APHL Overdose Biosurveillance Task Force

Assessing Polysubstance Overdoses

An Expanded Biosurveillance Strategy for Public Health Practice

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APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES®

Contents

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About the APHL Overdose Biosurveillance Task Force

In January 2019, the Association of Public Health Laboratories (APHL) Board of Directors established the Opioids Biosurveillance Task Force (OBTF) to consider the role of public health laboratories in the opioid epidemic response. Given the advanced analytical capabilities and expertise in public health laboratories to identify and measure chemical toxicants in clinical specimens, the OBTF acknowledged that there was an opportunity to fill critical data gaps in opioid overdose surveillance systems related to nonfatal opioids overdoses.

In July 2020, APHL published *Model Opioids Biosurveillance Strategy for Public Health Practice (Model Strategy)*, which outlines guidance and considerations for public health laboratories and state and local health agencies in designing and implementing opioids biosurveillance in their jurisdictions. The guidance document addresses various aspects of opioids biosurveillance program design, including specimen collection and testing strategies, data reporting and program evaluation. Several states have since initiated opioids biosurveillance programs which are at various stages of implementation and data sharing, with some programs utilizing biosurveillance information to inform public health intervention and policy.

In September 2022, APHL published *Laboratory Data in Neonatal Abstinence Syndrome (NAS) Surveillance*, which examined the value of laboratory data in NAS surveillance, discussed appropriate specimen types for NAS surveillance, and the legal, ethical and social implications of NAS surveillance. OBTF members who worked on the report included experts from neonatology, obstetrics and gynecology, analytical chemistry, birthing individual and child health, epidemiology, and clinical and diagnostic toxicology.

In October 2022, the OBTF was renamed the Overdose Biosurveillance Task Force and re-configured to provide multidisciplinary expertise in analytical chemistry, toxicology, medicine and epidemiology to explore the role of public health laboratories in polysubstance overdose biosurveillance. The reconfiguration included subject matter experts in forensic and medical toxicology, diagnostic and public health laboratory science, and emergency medicine . Public health partners representing national professional organizations and federal partners (The Association of State and Territorial Health Officials, Council of State and Territorial Epidemiologists, US Centers for Disease Control and Prevention's Division of Overdose Prevention and Division of Laboratory Sciences, and the American College of Medical Toxicology) provided valuable national perspective.

We recommend reading the previous biosurveillance strategy, *Model Strategy*, prior to reading this document, as we expand upon *Model Strategy* here and it will be referred to frequently for background information. While *Model Strategy* focused exclusively on opioids, this document provides recommendations for updated biosurveillance strategies to include additional substances implicated in drug overdoses. In this document, the OBTF has maintained the scope of public health laboratory biosurveillance of residual clinical specimens obtained from individuals who experienced a nonfatal overdose presenting to emergency departments. However, recommendations and conclusions related to polysubstance laboratory testing may be applicable to other related areas of interest.

Introduction

Assessing Polysubstance Overdose: An Expanded Biosurveillance Strategy for Public Health Practice (*Expanded Strategy*) serves as guidance for public health agencies interested in developing and implementing an effective and impactful nonfatal polysubstance overdose biosurveillance program in their jurisdiction. *Expanded Strategy* provides background information on polysubstance overdoses, collaboration and outreach efforts, analytical recommendations for polysubstance testing, and important considerations for the management, interpretation and dissemination of nonfatal overdose polysubstance biosurveillance data. Public health agencies should review the original *Model Strategy* and this *Expanded Strategy* in conjunction with other state and local laws, regulations and policies to develop plans specific to the needs of their jurisdictions.

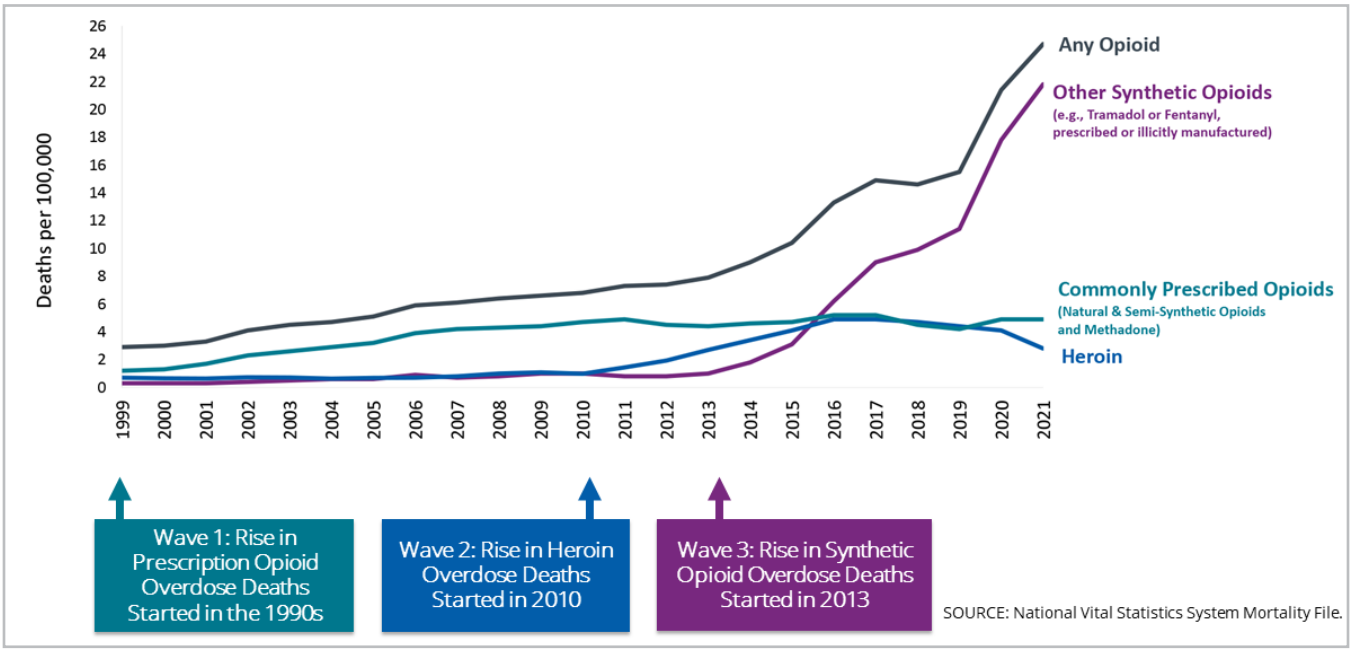
The Value of Biosurveillance for Nonfatal Polysubstance Overdoses

There is a critical lack of laboratory data that definitively identify substances that result in overdoses causing individuals to seek life-saving medical treatment. Biosurveillance—the analysis of clinical specimens, such as blood and urine for the purpose of public health surveillance—provides important exposure information not available in existing epidemiological, emergency medical services and seized drug data. While strides have been made in expanding opioids biosurveillance, a significant gap remains in surveillance for non-opioid substances, or mixtures of substances, implicated in overdoses.

Changing Overdose Trends

Since the formation of the OBTF, stark upward trends in nonfatal overdoses were driven primarily by opioids, including fentanyl and its many analogues. Age-adjusted rates of drug overdose deaths involving fentanyl rose 24.1% between 2020 and 2021.[1] In the 1990s, prescription opiates drove the first "wave " of opioid overdose deaths until the emergence of a second wave in 2010 with opioid overdose deaths primarily driven by heroin (**Figure 1**). Since 2013, the opioid epidemic has been fueled by synthetic opioids, namely fentanyl and its analogues, and opioid-involved death rates increased by 38% between 2019 and 2020 alone. However, in 2019, nearly half of all drug overdose deaths involved multiple drugs, and approximately one-third of deaths attributed to psychostimulant overdose also involved synthetic opioids.[2]

Figure 1. Three Waves of Opioid Overdose Deaths (source: CDC, Understanding the Opioid Overdose Epidemic)

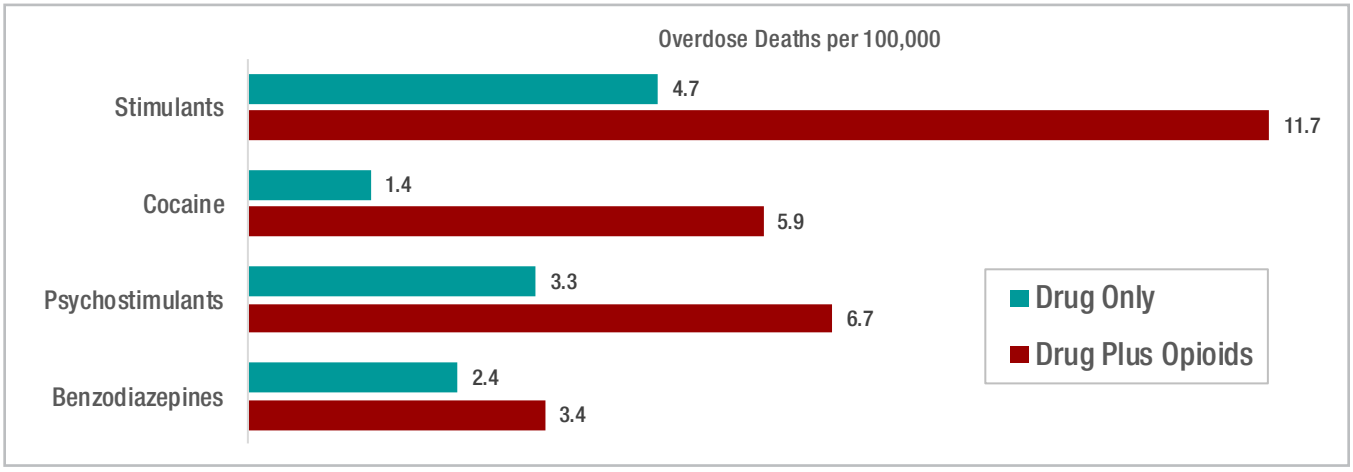


Polysubstance Use

Polysubstance (or polydrug) use is defined as the use of more than one drug consumed together or within a short time period.[2] Drugs implicated in polysubstance use may include opioids, stimulants, benzodiazepines, hallucinogens, and synthetic cannabinoids; novel psychoactive substances (NPS) are also prevalent. Polysubstance use can be both intentional—with an individual knowingly choosing to mix two substances together—or unintentional—where an individual may consume drugs that have been adulterated with substances unknown to the individual.[2]

Polysubstance use is more likely to lead to a fatal overdose than opioid use alone. **Figure 2** highlights the disparity in mortality between single substance use (opioids only) and polysubstance use (opioids and at least one other drug).

Figure 2. Age-adjusted rate of overdose deaths for single substance use (opioid-only) compared to polysubsatnce use.



Novel Psychoactive Substances

NPS, also known as new psychoactive substances, designer drugs or emerging drugs/substances, represent a group of heterogeneous drugs that affect mental processes. NPS began emerging as a widespread problem in the early 2000s. This catch-all drug category spans multiple pharmacological classes and includes opioids, benzodiazepines, cannabinoids, stimulants and hallucinogens.[2] NPS may be manufactured domestically or abroad and are made available in some instances, as an alternative to drugs that are currently illegal (e.g., synthetic cannabinoids vs. cannabis). The pharmacology and toxicity of NPS are often unknown at the time of their emergence, which leads to unknown health and safety implications. NPS challenge surveillance efforts and drug testing technology due to their novelty, variation in chemical structure, lack of incorporation into existing testing protocols, unavailability of calibration and QC materials, and prevalence.

The NPS opioid subclass has become increasingly diverse in recent years. Fentanyl analogues (e.g., furanyl fentanyl, acetyl fentanyl, para-fluorofentanyl) emerged in the wake of fentanyl's proliferation and now exist in a variety of chemical forms of varying activity and potency within the illicit opioid supply. A more recent subclass of synthetic opioids is nitazene analogues (e.g., isotonitazene, metonitazene) which vary in potency. Like fentanyl analogues, the potency of nitazene analogues can exceed the potency of fentanyl. Other subclasses of NPS include synthetic cathinones, also referred to as "bath salts" (e.g., dimethylpentylone, eutylone); synthetic cannabinoids, also referred to as "K2" or "spice" (e.g., 5F-ADB, MDMB-4en-PINACA); and novel benzodiazepines (e.g., etizolam, bromazolam, clonazolam).[3] NPS are often not detectable on routine clinical drug screening tests, which do not include these drugs in the scope of testing and are often not included in confirmatory test panels. This document will contain limited discussion of NPS; however, the analytical and interpretive strategies outlined below will apply to laboratory testing of NPS. Given the ever-changing landscape of NPS involvement in overdoses, public health laboratories are encouraged to expand their panel as they deem appropriate. Inclusion of NPS into testing methods can be facilitated using a variety of resources, please see appendix.

Adulterants and Other Substances

Adulterants are chemical substances that are added to drug products by illicit manufacturers or street level dealers to increase (or alter) drug effects or product weight;[4] however, not all adulterants induce a pharmacologically significant effect (e.g., sugar, baking soda) and are therefore more accurately classified as diluents. Adulterants appear in a variety of forms and quantities and can include everyday substances like acetaminophen or caffeine. Today, it is uncommon to find street drugs that have not been adulterated. In a 2017 study, street drug samples of opioids and cocaine from Vermont and Kentucky were found to contain additional pharmacologically active substances in 97% and 89% of samples, respectively, with as many as nine additional substances including acetaminophen, aminopyrine, levamisole, phenacetin and papaverine.[5] Each adulterant will have different pharmacology, toxicity, and effect profiles which may or may not be altered by other drugs present in a person's system. It is increasingly important to consider the presence of adulterants and their role in polysubstance overdoses, which begins with their inclusion in surveillance testing.

At the time of the writing of this document, a prominent trend in polysubstance use observed across the country was the adulteration of fentanyl with other central nervous system depressants (e.g., benzodiazepines) or sedatives (e.g., xylazine). Already dominated by illicitly manufactured fentanyl and fentanyl analogues, xylazine emerged as an adulterant to the illicit opioid supply in the early 2020s. Xylazine, a veterinary drug that can induce physiological dependence and severe withdrawal symptoms, was frequently found in combination with fentanyl in opioid drug products in several cities across the Northeast, Mid-Atlantic and Midwest. This drug combination was associated with adverse effects, including heavy sedation and severe skin wounds or ulceration.[6] The estimated number of xylazine-involved drug poisoning deaths grew 1,238% between 2018 and 2021 from 260 to 3,480 deaths.[6] In 2023, the White House Office of National Drug Control Policy (ONDCP) declared fentanyl adulterated with xylazine an emerging threat, the first declaration of its kind and the first to follow the criteria established in ONDCP's Dir. No. 2022-002 *Criteria for Designating Evolving and Emerging Drug Threats*. Xylazine, like other emerging adulterants, is not readily detected by current point-of-care testing methods and requires advanced, confirmatory (definitive) testing.[6]

Additional adulterants with notable clinical presence have been detected, including levamisole adulterated cocaine (multiple states and regions), synthetic cannabinoids (e.g., 5F-ADB), adulterated fentanyl and heroin (East coast), NPS benzodiazepine (e.g., etizolam, bromazolam), adulterated fentanyl (Midwest), quetiapine adulterated fentanyl (multiple regions), methamphetamine adulterated ecstasy (i.e., MDMA), and many others. While the roles of adulterants and adulteration in polysubstance overdose may not be clear at the time of sample collection or testing, adulterants are of increasing prevalence with significant toxicological relevance.

Using Laboratory Data to Inform Overdose Response Efforts: Rhode Island

In 2019, the Rhode Island Department of Health (RIDOH) implemented a public health requirement for all hospitals treating patients experiencing overdoses to retain residual urine specimens and forward them to the Rhode Island State Health Laboratories (RISHL), establishing a statewide opioid overdose biosurveillance initiative.

Between July 2019 and June 2021, RISHL received 1354 urine samples from 1197 unique individuals who were treated in emergency departments for suspected opioid overdoses, representing 45% of nonfatal overdoses reported to RIDOH during that time.[7]

Urine samples were extracted and then analyzed by liquid chromatography tandem mass spectrometry with a panel consisting of opiates, synthetic opioids, fentanyl analogues and select metabolites. At least one of the target analytes contained within RISLH's biosurveillance panel was detected in 1033 specimens for a total of 4251 detected opioids.

Analytes were grouped by the metabolic pathway of each potential parent substance in the panel to avoid double-counting of parent drugs and their metabolites, which identified 2049 unique substances. 58% of samples had more than one detected opioid substance, with an average of two opioid substances detected per overdose. A maximum of eight opioid substances was observed in samples.

Fentanyl was found in a monthly average of 79% of urine samples associated with nonfatal overdoses between July 2019 and June 2021. Biosurveillance enabled trend tracking of fentanyl analogues:

- Carfentanil was present in up to a quarter of all opioid-positive urine samples in August 2019, but this percentage declined rapidly in spring and summer of 2020.
- p-Fluorofentanyl was first detected in October 2020, and was found in 20% of all opioids-positive samples by December 2020.

Definitive laboratory identification of the opioids present in the samples offered a reliable source of information on current patterns of opioid drug use in RI that can be leveraged for timely and effective public health interventions. Demographic information collected from specimen submission data in combination with confirmatory laboratory results can enable research into factors that may contribute to or be protective from nonfatal overdose.[7] Following the success of the pilot biosurveillance program, RISHL has expanded their panel beyond opioids and fentanyl analogues to include central nervous system depressants and stimulants in whole blood samples to further characterize the nonfatal overdose landscape.

Collaboration and Outreach Efforts

Identifying Potential Partners

When developing or expanding a biosurveillance program, state public health laboratories and their public health partners should maximize community input into program design, seeking counsel from hospital partners, substance use prevention programs, relevant medical societies, medical examiners/coroners, epidemiologists, forensic toxicologists, state and local elected officials, social services, regional poison control centers and overdose prevention agencies. To begin a biosurveillance program, potential partner agencies and contacts within them must be identified to assist in obtaining specimens for testing.

Collaboration with healthcare providers with access to most relevant specimens is a common challenge within biosurveillance programs. Emergency departments are the suggested specimen source for biosurveillance programs as individuals most commonly present to the emergency department when experiencing a drug overdose. To perform toxicology testing, hospital staff will collect a blood or urine specimen from the patient to determine the substance(s) implicated in the overdose and guide treatment protocols. Following initial hospital laboratory testing of the specimen, residual blood or urine may be available for advanced, de-identified testing by public health laboratories.

Partnership building is integral to sourcing biosurveillance specimens and to the success of a biosurveillance program. As biosurveillance data, in combination with other data, inform public health action in states and localities, close collaboration with public health partners at state and local health departments is highly valuable. Such partners often can bridge connections between the public health laboratory and other partners like poison control centers, government-funded addiction treatment programs, overdose prevention organizations, community outreach workers, forensic partners, state and local politicians, and more. Biosurveillance programs are suggested to work with their state LRN-C coordinator who likely has established relationships with emergency departments at acute care hospitals. Partnerships with poison control centers can be beneficial as they may be affiliated with hospital-based clinical toxicologists, medical systems, legislative partners or other public health partners.

Potential Partners

Have you initiated collaboration efforts with these partners?

- ☑ **Hospitals and Healthcare Providers**
  Emergency departments, medical examiners/coroners, hospital associations, hospital leadership, medical societies, etc.
- ☑ **Social Services and Addiction Care**
  Substance use prevention programs, addiction treatment programs, overdose prevention organizations, community outreach workers, etc.
- ☑ **Government Partners**
  State and local health departments, politicians and other elected officials.
- ☑ **Public Health Partners**
  Poison control centers, epidemiologists, forensic toxicologists, state LRN-C coordinator, etc.
- ☑ **Organizations Outside Your Jurisdiction**
  Build a network of overdose response professionals to expand your scope and share resources.
- ☑ **Other Champions**
  Find trusted partners in the community who can advocate on behalf of biosurveillance programs.

Information on building initial collaborative partnerships is presented in *Model Strategy*.

Hospital associations, boards of directors or trustees, and other organizations comprising hospital leadership are suggested partners, as well as individual connections with emergency department clinicians and staff. Participation and interest may vary across hospital partners. When reaching out, it is advisable to contact all levels of hospital staff; this can be done in two different ways:

- **Approach senior leadership:** Approach hospital leadership, including a hospital's board of directors or trustees, or health agency executives who have signatory power and influence.
- **Approach patient care providers:** Approach care providers in emergency departments and/or hospital toxicology laboratories.

Merits for both approaches exist. A top-down approach to specimen sourcing may prove effective in some instances as individuals within executive positions are able to influence organization-wide decisions. However, a bottom-up approach is an opportunity to build connections with emergency department staff directly and with other staff members who will be performing tasks associated with supplying specimens to the public health laboratory. While taking a bottom-up approach to partnership building may be instinctive for many programs, an alternate top-down approach may be warranted if challenges in obtaining an adequate number of specimens arise. Experiences with sourcing specimens may vary between states, localities, hospital organizations, and individual hospitals, so an integrated approach of both top-down and bottom-up approaches may be required to achieve program goals.

In addition to state and county-wide connections, institutional systems or networks that include teaching hospitals are viable collaborators with potentially far-reaching networks. Poison centers or toxicology programs may be components of state educational institutions that can serve as valuable contributors and partners, may be involved in legislative-driven work, and may be involved with additional public health partners.

As overdose trends vary across the country, it is beneficial to maintain partnerships with organizations that are outside of your jurisdiction. Building relationships across the network of overdose response professionals can provide opportunities to discuss program design, challenges, scope and opportunities, and ensure that you are aware of local, regional, state, national or international trends or challenges occurring outside of your jurisdiction. When building such relationships, it is useful to group organizations by geographic region, scope, source of information, recipient of data or any combination most suitable to your program.

Throughout the process of building partnerships, prioritization must be placed on identifying "champions" that are groups or individuals who are trusted partners in the community and can advocate on behalf of biosurveillance programs. Such champions understand the utility and value of biosurveillance information and are eager to recruit other participants to inform overarching public health goals for which biosurveillance data can be used to inform. Champions can reside within any partners mentioned and may not always have the same form of influence. Individuals with substantial trust from the greater community are excellent champions to recruit to ensure success of the program. However, individuals who hold higher decision-making power, like hospital CEOs or board directors, can enhance the probability of successful hospital recruiting.

Strategies for Connection

When proposing partnership opportunities with hospitals or other patient-facing organizations, it is important to ensure the process of specimen submission to the public health laboratory is as simple as possible so that there is minimal interruption to regular hospital workflows. Specimens submitted for biosurveillance testing are residual or remnant from specimens collected by the hospital; collection of additional specimens or splitting of specimens before hospital testing is not recommended. Working with hospital partners to develop a system within their laboratories to ensure timely and appropriate specimen allocation for submission to the public health laboratory is critical to the acquisition of quality specimens for analysis.

Provide Resources

It is essential to provide hospitals with the necessary guidance and resources to facilitate residual specimen retention and transport. There are many logistical considerations involved in successful implementation of this process, including:

Specimen Collection

Rapid specimen submission is essential in ensuring minimal loss of drug concentration.[8]

- Provide hospital partners with detailed specimen collection instructions as an accessible reference for emergency department and hospital laboratory personnel.
- Solicit feedback from individual partners to ensure the requirements are feasible within their current workflows; APHL collates examples of such resources for member use.

Example: The gel in gel separator specimen collection tubes for serum or plasma separation may adsorb some drugs (including fentanyl, carfentanil and ketamine) and falsely lower drug testing results.

Packaging and Shipping

Consider providing:

- Resources to hospital partners for the acquisition of shipping materials, containers, vials and other necessary supplies.
- Specimen courier transportation to ensure safe and expedited delivery of specimens to the testing laboratory.

Support from Partner Leadership

- Develop a fact sheet that provides an overview of the project and its goals. This document can be shared with administrators and staff to introduce the project and gather internal hospital support. View an example of a concise fact sheet by the Minnesota Drug Overdose and Substance Use Surveillance Activity.
- Communicate your authority to conduct this type of testing as public health surveillance, as well as your data privacy and security standards.

Preserve Confidentiality

Common concerns include the necessity of human subjects review or the research-required institutional review board (IRB) review, which are discussed at length in the *Model Strategy*. Overdose biosurveillance is likely to be considered public health surveillance or public health investigation which does not necessitate IRB approval. Confidentiality and privacy concerns may arise when obtaining specimens from partners who do not consistently experience high patient volume or overdose prevalence. For example, a hospital may only have one nonfatal overdose specimen to submit for biosurveillance testing within the appropriate specimen submission time frame. In such situations, public health laboratories may recommend that no less than two specimens are submitted to the public health laboratory at a time.

Overdose biosurveillance programs introduce unique considerations for patient confidentiality, such as prosecution for drug use or possession and stigma associated with substance use disorder in healthcare settings. Care must be taken to ensure that patient confidentiality is preserved to the highest extent possible. Laboratory scientists must complete institutional privacy and confidentiality training and abide by standard protocols for the viewing, retention and sharing of data.

Create Flexible Systems

Approach the development of specimen submission systems with flexibility to best suit individual hospital practices.

Leveraging Technology to Ensure Flexibility With EHR

One possible approach to forming such systems may reside within hospital electronic health records (EHR), where residual blood and urine specimens could be flagged for submission to the state public health laboratory. An EHR approach could use ICD-10 or other medical coding/billing or diagnostic systems that provide specific indicators of patient diagnoses or symptoms. Depending on biosurveillance program design, ICD-10 codes that indicate overdose or poisoning from drug consumption (i.e., T40 and F11) could indicate appropriate specimens. Codes that indicate use of naloxone to reverse overdose may also be an effective way to identify appropriate specimens. As retention policies between hospitals may vary, be aware of retention policies for residual specimens to ensure timely procurement by the public health laboratory. If residual specimens are disposed shortly after processing, hospitals may need to make an exception to an existing retention policy to participate.

Establish MOUs/MOAs

Develop a memorandum of understanding (MOU) or memorandum of agreement (MOA) template for use when on-boarding a new hospital. Such agreements can help assist in a quick turnaround time and set program parameters early on.

Top-down Hospital Collaboration Success: South Carolina

The South Carolina Public Health Laboratory (SC PHL) found success with hospital collaboration by taking a top-down approach and conducting in-person meetings with key members in hospital leadership. This included but was not limited to laboratory managers, emergency department directors, quality assurance officers, chief nursing officers, chief executive officers, and/or chief operating officers. Each of these members played a key role in the partnership building process and provided valuable feedback that helped create processes to ensure hospitals would be able to participate.

As the SC PHL continued to successfully recruit hospitals into the SC Overdose Biosurveillance Program (OBP), the laboratory discovered other SC state government agencies running complementary programs such as prevention and education. Recognizing parallel efforts with similar goals, SC PHL contacted these agencies in the interest of forming a multi-agency working group. Through conversations and meetings, key agencies and representatives were recruited to participate.

The working group met monthly to discuss the various efforts and initiatives carried out by the different partners, and the working group was able to increase awareness on all overdose biosurveillance efforts. This allowed SC to take a unified approach in combating the overdose epidemic. With effective educational materials and clear, mutually beneficial goals, the SC PHL was able to quickly enlist hospitals to participate.

SC PHL continually assess its approach and relies on constant communication with its partners, including hospitals and agency partners. SC PHL also provides recognition to hospital participants so they may showcase their efforts in combating the overdose crisis to the communities they serve. These efforts solidify the partnerships that have been built and provide a solid foundation for the OBP as it continually pivots to support ever-changing needs.

Share Aggregate Data

Provide partners with aggregate data to inform local trends. Highly complex testing provided by the public health laboratories can identify substances that may not be detected by immunoassay testing. While not intended for diagnostic purposes, definitive laboratory data provided in aggregate can provide comprehensive surveillance information within their patient population, enabling a wide view of the scope of polysubstance use and array of substances in their area. Aggregate reports are delivered with the intention of heightening understanding of the overdose landscape for emergency departments.

Depending on individual program design and intention, programs may find benefit in supplementing aggregate reports with individual-level data for a “bifurcated” approach. Providing individual-level reports without identifiable information to

clinicians may help inform and improve treatment for future patients through definitive toxicology data.

Please note that reporting of individual-level data will prompt the need for a secure results transmission process. Additional activities such as confirmatory diagnostic testing, community-focused overdose prevention and research investigations are beyond the scope of this document and biosurveillance for nonfatal overdoses. Guidance contained in this *Expanded Strategy* pertains to reporting of aggregate biosurveillance results, although potential benefits of providing individual-level reports are recognized.

Maintaining Relationships with Key Partners

After establishing relationships with key partners, effort must be made to maintain these relationships and ensure mutual benefit. It may be appropriate to establish a forum through which continuous communication occurs between public health laboratories and the hospitals who submit specimens to them. Aggregate reports generated from biosurveillance data should be shared with hospital partners to keep them informed of data obtained from submitted specimens, as well as the benefit of continued participation. It can be helpful to incentivize partners to participate. Providing formal recognition, through the form of Certificates of Participation or Recognition, to hospitals and other program partners can increase participation in biosurveillance programs. Feedback from such partners should also be solicited throughout all stages of the program, and-regular informative meetings can be a tool in upholding this collaborative relationship. Such meetings should provide an outlet for all partners to convene and discuss relevant information, programmatic updates, quarterly reports, feedback, interpretation challenges, data dissemination and downstream impact of biosurveillance work. Virtual meeting platforms may assist in ensuring partners are able to attend meetings regularly.

Close partnership with overdose epidemiologists within health departments is essential to interpretation of biosurveillance results and generation of data products. Public health public information officers can assist in preparing reports and data products for dissemination, dashboards, alerts and general translation of data and trends into accessible information to be shared with the public, as well as actual dissemination of the data products through external communication methods like social media. State, county and regional health department partners can serve as valuable advocates for biosurveillance through heightened access and established relationships with related community groups like overdose prevention organizations, addiction treatment facilities, and organizations serving underserved populations. Such partners may use biosurveillance data to inform and evaluate public policy and interventions.

Public health laboratories must develop and nurture strong partnerships across health department offices and divisions, especially with epidemiologists who are critical to surveillance and quality assurance officers whose expertise is required for laboratory accreditation and certification. External audits of the laboratory are not an assessment of the overall quality of the laboratory's activities, but do assess the laboratory's capability to produce quality data. The process documents the technical competence of the laboratory against a set of accepted standards. In the United States, there are multiple accreditation and certification organizations. For clinical testing these include but are not limited to the College of American Pathologists, International Organization for Standardization accreditation, and CLIA certification.

Establishing a Biosurveillance Advisory Committee

Biosurveillance programs should consider the development of a multi-disciplinary group of individuals to provide input, discuss issues and challenges, and propose applications of biosurveillance data gleaned from public health laboratories which may be useful for activities in overdose prevention, quality improvement or other biosurveillance activities. Expertise suitable for membership in an advisory committee includes medical toxicologists, epidemiologists, overdose prevention specialists, informatics and communications experts, physicians, addiction treatment specialists, community outreach workers and other individuals engaging in work related to overdose prevention.

Emphasizing the advantages of participation in advisory committees can prove useful in recruiting committee members during group formation. Information gleaned from participation in advisory committees can provide data that informs practical implementation and prevention efforts. Biosurveillance provides an opportunity for correlation between clinical

presentation of patient overdoses with analytical confirmation of the substances which may have caused or contributed to overdose. Committee members are also instrumental in evaluation and management of program scope and expectations.

Data from biosurveillance programs require mindful, nuanced interpretation from multi-disciplinary expert panels such as the suggested advisory panel, which can work collaboratively to develop recommendations for best practices for analyzing data based on individual program needs and goals. Such recommendations provide insight to epidemiologists at federal, state, regional, county and local levels on proper interpretation of biosurveillance data. Advisory committee members can assist in communication of limitations of biosurveillance data and the necessary precautions to ensure proper data interpretation. Expertise from advisory committee members can assist in communication of limitations of biosurveillance data and necessary precautions to ensure proper data interpretation, help inform appropriate systematic sampling methods of hospital-acquired ED specimens to avoid selection bias or related challenges to ensuring representative data, and help address variation between geographic areas or demographic groups.

Biosurveillance Advisory Committee Members

Potential expertise for committee members:

- Epidemiologists
- Laboratory scientists
- Medical toxicologists
- Public health agency administrators
- Poison Center representatives
- Community members and individuals with lived experience of substance use disorder
- Academic partners

Analytical Considerations in Nonfatal Overdose Biosurveillance Programs

Since publication of the *Model Strategy*, the landscape for biosurveillance of drugs has changed significantly. NPS are constantly evolving in usage and type, resulting in an urgent need to incorporate NPS testing into current methods for analysis. To identify evolving drug usage trends, several public health laboratories have added high-resolution mass spectrometry (HRMS) to their analytical laboratories and are exploring how to utilize this technology for biosurveillance programs effectively. HRMS systems differ from other mass spectrometers in their ability to measure the mass of a substance with extremely high precision, down to 0.0001 atomic mass units for many substances. As opposed to more typical targeted MS analysis, in which only data for pre-selected analytes is acquired, HRMS in full scan mode acquires data for virtually all substances which are extracted from the sample and amendable to the chosen chromatography and MS ionization mode. By using a simple, generic extraction method and broad range chromatography, many thousands of substances can be detected in each HRMS analysis with an unlimited selection of analytes. Thus, analytes may be detected which were not originally suspected to be present in a sample. Furthermore, the data may be archived and interrogated months, or years, after the original analysis to determine the potential presence of analytes unidentified at that time. HRMS may be used in multiple ways to generate analytical data: targeted analysis, similar to current liquid chromatography-tandem mass spectrometry (LC-MS/MS), non-targeted analysis (NTA) for novel substance elucidation, or semi-targeted analysis (STA) for confirming substances in one or more chemical databases. Utilizing these various workflows, substances in surveillance samples can be identified and confirmed by comparison to certified analytical standards, giving public health laboratories and their partners the necessary analytical data to provide the missing context from nonfatal overdose events.

As with any analytical technique, it is important to note that HRMS will not detect all possible analytes of interest in a sample. Examples include analytes that are not amenable to the chosen extraction method, those which do not separate chromatographically, or those outside of the mass range chosen for the MS analysis or not ionized by the chosen ionization source. It is critical to understand the impact of analytical method choices and gaps or "blind spots" of the analysis on results interpretation. Nonetheless, a properly designed HRMS analysis offers the potential to detect an extraordinary range of drugs and metabolites.

Targeted Analysis

Targeted analysis is a technique commonly used in public health laboratories and advanced toxicology laboratories to identify and measure analytes of interest, including drugs and their metabolites. One main advantage to this technique is the ability to detect specific analytes of interest with great sensitivity. Targeted analysis uses optimized chromatographic separation followed by tandem mass spectrometry multiple reaction monitoring. Qualitative targeted analysis uses the comparison of unknown sample data with certified reference materials. To gain quantitative information, isotopically labeled versions of targeted analytes are incorporated into the analysis. Targeted testing is an integral component of current biosurveillance testing as methods are typically designed to identify the specific analytes at very low concentrations. The selectivity and sensitivity of these methods are essential for quantitative and targeted qualitative analysis. However, targeted analysis does limit public health laboratories' ability to identify an unknown substance that is outside of the testing panel.

Non-Targeted Analysis

Non-targeted analysis (NTA) with HRMS is a powerful tool that public health laboratories can use to collect information on analytes (known and unknown) within an analyzed sample. Unlike traditional mass spectrometry, the precision of HRMS provides improved substance mass specificity which is useful in distinguishing isobaric (compounds with the same nominal mass but different molecular formula) substances of interest. NTA methods have the advantage of collecting chemical measurements without any assumptions about the sample or chemical presence and will provide a more complete picture of the sample's composition. Because NTA methods do not have the limited target list of traditional LC-MS/MS, this technique may even be used to identify potentially never-before-detected chemical substances. Currently, NTA is primarily a qualitative investigative technique, which uses the comparison of patient specimens with matched controls. Patient-control comparisons can be used to identify unique or significantly elevated substances specific to cases for in-depth examination; mass spectral data can then be compared against reference libraries, or assessed by an expert user, to determine substance identity.

To perform NTA, public health laboratories must create a general extraction and analytical method that allows for the instrument to enable comprehensive data acquisition to provide qualitative identification. Due to the volume of data collected in NTA, data processing is labor intensive, relies heavily on data analysis software and informatics tools, and the number of samples analyzed per run will generally be lower compared to targeted analysis. Further, methods for quantifying substances via NTA, especially new and emerging substances without authenticated standards, are not currently established. Another important feature of NTA is that it allows for data mining of old data for new drugs to check for use histories.

Semi-Targeted Analysis

Semi-targeted analysis (STA) or suspect screening analysis with HRMS combines targeted and non-targeted approaches to sample analysis by comparing substances against a database containing retention times and MS information, such as the exact masses of analytes and their MS/MS fragments. This type of analysis is a subset of non-targeted analysis because although the data acquisition is non-targeted, the data analysis is targeted to the search database and does not interrogate novel substances. The databases used for STA are constructed via analysis of chemical standards and new analytes can be added over time. The size of suspect screening databases is functionally unlimited and such libraries may be used for many years if chromatographic and MS conditions are kept consistent. This allows public health laboratories a high degree of confidence in the identification of specific substances while still collecting a wide range of information for the identification of never-before studied substances. With the integration of boundaries, data processing time will improve, lessening the time burden on analysts. Data collected for STA analysis is available for NTA post-hoc analysis if needed, allowing for data mining of historic data for previously unidentified substances and new drugs as they are characterized.

Qualitative versus Quantitative Analysis

For overdose biosurveillance, qualitative analysis is sufficient to meet programmatic objectives, including the potential for rapid expansion of the target analyte list in response to newly identified drug trends. Qualitative reporting allows for expanded libraries because full calibration curves and concentration dependent quality control materials are not analyzed for every substance in every batch. Validation parameters for a qualitative method are similar to quantitative methods; however, qualitative methods do not determine analyte concentrations and have no concentration dependent acceptance criteria. For validation of a qualitative method, public health laboratories must assess their minimum acceptance criteria and should understand detection levels to facilitate informed discussions regarding potential non-detections of suspected substance, but limits of detection are not required for positive identification.

Quantitative datasets may provide powerful insights into the causative agents giving rise to an acute overdose event; however, interpretation of quantitative results is challenging and requires careful interpretation from experts well-versed in the field of toxicology. Specimen source and matrix type play an integral role in the utility of quantitative data as variables—such as antemortem vs. postmortem collection, whether blood or urine is analyzed, and individual patient characteristics—will weigh heavily on how a toxicological report is interpreted. One significant challenge associated with making medical use of quantitative data is the absence of reference ranges for illicit substances and the need to consider individual tolerance to such substances. With quantitative analysis, costs increase with the need to purchase certified reference materials of the targeted substance and its matched isotopically labeled standard for calibrations and concentration-specific quality control standards, leading to longer per batch analysis time and limitations on the number of analytes that can be detected in one method.

Urine Sample Quantitation is Not Recommended

This *Expanded Strategy* does not recommend providing quantitative results for urine samples because it will be impractical (if not impossible) to make epidemiological use of such information.

Due to the lack of reference ranges for the concentrations of any illicit substances, the quantity of a drug metabolite in urine, especially without creatinine corrections, will not provide valuable insight. If data from public health laboratories are being used to identify drug use trends and create local policies and decisions regarding intervention, drug concentration is not necessary.

Based on these considerations, it is recommended to utilize detect/non-detect as a qualitative reporting metric for urine samples.

Qualitative Substance Identification by HRMS

Substance identification significantly differs between low- and high-resolution instrumentation. Fragment ion collection using LC-MS/MS is determined by fixed, validated MS/MS transitions of the parent molecule to a single fragment. In contrast, HRMS systems (like Q-TOF and Orbitrap) offer several options for the collection of fragment ions and uniquely produce a full spectrum of MS/MS fragments used for qualitative substance assignment. The choice of a fragmentation mode involves trade-off in spectral quality, method complexity and suitability of data for future analysis.

In Data Dependent MS/MS (ddMS2, also known as Information Dependent MS/MS), the instrument selects precursor ions for fragmentation based on predetermined filters, such as a maximum signal intensity or a set screening list. This method provides clean MS/MS spectra for single precursor molecules, always provides exact mass MS1 measurements, and is well suited to MS/MS spectral library matching and possibly novel substance elucidation. However, because ddMS2 is dependent on triggering settings, optimization is necessary to ensure desired analytes always have associated MS/MS data, and it is possible to “miss” MS/MS for ions which are observable, making assignment challenging beyond the accurate mass and empirical formula.

In Data Independent MS/MS (DIA, sometimes called SWATH-MS) or All Ion Fragmentation (AIF, sometimes called MSE), the quadrupole mass selector is set to pass ranges of precursor ions, either small windows (e.g., m/z 150 – 200, 200 – 250, etc.) or the entire mass range (e.g., m/z 100 – 1200) respectively. All precursors within the given range are fragmented and their associated fragment ions detected simultaneously. This results in MS/MS fragmentation spectra from multiple precursors overlapping and requires alternative library matching algorithms for identification. This type of analysis has the advantage of not requiring instrument optimization to collect MS/MS data and being unbiased in its data collection, but the spectral matching process is less accurate because the association between any given fragment and any precursor is unclear. As a result, this data cannot be used for substance elucidation, only library matching. Instrument cycle time also becomes a consideration when maximizing the number of MS/MS spectra collected; smaller scan windows in DIA give less complex/overlapping spectra than AIF but the instrument cycle time for collecting sequential, narrow m/z ranges can limit the number of points collected across a chromatographic peak compared to AIF methods, which are straightforward to implement and do not require long cycle times but are imprecise for spectral library screening.

Developing an Expanded Test Panel

To better understand the substances involved in nonfatal overdoses, public health laboratories need to expand beyond the scope of opioids analysis and create an adaptable and robust testing method capable of detecting both common drugs of abuse and traditional therapeutic medications. A targeted approach using LC-MS/MS can be used for this analysis as most drugs and NPS are identifiable in positive ionization mode. However, there are limitations and challenges with panel expansion when utilizing traditional targeted analysis. Limitations of analyte type and number of detectable analytes are two primary weaknesses with a targeted approach. Building a method that encompasses a variety of substances is essential to the development of a sufficiently comprehensive testing panel. For biosurveillance, a more adaptable workflow could incorporate HRMS instrumentation with a semi-targeted analysis. Semi-targeted analysis with HRMS systems will allow public health laboratories to identify many substances in a single method and continuously expand their confirmation libraries only by adjusting data processing.

To construct a comprehensive library, public health laboratories should monitor reports from national and regional laboratories to ensure the testing scope stays relevant. The following are examples of groups which provide regular, timely drug trend data:

- Local forensic drug chemistry and toxicology laboratories
- Center for Forensic Science Research and Education's NPS Discovery program
- US Drug Enforcement Administration's National Forensic Laboratory Information System
- US Customs and Border Control
- National Institute on Drug Abuse's National Drug Early Warning System

For comparability across all regions, a public health laboratory's overdose biosurveillance testing panel should include a variety of substances (parent drugs and known metabolites) from different drug classes. The scope of comprehensive chromatographic-based screen and confirmatory analysis should include common over-the-counter, prescription/therapeutic and illicit drugs; for example: antidepressants, antihistamines, antipsychotics, antiseizure, hallucinogens, sedatives and stimulants. A comprehensive list like **Figure 3** is recommended for initial method development.

To help public health laboratories expand their confirmation libraries, this *Expanded Strategy* recommends the use of Traceable Opioid Material® Kits (TOM Kits®) developed by the CDC National Center for Environmental Health (NCEH) Division of Laboratory Sciences as reference material. The TOM Kits® product line contains the Fentanyl Analogue Screening Kit and Emergent Panels (FAS, FAS V1-V4 Kits) and the Emergency Drug Panel Kit which collaboratively support laboratory screening for 290 substances. TOM Kit contents are informed, in part, by data obtained from the US Drug Enforcement Administration Emerging Threat Reports, the National Forensic Laboratory Information System, and the Center for Forensic Science Research and Education Scope and Trend Reports.

Figure 3. Expanded Strategy Recommended Panel

Drug Class	Drugs (relevant metabolites)		
Stimulants	• **Amphetamine** • **Methamphetamine** (amphetamine)	• **MDA** • **MDMA** (MDA)	• **MDEA** • **Cocaine** (benzoylecgonine, cocaethylene)
Benzodiazepines	• **Alprazolam** (alpha-hydroxyalprazolam)* • **Clonazepam** (7-aminoclonazepam)	• **Diazepam** (nordiazepam) • **Oxazepam**	• **Temazepam** • **Lorazepam**
Opioids	**Standard Opioids** • **Fentanyl** (norfentanyl) • **Codeine** (codeine-6β-D-glucuronide) • **Hydrocodone** (norhydrocodone, hydromorphone) • **Hydromorphone** • **Morphine** (morphine-3β-D-glucuronide, morphine-6β-D-glucuronide) • **6-Acetylmorphine** • **Oxymorphone** • **Oxycodone** (noroxycodone, oxymorphone) • **Methadone** (EDDP) • **Buprenorphine** (norbuprenorphine) • **Tramadol** (o-desmethyltramadol)	**Fentanyl Analogue/ Precursor Testing**** • **4-ANPP^** • **Acetyl fentanyl** • **Fluorofentanyl, all isomers** • **Carfentanil** (norcarfentanil)	**Other Illicit Synthetic Opioids** • **2-Methyl-AP-237** • **Brorphine** • **Etodesnitazene** • **Isotonitazene** • **Metonitazene** • **N-Pyrrolidino etonitazene** • **Protonitazene**
Cannabinoids	• **Delta-9-THC** (11-hydroxy-THC, carboxy-THC)	• **THC isomers,** such as delta-8, delta-10	• **THC derivatives,** such as THC-O, THC-P
Other Relevant Substances Non-standard emerging substances, may vary in prevalence by location.	**Illicit Stimulants/Hallucinogens** • **2F-Deschloroketamine** • **3-HO-PCP/4-HO-PCP** • **Alpha-PHP/alpha-PiHP** • **Eutylone** • **N,N-Dimethylpentylone**	**Illicit Benzodiazepines/ Benzodiazepine Analogs** • **Clonazolam** • **Etizolam** • **Flualprazolam** • **Flubromazolam**	**Other** • **Gabapentin** • **Medetomidine** • **Xylazine**

* This substance was previously listed as 6-alpha-hydroxyalprazolam. Laboratories conducting overdose biosurveillance under OD2A-S Strategy 4 should continue to use 6-alpha-hydroxyalprazolam when reporting data to CDC.

** Fentanyl analogue testing to include relevant analogues common at the time and to the region.

Method Validation for HRMS Testing

Many approaches exist for the validation of an analytical method for biosurveillance, which involves consideration of several variables, including drugs included in the testing panel, specimen type, targeted versus semi-targeted analysis, and whether the method will be qualitative or quantitative. Additionally, with the ever-changing landscape of trending drugs, laboratories are faced with the challenge of regularly updating their testing panel in a short period of time. To accommodate rapid changes to the testing panel, validation plans conducive to timely addition of new substances are recommended.

Method validation for the non-targeted analysis of many substances using HRMS is significantly different from validation for quantitative targeted analysis using HPLC with LC-MS/MS. Targeted method validation focuses on analytical figures of merit such as detection limits, reproducibility, and quantitative accuracy, while non-targeted HRMS validation considers chemical identification in addition to analytical figures. HRMS validation must consider characteristics of the instrumentation, goals of analytical analysis, and practical limitations involved with analyzing for potentially hundreds of substances with an ongoing need to expand.

Due to the complexity of the substances being analyzed for, this *Expanded Strategy* recommends that public health laboratories include an internal quality control mix containing a representative subset of substances for use in monitoring long-term method performance. It is recommended that an initial performance evaluation be conducted to validate the inclusion of a substance within the method, but due to the complexity of the sample panel being analyzed, a representative subset should be used for monitoring long-term method performance.

HRMS Identification Criteria

Analytical Metrics for Qualitative Analysis of Substance Detections/Identifications

For qualitative analysis using HRMS, analytical metrics can be used to grade substance detections/identifications. Presence of a match is considered assuming:

- **Precursor Mass Accuracy Match:** ± 5 ppm of theoretical
- **Retention Time Match:** ±0.05 min of the expected RT
- **Isotope Pattern Match:** Instrument software scores for precursor isotope pattern match to theoretical should be set based on the performance of standard mixes due to vendor software differences. A suggested pass threshold is >80, depending on the software package.
- **MS2 Spectrum Match:** If Data Dependent MS/MS is used, then the MS2 spectrum provides additional identification confidence. MS2 scores > 70 can be considered passing. If either Data Independent MS/MS or All Ion Fragmentation are used, then the measurement of at least one fragment ion must be within +/- 10 ppm of its accurate mass for the analyte to be identified.

Individual Metrics for Instrument Performance

Detection Level/Low Spike Concentration

Formal determination of Limits of Detection for all analytes is unnecessary for screening purposes, but a minimum desired detection level for spiking should be determined which meets the needs of the program and is achievable under routine analytical conditions. This concentration may be estimated via standards analysis as a traditional limit of detection determination with a few spikes. This is then used as the lowest spiking level for validation experiments and ongoing QC testing. It may be considered the Reporting Limit for the test, being a concentration demonstrated to have been achieved for each analytical batch but is practically the minimum level at which the laboratory will validate presence. Note that this concentration may vary for analytes of different chemical classes.

Accuracy/Detectability

Positive control spikes to validate the detectability and accurate assignment of screening substances should be prepared as matrix spike replicates at multiple concentration levels. Three replicates at three concentrations are suggested. The low-level spike should be selected based on the detection level set above, with higher spike concentrations chosen based on experience with the practical range of real-world samples or as a simple ratio (e.g., 5x, 50x) of the low concentration. The control analytes are evaluated with a performance criterion of 100% detectability and chemical substance identification for all substances in the spikes.

An initial sensitivity analysis should assess positive detections vs non-detections in the low-level spike samples and matrix blanks using percent positive agreement (PPA, **Figure 4**).

If one or more of the control analytes are not detectable in two or more of the low-level spikes, then further work on method optimization may be required and/or the sample preparation procedure may require revision.

Figure 4. Positive Predictive Agreement

PPA= a/(a+c) *100		Expected	
		Present	Not Present
Test	Detected	a	b
	Not Detected	c	d

Precision

Replicates should be assessed for reproducibility of the chemical analytical performance as in a quantitative assay. This includes maximum RSDs for retention time, mass accuracy, and spectral library match score.

Selectivity and Specificity

Specificity should be assessed by examining at least three matrix blanks to determine the presence and abundance of interferents resulting in false positive detections. Credible detection in a sample should only be assigned for substances exceeding the median blank abundance level by a defined threshold (e.g., blank abundance + 10xSD of blank, 3x blank abundance, etc.). Accurate mass measurements with MS/MS validation yields identifications that are unlikely to exhibit interference in a manner typical for low resolution LC-MS/MS but initial validation should note the presence of matrix interferents. Carryover assessment should also be performed as part of the initial validation by examination of a matrix blank sample immediately following the highest matrix spike described in the **Accuracy** section.

Long-Term Method Performance

It may be impractical for the laboratory to validate performance for every analyte in the method in every analysis. The laboratory should select a subset of analytes based on chemical class, polarity, molecular weight, and chromatographic behavior which are representative of all the analytes in the method. While standard mixtures containing all the analytes may be used for validation spiking experiments, only the representative control analytes are evaluated to ensure continued performance. Sample and extract stability can be assessed in a manner consistent with targeted quantification by sample reinjection over time to ensure reproducible detection. The use of QC mixtures and/or internal standard can be used to monitor the instrument response sensitivity over time using control charts to identify degradation of mass accuracy or instrument response before it exceeds performance criteria.

Stability of the Chemical In and Out of Matrix

There are several types of stability that need to be addressed: freeze thaw stability, short-term stability at room temperature, long-term stability in storage conditions, and stability of the chemical in solution as opposed to matrix.

Quantitative Analysis

For labs considering quantitative analysis, method validation should incorporate the same metrics as qualitative analysis plus the following:

- Reportable (analytical measurement) range (AMR)/calibration model for new drug(s), or for all drugs if changed from current method
- Linearity
- Limit of detection
- Limit of quantitation
- Dilution integrity for new drug(s)

An additional option for adding substances to a validated method is Standard Addition. This type of methodology may provide more flexibility for new and emerging drug threats and would allow for a much less rigorous validation process. Standard Addition is a quantitative analysis technique for complex samples which can minimize interfering matrix effects. Standard Addition may also be used to estimate concentration for an analyte not in a panel, but this technique is primarily recommended by this *Expanded Strategy* for method validation.

Best practice for Standard Addition validations may include:

- **Primary recommendations:**
  - The sample to be quantitated should be run unfortified as well as spiked with (at a minimum) three concentrations of sample matrix fortified with the drug(s) of interest.
  - The concentration levels for spikes should be at approximately 50%, 100% and 150% of the expected sample concentration.
  - A linear regression analysis should be used with a coefficient of determination (r^2) of 0.990 or greater as the acceptance criteria.
  - Toxicity data of similarly structured substances within a class will assist in defining the range of concentrations needed to perform the standard addition quantification.
- **Secondary Recommendation:** Additional spike concentration levels may be added to cover a larger concentration range or to duplicate the current range, though sample volume may limit this practice. This will allow for flexibility should any spike issues arise.

Ongoing Method Validation

The ability to quickly add substances of interest to overdose biosurveillance panels is a valuable part of all programs. Once the analytical method is validated, a full method validation is not required for the addition of these new substances of interest. The following method validation parameters will aid in the testing and inclusion as new substance trends arise:

- Add drug(s) of interest by chemical formula to collect data on potential exposures.
- Purchase certified reference material (CRM) for all drug(s) identified for addition to library.
- Analyze a diluted stock of the CRM to collect the following data points:
  - Retention time
  - Isotope Pattern
  - Adducts
  - Characteristic fragments
  - Possible Interferences.
- If the analyte is of the same chemical class as one of the control analytes, then spike the CRM into relevant matrices and analyze using the validated method.
- Analysis should be performed over three analytical runs.
- If the analyte represents a new chemical class or cannot be related back to one of the control analytes for other reasons, then a complete validation should be conducted for the analyte.

Ongoing Review of Method Validation

There are several ways to ensure the integrity of method validation:

Update Protocols as Needed

Method validation protocols should be reviewed at least annually and revised whenever necessary due to changes in the procedure, equipment, extension to a new sample matrix or other significant change. If significant changes are made to a method, it may be necessary to revalidate the protocol to ensure that accuracy, precision and sensitivity have been maintained. New equipment should be verified by comparison studies with analysis of split samples on the existing and new instrumentation when possible.

Staff Training

Analysts must demonstrate competency in the method prior to analyzing study samples. Initial competency may be established through training by an experienced analyst (i.e., performance of the method under the guidance of a competent analyst and analysis of samples of known concentration). The samples may consist of certified reference materials (CRMs), fortified matrix, previously analyzed PT samples or other samples for which the concentration of analyte has been well established.

Quality Assurance

Quality control (QC) and proper laboratory techniques help ensure that biosurveillance results are valid and scientifically defendable. QC also enhances the confidence with which data from different sources may be compared.

Quality control samples analyzed with each analytical batch are a continuous indicator of accuracy and precision of the instrumental portion of the method. Consistent QC results address potential quality issues like build-up of materials in the instrument or environmental conditions like humidity or room temperature fluctuations that can lead to instrument drift and erroneous results. QC materials must be stable and well-characterized so QC data can be tracked over time. For HRMS analysis, it is necessary for QC materials to include multiple classes of drugs that span the gradient of the LC method. For quantitative analysis, utilizing QC samples with concentrations that include the limit of detection (LOD) and separate QC samples that exceed the LOD will provide more potential interpretation data by public health laboratory partners.

Within each analytical batch, laboratories should run a minimum of one QC sample for every 20 unknown samples. QC material should be a match for the matrix in which the testing is occurring, for example, urine, blood or serum/plasma. For blood and serum/plasma, matrix matching is less important and whole blood can be used for all blood matrices. It is important to understand that some drugs partition differently between whole blood and serum/plasma making the identity of the sample matrix imperative for appropriate result analysis by public health laboratory partners, but for many analytes matrix matching may be less critical.

Ideally, each analytical sequence for qualitative HRMS testing would begin and end with analysis of a standard containing all analytes at a concentration equivalent to that of the low-level spike from the method validation. However, for laboratories with panels including over 1000 analytes, it is impractical to do so. A QC sample containing as many analytes as determined reasonable by your laboratory should be run at the beginning and ending of each analytical sequence. It should also contain at least one matrix blank and one matrix spike, again at the low concentration from the method validation. The matrix blank is evaluated for contamination by analytes or interferences. The control analytes are evaluated in the standard and matrix spike to ensure that all are detectable. If any analyte is detected in a sample, then that analyte may also be specifically evaluated in the standard, matrix blank and matrix spike.

Use of internal standards for qualitative testing by HRMS: Isotopically labeled internal standards are typically used for quantitative analysis, but they are also useful for qualitative work. The addition of one or more internal standards prior to sample extraction provides the opportunity to evaluate each analysis for proper extraction and injection of the sample. If the internal standard is not detected, then the analysis should be carefully reviewed and re-extraction and/or re-analysis performed as needed.

Demonstration of Analytical Proficiency

Proficiency testing (PT) establishes the capability of a laboratory to accurately perform testing under a given set of circumstances. It is an important comparison and objective assessment of staff competencies, specimen handling, equipment functionality and results reporting (CDC/ATSDR, see appendix). Unknown samples are submitted to a laboratory through a PT program provider or internal accuracy checks. Enrollment in an external PT program is the preferred method for confirming the quality of laboratory measurements as well as pre-analytical and post-analytical laboratory procedures. Laboratories complete a standardized testing and analyte identification process in accordance with their validated standard operating procedures. By routinely meeting PT requirements, laboratories can ensure consistency in the performance and reliability of their method(s). This document provides available resources and support of needed public health testing on drugs of abuse (see **Appendix**). Note that the evolving drug landscape and use of HRMS may present emerging analytical challenges with proficiency testing, including unintentional identification of substances or contaminants in specimens provided by PT programs.

Inter-laboratory comparison via round-robin or split sample testing is a good way to demonstrate analytical proficiency in the absence of a formal external program. Homogeneity of specimens is critical to a successful inter-laboratory assessment and should be initiated only after careful planning and preparation. Cross-laboratory collaboration and evaluation are encouraged and provide a unique opportunity to harmonize measurements.

Blinded internal challenges in which the analytes and/or target concentrations are unknown to the analysts are an acceptable way to demonstrate analytical proficiency. This approach may be particularly important during method development and prior to the establishment of an external proficiency testing program or inter-laboratory assessments.

Data Analysis Interpretation

In biosurveillance testing programs, the public health laboratory does not always have complete information for the patient from which the sample was obtained. Drugs administered during the treatment and care of the individual are not known to the public health laboratory. It is important for the public health laboratory to document the data elements collected from the patient and convey this to the partner compiling the results data. The *Model Strategy* outlines the various data elements that are collected as part of the biosurveillance program. The interpretation of results by the public health laboratory will be limited to the testing performed, instrumentation used and other analytical/technical aspects of the testing.

HRMS Data Management

HRMS data storage and file size are significantly larger than LC-MS/MS data. To ensure the instrument data requirements, capacity, and output are met, it is vital to involve the Information Technology (IT) department in your laboratory. If allowed by the public health laboratory's IT department, working up the data from an HRMS instrument on a separate, secondary workstation is beneficial. This will free up the instrument for use during analytical runs and provide flexibility for remote data analysis. Consideration should be given to the need for data backup and storage as data files can easily exceed 150MB per analysis. Oftentimes a separate data server is necessary to retain all HRMS data. A retrieval method will need to be implemented to recover data for historical data investigations.

Interpretation, Management and Dissemination of Data

Biosurveillance data are generally sorted into three categories: aggregate, individual de-identified and individually identifiable. Aggregate data is generally the most appropriate data form to share with the public, governmental, law enforcement, or public safety organizations. See **Figure 5** for the minimum and desirable data elements to include in biosurveillance specimen submission and **Figure 6** for the minimum data elements to include in the laboratory report. For more information on categories of biosurveillance data, security, utility and additional data sharing considerations, please refer to the *Model Strategy*.

Figure 5. Biosurveillance Specimen Submission Data Elements

Minimum Data Elements	Desirable Data Elements
• Gender • Age group • Three-digit zip code (patient's residence) • Submitting facility (provider information) • Date of specimen collection • Time of specimen collection • Specimen type • Medical record number or other patient identifying information	• Race • Ethnicity • Drug test results • Drug test methods • Clinical presentation • Pregnancy status

Figure 6. Minimum Data Elements to Include in the Laboratory Report

Specimen Collection Data	Specimen Analysis Data
• Laboratory name • Laboratory address • Specimen ID number • Specimen type • Collection date • Collection time	• Analyte • Analytical method • Result • Result units • Reporting limit units • Date of analysis • Time of analysis

Analyzing and Interpreting Aggregate Polysubstance Biosurveillance Data

Analytical laboratory testing produces considerable complex data that requires careful interpretation, management and dissemination to provide actionable data to promote and propel public health actions. Definition of terms varies by discipline; for the purpose of this *Expanded Strategy*, the following definitions will be used:

- **Laboratory data analysis:** Analysis of raw analytical data that must be analyzed and assessed prior to reporting to epidemiologists.
- **Results analysis:** Transformation of laboratory data using epidemiologic methods into information that is reliable, easily disseminated, and valuable in informing decision making of partners.

Before epidemiologists can utilize laboratory data, laboratory scientists must analyze complex results yielded from the laboratory instrument, such as chromatograms, with detailed information on retention time, MRM transitions, mass accuracy, isotope patterns, intensity counts or concentration for quantitative analysis, which is then used to determine "results" on whether a specific analyte was detected in the specimen. In this document, this process is referred to as **data analysis**, referring to analysis of raw analytical data that must be analyzed and assessed prior to reporting to epidemiologists.

Once this laboratory data analysis is completed, epidemiologists can begin **results analysis**. The purpose of results analysis of laboratory biosurveillance data is to utilize epidemiologic methods to transform the reported laboratory data into information that is reliable, easily disseminated, and valuable in informing decision making of partners. Appropriate results analysis requires an understanding of your target audience and the goals for data products yielded from the analysis. Some public health agencies may require reporting of overdose biosurveillance data as reportable conditions, subject to local reporting guidelines.

Ensuring Accuracy in Interpretation of Polysubstance Biosurveillance Data

Along with providing results to epidemiologists for results analysis, laboratory scientists should continue to provide input on results interpretation. Results transferred from the public health laboratory to epidemiology partners will include findings of analytes or substances detected in patient samples and may be reported in qualitative or quantitative values.

Metadata, commonly described as "data about data," is additional information provided alongside datasets which assist in describing, explaining, locating and using data. Examples of metadata are the instrument technology and software, sampling method information, geographic location or a list of analytes included in the laboratory's panel. When completing results analysis, metadata management must be informed by program goals to guide analysis and interpretation. Proper metadata management is especially important for programs seeking to gain insight on overdose trends within a particular geographic area, or regarding particular substances or combinations of substances.

Metadata to Include

- Sampling method information
- Data completeness information
- Testing method information (instrument, platform, software)
- Geographic location (zip code or county)
- Analytes included in test panel

Biosurveillance data is useful in identifying substances implicated in overdose trends within the selected population. When communicating findings gleaned from biosurveillance data, it is important to communicate the scope of the testing panel transparently and accurately at the time of results analysis. As laboratories frequently expand or scale down their testing panels, findings may fluctuate over time. For example, if a laboratory's panel does not include methamphetamine at the time of sample analysis, it is not appropriate to determine conclusions regarding the prevalence of methamphetamine within the population from this data. This does not indicate that methamphetamine usage is absent within the group of patients presenting with nonfatal overdose included in biosurveillance data, only that the laboratory is not currently including methamphetamine within their test panel. When reporting information out, it is important to ensure that such limitations are echoed in any findings disseminated.

When planning analyses from which findings can be derived to inform program goals, adequate biosurveillance testing volumes relative to population size, geographic area, region or other characteristics are necessary to ensure representativeness and accurate trend reporting. Consider whether the volume of specimens included in testing is high enough to support planned analyses, and if techniques like data suppression should be employed if testing volumes are not sufficient. Care must be taken to ensure that data provided has been obtained from a dataset with a volume that supports robust analysis for a particular area, region, or other population characteristic. Any conclusions provided should be accurately contextualized within the limitations of the data.

Interpretive Considerations

Care must be taken to ensure that data interpretation is accurately contextualized within limitations of analytical drug testing employed in biosurveillance.

Laboratory testing can yield a complex mixture of detected chemical substances, which can include both individual and mixtures of parent (i.e., unchanged) drugs, precursors and metabolites. An analyte detected may be a combination of metabolite and parent drug or a metabolite and precursor, such as the metabolite norhydrocodone and parent drug hydrocodone. Alternatively, an analyte detected may be both a metabolite and precursor, such as 4-anilino-N-phenethyl-piperidine (4-ANPP), which is both a precursor used in the manufacture of fentanyl and an inactive metabolite of fentanyl. Substances detected may also include a combination of metabolite and parent drug where the metabolite is also a parent drug, such as hydrocodone and hydromorphone. When interpreting laboratory data, it is important to ensure that parent drugs and their associated metabolites are not "double counted." To avoid double counting, analytes may be grouped based on the metabolic pathway (**right**) of each potential parent substance in the public health laboratory's panel.

Metabolic Pathway Groupings

Parent drug

The substance at the beginning of a biotransformation reaction. Parent drugs can be biologically active or inactive. Some parent drugs may require activation by metabolic pathways before they become active. Examples:

- Fentanyl, furanyl fentanyl, acetyl fentanyl
- Methamphetamine

Precursor

Chemical utilized in the manufacture of illicit substances. Precursors may be active or inactive and may be detected in patient samples due to improper illicit synthesis of a drug product. Many precursors also have legitimate commercial uses. Examples:

- 4-ANPP, norfentanyl (inactive)
- Ephedrine, pseudoephedrine (active)

Metabolite

A byproduct of the metabolism of a drug. Examples:

- 4-ANPP, norfentanyl (inactive)
- 4-hydroxymethamphetamine, amphetamine (active)

As indicated in polysubstance overdoses, co-occurrence of more than one drug may be present in patient specimens. For example, fentanyl and methamphetamine, cocaine and amphetamines or other combinations may be detected in the same patient sample. Samples may also include analytes for parent drugs, analogues, and adulterants, like fentanyl, furanyl fentanyl and xylazine. Adulterants or contaminants may have been intentionally or unintentionally added to a drug product consumed by a patient (i.e., intentional adulteration of drug product by supplier versus drug residue remaining on drug product preparation surfaces/tools). Patients may be treated with medications during their treatment course while hospitalized or may be prescribed medications for routine use that may be detected. Some substances, like amphetamine, can be a prescription drug, an illicitly manufactured drug, or a metabolite of a parent drug like methamphetamine. Prodrugs, biologically inactive substances that are converted to active form *in vivo*, may metabolize into active metabolites of illicit substances. Selegiline, a drug prescribed for the treatment of Parkinson's disease, is metabolized into methamphetamine and amphetamine.[9] Drug-drug interactions also have the potential to influence results yielded, for example, concentrations of both methadone and buprenorphine are reduced by cocaine.[10] Patient intent or behavior cannot be accurately determined from laboratory results alone and must be corroborated by product matching and/or patient self-reporting of intention. Care should be taken not to assume that all detected drugs and drug metabolites are associated with illicit use or as contributing to the patient's overdose.

Specimen type must also be considered as results obtained from urine and blood are contingent upon several factors which significantly influence interpretation of results. Drug metabolites present in urine may vary based on dose, drug half-life, time since ingestion or consumption, duration of use (chronic versus sporadic), drug-drug interactions, sample dilution, and individual patient characteristics like genetics, weight and pH of urine.[9] For urine specimens, co-occurring substances can be detected for many hours after initial use and elimination time may vary depending on analyte. As

half-lives and elimination times vary between drugs, it is possible that one substance that an individual consumed will no longer be detectable while another substance may remain detectable for a prolonged window of time. Such considerations also significantly impact the viability of interpretation of quantitative urine test results.

Drugs may be eliminated from blood specimens quicker than that of urine and will not reflect substances consumed for the same duration of time as urine specimens. Blood testing generally detects drug substances themselves, while urine testing detects the biotransformed metabolites of drugs. Due to biotransformation of substances once metabolized and subsequently eliminated in urine, both biologically active and inactive metabolites resulting from such processes may be present in urine, while the same substances may not be found present in blood. Furthermore, gel in gel separator specimen collection tubes commonly used for blood and serum samples may adsorb some drugs, resulting in underestimation of drug concentration at time of sample collection. Substantial loss of concentration through drug adsorption was observed as quickly as one day after specimen collection for numerous substances, including fentanyl, carfentanil, methadone, clonazepam, and ketamine; stimulants like cocaine, methamphetamine, and amphetamine were less susceptible to drug adsorption from gel separator tubes.[8]

Each of the examples in this section are limitations to be aware of that may impact your interpretation and underscore the value of utilizing appropriate advisory board members, including laboratory scientists and medical toxicologists, in ensuring accurate results interpretation.

Case Study: Interpretation

Consider a patient who presents to the ED with a nonfatal overdose and has their specimen submitted to a public health laboratory for biosurveillance testing.

Upon testing, laboratory results show that the patient's urine is positive for both fentanyl and THC. It was not known whether the patient used cannabis and fentanyl together, used fentanyl and cannabis separately or at different points in time, or if they were consumed together as part of one drug product through intentional or unintentional adulteration or contamination ("lacing").

It is important to only make conclusions that are consistent from the information available at the time of interpretation. From the information available, it is not possible to conclude that the cannabis consumed was laced with fentanyl without conclusive matching with the drug product.

Thus, when reporting results from such a patient specimen, it is best practice to acknowledge the presence of both drugs in the patient sample rather than infer that cannabis laced with fentanyl was the source of the overdose.

Reporting and Communication of Aggregate Polysubstance Biosurveillance Data

One goal of biosurveillance is to provide definitive laboratory data that can be used to guide and inform public health interventions aimed at preventing overdoses. External communication of biosurveillance data may include both internal and external partners as well as the general public as target audiences. To ensure successful communication, complex laboratory results must be simplified into information that is concise, correct and clear. Reports, data dashboards and all communications should be crafted in plain language. Such information should arrive at conclusions that are appropriate to the type of analysis performed and should be accurately representative of observed trends in results analysis. Information disseminated to the public must be accessible to all audiences.

When reporting or communicating biosurveillance data, a variety of approaches are available and vary by intended audience. If your audience is the general public, consider the use of public-facing online dashboards that are regularly updated. Care should be taken to ensure the use of accessible communication strategies for public dissemination of biosurveillance data, like utilizing appropriate visual aids and plain language. Close, ongoing collaboration with partners in epidemiology, communications and data visualization can aid in dashboard design, maintenance and promotion. In disseminating information through partner organizations, it may be appropriate to develop semi-regular reports that are distributed on a biannual, quarterly, or monthly basis. If program goals include sharing of biosurveillance results with individuals who use drugs or those who engage in outreach to them, mass text messages and social media posts to share information in a rapid and timely manner may be appropriate to aid in overdose prevention. Mass emails may also be beneficial in ensuring partners and outreach workers are able to quickly receive actionable findings to rapidly respond.

Once trends have been identified through results analysis, consider discussion of observed trends with partners who are performing testing related to illicit drug use or overdose. Medical examiners and coroner's offices may observe similar trends in fatal overdoses, or forensic/crime laboratories may have identified trends within drug product seizures or other related testing. If organizations are performing overdose prevention efforts, they may also have observed similar trends. Discussion of observed trends amongst relevant partners can corroborate findings and can enable swift action.

Data Dissemination Methods

Examples of effective means of biosurveillance data dissemination:

- Minnesota Drug Overdose and Substance Use Surveillance Activity
- Rhode Island Nonfatal Overdose Biosurveillance Data
- Overdose Spike Alerts
- Health Alert Network notifications
- Quarterly reports
- Print and social media
- Peer-reviewed publications
- Presentations at scientific meetings

Expanded Strategy Recommendations

Collaboration and Outreach Efforts

- Seek community input into the biosurveillance program designs. Hospitals, substance use prevention programs, relevant medical societies, medical examiners/coroners, epidemiologists, forensic toxicologists, state and local elected officials, social services, regional poison control centers and overdose prevention agencies bring unique insights and perspectives that will benefit the program.
- Work with your state's LRN-C coordinator who likely has established relationships with emergency departments at acute care hospitals.
- Contact various levels of hospital staff when seeking collaboration, including both patient care providers and senior leadership.
- Identify trusted partners in the community who understand the value and utility of biosurveillance and are eager to share information.
- Provide hospitals with necessary guidance and resources to facilitate specimen transfer. Consider working with hospital partners to develop an internal system that is suited to individual facility needs.
- Prioritize and sustain close partnerships with health department epidemiologists, quality assurance officers, and communications offices.
- Consider the development of a multi-disciplinary group of individuals to provide input, discuss opportunities and challenges, and propose public policy initiatives and public health interventions that biosurveillance data helped inform.

Analytical Considerations in Nonfatal Overdose Biosurveillance Programs

- Broaden the scope of biosurveillance beyond opioids by creating an adaptable and robust testing method capable of detecting both drugs of abuse and therapeutic medications, to better understand substances involved in nonfatal overdoses.
- Monitor reports from national and regional laboratories to ensure the testing scope (analyte list) stays relevant.
- Validate analytical methods to identify substances contained in the Expanded Strategy's suggested panel. Update the analyte list with emerging fentanyl analogues and novel psychoactive substances, as appropriate for the region. Follow the Method Validation Plan described in the Expanded Strategy.
- Use TOM Kits® supported by the CDC Division of Laboratory Sciences as a common reference material and to expand confirmation libraries. The TOM Kits® product line contains the Fentanyl Analogue Screening Kit and Emergent Panels (FAS, FAS V1-V4 Kits) and the Emergency Drug Panel Kit.
- Include multiple classes of drugs that span the gradient of the LC method in QC materials used for HRMS analysis. Within each analytical batch, run a minimum of one QC sample for every 20 unknown samples. QC material should be matched to the matrix of specimens being tested, for example, urine, blood, or serum/plasma.
- Engage the IT department in your laboratory to ensure the instrument data requirements, capacity, and output are met since HRMS data storage and file size are significantly larger than LC-MS/MS data.

Interpretation, Management and Dissemination of Data

- Interpret laboratory data within the limitations of analytical drug testing .
- Seek guidance from laboratory scientists, medical toxicologists or other appropriate experts to ensure accuracy when interpreting the complex results yielded by biosurveillance.
- Ensure representative and accurate biosurveillance information reporting through careful design of statistical analysis and consideration of characteristics that influence data interpretation, including testing volume, population size, geographic area, region or others.
- Do not attempt to determine patient intent or behavior from laboratory results alone as such inferences must be corroborated by product matching and/or patient self-reports. Not all drugs or metabolites detected should be assumed as contributing to the patient's overdose. They may be prescribed medications for regular use or may have

received a medication during treatment in the emergency department.

- Simplify complex laboratory results into information that is concise and clear to ensure successful communication. Such information should arrive at conclusions that are appropriate to the type of analysis performed and should be accurately representative of trends observed in results analysis.
- Report and communicate biosurveillance data to the public using accessible communication strategies, such as plain language and appropriate visuals and graphics. See CDC's Plain Language Materials and Resources.

Conclusion

Polysubstance biosurveillance conducted in public health laboratories contributes valuable information to inform national and jurisdictional surveillance of nonfatal overdoses. Definitive laboratory data complement and supplement epidemiological information gathered via medical record abstraction and syndromic surveillance, resulting in a more complete understanding of polysubstance use associated with nonfatal overdoses. The aim of these surveillance systems is to inform public health interventions and/or public policy to reduce the incidence of overdose.

Future Considerations

Biosurveillance programs inform a holistic and unified approach to overdose surveillance. As the overdose epidemic continues to evolve, the overdose surveillance landscape is ever-expanding. A variety of surveillance approaches including product and paraphernalia testing, wastewater surveillance, and retroactive data mining for emerging substances are potential valuable partnership opportunities.

Appendix

PT Programs

The following are providers for PT programs (as of time of publication):

- College of American Pathologists (CAP): Proficiency Testing
- American Association of Bioanalysts (AAB)
- Pennsylvania Department of Health (PA DOH): Proficiency Testing
- Research Triangle Institute: Proficiency Testing and Reference Materials Services and Capabilities
- LGC Standards (AXIO): Reference Materials, Standards & Testing
- BIPEA-International Proficiency Testing Provider: Proficiency Testing Programs

Figure 7. Providers PT by Drug Type (Number of Analytes Available)

Matrix	Synthetic Cannabinoids	Synthetic Stimulants	Benzodiazepines	Opioids
Urine	• AAB: Urine Drug Screening (2) • CAP: Synthetic Cannabinoids and Designer Drugs (27) • PA DOH: Drugs of Abuse (2)	• CAP: ○ Toxicology and Urine Toxicology (12) ○ American Association for Clinical Chemistry (5) • PA DOH: Drugs of Abuse (15)	• CAP: ○ Toxicology and Urine Toxicology (18) ○ American Association for Clinical Chemistry (11) • PA DOH: Drugs of Abuse (15)	• AAB: Urine Drug Screening (2) • CAP: ○ Toxicology and Urine Toxicology (19) ○ American Association for Clinical Chemistry (11) • PA DOH: Drugs of Abuse (20)
Serum & Whole Blood	CAP: • Toxicology and Urine Toxicology (1) • Forensic Toxicology and Criminalistics (2) • Blood Cannabinoids (3)	CAP: • Toxicology and Urine Toxicology (12) • Forensic Toxicology and Criminalistics (16)	• CAP: ○ Toxicology and Urine Toxicology (19) ○ Forensic Toxicology and Criminalistics (17) ○ Novel Opioids and Benzodiazepines (20) • AXIO: (10)	CAP: • Toxicology and Urine Toxicology (19) • Forensic Toxicology and Criminalistics (17) • Novel Opioids and Benzodiazepines (20)

Resources

Collaboration and Outreach Efforts

- National Overdose Prevention Network: Partnerships in Action
- CDC: Strategies and Partnerships
- Association of State and Territorial Health Officials: A Comprehensive Public Health Framework to Address the Opioid Crisis
- The Overdose Response Strategy: Reducing Drug Overdose Deaths Through Strategic Partnership Between Public Health and Public Safety
- US Substance Abuse and Mental Health Services Administration: Engaging Community Coalitions to Decrease Opioid Overdose Deaths Practice Guide 2023

Analytical Considerations in Nonfatal Overdose Biosurveillance Programs

- TOM Kits®: Fentanyl Analogue Screening Kit and Emergent Panels
- Center for Forensic Science Research and Education: NPS Discovery
- Monographs: GCMS and LC-QTOF-MS Library Databases
- American Academy of Forensic Sciences: Academy Standards Board
- US Drug Enforcement Agency: National Forensic Laboratory Information System
- US Customs and Border Protection: USBP and OFO Drug Seizure Statistics and AMO Drug Seizure Statistics
- National Institute on Drug Abuse: National Drug Early Warning System

Interpretation, Management and Dissemination of Data

- Minnesota Drug Overdose and Substance Use Surveillance Activity
- Rhode Island Department of Health Drug Overdose Surveillance Data Hub: Nonfatal Overdose Biosurveillance Data
- CDC: Plain Language Resources

General Resources

- APHL: Model Opioids Biosurveillance Strategy for Public Health Practice
- CDC: Overdose Data to Action in States
- University of Maryland: Center for Substance Use, Addiction & Health Research
- Toward a National System of Expanded Testing of Existing Urine Specimens: The Drug Outbreak Testing Service
- American College of Medical Toxicologists: Drug Overdose Toxico-Surveillance Reporting Program
- The Fentalog Study: A Subset of Nonfatal Suspected Opioid-Involved Overdoses with Toxicology Testing

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Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

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