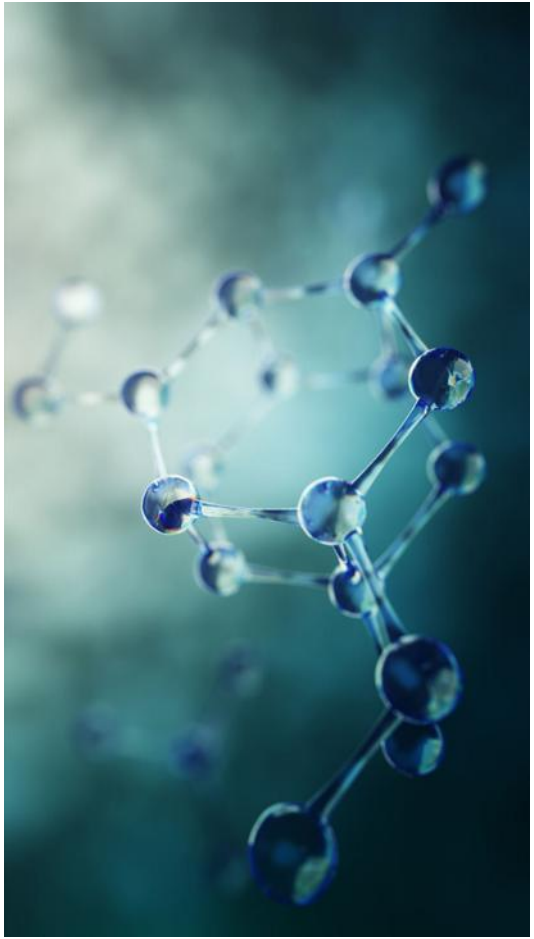
Toxicological Outbreak Investigation

Module 3: Toxicological Laboratory Principles

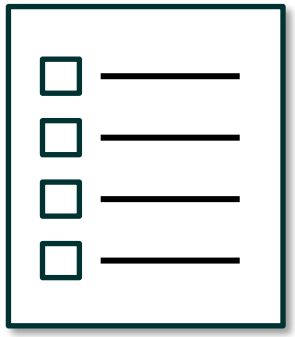
Welcome

- Welcome to Module 3 of Toxicological Outbreak Investigation.
- In this module, we will explain the importance of early sample collection and the information needed in sample collection protocols.
- We will also discuss what needs to be considered when a need for laboratory testing is identified, as well as approaches for the collection of comparison samples.
- This module should take about 60 minutes to complete.



Objectives

- After completing this module, you will be able to
  - Explain the importance of early sample collection
  - Identify information needed in sample collection protocols
  - List laboratory testing considerations
  - Discuss collecting comparison samples in an outbreak scenario



Early Sample Collection

- Collect environmental and biological samples as soon as possible during a toxicological outbreak.
- Many toxic agents degrade in the environment or leave the body within hours.
- Early collection improves the chance of detecting the agent.
- Consult the testing laboratory beforehand to confirm sample types and storage methods.

Develop a Sample Collection Protocol

- Identify and work with a laboratory that will analyze the samples to develop a collection protocol.

- Ask the laboratory
  - What types of samples should be collected?
  - How much sample should be collected?
  - How should samples be collected?
  - What types of containers should be used?
  - What needs to happen to the samples after collection?
  - At what temperature should they be stored?
  - How long can they be stored?
  - How and where should samples be shipped?

Sample Collection Protocol: Sample Types

- Broadly, there are two types of samples
  - Environmental (e.g., soil, water, food)
  - Biological (e.g., blood, urine)
- As you think about what types of samples to collect, consider the following:
  - Some analytes can only be detected in specific media or biological samples.
  - Depending on the amount of time that has passed, the toxic agent or its metabolites might be more detectable in a specific medium.

Sample Collection Protocol: Sampling Considerations

- Environmental sampling is most useful when you can narrow your hypothesis to a small number of potential exposures, for which specific types of samples can be collected (e.g., water, food, soil).
- Considerations for biological samples
  - When a specific hypothesis has not yet been developed, it is usually best to collect both urine and whole blood.
  - If a lot of time has elapsed since exposure, there might be a better chance of finding the toxic agent or its metabolite in urine rather than in blood.

Sample Collection Protocol: Amount of Sample

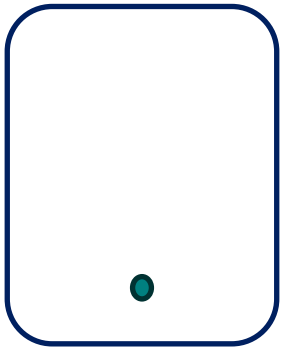
- Be sure that you know the minimum sample amount needed by the laboratory for the type of testing that you might need to do.
- The more tests you plan to do, the more sample (volume or mass) you'll have to collect.
- Having extra sample will allow the laboratory to perform repeat testing or validation.

Sample Collection Protocol: Collection of Samples

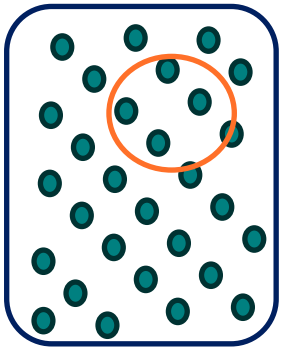
- The laboratory should provide a protocol that specifies when and how to collect the sample. Protocols will vary.
- For example
  - If you are collecting urine, does it need to be a first morning void?
  - If you are collecting a soil sample, does it need to be taken from the top layer?
- Regardless of whether environmental or biological samples are being collected, personal protective equipment should be worn.

Heterogeneity in Environmental Samples

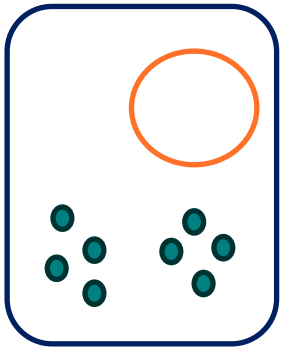
- When collecting an environmental sample, consider how a toxic agent might be distributed in the air, water, soil, etc.
- For example, consider a bag of corn/maize flour that contains a toxic agent:



The rectangle represents the bag of flour. The small circle represents a toxic agent.



If a toxic agent is distributed evenly, this is a **homogenous** distribution. In this case, a single sample might be sufficient for collection.



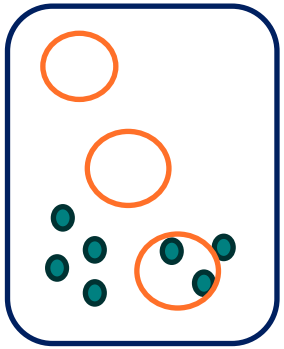
If a toxic agent is NOT distributed evenly, this is a **heterogenous** distribution. A single sample might miss the toxic agent.

- If you are unsure, assume the distribution could be heterogeneous.

Sampling Strategy for Heterogenous Distribution

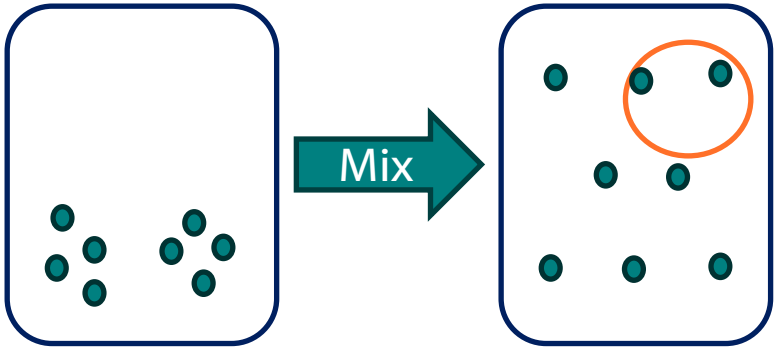
- A heterogenous distribution requires a different sampling strategy.

  Consider two options:



Collect multiple smaller samples from different locations in the bag.

- This could provide information about how the toxic agent is distributed in the bag.
- It would likely require several samples.
- You might need to record information about the location of each sample.



Alternatively, mix the bag to more evenly distribute the toxic agent prior to sample collection.

- This might require fewer samples.
- It would not provide information about how the toxic agent was distributed in the bag
- It might result in dilution of the toxic agent, which could make the agent harder to detect.

Sample Collection Protocol: Container Type

- Some toxic agents react with specific materials; therefore, the type of container or collection material needs to be considered.
  - For example, blood should be collected in the appropriate tubes for the desired test.
  - Some tubes contain additives that could interfere with certain tests.
- In some cases, it is important to make sure that the container used for sample collection is not potentially contaminated with the toxic agent of interest.
  - An example is the need to use metal-free specimen containers to collect urine for metal testing.

Sample Collection Protocol: Post-collection Processing

- Wear personal protective equipment during sample processing.
- Label the samples with an ID sticker or a small piece of paper with the necessary information. Ensure the label is securely taped to the sample. Information on the label should include
  - Who/what the sample was drawn from
  - ID or barcode
  - Date and time when the sample was collected
- Many samples need processing after collection. For example
  - A blood specimen might need to be centrifuged to separate the serum.
  - A water sample might need an acid preservative added.
- A log sheet or sample log should be completed. A sample log is a systematic way to record descriptive information about each sample.

Sample Collection Protocol: Sample Log

- The following information should be included on a sample log.
  - Who: ID#, name, or both
  - What: Type of medium and any pertinent details on the sample
  - When: Collection date and time
  - Where: Collection location
- A modifiable Sample Log is available in the Tool Kit.

Institution name:

Institution address:

Institution phone:

Sample ID	Collection date	Collection time	Person collecting the sample (Name)	Location where collected (*GIS coordinates if possible*)	Type of sample (*urine, blood, water, food, etc.*)	Amount of sample (units)	Collected from (Name)	Collected from (ID)	Observations (*visible debris, tampering, etc.*)

Sample Protocol: Potential Problems in Sample Labeling and Logs

- If samples are missing a label or a log, then investigators might be
  - Unable to determine who or what the samples came from
    - For example, did they come from cases or controls?
  - Unable to determine what the sample is (particularly if it is an environmental sample)
  - Unable to determine how many half lives might have passed between exposure and sample collection

Sample Protocol: Storage

- Storage Conditions
  - Sometimes, placing a sample in the refrigerator or freezer, or preventing its exposure to light, is required to slow the degradation or metabolism of a potentially present toxic agent.
  - The laboratory you are working with will be able to provide you with the correct storage conditions for your samples.
- Storage Duration
  - Some samples will only last a certain amount of time, depending on the storage conditions.
  - The laboratory you are working with can tell you about storage duration based on your storage conditions.

Challenge #1

When sampling a heterogeneous distribution, which of the following strategies could be legitimate options? Select all that apply.

A. Collect a single sample at a random location.

B. Mix the medium, then collect multiple samples.

C. Collect multiple samples from different locations.

D. Mix the medium, then collect a single sample from a random location.

Challenge #1 Answer

The correct answers are B, C, and D.

When you have a heterogenous distribution of a suspected toxic agent, multiple samples should be taken from random locations, or else the medium should be mixed before a single sample, or multiple samples, are taken.

Challenge #2

True or false. Depending on the amount of time that has passed, a toxic agent might be detectable only in a specific medium or type of biological sample. Select the best response.

- ❑ True
- ❑ False

Challenge #2 Answer

The correct answer is true.

A toxic agent (or its metabolite) might be detectable only in a specific medium or type of biological sample depending on the amount of time that has passed.

For example, if a significant amount of time has elapsed since exposure, then there might be a better chance of finding the toxic agent or its metabolite in urine than in blood.

Laboratory Testing Considerations

- After you have collected samples, you might still need to make decisions about testing those samples for toxic agents.
- Toxicological lab testing can be expensive and resource intensive.
- When you are thinking about doing laboratory testing, some important considerations include the following:
  - Is laboratory testing needed?
  - Can the etiology be narrowed to a small number of likely toxic agents?
  - Is there an accredited laboratory with validated methods that can test for the suspected toxic agents?
  - Is there identifying information to help you interpret the results of testing done on a biological specimen or environmental sample?
  - Will you be able to interpret the results?

Is laboratory testing needed?

- Results can sometimes take weeks or months to receive. By that time, the outbreak might be over, and cases might have stopped occurring.
- If samples can be collected and preserved, decisions about testing might be delayed until more is known from the epidemiological investigation or initial laboratory testing results are available. Each investigation will be different in this regard.
- Biological testing might not be needed if environmental samples are found to contain the hypothesized toxic agent and these conditions are met:
  - That toxic agent and dose are clinically compatible with the illness.
  - The epidemiologic data show an association between illness and exposure.
- Environmental testing might not be needed if biological specimens are found to contain the hypothesized toxic agent, and these conditions are met:
  - That toxic agent and dose are clinically compatible with the illness.
  - The epidemiologic data show an association between illness and exposure.

Can the etiology be narrowed to a small number of likely toxic agents?

- Thousands of toxic agents can cause illness.
- Generally, no single test can screen for all possible toxic agents at one time.
  - Each agent requires a specific, validated method for detection and confirmation.
  - Each sample type, whether biological or environmental (such as urine, blood, or water), requires a different validated method.
- Investigators need a specific idea of what to test for before they start testing.
- The amount of sample available for testing is typically limited, so decisions must be made about how to best use the available sample.

Is there an accredited laboratory with validated methods that can test for the suspected toxic agents?

- Not all laboratory capabilities are the same.
  - Some laboratories have more resources and technical expertise than others.
  - Consider how much precision and accuracy is needed when exploring laboratory options.
  - Cost might also be a consideration when selecting a laboratory.
- Where is the nearest laboratory that can perform the testing?
  - It might be in another country.
  - Are they willing and able to perform the test?
  - What are the export/import requirements for shipping?
- There are many toxic agents for which no laboratory test exists.

Is there identifying information to help you interpret the biological specimen or environmental sample?

- The sample log is important for both the environmental samples and biological specimens.
- At a minimum, you need to know
  - Who or where did the sample come from?
  - What is it?
  - How was it collected?
  - When was it collected?
  - Was a chain of custody form completed (if needed)?

Will you be able to interpret the results?

- Finding a toxic agent in an environmental sample and/or a biological specimen does not always mean that it caused the outbreak.
- Data on what are considered “normal” background levels in the population, and the levels that could cause acute illness, are helpful for interpretation.
- Were comparison samples collected, or are known mean/average values in the population available for comparison?

Background Levels of Toxic Agents

- People are continuously exposed to various toxic agents.
- Toxic agent exposures depend on
  - Where people live
  - What people do
  - What people eat

Hypothetical Laboratory Results from a Healthy Adult

- If a randomly selected person living in the United States was tested for exposure to metals, their lab results might look like the table shown to the right.
- Individuals might have detectable levels of metals indicating background exposures in the body without having any signs of illness.
- These background levels can vary depending on a person's diet, occupation, and other factors.

Analyte	Level
Antimony	0.066 µg/L
Arsenic	6.85 µg/L
Barium	1.49 µg/L
Cadmium	0.412 µg/L
Cesium	4.35 µg/L
Cobalt	0.316 µg/L
Lead	0.766 µg/L
Molybdenum	39.7 µg/L
Thallium	0.155 µg/L
Tungsten	0.071 µg/L

Laboratory Data: Comparison Values

- When investigating a toxicological outbreak, it can be important to determine whether the levels of a toxic agent found in samples associated with people who became ill are higher than the levels that might be expected because of background exposures.
- Comparison values might come from testing samples collected as part of an epidemiological study or might come from community reference values that are available for the affected population.
- When comparing lab values, make sure laboratory methods used are consistent between tests, or fit a recognized standard (e.g., EPA or CLIA method).
- Three types of epidemiologic study designs are commonly used for collection of comparison samples: case-control studies, cohort studies, and case-crossover studies. Other epidemiologically sound approaches could also be used.

Comparison Values: Case-Control Study

- In a case-control study, samples are collected from people who have the outcome of interest (case-patients) and people who do not have the illness of interest (control participants).
- Measurements done on samples from control participants provide an estimate of the expected levels of an agent in the population from which the cases came, so care needs to be taken to ensure that the control group is representative of that population.
- If levels of a toxic agent in biological samples collected from case-patients are substantially higher than levels in the same types of samples collected from control participants, then the outcome might be associated with exposure to that toxic agent.
- If levels of a toxic agent are substantially higher in environmental samples associated with case-patients than in samples associated with control participants, then the environmental medium sampled (e.g., flour from homes, soil from yards) might be the source of the toxic agent that caused the outcome.

Comparison Values: Retrospective Cohort Study

- In a cohort study, samples are collected for everyone (or as many people as possible) in a defined population that has been affected by an outbreak (e.g., a school, employees at a specific facility).
- Levels of a toxic agent in biological or environmental samples associated with people who did and did not have the outcome of interest are compared.
- If levels of a toxic agent in biological samples collected from people who had the outcome of interest are substantially higher than levels in samples collected from people who did not have the outcome, then the outcome might be associated with exposure to that toxic agent.
- If levels of a toxic agent are substantially higher in environmental samples associated with people with the outcome than in samples associated with people who did not have the outcome, then the environmental medium sampled (e.g. flour from homes, soil from yards) might be the source of the toxic agent that caused the outcome.

Comparison Values: Case-Crossover Study

- In a case-crossover study design, multiple samples are collected from case-patients, representing times when they were ill and when they were not ill.
- Biological Samples
  - For biological specimens, this study design is useful only if the suspected toxic agent has a short half-life.
  - If measured levels of a toxic agent are substantially higher in specimens collected when a person was ill than in specimens collected when the person was not ill, then illness might be associated with the toxic agent.
- Environmental Samples
  - Multiple samples could be collected at different times during the investigation, or they could represent different times (e.g., if you suspect contaminated flour, you could collect samples from bags that case-patients were eating from when they started to experience symptoms and from other bags of flour in the household).
  - If measured levels of a toxic agent are substantially higher in samples that are associated with times when people were ill, you might conclude that the environmental medium sampled (e.g. flour) was the source of the toxic agent that caused the illness.

Comparison Values: Community Reference Levels

- Information about typical background levels of a toxic agent in biological samples among the general population might be available from existing surveillance sources, such NHANES (the National Health and Nutrition Examination Survey) in the United States.
- Care must be taken to ensure that the community reference levels are appropriate for the population involved in the outbreak.
- If levels of a toxic agent in biological specimens or environmental samples associated with people who have the outcome of interest are substantially higher than reference levels that are available for the community in general, you might conclude that the outcome was associated with the exposure.

Caution about Comparison Values

- For any of these types of comparison values, it is still important to interpret results with consideration of the biologic plausibility of the toxic agent causing the observed symptoms and signs.
- Remember that although observing an association between people having an illness and measured levels of a toxic agent can be helpful in potentially implicating that toxic agent as the cause of the illness, associations do not always indicate causation.

Challenge #3

Which of the following are true statements regarding sample collection? (Select all that apply.)

A. Biological samples should be collected as soon as possible.

B. Environmental samples should be collected as soon as possible.

C. There is one single test that can screen for all possible toxic agents at one time.

Challenge #3 Answer

- **The correct answers are A and B.**

  Generally speaking, both environmental and biological samples should be collected as soon as possible, as some toxic agents can begin to break down or be eliminated from the body with time.

Module Summary

- This concludes Module 3.
- In this module, we explained the importance of early sample collection and the information needed in sample collection protocols. We also discussed what needs to be considered for laboratory testing and sample collection.
- You should now be able to
  - Explain the importance of early sample collection
  - Identify information needed in sample collection protocols
  - List laboratory testing considerations
  - Discuss collecting comparison samples in an outbreak scenario

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