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technical BRIEF
INNOVATIVE RESEARCH FOR A SUSTAINABLE FUTURE

Sample Processing Method for Ricin Analysis

Background

Ricin, the *Ricinus communis* (castor bean) toxin, is known as one of the most lethal natural poisons. It is a Category B bioterrorism agent, Schedule 1 chemical warfare agent, and is periodically used in the U.S. and abroad for nefarious intentions and homicide attempts [1]. The toxin is manufactured in a powder form by partial purification or refinement of the castor bean pulp. Ricin toxicity can occur from inhalation, ingestion, or injection, and, if ingested, as few as 8 castor beans can contain a lethal dose. For inhalation, symptoms including respiratory distress, fever, cough, nausea, and chest tightness can appear as early as 4–8 hours post-exposure, whereas symptoms from ingestion (nausea, vomiting, and diarrhea) typically develop in less than ten hours. Death usually occurs after 36–72 hours depending on the exposure route and the dose received.

The U.S. Environmental Protection Agency (EPA) is the lead federal agency to support states and local authorities in the cleanup of facilities/sites contaminated with ricin. In the last five years, EPA responded to four separate ricin incidents. EPA supported the sampling, analysis and decontamination work, with final clearance for re-entry and reuse of the sites performed by local or state health departments. In June 2013, ricin-containing letters were sent to then President Barack Obama from Tupelo, Mississippi [2]. EPA was called upon to decontaminate and clear for reuse the two sites where the ricin used in the letters was prepared. During 2014, EPA responded to ricin-contaminated sites in Oklahoma City, Oklahoma and Oshkosh, Wisconsin. During a 2017 ricin incident in Boulder, Colorado, EPA was called upon to decontaminate the affected site. During these ricin incidents,

samples were collected to determine the extent of contamination and, after decontamination, to determine if the sites could be released to the public for reuse.

The time-resolved fluorescence (TRF) immunoassay is one of the primary screening methods for ricin in environmental samples. It is also a method used by the Laboratory Response Network of the Centers for Disease Control and Prevention (CDC). However, during the EPA response to the Tupelo, Mississippi ricin incident, unsatisfactory results were obtained due to high fluorescence backgrounds. As a result, the TRF immunoassay could not be used for samples collected from surfaces to which chlorine bleach had been applied for decontamination [3]. The assay interferences were attributed to various potential factors including impurities in reagents used for the TRF assay, residuals resulting from bleach application, sampling material, and the buffer used for wetting the sampling devices.

Without appropriate sample processing prior to analysis, bleaching residuals and/or other matrix effects from environmental samples could cause assay interferences that could ultimately lead to false negative or false positive results. False negative results could occur if high background fluorescence masked actual ricin presence, possibly leading to human exposure if facilities were re-opened prematurely. False positives could occur if controls were within range, but samples showed elevated fluorescence due to the assay interfering materials present in the sample, thereby triggering additional, unwarranted decontamination activities. Furthermore, without sample concentration, ricin could be present below the analytical method's ability to detect it, while still being present at hazardous levels.

Decision-makers with local, state, federal, and tribal governments require rapid and high-confidence results that are not unduly impacted by false positives or false negatives to safely clear areas for re-entry and reuse and to reopen facilities. Therefore, ricin analytical methods must be reproducible, sensitive, and specific, even in complex environmental backgrounds. To mitigate the TRF immunoassay interference issue for post-decontamination ricin analysis, the Homeland Security Research Program of the EPA's Office of Research and Development, in partnership with the Lawrence Livermore National Laboratory, developed a sample processing approach to enable accurate ricin analysis.

Development of a Ricin Sample Processing Method

A ricin sample processing method (Appendix B & C) including sample cleanup and ricin concentration was developed for preparing ricin in environmental samples for analysis [4]. The method is performed in two steps. Step 1 is a sample extract pre-filtration using a 0.22 micrometer syringe filter to remove potential assay interferences by particulate matter that could be present in the sample. Step 2 is a further cleanup of the sample extract and ricin concentration using a centrifugal ultrafiltration (UF) device (Amicon® Ultra centrifugal filter devices, Millipore, Inc., Billerica, MA). Such devices are used for sample cleanup for many substances. The UF devices are selected based on their nominal molecular weight limit cutoff to retain the target analyte, such as ricin, while allowing other soluble materials with lower molecular weight to pass through. Basically, the UF devices allow washing out or removal of soluble materials present in the sample extract that could interfere with analysis while also enabling several-fold concentration of the target analyte due to volume reduction by centrifugation. The processed sample is then analyzed by analytical methods such as

the TRF immunoassay. Both the 0.5-mL and 2-mL UF devices were evaluated. The results indicated that there was no loss of ricin from the sample while using the UF device-based sample processing method. Using this sample processing method, a 1-mL sample could be concentrated to 100 µL (10-fold concentration) with a 0.5-mL UF device (by using multiple loadings on the same device). Relatedly, a 2-mL sample could be concentrated to 100 µL (20-fold concentration) with a 2-mL UF device. Such a volume-reduction-based concentration also concentrated ricin in the sample. Further, the sample processing method was also tested for sponge-sticks sample extracts containing bleach residues and reference test dust [Arizona Test Dust (ATD), selected to be representative of dust found on sampled surfaces]. The results indicated that this sample processing method is effective even for dirty samples

Conclusions

The ricin sample processing method based on the application of a pre-filter and centrifugal UF devices allows sample extract cleanup and ~10-fold to 20-fold concentration of ricin, and thereby, enhances the performance and sensitivity of the ricin TRF immunoassay. Thus, this method could minimize false positive and false negative results, especially for the samples collected for analysis during the post-decontamination phase of the response to a ricin incident. Because the sample processing procedure developed here is intended for use following the sample extraction steps, it could be used for both the pre- and post-decontamination phase samples. Additionally, it could be used with any other fluorescence- and electrochemiluminescence-based immunoassays, as well as other analytical methods (e.g., PCR assays) used for ricin detection, although further verification and validation would be required (i.e., for different surface types and potential interferences).

It is important to note how the generation of false positives and false negatives is related to how specific assays function. For example, some assays, like TRF, are based on the binding of an antibody to a specific part of the ricin molecule. However, this part of the ricin molecule can be present even if the ricin cannot produce a toxic effect. On the other hand, absence of the response is often considered to suggest that the ricin molecule has been sufficiently degraded, especially by decontamination agent such as bleach, to preclude a toxic effect. Thus, improvement in false positive and false negative rates, regardless of what they mean in context of a particular assay, is an important goal, which this sample processing method helped meet.

This sample processing method was successfully used to analyze post-bleach-decontamination samples during the recent (2017) ricin incident in Boulder, Colorado. Data quality objectives, including those related to false positives and false negatives, were met, enabling the site — an apartment building where small children lived — to be returned to its intended use in a timely manner.

References

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Additional Information

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Disclaimer

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