Data/Metadata

**Manuscript: “Sample Processing Approach for Detection of Ricin in Surface Samples”**

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**Figure 1.**

**Figure 1.** Fluorescence counts vs. ricin holotoxin concentration with background fluorescence (from negative controls, PBS buffer) subtracted. Data are from two separate experiments with triplicate TRF analyses per data point. Error bars represent ± one standard deviation. For each TRF analysis, 10 µL of solution was added to 100 µL of assay buffer.

**TRF:** Time Resolved Fluorescence. This assay is used for Ricin biotoxin detection/measurement with the Perkin Elmer Victor X4 plate reader/analytical instrument.

**Fluorescence counts:** Fluorescence counts generated from the Time Resolved Fluorescence assay of Ricin biotoxin and measured by the Perkin Elmer Victor X4 plate reader/analytical instrument.

**Avg. Fluorescence counts:** Average of fluorescence counts from triplicate TRF analyses.

**Ricin Concentration:** 1, 10, 100, and 1000 nanograms per mL concentrations of Ricin were analyzed in triplicate TRF assays.

**ng/mL:** Nanograms per milliliter.

**1.E+03 =** 1000, **1.E+04 =** 10000, **1.E+05 =** 100000, **1.E+06** = 1000000

**Holotoxin:** Complete toxin and not a part or a subunit/chain

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| --- | --- | --- | --- | --- |
| Figure 1 Raw Data |  |  |  |  |
| **Buffer Type** | **Expt Replicate - Sample Replicate** | **Fluorescence Count by Ricin Concentration (ng/mL)** |
| **1000** | **100** | **10** | **1** |
| **Phosphate Buffered Saline (PBS)**  | **1 - 1**  | 1.190E+06 | 1.370E+05 | 1.587E+04 | NA |
| **1 - 2** | 1.134E+06 | 1.382E+05 | 1.472E+04 | NA |
| **1 - 3** | 1.039E+06 | 1.372E+05 | 1.445E+04 | NA |
| **Avg** | 1.121E+06 | 1.375E+05 | 1.502E+04 | NA |
| **SD** | 7.602E+04 | 6.469E+02 | 7.535E+02 | NA |
| **2 - 1** | NA | 1.795E+05 | 2.024E+04 | 2.799E+03 |
| **2 - 2** | NA | 1.777E+05 | 2.114E+04 | 3.033E+03 |
| **2 - 3** | NA | 1.743E+05 | 1.987E+04 | 2.568E+03 |
| **Avg** | NA | 1.772E+05 | 2.042E+04 | 2.800E+03 |
| **SD** | NA | 2.618E+03 | 6.529E+02 | 2.325E+02 |
|  |  |  |  |  |  |
| Expt 1 PBS Background = 1516 |  |  |  |
| Expt 2 PBS Background = 1600 |  |  |  |

|  |  |  |  |
| --- | --- | --- | --- |
| Figure 1 | Data with Background Subtracted |  |  |
|  |  |  |  |  |
| **Buffer Type** | **Expt Replicate - Sample Replicate** | **Fluorescence Count by Ricin Concentration (ng/mL)** |
| **1000** | **100** | **10** | **1** |
| **Phosphate Buffered Saline (PBS)**  | **1 - 1**  | 1.188E+06 | 1.355E+05 | 1.436E+04 | NA |
| **1 - 2** | 1.132E+06 | 1.367E+05 | 1.321E+04 | NA |
| **1 - 3** | 1.038E+06 | 1.357E+05 | 1.294E+04 | NA |
| **Avg** | 1.119E+06 | 1.360E+05 | 1.350E+04 | NA |
| **SD** | 7.602E+04 | 6.469E+02 | 7.535E+02 | NA |
| **2 - 1** | NA | 1.779E+05 | 1.864E+04 | 1.199E+03 |
| **2 - 2** | NA | 1.761E+05 | 1.954E+04 | 1.433E+03 |
| **2 - 3** | NA | 1.727E+05 | 1.827E+04 | 9.680E+02 |
| **Avg** | NA | 1.756E+05 | 1.882E+04 | 1.200E+03 |
| **SD** | NA | 2.618E+03 | 6.529E+02 | 2.325E+02 |

**ng/mL:** Nanograms per milliliter.

**1.E+03 =** 1000, **1.E+04 =** 10000, **1.E+05 =** 100000, **1.E+06** = 1000000

**Avg =** Average

**SD** = Standard Deviation

**Figure 2.**

**Figure 2.** Fluorescence counts vs. ricin holotoxin concentration (ng/well) for dilutions made in different phosphate-buffered saline (PBS) solutions. T = Tween 80 at 0.05% final concentration; BSA = Bovine Serum Albumin, at 3% final concentration. Bars represent the average of triplicate TRF analyses, and error bars are ± one standard deviation. For each TRF analysis, 10 µL of solution were added to 100 µL of assay buffer. Data for 1 and 0.1 ng ricin per well are from two separate experiments with triplicate TRF analyses per data point; whereas, data for 10 and 0.01 ng per well are from a single experiment with triplicate TRF analyses per data point.

**TRF:** Time Resolved Fluorescence. This assay is used for Ricin biotoxin detection/measurement with the Perkin Elmer Victor X4 plate reader/analytical instrument.

**Fluorescence counts:** Fluorescence counts generated from the Time Resolved Fluorescence assay of Ricin biotoxin and measured by the Perkin Elmer Victor X4 plate reader/analytical instrument.

**Avg. Fluorescence counts:** Average of fluorescence counts from triplicate TRF analyses.

**ng/well:** Nanograms per well on a plate used to run the assay.

**Ricin Concentration:** 0.01, 0.10, 1.00, and 10.00 nanograms per well concentrations of Ricin were analyzed in triplicate TRF assays.

**1.E+03 =** 1000, **1.E+04 =** 10000, **1. E+05 =** 100000, **1.E+06 =** 1000000

**Holotoxin:** Complete toxin and not a part or a subunit/chain.

**PBS:** Phosphate-buffered saline. It is a common buffer for Ricin dilution, sample collection, and as a buffer, in general.

**PBST:** Phosphate-buffered saline containing Tween 80.

**PBS/BSA:** Phosphate-buffered saline containing Bovine Serum Albumin.

**PBST/BSA:** Phosphate-buffered saline containing Tween 80 and Bovine Serum Albumin.



**Figure 2 Fluorescence Counts Average and SD**



**ng/mL:** Nanograms per milliliter.

**1.E+03 =** 1000, **1.E+04 =** 10000, **1.E+05 =** 100000, **1.E+06** = 1000000

**Avg =** Average

**SD** = Standard Deviation

**TRF:** Time Resolved Fluorescence. This assay is used for Ricin biotoxin detection/measurement with the Perkin Elmer Victor X4 plate reader/analytical instrument.

**Treatment:** Bleach residue and Water residue (control for bleach residue treatment) were tested in the TRF assay to see their effect on the assay in the absence of Ricin. This helps to see whether bleach residue in post-decontamination samples would interfere with the TRF assay by generating high background fluorescence counts leading to false results.

**Bleach Residue:** Bleach is a common Ricin decontamination agent/chemical. Its residues were collected following the decontamination procedure and tested for their effect on the Ricin analysis using the TRF assay.

**Water Residue:** It is a treatment negative control and should not give high background fluorescence. Its fluorescence counts are compared with those of the bleach treatment. If there is no statistically significant difference between the counts, it indicates that bleach residue does not interfere with the TRF assay.

**PBS (Control):** PBS is the TRF assay negative control. Its fluorescence counts are considered as the assay background and are compared with those of the bleach treatment. If there is no statistically significant difference between the counts, it indicates that bleach residue does not interfere with the TRF assay. No statistically significant difference in the fluorescence counts for the water residue and the PBS control should be observed.

**Experiment/Sample Replicate:** Replicate experiments were performed and each experiment included three sample replicates. e.g. “1-3” represents Experiment 1 and sample replicate number 3. Also, for each sample replicate, the TRF assay was performed in triplicate.

**Swab and Sponge-sticks:** Both are used to sample Ricin from surfaces contaminated with the biotoxin.

**Neutralizing Buffer:** This buffer is used for post-decontamination sample collection and preservation before the sample is analyzed.

**Fluorescence counts:** Fluorescence counts generated from the Time Resolved Fluorescence assay of Ricin biotoxin and measured by the Perkin Elmer Victor X4 plate reader/analytical instrument.

**Avg. Fluorescence counts:** Average of fluorescence counts from triplicate TRF analyses.

**TRF:** Time Resolved Fluorescence. This assay is used for Ricin biotoxin detection/measurement with the Perkin Elmer Victor X4 plate reader/analytical instrument.

**0.5 mL 10 kDa UF Device:** An Ultrafiltration Device that has a 10000 Dalton nominal molecular weight cut-off value (i.e. it will retain from a liquid sample or a solution any chemical/biochemical with a molecular weight equal to or higher than 10000) and it can accept no more than a volume of 0.5 milliliter. These devices are used to remove any aqueous phase-soluble assay interferences present in an environmental sample and also to concentrate the Ricin in the sample which can help improve the assay sensitivity of detection. The concentration of ricin is mainly due to the volume reduction after ultrafiltration (UF) from the original volume of 1.0 mL, reduced to 0.1 mL.

**Treatment:** Ricin concentration used with and without ultrafiltration (UF).

**10 ng/mL Ricin –UF:** 10 ng/mL Ricin was used and ultrafiltration with 10 kDa UF devicewas performed before TRF assay. Ultrafiltration concentrates the Ricin.

**100 ng/mL Ricin – No UF:** 100 ng/mL Ricin was used and no ultrafiltration was performed before TRF assay. The fluorescence counts of this treatment are compared with those of the 10 ng/mL Ricin – 10K UF treatment.

**PBS (Control):** PBS is the TRF assay negative control. Its fluorescence counts are considered as the assay background and are compared with those of the other two treatments.

**Experiment/Sample Replicate:** Replicate experiments were performed and each experiment included three sample replicates. e.g. “1-3” represents Experiment 1 and sample replicate number 3. Also, for each sample replicate, the TRF assay was performed in triplicate.

**ng/mL:** Nanograms per milliliter.

**Fluorescence counts:** Fluorescence counts generated from the Time Resolved Fluorescence assay of Ricin biotoxin and measured by the Perkin Elmer Victor X4 plate reader/analytical instrument.

**Avg. Fluorescence counts:** Average of fluorescence counts from triplicate TRF analyses.

**Avg. Fold Difference from No UF Treatment:** Upon the TRF analysis, the average fluorescence counts of the UF treatment samples are compared with those of the No UF treatment samples and the fold difference in the fluorescence counts was calculated and expressed.

**Concentration Factor:** A concentration factor based on the average fluorescence counts was derived for Ricin after UF treatment. Since Ricin gets concentrated by the UF treatment due to the volume reduction (from original volume of 1.0 mL to 0.1 mL), 10 ng/mL was used for the UF treatment while 100 ng/mL was used as a control (No UF), i.e. 10-fold less Ricin underwent UF treatment. Upon the TRF analysis, the concentration factor was derived by comparing the average fluorescence counts of the UF treatment of 10 ng/mL Ricin with those of the No UF Treatment – 100 ng/mL Ricin.

**TRF:** Time Resolved Fluorescence. This assay is used for Ricin biotoxin detection/measurement with the Perkin Elmer Victor X4 plate reader/analytical instrument.

**2.0 mL 10 kDa UF Device:** An Ultrafiltration Device that has a 10000 Dalton nominal molecular weight cut-off value (i.e. it will retain from a liquid sample or a solution any chemical/biochemical with a molecular weight equal to or higher than 10000) and it can accept no more than a volume of 2.0 milliliters. These devices are used to remove any aqueous phase-soluble assay interferences present in an environmental sample and also to concentrate the Ricin in the sample which can help improve the assay sensitivity of detection. The concentration of ricin is mainly due to the volume reduction after ultrafiltration (UF) from the original volume of 1.0 mL, reduced to 0.1 mL.

**ATD:** Arizona Test Dust. It is a commonly used reference dust to study the inhibition or interference of analytical methods.

**Treatment:** SS = Sponge-Stick. This is a commonly used sampling tool for Ricin contaminated surfaces. The Sponge-Stick comes pre-wet with Neutralizing Buffer. The SS Expressed solution is the buffer collected by squeezing the Sponge-stick.

**SS Expressed Solution:** No ATD was added to this expressed solution.

**SS Expressed Solution with 250 mg ATD:** Sponge-Stick expressed solution with 250 mg ATD added.

**No UF:** The samples did not go through ultrafiltration.

**UF:** Samples containing Ricin went through ultrafiltration. The 10 kDa UF Device with 2 mL capacity was used.

**Fluorescence counts:** Fluorescence counts generated from the Time Resolved Fluorescence assay of Ricin biotoxin and measured by the Perkin Elmer Victor X4 plate reader/analytical instrument.

**Avg. Fluorescence counts:** Average of fluorescence counts from triplicates TRF analyses.

**Concentration Factor:** A concentration factor based on the average fluorescence counts was derived for Ricin after UF treatment. Since Ricin gets concentrated by the UF treatment due to the volume reduction (from original volume of 1.0 mL to 0.1 mL), 10 ng/mL was used for the UF treatment while 100 ng/mL was used as a control (No UF), i.e. 10-fold less Ricin underwent UF treatment. Upon the TRF analysis, the concentration factor was derived by comparing the average fluorescence counts of the UF treatment of 10 ng/mL Ricin with those of the No UF Treatment – 100 ng/mL Ricin.

**Neutralizing Buffer:** The Sponge-sticks are supplied pre-wet with this buffer by the manufacturer.

**mg:** Milligrams.