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Method Article

A culturally appropriate method for validating self-reported drug administration among indigenous people who use injection drugs

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A R T I C L E I N F O

Method name:
Using syringe washes from Indigenous people who use injection drugs to validate self-reported drug administration

Keywords:
Injection drugs
Polysubstance use
American Indians/Alaska natives
Mass spectrometry

A B S T R A C T

Compared with other racial/ethnic groups in the United States (US), American Indians/Alaska Natives have one of the fastest climbing rates of drug overdose deaths involving stimulants. Validating the substances self-reported by Indigenous people who use injection drugs (IPWIDs) can present logistical and cultural challenges. While the collection of biospecimens (e.g., urine, blood, hair follicle) can be one way to cross-validate the substances self-reported by IPWIDs, the collection of biospecimens has been historically problematic when conducting substance use research with Indigenous North Americans. In our National Institutes of Health (NIH)-supported pilot research conducted with IPWIDs, we have documented low willingness to provide a biospecimen to a research team. This article demonstrates an alternative method for validating self-reported substances injected by IPWIDs that does not require the extraction of biospecimens from Indigenous bodies and spaces. The method described includes:

- Collecting used, unwashed syringes from IPWIDs at the time of behavioral assessment,
- Sampling the used syringe by washing the syringe needle/barrel with methanol,
- Analyzing the samples with gas chromatography mass spectrometry (GC–MS) and liquid chromatography coupled to triple-quadrupole mass spectrometry (LC-QQQ-MS).

This method offers a more culturally appropriate alternative to validate substances self-reported by IPWIDs during behavioral assessments.

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Specifications table

Subject area:	Chemistry
More specific subject area:	Substance use
Name of your method:	Using syringe washes from Indigenous people who use injection drugs to validate self-reported drug administration
Name and reference of original method:	Néfau, T., Charpentier, E., Elyasmino, N., Duplessy-Garson, C., Levi, Y., & Karolak, S. (2014). Drug analysis of residual content of used syringes: A new approach for improving knowledge of injected drugs and drug user practices. The International Journal of Drug Policy, 26(4), 412–419. Brunt, T., Lefrançois, E., Gunnar, T., Arponen, A., Seyler, T., Goudriaan, A. E., McAuley, A., McKeown, D. A., Detrez, V., Csorba, J., Deimel, D., Auwärter, V., Kempf, J., Karolak, S., & Nefau, T. (2021). Substances detected in used syringes of injecting drug users across 7 cities in Europe in 2017 and 2018: The European Syringe Collection and Analysis Project Enterprise (ESCAPE). The International Journal of Drug Policy, 95, 103,130–103,130.
Resource availability:	Methanol (CH4O, LC/MS Grade) Agilent 7890 GC coupled to a 5975C MS Agilent 1290/6470 LC-QQQ-MS

Method details

Compared with other racial/ethnic groups in the United States (US), American Indians/Alaska Natives have the fastest climbing rates of drug overdose deaths involving stimulants [1]. Indigenous people who use injection drugs (IPWIDs) experience elevated risks for polysubstance use and overdose given their sociodemographic profile, unique health risks, and limited access to treatment and harm reduction services [2]. Validating the substances self-reported by IPWIDs in behavioral research may be necessary, as interviewees may be under the influence of a substance at the time of the research activity, and given that the target substance being injected by IPWIDs may be adulterated with compounds unknown to the individual. While the collection of biospecimens (e.g., urine, blood, hair follicle) can be one way to cross-validate the substances self-reported during an assessment, the collection of biospecimens is a historically and socially contentious issue when conducting research in North American tribal communities. Indigenous people in the US have historically been subject to research that provides little to no benefit to them, which has fomented resistance to large-scale research projects using biospecimens [3]. There is limited literature on factors related to the decision of Indigenous people to provide biospecimens to researchers [4]. However, simply excluding Indigenous North Americans from research protocols including the collection of biospecimens could result in the under-identification of risk factors and unequitable distribution of research that contributes to health disparities [4]. In conducting community-engaged research with IPWIDs (Assiniboine and Sioux) living on the Fort Peck Reservation in Northeastern Montana, we sought to identify an alternative and culturally appropriate method for cross-validating self-reported substances injected by IPWIDs.

This current study builds on ongoing community based participatory research (CBPR) between members of the study team and the Fort Peck tribes over the past 15 years. The current study was supported by the National Institute On Minority Health and Health Disparities of the National Institutes of Health Under Award Number NIMHD (U54MD012393), Florida International University Research Center in Minority Institutions. In the spirit of determining the appropriateness of future research practices, 40 IPWIDs in Northeastern Montana were asked about their perspectives regarding the collection of biospecimens that would be informative to future research efforts. Only 22.5% of participants reported that they would be willing to provide biospecimens to researchers in a future research project (Table 1). However, some participants described that they would be willing to provide a used syringe to the research team. A used syringe is considered a non-medical device and collecting it for the purposes of validating the target substance of use within the syringe avoids the historically contentious issue of collecting/extracting biospecimens from tribal members' bodies and communities. Beyond its application for research with Indigenous North Americans, this method may also be applicable for use with PWIDs more broadly. As part of the pilot research project, a sub-sample of IPWIDs ($n = 10$) were asked to bring a used, unwashed syringe to the study before receiving a behavioral assessment. This manuscript describes the methods that were used to 1. Collect behavioral data and used syringes from IPWIDs, 2. Collect a sample from the syringe, and 3. Cross-validate the self-reported behavioral data using biochemical analysis in a subsample of 10 IPWIDs.

Eligibility

Eligible participants were individuals who were: (1) currently injecting drugs or who previously injected substances for ≥12 months; (2) A registered member of a federally recognized tribe, or an associate tribal member; and (3) Greater than 13 years of age. The study's only exclusion criterion was being currently incarcerated and/or in police custody at the time of the study. All participants provided verbal consent prior to participating in the study. All study participants received $50 for participating in the research and were offered clean syringes in exchange for the used syringe. Human Subjects approval was obtained from the Fort Peck Institutional Review Board (IRB) and the Florida International University IRB (Protocol Approval # IRB-21–0177). Ten IPWIDs provided a used syringe to the study team.

Recruitment

Participants were recruited through a chain-referral sampling procedure. In chain-referral sampling, seed participants are selected and study participants recruited other IPWIDs through word of mouth and invitation by the previous participant. This sampling

Table 1
Perspectives regarding researchers' use of biospecimens in a pilot sample of IPWIDs, $n = 40$.

Questionnaire Item	Total N (%)
Biospecimen collection	
Has heard of researchers doing this	24 (60.0%)
Has never heard of researchers doing this	16 (40.0%)
Willingness to provide biospecimens to researchers in a future research project	
Willing	9 (22.5%)
Unwilling	5 (12.5%)
It depends	26 (65.0%)

strategy was chosen given the assumption that members of the target population know one another and are densely interconnected [5,6].

As part of the recruitment protocol, participants were asked to bring an unwashed, used syringe to the study site. The syringe was tagged with a code by a data collector who wrote the code on tape that was attached to the barrel of the syringe, and then placed into a sharps disposal container using tweezers. The code was then applied to each participants' behavioral data. Participants then responded to a questionnaire to collect information regarding demographic information, substance use history, and willingness to try an Injection Drug Use Syringe Filter (DUSF) at a future point in time. For this portion of the pilot research, data were collected during July of 2022.

Behavioral data collection

Participants were asked to provide self-reported information on gender identity, age, year of birth, relationship status, tribal registration or associate tribal member status, and tribal identification (Assiniboine, Sioux, both, other). To collect data on drug use history, the drug/alcohol use module of the Addiction Severity Index (ASI) 5th Edition adapted for use with American Indians was administered [7]. The module screens participants for use of alcohol, heroin, methadone, other opiates/analgesics, barbiturates, sedatives/hypnotics/tranquilizers, cocaine, amphetamines, cannabis, hallucinogens, inhalants, and more than one substance used per day. For each substance, participants were asked whether they had ever used the substance in their lifetime (yes/no), whether they had used the substance in the past 30 days (yes/no), age at first use, years of use, route of administration, and age of last use. Participants were also asked to indicate the substance that they last used in the syringe prior to bringing it to the study site.

Sample extraction and preparation

At the end of each data collection day, sharps disposal containers were brought to a local chemistry lab. The sample preparation protocol was informed by that used by Néfau et al. (2014), where methanol was used to pump the used syringe in order to dissolve the compounds in the syringe prior to depositing the solution back into a clean vial [8]. In a subsequent related study, the syringe was pumped five times to wash the syringe [9]. Safety measures were taken when preparing the samples, including the use of extra-long forceps to handle syringes, thick nitrile gloves, and splash-resistant laboratory gowns (the researcher sampling the syringe used a splash shield instead of goggles when pumping the syringe) [9]. (See Figs. 1 and 2). To wash the syringe needle and barrel, 1.5 mL of methanol (CH_3OH, LC/MS Grade) was pumped five times with the used syringe and then deposited into a 2 mL glass vial. Two syringes were broken and could not be pumped, which from visual examination appeared to be due to overuse of the syringe. For these damaged syringes, methanol was deposited directly into the barrel of the syringe and then poured from the barrel of the syringe directly into the vial without pumping the methanol through the needle. Overuse of syringes is likely among IPWIDs in this region due to limited accessibility of new syringes and the absence of a local harm reduction program [10,11]. For each day of data collection, a clean (unused) syringe was pumped five times with 1.5 mL of methanol and deposited into a vial to obtain a control sample for that day of collection/extraction. Vials were coded, wrapped in parafilm to prevent evaporation, and subsequently refrigerated. Samples were packed in a Styrofoam container that was surrounded by dry ice and shipped overnight (from Williston, North Dakota to the Forensic and Analytical Toxicology Facility at Florida International University) for analysis. The samples arrived within 24 h and were still cool upon delivery to the facility. The samples were immediately refrigerated the day that they arrived.

Biochemical analysis

Laboratory technicians receiving the samples were blind to the substance that was self-reported by research participants during the behavioral assessment. Samples were analyzed by gas chromatography mass spectrometry (GC–MS) and liquid chromatography coupled to triple-quadrupole mass spectrometry (LC-QQQ-MS).

To prepare samples for GC–MS analysis, a 10 μL aliquot was transferred into a 2 mL amber glass vial with a 250 μL glass insert. A 1:10 dilution of the sample with methanol was performed to reduce high concentrations of analytes. Instrument specification and parameters for GC–MS are shown in Table 2.

Fig. 1. Extra-long forceps were used to handle the tagged syringes.

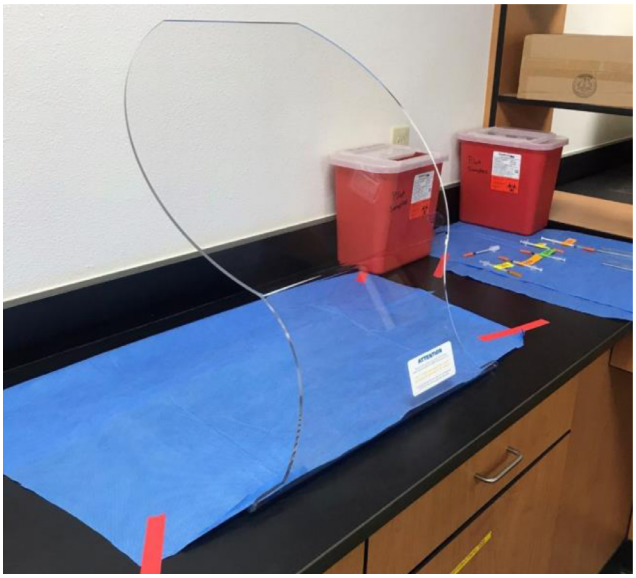

Fig. 2. A splash guard was used as PPE to protect the technician while pumping the used syringes with methanol.

Table 2
Instrument specification and parameters for GC–MS.

Instrument	Agilent 7890 GC coupled to a 5975C MS
Analytical Column	Restek RTX-Volatiles (30 m x 0.25 mm x 0.25 μm)
Carrier gas	Helium with a flow rate of 1.2 mL/min
Oven Ramp Temperatures	60 °C hold for 1 min and the temperature increases to 260 °C at a rate of 10 °C/min and hold for 8 min
Injection	1 μL sampling volume, Split 20:1, injection port at 280 °C
Mass Analyzer	MS, Transfer line at 300 °C, full scan mode 45–570 m/z
Ionization Source	EI, source temperature at 230 °C, quadrupole temperature at 150 °C

Table 3
Instrument specification and parameters for LC-MS.

Instrument	Agilent 1290/6470 LC-QQQ-MS
Analytical Column	HPLC Zorbax reversed phase C18 column (3.0 × 50 mm, 1.8 μm)
Flow rate	0.300 mL/min
Injection	1 μL sampling volume, column temperature at 40 °C
Mass Analyzer	QQQ, MS2 Scan
Ionization Source	AJS ESI, gas temperature 325 °C, gas flow 11 L/min, nebulizer 35 psi, sheath gas temperature 350 °C, sheath gas flow 11 L/min, capillary voltage 4000 V, nozzle voltage 1500 V
Mobile Phase	Aqueous phase (A): 5 mM ammonium formate in water with 0.1% formic acid Organic phase (B): 0.1% formic acid in methanol
Gradient	Starts at 5% B for 1 min, then increases to 95% B at 6 min, and ends at 9 min with the final gradient composition of 95% B. Total run time 6 min and 3 min of post time for column conditioning.

To prepare samples for LC-QQQ-MS analysis, a 10 μL aliquot was transferred into a 2 mL amber glass vial with a 250 μL glass insert. A 1:100 dilution of the sample with methanol was performed on each sample to reduce high concentrations of analytes. Instrument specification and parameters for the LC-QQQ-MS are shown in Table 3.

Peak splitting was observed in GC–MS analyses with samples that had very high levels of methamphetamine. For these samples, a dilution with additional methanol was performed before reinjection. All samples required a dilution before injection into the LC-QQQ-MS instrument. In addition to a dilution (1:10), a longer analytical method was used with samples for GC–MS analysis in attempts to elucidate other potential target compounds that may have been masked by a large methamphetamine peak. As a result, the diluted samples showed a retention time (RT) shift of ~4 min. A methamphetamine standard was injected to determine RT and mass spectrum for comparison to sample results. Other drugs of interest and adulterants were scanned for via extracted ion chromatograms (EIC) in both GC and LC analyses. These included methamphetamine, clonitazene, N-benzylamphetamine, amphetamine, ephedrine/pseudoephedrine, and benzene. Results from the standard (RT and mass spectrum) were used to confirm the presence of the analytes in the samples.

Method validation

According to the behavioral assessment, each of the ten IPWIDs reported that their syringes were used to inject only methamphetamine. According to the analysis by GC–MS and LC-QqQ-MS, all samples (except for the control samples) contained methamphetamine (see Supplementary Material). No other target analytes were detected. The lack of identification of adulterants likely reflects a confluence of factors including probable local production of the methamphetamine tested, the relatively lower cost of not adulterating the methamphetamine from which these samples were most likely drawn, and the chain-referral sampling technique used to recruit participants that likely produced homogeneity among the ten IPWIDs who formed part of this pilot. Nonetheless, methamphetamine, clonitazene, N-benzylamphetamine, amphetamine, ephedrine/pseudoephedrine, and benzene were scanned for via EIC in both GC and LC analyses, and adulterated methamphetamine may be detected in the broader population of IPWIDs. GC–MS and LC-QQQ-MS results by individual IPWID are included in the supplementary materials that accompany this manuscript.

In addition to cross-validating the behavioral data with the results of the biochemical analysis, the validation of the 10 samples illustrates that asking IPWIDs to bring an unwashed syringe to the study site worked in the context of the chain-referral sampling strategy. Participants will often wash their syringes with bleach before disposing of them. In this case, it was demonstrated that the study protocol was feasible for participants and the research team, as well as the tribal community in which the research took place. These findings contribute to our assertion that this method of cross-validating behavioral data with syringe washes from IPWIDs to validate self-reported substance injected is a more culturally appropriate method than validation via the collection of biospecimens for this community.

Ethics statements

Informed consent was obtained from all subjects involved in the study.

Supplementary material *and/or* additional information [OPTIONAL]

GC–MS and LC-QqQ-MS results by individual are included in the supplementary materials that accompany this manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Michael Anastario: Conceptualization, Methodology, Investigation, Writing – original draft. **Leonardo B. Maya:** Validation, Formal analysis, Investigation, Writing – original draft. **Kaylyn A. Keith:** Conceptualization, Methodology. **Anamary Tarifa:** Validation, Investigation. **Paula Firemoon:** Investigation. **Jordan Quintana:** Project administration. **Anthony P. DeCaprio:** Conceptualization, Methodology, Investigation. **Elizabeth Rink:** Conceptualization. **Eric Wagner:** Conceptualization, Funding acquisition.

Data availability

Data are shared in supplementary materials.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2023.102067.

References

[1] M. Kariisa, L. Scholl, N. Wilson, P. Seth, B. Hoots, Drug overdose deaths involving cocaine and psychostimulants with abuse potential - United States, 2003-2017, Morb. Mortal. Wkly. Rep. 68 (17) (2019) 388–395, doi:10.15585/mmwr.mm6817a3.
[2] M. Anastario, J. Leston, C. Crisp, C. Lee, E. Rink, A qualitative study of services accessibility for indigenous persons who use injection drugs across three communities in the United States, J. Ethn. Subst. Abuse (2022) 1–23, doi:10.1080/15332640.2022.2043798.
[3] J. Bardill, N.A. Garrison, Naming indigenous concerns, framing considerations for stored biospecimens, Am. J. Bioeth. 15 (9) (2015) 73–75.
[4] K.A. Sinclair, C. Muller, C. Noonan, C. Booth-LaForce, D.S. Buchwald, Increasing health equity through biospecimen research: identification of factors that influence willingness of Native Americans to donate biospecimens, Prev. Med. Rep. 21 (2021).
[5] D. Heckathorn, Respondent-driven sampling II: deriving valid population estimates from chain-referral samples of hidden populations, Soc. Probl. 49 (1) (2002) 11–34, doi:10.1525/sp.2002.49.1.11.
[6] B.H. Erickson, Some problems of inference from chain data, Sociol. Methodol. 10 (1979) 276–302, doi:10.2307/270774.
[7] D. Carise, K. Wicks, A. McLellan, P. Olton, Addiction Severity Index 5th Edition–North Dakota State Adaptation For Use With Native Americans, Treatment Research Institute at University of Pennsylvania, 1998.
[8] T. Néfau, E. Charpentier, N. Elyasmino, C. Duplessy-Garson, Y. Levi, S. Karolak, Drug analysis of residual content of used syringes: a new approach for improving knowledge of injected drugs and drug user practices, Int. J. Drug Policy 26 (4) (2014) 412–419.
[9] T. Brunt, E. Lefrançois, T. Gunnar, A. Arponen, T. Seyler, A.E. Goudriaan, A. McAuley, D.A. McKeown, V. Detrez, J. Csorba, D. Deimel, V. Auwärter, J. Kempf, S. Karolak, T. Nefau, Substances detected in used syringes of injecting drug users across 7 cities in Europe in 2017 and 2018: the European syringe collection and analysis project enterprise (ESCAPE), Int. J. Drug Policy 95 (2021) 103130 103130–.
[10] M. Anastario, P. Firemoon, A.M. Rodriguez, C. Wade, C. Prokosch, E. Rink, E. Wagner, A pilot study of polysubstance use sequences across the lifespan among assiniboine and sioux people who use injection drugs, Int. J. Environ. Res. Public Health 20 (1) (2023).
[11] M. Anastario, K. FourStar, A. Ricker, R. Dick, M. Skewes, E. Rink, A preliminary needs assessment of American Indians who inject drugs in northeastern Montana, Harm Reduct. J. 14 (22) (2017).