جامعة نايف العربية للعلوم الأمنية
NAIF ARAB UNIVERSITY FOR SECURITY SCIENCES
تأسست ١٩٧٨ Est. 1978

Naif Arab University for Security Sciences
Arab Journal of Forensic Sciences and Forensic Medicine
المجلة العربية لعلوم الأدلة الجنائية والطب الشرعي
https://journals.nauss.edu.sa/index.php/AJFSFM

الجمعية العربية لعلوم الأدلة الجنائية والطب الشرعي
Arab Society for Forensic Sciences and Forensic Medicine

Processing the Backlog of Sexual Assault Evidence From a Brazilian Forensic Genetics Laboratory Using Automation

معالجة الأدلة المتراكمة لقضايا الاعتداءات الجنسية في مختبر الوراثة الجنائية البرازيلي باستخدام الأتمتة

Gustavo Lucena Kortmann[1]*, Ana Paula Magalhães Leboute[2], Mara Regina Netto Benetti[2], Tatiana Pereira Gonzalez[2]

[1]Forensic Laboratory Department of Instituto-Geral de Perícias do Rio Grande do Sul, RS, Brazil.
[2]Forensic Genetics Division of Instituto-Geral de Perícias do Rio Grande do Sul, RS, Brazil.

Received 28 Feb. 2024; Accepted 30 Mar. 2024; Available Online 18 Jun. 2024.

Abstract

The accumulation of untested sexual evidences is a common problem for many forensic laboratories. In Brazil, there are around 150 thousand sexual evidences awaiting DNA processing. At the same time, manual extraction by differential lysis of these evidences is laborious and time-consuming. In this regard, the Brazilian federal government has structured and sponsored forensic genetics laboratories, providing automated platforms for evidence processing, with the objective of clearing the backlogs of sexual evidences and feeding the national DNA database. This study aims to evaluate the results from processing the backlog of sexual evidences in the forensic genetics laboratory of the Instituto-Geral de Perícias do Rio Grande do Sul to verify if the use of automation is an efficient strategy to manage large amounts of stored evidences. A total of 614 cases of sexual assault (1026 sexual evidences) from the last 10 years (2013 to 2023) with at least one sample with positive result for spermatozoa detection were analyzed. Samples were submitted for DNA extraction by differential lysis on the Hamilton Microlab Autolys STAR SAE platform, followed by the amplification of STR autosomal markers, capillary electrophoresis analysis in the ABI 3500 genetic analyzer and insertion in the BPG-RS

Keywords: Forensic sciences, Rape, DNA Database, Untested evidence, Crime resolution.

المستخلص

يعتبر تراكم الأدلة الجنسية غير المفحوصة مشكلة مشتركة للعديد من المختبرات الجنائية. في البرازيل، حيث يوجد حوالي 150 ألف دليل من الأدلة الجنسية تنتظر عمل تحليل للحمض النووي. في الوقت نفسه، يعتبر الاستخلاص اليدوي باستخدام التحلل التفاضلي لهذه الأدلة عملية شاقة وتستغرق وقتًا طويلاً. ومن ثم، قامت الحكومة الفيدرالية البرازيلية بتنظيم وتمويل مختبرات الأدلة الجنائية، وتوفير منصات آلية لمعالجة الأدلة، بهدف التخلص من تراكم الأدلة الجنسية وإدخالها في قاعدة بيانات الحمض النووي الوطنية.

وتهدف هذه الدراسة إلى تقييم نتائج معالجة تراكم الأدلة الجنسية في مختبر الأدلة الجنائية التابع لمعهد الطب الشرعي العام في ريو غراندي دو سول للتحقق مما إذا كان استخدام الأتمتة إستراتيجية فعالة لإدارة كميات كبيرة من الأدلة المخزنة. وقد تم تحليل ما مجموعه 614 حالة اعتداء جنسي (1026 دليلاً جنسياً) من السنوات العشر الأخيرة (2013 إلى 2023) مع وجود عينة واحدة على الأقل بنتيجة إيجابية لاكتشاف الحيوانات المنوية. وأُرسلت العينات لعمل استخلاص للحمض النووي عن طريق التحلل التفاضلي باستخدام جهاز Hamilton Microlab Autolys STAR SAE، تلتها عملية تكثير للمواقع الوراثية STR الجسدية، ثم تحليل الهجرة الكهربائية الشعيرية في جهاز تحليل الجينات ABI 3500 والإدخال في قاعدة بيانات الحمض النووي لولاية ريو غراندي دو سول باستخدام برنامج CODIS 7.

الكلمات المفتاحية: علوم الأدلة الجنائية، الاغتصاب، قاعدة بيانات الحمض النووي، الأدلة غير المفحوصة، حل الجريمة.

Production and hosting by NAUSS

* Corresponding Author: Gustavo Lucena Kortmann
Email: gustavo-kortmann@igp.rs.gov.br

doi: 10.26735/ADAW2637

using CODIS 7 software. The cases were stratified into three groups, according to the year in which they occurred (182 cases from 2013 to 2016; 123 cases from 2017 to 2019 and 151 cases from 2020 to 2023), in order to verify whether there were differences in the efficiency of differential extraction due to the storage time. 374 cases (61%) presented at least one sample with an eligible autosomal genetic profile for DNA national database. In 64 cases (10.4%) it was possible to obtain only the Y chromosome haplotype profile. From each sexual assault case, only one sample was inserted in the DNA national database. Cases from the years 2020 to 2023 presented 68% of autosomal eligible profiles, followed by cases from 2017 to 2019 (58%) and by cases from 2013 to 2016 (54.9%). The results of this study support the importance of processing the backlog of sexual samples as soon as possible in order to get more eligible autosomal profiles for CODIS ($p < 0.05$). Using automation to reduce the size of the backlog has been effective, allowing the simultaneous processing of a large number of samples, with reliability and less chance of contamination.

وقد صُنفت الحالات إلى ثلاث مجموعات حسب السنة التي حدثت فيها (182 حالة من 2013 إلى 2016 و123 حالة من 2017 إلى 2019 و151 حالة من 2020 إلى 2023) وذلك للتحقق مما إذا كانت هناك اختلافات في كفاءة الاستخلاص التفاضلي بسبب مدة التخزين. وأظهرت 374 حالة (61٪) عينة واحدة على الأقل تحتوي على ملف تعريف جيني صبغي جسمي مؤهل لقاعدة بيانات الحمض النووي الوطنية. وفي 64 حالة (10.4٪) كان من الممكن الحصول فقط على ملف تعريف نمط كروموسوم Y.

من كل حالة اعتداء جنسي، تم إدخال عينة واحدة فقط في قاعدة بيانات الحمض النووي الوطنية. وأظهرت الحالات من عام 2020 إلى 2023 نسبة 68٪ من ملفات التعريف الشخصية الصبغية الجسدية المؤهلة، تليها الحالات من عام 2017 إلى 2019 (58٪) ومن ثم الحالات من عام 2013 إلى 2016 (54.9٪).

وتدعم نتائج هذه الدراسة أهمية معالجة تراكم العينات الجنسية في أقرب وقت ممكن من أجل الحصول على المزيد من ملفات التعريف الشخصية للكروموسومات الجسدية المؤهلة لنظام CODIS (قيمة الاحتمالية أقل من 0.05). لقد كان استخدام الأتمتة لتقليل حجم المتراكم فعالاً، حيث سمح بالمعالجة المتزامنة لعدد كبير من العينات، مع موثوقية وفرصة أقل للتلوث.

1. Introduction

Sexual crimes are a worldwide problem with some relevant similarities, such as the lack of notification to authorities [1] by the victim and recidivism by the sexual offenders [2]. A worldwide systematic review and meta-analysis estimated that nine girls and three boys out of 100 are victims of forced intercourse [3]. Moreover, every minute, two person Experience sexual assault in Brazil [4]. These cases are generally underrated, with less than 10% of cases reported to police authorities [4]. Most victims are children under 14 years old in 75% of these cases. In 85% of these sexual violence cases, the perpetrators are known by the victim, a fact that reinforces underreporting [5].

Despite the worrying scenario for sexual assault, only some of the victims have been able to have their sexual assault evidences processed for DNA profiling [6, 7]. The accumulation of untested sexual evidences is a common problem for many forensic laboratories [1, 8]. In Brazil, there are around 150.000 sexual evidences awaiting DNA processing [9]. A manually differential lysis of these evidences is a laborious and time-consuming [10]. While jurisdictions may vary in their definition of a backlog based on legislation or policy, it is generally considered as a case received by the laboratory that exceeds the laboratory's capacity and is awaiting testing [11]. In this regard, the Brazilian National Public Security Secretariat (SENASP) created a working group in 2018 to study alternatives to reduce sexual evidences backlogs in Brazilian laboratories. SENASP has also structured and sponsored forensic genetics laboratories, providing automated platforms for evidence processing, with the objective of clearing the backlogs of sexual evidences and feeding the national DNA database.

The Forensic Genetics Division of the Instituto-Geral de Perícias do Rio Grande do Sul (IGP-RS) is one of the oldest official criminal laboratories in Brazil and is responsible for processing all evidence from criminal cases in Rio Grande do Sul, the southernmost state of Brazil. In 2020, IGP-RS received a Hamilton MicrolabAutolys STAR SAE platform from SENASP to help to process all of its backlog of sexual evidences, estimated at about 4.500 cases and 17.000 evidences.

There are many recommendations about DNA processing of sexual assault evidence kits [11]. However, different approaches still remains as particular decisions of the criminal laboratories and of the local policies impact forensic work [7]. This study aims to evaluate the results of processing sexual evidences in the forensic genetics laboratory of the IGP-RS to verify if the use of automation has been an efficient strategy to manage large amounts of stored evidences. Specifically, we aim to estimate the most efficient procedure to process the entire sexual assault backlog.

2. Materials and Methods

2.1. Setting and samples

A total of 614 cases of sexual assault (1026 sexual evidences) from the last 10 years (2013 to 2023) of the IGP-RS backlog with at least one sample with positive result for spermatozoa detection (Papanicolaou stain) were analyzed. Cases in which CODIS-eligible genetic profiles were not obtained in the first cycle and with sufficient material for repetition were reprocessed following the same conditions.

The cases were stratified into three groups, according to the year in which they occurred (182 cases from 2013 to 2016; 123 cases from 2017 to 2019 and 151 cases from 2020 to 2023), in order to verify whether there were differences in the efficiency of differential extraction due to the storage time. We followed SWGDAM recommendation about processing first the swabs contained in the sexual assault kits [11]. However, for some cases, underwear, condoms and other items were the only available evidences.

At least one type of evidence from each case was sampled in the Autolys A-tube and worklists with 42 sexual assault samples positive for spermatozoa were created.

2.2. DNA typing

Samples were submitted to differential separation process of sperm and non-sperm cells on the Hamilton MicrolabAutolys STAR SAE platform [10]. The equipment is set to process up to 42 sexual assault samples per cycle, resulting in 84 fractions (42 sperm and 42 non-sperm fractions). Each resulting sample were submitted to DNA extraction and purification by magnetic beads on the Hamilton MicrolabAutolys STAR SAE platform, using the PrepFiler™ Automated Forensic DNA Extraction Kit from Applied Biosystems. Quantification was performed using Quantifiler Trio (Applied Biosystems) and/or Quantiplex PRO (Qiagen) on the ABI 7500 RealTime PCR System. Normalization was performed also by Hamilton MicrolabAutolys STAR SAE platform in an integrated workflow from the quantification output data. Amplification of STR autosomal markers and Y-STR markers were performed, respectively, with PowerPlex Fusion 6C kit and from PowerPlex Y23 kit (Promega) on Veriti Thermal Cyclers (Applied Biosystems). Capillary electrophoresis analysis were run in the ABI 3500 genetic analyzer. Genetic profiles were analyzed in the Genemapper ID-X (Applied Biosystems) software and inserted in the Rio Grande do Sul DNA Database using CODIS 9 software. All the steps in DNA typing followed the manufacturers' recommendations.

2.3. Statistical analysis

The three sexual assault case groups were compared using Pearson's chi-square analysis to evaluate the relation between the samples storage time and the success in obtaining eligible autosomal genetic profiles for CODIS. Significance was evaluated as $p < 0.05$.

3. Results and Discussion

Processing the sexual assault evidences at Hamilton MicrolabAutolys STAR SAE platform re-

Table 1- *Sexual assault cases of IGP-RS backlog grouped by age of occurrence and by success of at least one sample with eligible autosomal genetic profile for CODIS after processed with automation.*

Year group of case occurrence	Eligible for CODIS	Non eligible for CODIS	Row Totals
2013 - 2016	100 (110.86) [-1.03]	82 (71.14) [1.29]	182
2017 - 2019	123 (129.13) [-0.54]	89 (82.87) [0.67]	212
2020 - 2023	151 (134.01) [1.47]	69 (85.99) [-1.83]	220
Column Totals	374	240	614 (Grand Total)

$\chi^2 = 8.98$. p-value = 0.011.

(in parentheses) = expected number of sexual assault cases calculated on chi-square test.

[in brackets] = standardized residual analysis after chi-square result for each year group of sexual assault case occurrence and eligible status for CODIS insertion.

sulted in good quality genetic profiles for CODIS and demonstrated to be a great strategy to clear backlog evidences from the laboratory with few manual steps by the analyst and possibility to integrate all the DNA processing workflow. 374 cases (61%) of the total amount presented at least one sample with an eligible autosomal genetic profile for CODIS and were submitted to local database (including forensic unknown and forensic mixtures). Single source samples (forensic unknown) containing at least the 13 CODIS core loci (D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX and VWA) were also uploaded to the DNA national database. In 64 cases (10.4%) it was possible to obtain only the Y chromosome haplotype profile. In the last situation, a report was send to the police asking for suspects for comparison. We verified that in most of these cases the quantification showed that the male-female ratio in the sperm fraction was unfavorable to obtain the male autosomal genetic profile (greater than male-female ratio 1:4, as observed in our internal validation). In few cases, quantification pointed to a low autosomal DNA template, yielding only Y chromosome haplotype profiles. However, the number of samples that resulted only in Y haplotype profiles is insufficient to establish a correlation between the obtaining of Y profiles and the years of sample storage in the backlog. From each sexual assault case, only one sample was inserted in the DNA national database. Table 1 indicates the effect of the sample age of sexual assault cases of IGP-RS backlog on the success of obtaining at least one sample with eligible autosomal genetic profile for CODIS after processed with automation.

Cases from the years 2020 to 2023 presented 68% of autosomal eligible profiles for CODIS, followed by cases from 2017 to 2019 (58%) and by cases from 2013 to 2016 (54.9%) ($p = 0.01$), Table 1. The standardized residual analysis was not significant for an $\alpha = 0.05$, but the residual analysis point to a larger difference between the obtained and expected data for the more recent cases group (2020 to 2023), in which the autosomal genetic profiles for CODIS were clearly more frequent than in the other groups. Results obtained in this study are similar to those obtained in other Brazilian forensic genetics laboratories [12, 13]. This similarity might be partially explained by the standardization of methodologies and reagents used in the Brazilian forensic genetics laboratories, which follow policy statements of the Integrated Network of DNA Databases (RIBPG) [14]. However, the results also highlight the need of a national discussion about strategies to improve these success rates of eligible genetic profile for CODIS of sexual assault evidences.

Some cases with no results in the first sample analysis resulted a genetic profile eligible for

CODIS in the second sample processing, indicating heterogeneity in the collection or in the preservation conditions of the sexual assault samples. The more recent cases presented the best average success rate for eligible male autosomal profiles for CODIS. This may be explained by better overall integrity of samples or evolution in the conditions of sample collection and storage [15]. Despite the fact that most sexual assault samples came from more populous cities near the state capital Porto Alegre, our institution have faced some difficulties to access victims who live far from the forensic legal medicine units spread in the state. Hospitals in Brazil generally do not collect sexual assault evidences for forensic DNA analysis. Thus, sexual assault victims frequently arrive at forensic legal medicine units some days after the crime occurred, preventing the sexual assault evidences to be collected within the proper interval of the first 72 hours after violence [11, 16].

In this context, forensic institutions should play a major role in identifying promptly these perpetrators, in order to prevent them from continuing to commit crimes. Crime evidences awaiting DNA processing are also a common worldwide problem and requires authorities attention because each sexual victim should receive the same respect considering sample processing by the forensic science community.

4. Conclusion

Our results support the importance of processing the backlog of sexual samples as soon as possible in order to get more eligible autosomal profiles for CODIS. We suggest that the strategy of processing evidence from most recent to oldest is suitable for populating the genetic profile databases more quickly in order to benefit sooner from their efficiency. Using automation to reduce the size of the backlog has been effective, allowing the simultaneous processing of a large number of samples, with reliability and less chance of contamination. Moreover, the use of automation to process the sexual evidences backlog allows the forensic lab staff to be available for other tasks that require greater expertise from the analysts, such as analysis and interpretation of genetic profiles, statistical calculations and report.

Automating sexual evidences processing steps may also represent a solution for forensic genetics laboratories with few analysts or that are experiencing periods of restricted in-person work, as occurred during the COVID-19 pandemic. The integration of all equipment in a network workflow allows the processing and operation of equipment to be carried out even if working remotely, providing alternatives for modernizing laboratories.

Despite the successful results obtained through automation of the sexual assault backlog processing, our study has some limitations. Only samples with positive result for spermatozoa detection were included in this study. It remains necessary to evaluate viable approaches for samples with negative spermatozoa results, especially when they are most of backlog samples. Moreover, more studies are necessary in order to access how the degradation status of these samples affect the genetic profiles recovery efficiency over various storage years. Additional studies are also important to evaluate which techniques would be interesting to laboratories with different automated platforms or when there is no automation available.

Conflict of interest

The authors declare no conflicts of interest.

Source of funding

This study was supported by Brazilian National Public Security Secretariat (SENASP/MJSP) with collaboration from the Public Security Secretariat of Rio Grande do Sul (SSP/RS).

Acknowledgement

We would like to thank Janaína Pacheco Jaeger for the critical review of the related statistics.

References

1. Sulley C, Wood L, Cook Heffron L, et al. "At Least They're Workin' on My Case?" Victim Notification in Sexual Assault "Cold" Cases. J Interpers Violence. 2021;36(9-10):4360-4380. DOI:10.1177/0886260518789905
2. Furby L, Weinrott MR, Blackshaw L. Sex offender recidivism: a review. Psychol Bull. 1989;105(1):3-30. DOI:10.1037/0033-2909.105.1.3
3. Barth J, Bermetz L, Heim E, Trelle S, Tonia T. The current prevalence of child sexual abuse worldwide: a systematic review and meta-analysis. Int J Public Health. 2013;58(3):469-483. DOI:10.1007/s00038-012-0426-1
4. Ferreira H, Coelho DSC, Cerqueira D, Alves P, Semente M. Elucidando a prevalência de estupro no Brasil a partir de diferentes bases de dados. Instituto de Pesquisa EconômicaAplicada – IPEA 2023 [cited 2024, Feb 14]. Available from: https://repositorio.ipea.gov.br/bitstream/11058/11814/4/TD_2880_web.pdf
5. ANUÁRIO BRASILEIRO DE SEGURANÇA PÚBLICA. São Paulo: Fórum Brasileiro de SegurançaPública, ano 16, 2022. ISSN 1983-7364.
6. Lovrich NP, Pratt TC, Gaffney MJ, Johnson CJ, Asplen CH, Hurst LH, et al. National DNA study report, final report. Washington, DC: U.S. Department of Justice, 2004.
7. Strom KJ, Hickman MJ. Unanalyzed evidence in law-enforcement agencies: a national examination of forensic processing in police departments. Criminol Public Policy 2010;9:381–404.
8. Spohn C. Untested sexual assault kits: a national dilemma. Criminol Public Policy 2016;15:551–4.
9. BRASIL. Comitê Gestor da Rede Integrada de Bancos de PerfisGenéticos. Manual de procedimentosoperacionais da rede integrada de bancos de perfisgenéticos [Internet]. Ministério da Justiça e SegurançaPública. 2019 [cited 2024, Feb 14] Available from: https://www.gov.br/mj/pt-br/assuntos/sua-seguranca/seguranca-publica/ribpg/manual
10. Timken MD, Klein SB, Kubala S, Scharnhorst G, Buoncristiani MR, Miller KW. Automation of the standard DNA differential extraction on the Hamilton AutoLys STAR system: A proof-of-concept study. Forensic Sci Int Genet. 2019;40:96-104. DOI: 10.1016/j.fsigen.2019.02.011
11. SWGDAM. Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits in a Laboratory [Internet]. 2016 [cited 2024, Feb 14] Available from: https://www.swgdam.org/publications
12. Carvalho NR, Arão GOL, Lima YAR, Godinho NMO, Mota MF, Gigonzag TCV. The contribution of DNA databases for stored sexual crimes evidences in the central of Brazil. Forensic science international. Genetics. 2020;46:102235. DOI: 10.1016/j.fsigen.2020.102235
13. Kobachuk LDG, Castro TC, Eidt FP, Marina SBCC, de Moraes BAC, Andressa MTLJ, Brodzinski J, Maia TRM, Maia R, Martins SC, Leonardo SRF, Malaghini M. Processamento de backlog de vestígios de crimes sexuais no Estado do Paraná. Rev. Bras. Crimin. 2023, 12(2):55-60.
14. da Silva Junior RC, Wirz LN, Solares Reyes E, Del Moral Stevenel MA. Development of DNA databases in Latin America. Forensic Sci Int. 2020;316:110540. DOI:10.1016/j.forsciint.2020.110540
15. Williams CM. Evidentiary discrepancies in sexual assault casework within the US. Forensic Sci Res. 2021;6(3):189-194. DOI:10.1080/20961790.2021.1960465
16. Hall A, Ballantyne J. Novel Y-STR typing strategies reveal the genetic profile of the semen donor in extended interval post-coital cervicovaginal samples. Forensic Sci Int. 2003;136(1-3):58-72. DOI:10.1016/s0379-0738(03)00258-5.