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Evaluation of automated and manual DNA purification methods for detecting *Ricinus communis* DNA during ricin investigations

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ABSTRACT

In April of 2013, letters addressed to the President of United States and other government officials were intercepted and found to be contaminated with ricin, heightening awareness about the need to evaluate laboratory methods for detecting ricin. This study evaluated commercial DNA purification methods for isolating *Ricinus communis* DNA as measured by real-time polymerase chain reaction (PCR). Four commercially available DNA purification methods (two automated, MagNA Pure compact and MagNA Pure LC, and two manual, MasterPure complete DNA and RNA purification kit and QIAamp DNA blood mini kit) were evaluated. We compared their ability to purify detectable levels of *R. communis* DNA from four different sample types, including crude preparations of ricin that could be used for biological crimes or acts of bioterrorism. Castor beans, spiked swabs, and spiked powders were included to simulate sample types typically tested during criminal and public health investigations. Real-time PCR analysis indicated that the QIAamp kit resulted in the greatest sensitivity for ricin preparations; the MasterPure kit performed best with spiked powders. The four methods detected equivalent levels by real-time PCR when castor beans and spiked swabs were used. All four methods yielded DNA free of PCR inhibitors as determined by the use of a PCR inhibition control assay. This study demonstrated that DNA purification methods differ in their ability to purify *R. communis* DNA; therefore, the purification method used for a given sample type can influence the sensitivity of real-time PCR assays for *R. communis*.

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1. Introduction

The protein toxin ricin, which is present in the seeds of the castor oil plant, *Ricinus communis*, is one of most potent biologic toxins. *R. communis* is a common decorative garden plant found in warm climates. The toxin is estimated to comprise 1–5% of the castor bean seed protein material [1]. Ricin belongs to the A–B family of toxins that also includes bacterial toxins, such as diphtheria, Shiga, and anthrax toxins. All clinical reports of ricin poisoning refer to castor bean ingestion for which the lethal dose in humans has been estimated to be 1–20 mg of ricin/kg body weight (approximately 8 beans) [2].

The toxicity of ricin and associated symptoms depends on the route of exposure, which includes ingestion, inhalation, parenteral delivery, and dermal application. Ingestion of ricin causes non-specific symptoms, such as nausea, vomiting, diarrhea, and abdominal pain and may progress to multi-organ failure [2]. Inhalation would also lead to a large number of causalities because of the high toxicity [3]. Inhalation of ricin is expected to produce symptoms such as cough, dyspnea, arthralgias, fever, respiratory distress, and death. Documented cases of ricin poisoning by parenteral delivery, specifically injection, have been associated with even greater mortality rates [4]. The initial symptoms of injection include generalized weakness and myalgia; progression of the illness can include vomiting, fever, hypotension, and death [2]. The literature does not indicate that a dermal application of ricin in which the toxin is mixed with a solvent is effective [4]. Currently, no specific treatment exists for ricin poisoning; instead clinicians provide supportive care for the symptoms.

The ease of procuring castor bean seeds, the high toxicity of the seed extract, and the ease with which it can be disseminated make ricin ideal for biologic crimes or bioterrorism [5]. Ricin has been used to cause harm and terrorize in several documented cases. Most notably, ricin was suspected to be used in 1978 in the assassination of the Bulgarian dissident Georgi Markov in London

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and in the attempted murder of Vladimir Kostov in Paris [4,5]. In 2003 and 2004, ricin was found at a mail sorting facility in South Carolina [2,6]. More recently, ricin was found in letters addressed to the President of the United States [7]. Because of its moderate ease to disseminate and moderate morbidity rates, ricin has been classified as a category B biological threat agent by the Centers for Disease Control and Prevention (CDC) [8].

Real-time PCR is routinely used to amplify *R. communis* DNA, which is present in castor bean seeds along with the toxin [9,10]. Although this technique detects the plant DNA rather than the toxin protein, this rapid diagnostic tool has proven to be useful during investigations of ricin in suspicious samples [11]. Moreover, public health laboratories routinely use real-time PCR for rapid screening of environmental samples for the presence of *R. communis* DNA [12]. The sensitivity of PCR assays has been reported to be influenced by the method of DNA purification [13–15]; hence, several studies have evaluated methods to isolate DNA from potential bioterrorism agents using a variety of sample types [16–19]. However, we have not found any studies that have evaluated commercial methods specifically for the recovery of *R. communis* DNA from sample types that are tested during ricin investigations, which suggests the need for such a study.

The purpose of this study was to evaluate commercial DNA purification methods for isolation of *R. communis* DNA as measured by real-time PCR. Four methods were compared, including two automated methods and two manual methods, which represent three different methods for DNA purification: magnetic bead purification, filter membrane purification, and alcohol precipitation. In addition, these methods were selected for evaluation because of their varying sample throughput capacities and availability to forensic and public health laboratories. The evaluation criteria included real-time PCR limit of detection (LOD) studies with castor bean suspensions and crude ricin preparations, as well as analyses in which *R. communis* DNA was isolated from spiked swabs and spiked powders, which are commonly tested during ricin investigations.

2. Materials and methods

2.1. Biosafety procedures

All work with castor beans and spiked environmental samples was conducted in biosafety level 3 (BSL-3) facilities at the North Carolina State Laboratory of Public Health or CDC. Extraction of ricin toxin, spiking of environmental samples, and manual DNA purification were performed in biological safety cabinets (BSC), using practices outlined in the "Biosafety in Microbiological and Biomedical Laboratories, 5th Edition" [20], including the use of respiratory protection and protective laboratory clothing. PCR assays were conducted in BSL-2 facilities.

2.2. Preparation of castor bean suspensions

Two representative types of castor beans were used in this study, one that was provided to CDC by the United States Department of Agriculture (USDA), (PI 204324 India, USDA/ARS Griffin, GA) and one that is commercially available (Wyatt Quarles Seed Company, Garner, NC). A single castor bean was cracked with a pair of pliers, and the hard outer shell was removed using disposable forceps. Approximately 20 mg of the hulled seed was crushed in 0.25 ml of phosphate buffered saline [PBS, (0.01 M, pH 7.4)] in a pellet pestle microcentrifuge tube. The seed material was centrifuged at 12,000 × *g* for 2 min. The supernatant, henceforth referred to as castor bean suspension, was diluted 1:10 in PBS. Eight additional 10-fold serial dilutions were performed. The original castor bean suspension and all dilutions were retained at 2–8 °C for up to 30 days and then destroyed by autoclaving.

2.3. Extraction of ricin toxin from castor beans

To simulate samples that could potentially be tested during suspected bioterrorism incidents or biologic crimes, ricin was extracted from castor beans using a crude method described in "The Poisoner's Handbook" [21]. Five castors beans were processed by two rounds of the precipitation procedure as previously described [21]. The precipitate (ricin extract) was resuspended in 5 ml of PBS and 10-fold serial dilutions in PBS were performed. All of the ricin extracts were stored at 4 °C for up to 30 days and then destroyed by autoclaving.

2.4. Spiking and processing of environmental samples

Swabs were included as a sample type because they are used for environmental sampling during ricin investigations and submitted to public health and forensic laboratories for testing [6,11,12]. Dacron® swabs (Fisher Scientific, Suwanee, GA) were spiked in triplicate with 10-μl of the ricin extracts at each dilution and were allowed to air dry overnight at room temperature inside a BSC. The swabs were then transferred to 15-ml polypropylene tubes (Fisher Scientific, Suwanee, GA), each containing 1 ml of PBS and were then vortexed at high speed for 2 min. Residual liquid was expressed from each swab by pressing the swabs against the side of the tubes, and the eluted swab suspensions were used for subsequent DNA purification experiments.

Two powders were selected because of their reported use in hoaxes and suspected bioterror incidents or because they are listed in the United States Department of Defense suspicious powders reference material panel, flour, and baking soda [22]. For both powders, a total of 50 mg each was measured in 2-ml polypropylene tubes (Fisher Scientific, Suwanee, GA). Each powder was spiked in triplicate with 10 μl of the ricin extract at each dilution as described above and then allowed to air dry overnight at room temperature inside a BSC. The spiked powders were resuspended in 1 ml of PBS, vortexed at high speed for 1 min, and then centrifuged for 1 min at 8000 × *g*. The supernatants were used in subsequent DNA purification experiments.

2.5. Nucleic acid purification

Four commercial methods, including two automated systems and two manual kits, were evaluated in this study. Automated nucleic acid purification was performed using the MagNA Pure Compact, which has a throughput capacity of 8 samples per run, and the MagNA Pure LC instrument, which has a throughput capacity of 32 samples per run (Roche Applied Science, Indianapolis, IN). Both automated instruments use magnetic bead technology. The MasterPure complete DNA and RNA purification kit (Epicenter, Madison, WI) uses a precipitation method, and the QIAamp DNA blood mini kit (Qiagen, Inc., Valencia, CA) uses silica spin filter technology. To compare their efficiency at isolating DNA, the four methods were performed in triplicate on dilutions of the following four sample types: (1) castor bean suspensions; (2) ricin extracts; (3) spiked swabs; and (4) spiked powders. Following nucleic acid purification procedures, samples were stored at −20 °C in the elution or resuspension buffers provided with the kits, as recommended by the manufacturers.

2.6. DNA yield and purity

A NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE) was used to determine the yield and purity of DNA isolated with each purification method. DNA absorbances were measured in the elution buffer provided with each kit, and the spectrophotometer was blanked with the corresponding buffer