. More studies are needed to precisely determine the effect of time on the detection of spermatozoa in urine samples after an alleged non-consensual sexual act; however, evidence shows that spermatozoa can be observed after sexual activity in the urine of patients during menstruation, as well as in patients who have previously urinated or bathed before urine collection.[6,7]

This article should be cited as:

Alves, Hemerson Bertassoni; Anghebem, Mauren Isfer; Franz, Ana Paula Giolo; Martinello, Flávia; Menezes, Maria Elizabeth. Recommendations from the Brazilian Society of Clinical Analysis – SBAC regarding the preservation of urine samples for forensic purposes from female individuals suspected of sexual abuse. RBAC. 2024; 56(3):146-148.

Received on Sep 10, 2024 | Approved on Sep 12, 2024 | DOI: 10.21877/2448-3877.202400196.en

Due to the scarcity of scientific material, this article aims to propose procedures and guidelines regarding the chain of custody and preservation of urine samples for forensic purposes of human genetic identification.

CHAIN OF CUSTODY OF THE SAMPLE

The chain of custody of forensic evidence, according to Law number 13,964/2019, which updated the Code of Criminal Procedure, is defined as the "set of all procedures used to maintain and document the chronological history of the evidence collected at crime scenes or from victims, to trace its possession and handling from its recognition to its disposal."[8]

If the Clinical Analysis Laboratory is notified to preserve a urine sample for forensic human identification purposes, it must adhere to the principles outlined in Articles 158-A to 158-F of the Brazilian Code of Criminal Procedure, the rules of which were published and established by Federal Law number 13,964/2019.[8] The laboratory becomes the faithful custodian of the sample under judicial demand, meaning it assumes responsibility for the sample and may not discard it until the legal proceedings are concluded, under penalty of liability for loss and damages (Article 640 of the Civil Code).[9]

In summary, the urine sample must be registered in an electronic system by the laboratory's registration system, including all possible identifying information, such as: first name, last name, date, time of collection, and other relevant details about the urine and its donor, describing how the sample arrived at the laboratory and what analytical procedures were performed, allowing for the tracking of its possession and handling from its recognition to its disposal.

PRESERVATION OF SAMPLES FOR GENETIC TESTING

Proper preservation of urine samples is crucial to ensure the DNA integrity for genetic testing used in human identification. Storage under cold conditions, and aseptic handling techniques are essential for obtaining accurate and reliable results. Additionally, clear communication with the laboratory that will conduct the genetic analysis is important to ensure that the preservation and transport methods are appropriate for the specific needs of the test.[10]

Technically, any frozen matrix that is not influenced by inhibitors of the polymerase chain reaction (PCR), including urine, can be stored for long periods.[10]

Technical Procedure

Depending on the situation, the clinical analysis laboratory should proceed as follows, always ensuring the chain of custody:

1. Primary urine sample for genetic identification collected at the laboratory or elsewhere:

Urine samples from female children and adolescents under 14 years old suspected of being contaminated by seminal material, collected at the laboratory or another location (hospital, clinic, school, daycare, residence). These samples should then be sent to the clinical analysis laboratory for forensic analysis, and processed as follows:

1. Separate a 10 mL aliquot of urine into a sterile tube and, if any urine sample remains, store the properly labeled primary tube in a freezer at -20 ºC until the conclusion of the process.
2. Centrifuge the sterile tube at 2000-3000 g for 10-15 minutes.
3. Discard the supernatant with a pipette and resuspend the pellet (sediment) in the remaining urine.
4. Freeze this sample in an Eppendorf-type tube in a freezer at least-20 ºC or, preferably, at -80 ºC.
5. Register this frozen sample in the laboratory's system for submission to the laboratories indicated by the court for testing, following the recommendations contained in RDC ANVISA 504/2021,[11] or its updates, which stipulate good practices for the transporting of human biological material.
6. Repeat steps 1 to 5, storing one sample in the laboratory for counter-testing analysis.

2. Processed urine sample for subsequent genetic identification

If the presence of spermatozoa is identified during the microscopic analysis of the urinary sediment from female children and adolescents under 14 years old, the finding must be reported as suggested in the Positioning of the Brazilian Society of Clinical Analysis (SBAC) regarding the description of the presence of spermatozoa in urine samples[2] and an aliquot of this material must be preserved for genetic identification testing.

The procedure for storage is as follows:

1. Separate two aliquots of the pellet (sediment) into Eppendorf-type tubes, one aliquot for the test and another for counter-testing analysis. If any urine sample remains in the primary tube, store it properly labeled in a freezer at -20 ºC until the conclusion of the process.
2. Freeze both tubes in a freezer at -20 ºC or, preferably, in a freezer at -80 ºC.
3. Register these frozen samples in the laboratory's system for submission to the laboratories indicated by the court*, following the recommendations contained in RDC ANVISA 504/2021.[10]

* The laboratory notified by the court must contact the Clinical and/or Forensic Analysis Laboratory designated by the court to conduct the analyses, in order to establish guiding procedures for the forwarding of these samples.

REFERENCES

1. Conselho Federal de Medicina. Parecer CFM nº 18/2024, de 27 de julho de 2024. Available at: https://sistemas.cfm.org.br/normas/visualizar/pareceres/BR/2024/18.
2. Anghebem, Mauren Isfer; Franz, Ana Paula Giolo; Martinello, Flávia. Posicionamento da Sociedade Brasileira de Análises Clínicas (SBAC) sobre o relato da presença de espermatozoides em amostra de urina – 2024. Comissão de Publicações da Sociedade Brasileira de Análises Clínicas – SBAC. RBAC. 2024; 56(3):133-137.
3. Brasil. Lei nº 8.069, de 13 de julho de 1990. Dispõe sobre o Estatuto da Criança e do Adolescente e dá outras providências. Diário Oficial da União, 27 set. 1990.7.
4. Brasil. Decreto-Lei nº 2.848, de 7 de dezembro de 1940. Código Penal. Diário Oficial da União, 3 jan. 1941.
5. Kouri TT, Hofmann W, Falbo R, Oyaert M, Schubert S, Gertsen JB, Merens A, Pestel-Caron M; Task and Finish Group for Urinalysis (TFG-U), European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). The EFLM European Urinalysis Guideline 2023. Clin Chem Lab Med. 2024 Jun 5;62(9):1653-1786.
6. Smith DA, Webb LG, Fennell AI, Nathan EA, Bassindale CA, Phillips MA. Early evidence kits in sexual assault: an observational study of spermatozoa detection in urine and other forensic specimens. Forensic Sci Med Pathol. 2014 Sep;10(3):336-43.
7. Joki-Erkkilä M, Tuomisto S, Seppänen M, Huhtala H, Ahola A, Karhunen PJ. Urine specimen collection following consensual intercourse - A forensic evidence collection method for Y-DNA and spermatozoa. J Forensic Leg Med. 2016 Jan; 37:50-4.
8. Brasil. Lei nº 13.964, de 24 de dezembro de 2019. Aperfeiçoa a legislação penal e processual penal. Diário Oficial da União, 29 abril 2021.
9. Brasil. Lei nº 10.406, de 10 de janeiro de 2002. Institui o Código Civil. Diário Oficial da União, 11 jan. 2002.
10. Sambrook, Joseph; Russell, David William. Molecular cloning: a laboratory manual. 3. ed. New York: Cold Spring Harbor Laboratory Press, 2001.
11. Resolução RDC nº 504, de 27 de maio de 2021. Dispõe sobre as boas práticas para o transporte de material biológico humano. Publicação: Diário Oficial da União, 31 maio 2021.