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EVALUATION OF COMMERCIALLY AVAILABLE CYANIDE TEST KITS AGAINST VARIOUS MATRICES

Darren W. Hicklin
Wilfred M. Willingham

DIRECTORATE OF PROGRAM INTEGRATION

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14. ABSTRACT:
Five commercially available cyanide-detection test kits or strips were selected for evaluation based upon a premarket survey: Quantofix test strips, Cyantesmo test paper, and Visocolor ECO test kits (Macherey-Nagel; Düren, Germany); eXact test strips (Industrial Test Systems; Rock Hill, SC); and MQuant test strips (EMD Millipore; Billerica, MA). Kits were tested against spiked matrices of flour, powdered sugar, and talcum powder with potassium cyanide at concentrations of 0 to 1% weight-to-weight ratio. Cyantesmo paper and Visocolor ECO test kits yielded the most consistent results across all concentration ranges, even for sample sizes as low as 5 mg. Based upon these results and the mechanics of performing the analyses, Cyantesmo paper was selected for further evaluation in a second phase of testing. Cyantesmo paper was tested against 15 matrices, including baking soda, boric acid, brewer's yeast, chalk dust, chitin, coffee powder (instant coffee), cornstarch, drywall dust, flour, kaolin, γ-aminobutyric acid, L-glutamic acid, powdered coffee creamer, powdered milk, and talcum powder spiked with potassium cyanide at concentrations of 0 to 1% weight-to-weight ratio. In the final results, Cyantesmo paper successfully detected the presence of 0.1% cyanide in 50 mg sample sizes; however, at the 5 mg sample size, the test was unreliable.

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PREFACE

The work described in this report was authorized under contract number DHS S&E MIPR HSSS01-16-X-0022. The work was started in March 2015 and completed in September 2015.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

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1. INTRODUCTION

1.1 Background

Commercially available test kits were evaluated in a two-phase process. In Phase I, a market survey was performed, and five test kits were selected on the basis of the manufacturer's claims for lowest detectable cyanide concentration, the sample mass required to perform the analysis, and the amount of time required to complete the analysis. Commercially available cyanide test kits and strips were evaluated against dry mixtures of potassium cyanide in three matrices at a 1% weight-to-weight ratio (w/w) and at 0.1% w/w. Those matrices were flour, powdered sugar, and talcum powder. Additionally, the test strips and kits were tested against a known cyanide standard solution. The selected strips or kits were as follows: Quantofix cyanide test strips, Cyantesmo paper, and Visocolor ECO cyanide test kit (all three from Macherey-Nagel; Düren, Germany); eXact cyanide test strips (Industrial Test Systems; Rock Hill, SC); and MQuant cyanide test strips (EMD Millipore; Billerica, MA). Another test kit, the MColortest system (EMD Millipore), was planned for but not included in testing because it was backordered and unavailable.

Based upon Phase I results, Phase II consisted of further evaluation of Cyantesmo paper against dry mixtures of potassium cyanide in 15 matrices at 1 and 0.1% w/w. Those matrices were baking soda, boric acid, brewer's yeast, chalk dust, chitin, coffee powder (instant coffee), cornstarch, drywall dust, flour, kaolin, γ-aminobutyric acid, L-glutamic acid, powdered coffee creamer, powdered milk, and talcum powder.

1.2 Materials

Following is a list of materials used for the evaluation of cyanide test kits. Manufacturer or distributor information and lot or batch numbers have been included where applicable.

- Quantofix test strips (Lot N25B050);
- MQuant cyanide test (Lot HC411503);
- Cyantesmo paper (Batches 6044414 and 6041415);
- Visocolor ECO cyanide test (Lot 1137);
- eXact cyanide test strips (Lots 020514 and 020414I);
- Potassium cyanide, technical grade (Lot 138178A; Fisher Chemical; Waltham, MA);
- Certipur 1000 mg/L cyanide standard solution (Lot HC42125833; EMD Millipore);
- Optima sulfuric acid (Lot 3203040; Fisher Chemical);
- MColorpHast pH strips (Lot HC431579; EMD Millipore);

- Smart & Simple enriched wheat flour (Lot 01A 08:26; Dollar General [DG]; Goodlettsville, TN);
- DG powdered sugar;
- DG talcum powder (Lot 14312THW);
- DG Clover Valley baking soda (Lot PMHB51);
- Boric acid (Lot 766965; Fisher Scientific; Fair Lawn, NJ);
- Brewer's yeast (Lot SLBK4518V; Sigma Aldrich; St. Louis, MO);
- DG chalk dust;
- Chitin from shrimp shells (SLBL2694V; Sigma-Aldrich);
- Folgers instant coffee (J.M. Smucker Company; Orrville, OH);
- DG Clover Valley cornstarch (Lot PMHCS2);
- Drywall dust; (Lowes; Mooresville, NC);
- Kaolin (Lot MKB51103V; Sigma-Aldrich);
- γ-Aminobutyric acid (Lot BCBM1065V; Sigma-Aldrich);
- L-glutamic acid (Lot SLBM4966V; Sigma-Aldrich);
- DG Clover Valley coffee creamer (Lot 09603235 9864 2048); and
- Powdered milk (Nestle; Vevey, Switzerland).

2. PHASE I TESTING

Phase I testing consisted of selecting cyanide test kits for evaluation based upon client requirements. Those requirements were (1) sensitivity at or below the Quantofix cyanide test strip detection limits, (2) sample mass of less than 500 mg or sample volume of less than 0.5 mL, and (3) analysis time of less than the standard 8 h work day. A client survey resulted in five qualifying test kits. Quantofix cyanide test strips, which have a 1 mg/mL limit of detection, were evaluated as a control. Based upon the detection limit of the Quantofix cyanide test strips, the following kits were selected: Quantofix cyanide test strips, Cyantesmo paper, eXact cyanide test strips, MQuant cyanide test strips, and Visocolor ECO cyanide test kit.

2.1 Quantofix Cyanide Test Strips: Procedures and Results

Quantofix cyanide test strips were tested using enriched wheat flour, powdered sugar, and talcum powder with potassium cyanide at 0, 0.1, and 1.0% w/w, respectively. Each matrix was evaluated in triplicate at three different masses: 500 mg (Table 1), 50 mg (Table 2), and 5 mg (Table 3).

The spiked or unspiked samples were weighed in culture tubes, and 5 mL of deionized (DI) water was added to each tube. The pH was measured to confirm the samples were below pH 10, in accordance with manufacturer's specifications. One spoonful (using the spoon included in the test kit) of cyanide-1 powder (proprietary phosphate buffers) was added to each tube along with 5 drops of cyanide-2 (1–5% chloramine T and 5–44% pyridine). The samples were capped and agitated until all reagents were dissolved. After agitation, test strips were submerged in the solutions for 45 s and then removed. The test strips were visually compared with the Quantofix reference chart, and approximate concentrations were recorded. The

Quantofix reference chart range was from 0 to 30 mg/L. Any results observed to be outside the chart range were reported with a < or > symbol. Visual color changes observed to be between concentration ranges were reported with a ~ symbol based upon the closest color match.

For the 500 mg samples, all negative controls showed no false-positive results. Spiked samples, once extracted in DI water, yielded a theoretical cyanide solution concentration of either 100 or 1000 mg/L. Therefore, all spiked samples yielded positive results that were above the highest range of the Quantofix test.

Table 1. Quantofix Cyanide Test Strips: 500 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.5000	0	5.00	0	0	0	0	0
	0.5000	1	5.00	100	>30	>30	>30	>30
	0.5000	10	5.00	1000	>30	>30	>30	>30
Powdered sugar	0.5000	0	5.00	0	0	0	0	0
	0.5000	1	5.00	100	>30	>30	>30	>30
	0.5000	10	5.00	1000	>30	>30	>30	>30
Talcum powder	0.5000	0	5.00	0	0	0	0	0
	0.5000	1	5.00	100	>30	>30	>30	>30
	0.5000	10	5.00	1000	>30	>30	>30	>30

For the 50 mg samples, all negative controls showed no false-positive results. The flour and talcum powder matrices showed results consistent with the expected final concentration. However, the powdered sugar matrix produced results that were lower than anticipated.

Table 2. Quantofix Cyanide Test Strips: 50 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0500	0	5.00	0	0	0	0	0
	0.0500	1	5.00	10	~10	~10	~10	~10
	0.0500	10	5.00	100	>30	>30	>30	>30
Powdered sugar	0.0500	0	5.00	0	0	0	0	0
	0.0500	1	5.00	10	~3	~10	~3	~8.3
	0.0500	10	5.00	100	>30	>30	>30	>30
Talcum powder	0.0500	0	5.00	0	0	0	0	0
	0.0500	1	5.00	10	~10	~10	~10	~10
	0.0500	10	5.00	100	>30	>30	>30	>30

Note: Yellow highlighting indicates results that were inconsistent with theoretical calculations.

For the 5 mg samples, all negative controls showed no response or interference. Additionally, the spiked matrices were negative for cyanide at all concentration ranges.

Table 3. Quantofix Cyanide Test Strips: 5 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0050	0	5.00	0	0	0	0	0
	0.0050	1	5.00	1	0	0	0	0
	0.0050	10	5.00	10	0	0	0	0
Powdered sugar	0.0050	0	5.00	0	0	0	0	0
	0.0050	1	5.00	1	0	0	0	0
	0.0050	10	5.00	10	0	0	0	0
Talcum powder	0.0050	0	5.00	0	0	0	0	0
	0.0050	1	5.00	1	0	0	0	0
	0.0050	10	5.00	10	0	0	0	0

Note: Yellow highlighting indicates results that were inconsistent with theoretical calculations.

In addition to being used to test different spiked matrices, the Quantofix kit was evaluated against a known cyanide standard concentration to determine the lowest detectable concentration in water. A 100 mg/L cyanide standard was prepared and then spiked into DI water to yield a final cyanide concentration solution at the lower detection limit of the test (Table 4).

Quantofix samples were prepared by spiking 50 µL of a 100 mg/L cyanide standard into DI water and then adding DI water to a final volume of 5 mL to yield a water sample at 1.0 mg/L cyanide. The final solution concentrations were increased until the test yielded a positive result. Although the Quantofix color comparison chart has a 1 mg/L reference window, no color changes were observed until the final cyanide concentration in water was 4 mg/L. The 1 mg/L level was tested in triplicate, twice, by two different analysts, to confirm that the results were consistent with no recordable change.

Table 4. Quantofix Cyanide Test Strips: Spiked Test

Matrix	100 mg/L CN Spike Volume (µL)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result 4 (mg/L)	Result 5 (mg/L)	Result 6 (mg/L)	Result Average (mg/L)
DI H_2O	50	4.95	1	0	0	0	0	0	0	0
	100	4.90	2	0	0	0	NA	NA	NA	0
	200	4.80	4	~3	~3	~3	NA	NA	NA	~3

Note: Yellow highlighting indicates results that were inconsistent with theoretical calculations.

Finally, the kit was evaluated with 50 mg of a 0.1% w/w spiked matrix but using one-half and one-fourth of the required amount of DI water (Table 5). This effectively increased the concentration of the sample by 2- or 4-fold. Quantofix samples were prepared in the same manner that was used for the previous 50 mg 0.1% spiked matrix tests, with the exception that either 2.5 or 1.25 mL of DI water was used. The sample results were inconsistent when performed outside the kit manufacturer's recommendations.

Table 5. Quantofix Cyanide Test Strips: Reduced-Volume Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0500	1	2.50	20	~10	~10	~10	~10
	0.0500	1	1.25	40	>30	>30	>30	>30
Powdered sugar	0.0500	1	2.50	20	0	0	0	0
	0.0500	1	1.25	40	>30	>30	>30	>30
Talcum powder	0.0500	1	2.50	20	0	0	0	0
	0.0500	1	1.25	40	0	0	0	0

Note: Yellow highlighting indicates results that were inconsistent with theoretical calculations.

2.2 Cyantesmo Paper: Procedures and Results

Cyantesmo paper was tested using enriched wheat flour, powdered sugar, and talcum powder with potassium cyanide at 0, 0.1, and 1.0% w/w, respectively. Each matrix was evaluated in triplicate at three different masses: 500 mg (Table 6), 50 mg (Table 7), and 5 mg (Table 8).

The spiked or unspiked samples were weighed in culture tubes, and 10 mL of DI water was added to each tube. One drop of concentrated sulfuric acid was added to each sample. Tubes were capped and agitated, and the pH was checked to confirm that the samples were weakly acidified (pH 2–7). A strip of Cyantesmo paper approximately 75 mm long was submerged approximately 10 mm into the test solution, and the remaining portion of the paper was positioned above the solution, taking care to ensure that a portion of the paper remained in the gas zone immediately above the solution. After 15 min, samples were observed for a blue color change in the portion of the paper immediately above the solution. Because the Cyantesmo paper is a semiquantitative test method that reacts only when cyanide is present at concentrations of 0.2 mg/L or greater, any color change was reported as ≥0.2 mg/L, and no color change was reported as <0.2 mg/L.

For the 500 mg samples, all negative-control test matrices showed no false-positive results. All spiked samples resulted in positive test results.

Table 6. Cyantesmo Paper: 500 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.5000	0	10.00	0	<0.2	<0.2	<0.2	<0.2
	0.5000	1	10.00	50	≥0.2	≥0.2	≥0.2	≥0.2
	0.5000	10	10.00	500	≥0.2	≥0.2	≥0.2	≥0.2
Powdered sugar	0.5000	0	10.00	0	<0.2	<0.2	<0.2	<0.2
	0.5000	1	10.00	50	≥0.2	≥0.2	≥0.2	≥0.2
	0.5000	10	10.00	500	≥0.2	≥0.2	≥0.2	≥0.2
Talcum powder	0.5000	0	10.00	0	<0.2	<0.2	<0.2	<0.2
	0.5000	1	10.00	50	≥0.2	≥0.2	≥0.2	≥0.2
	0.5000	10	10.00	500	≥0.2	≥0.2	≥0.2	≥0.2

For the 50 mg samples, all negative-control test matrices showed no false-positive results. All spiked samples resulted in positive test results.

Table 7. Cyantesmo Paper: 50 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0500	0	10.00	0	<0.2	<0.2	<0.2	<0.2
	0.0500	1	10.00	5	≥0.2	≥0.2	≥0.2	≥0.2
	0.0500	10	10.00	50	≥0.2	≥0.2	≥0.2	≥0.2
Powdered sugar	0.0500	0	10.00	0	<0.2	<0.2	<0.2	<0.2
	0.0500	1	10.00	5	≥0.2	≥0.2	≥0.2	≥0.2
	0.0500	10	10.00	50	≥0.2	≥0.2	≥0.2	≥0.2
Talcum powder	0.0500	0	10.00	0	<0.2	<0.2	<0.2	<0.2
	0.0500	1	10.00	5	≥0.2	≥0.2	≥0.2	≥0.2
	0.0500	10	10.00	50	≥0.2	≥0.2	≥0.2	≥0.2

For the 5 mg samples, all negative-control test matrices showed no false-positive results.

Table 8. Cyantesmo Paper: 5 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0050	0	10.00	0	<0.2	<0.2	<0.2	<0.2
	0.0050	1	10.00	0.5	≥0.2	≥0.2	≥0.2	≥0.2
	0.0050	10	10.00	5	≥0.2	≥0.2	≥0.2	≥0.2
Powdered sugar	0.0050	0	10.00	0	<0.2	<0.2	<0.2	<0.2
	0.0050	1	10.00	0.5	≥0.2	≥0.2	≥0.2	≥0.2
	0.0050	10	10.00	5	≥0.2	≥0.2	≥0.2	≥0.2
Talcum powder	0.0050	0	10.00	0	<0.2	<0.2	<0.2	<0.2
	0.0050	1	10.00	0.5	≥0.2	≥0.2	≥0.2	≥0.2
	0.0050	10	10.00	5	≥0.2	≥0.2	≥0.2	≥0.2

In addition to being used to test different spiked matrices, Cyantesmo paper was evaluated against a known cyanide standard concentration to determine the lowest detectable concentration (Table 9). A 100 mg/L cyanide standard was prepared and then spiked into DI water to yield a final cyanide concentration solution at the lower limit of the test.

Cyantesmo samples were prepared by spiking 20 µL of a 100 mg/L cyanide standard into DI water and then adding DI water to produce a final volume of 10 mL; this yielded a water sample with a 0.2 mg/L cyanide concentration. After 15 min, no color changes were observed, and higher final-concentration cyanide samples were prepared. However, after 30 min, both the original samples and the higher-concentration samples showed as positive. The results were recorded as positive in accordance with manufacturer's instructions, which indicate that the test be allowed to run up to overnight. Solutions prepared at 0.1 mg/L yielded no positive results. All color changes were reported as ≥0.2 mg/L, and no color change was reported as <0.2 mg/L.

Table 9. Cyantesmo Paper: Spiked Test

Matrix	100 mg/L CN Spike Volume (µL)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
DI H_2O	30	9.97	0.3	≥0.2*	≥0.2*	≥0.2*	≥0.2
	20	9.98	0.2	≥0.2*	≥0.2*	≥0.2*	≥0.2
	10	9.99	0.1	<0.2†	<0.2†	<0.2†	<0.2†

*Test paper was negative after 15 min but indicated positive result after 30 min.
†Results were negative after 90 min.

Finally, the kit was evaluated with 50 mg of 0.1% w/w spiked matrix but using one-half and one-fourth of the required amount of DI water (Table 10). This effectively increased the concentration of the sample by 2- or 4-fold. Cyantesmo samples were prepared in

the same manner that was used for the previous spiked matrix tests, with the exception that either 5.0 or 2.5 mL of water was used. All test samples yielded positive results regardless of sample matrix, percent cyanide, or amount of water used.

Table 10. Cyantesmo Paper: Reduced-Volume Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0050	1	5.00	1	≥0.2	≥0.2	≥0.2	≥0.2
	0.0050	1	2.50	2	≥0.2	≥0.2	≥0.2	≥0.2
Powdered sugar	0.0050	1	5.00	1	≥0.2	≥0.2	≥0.2	≥0.2
	0.0050	1	2.50	2	≥0.2	≥0.2	≥0.2	≥0.2
Talcum powder	0.0050	1	5.00	1	≥0.2	≥0.2	≥0.2	≥0.2
	0.0050	1	2.50	2	≥0.2	≥0.2	≥0.2	≥0.2

2.3 eXact Cyanide Test Strips: Procedures and Results

The eXact cyanide test strips were tested using enriched wheat flour, powdered sugar, and talcum powder with potassium cyanide at 0, 0.1, and 1.0% w/w, respectively. Each matrix was evaluated in triplicate at three different masses: 500 mg (Table 11), 50 mg (Table 12), and 5 mg (Table 13).

The spiked or unspiked samples were weighed in culture tubes, and 2 mL of DI water was added to each tube. After samples were agitated and separated in a centrifuge, the supernatant of each sample was retained and transferred to a 2 mL cuvette that was provided with the test kit. The pH of each sample was checked to verify that samples were between pH 5 and 11. Cyanide ReagentStrip no. 1 was inserted and removed from the cuvette at a rate of approximately once per second for 30 s. Afterward, Cyanide ReagentStrip no. 2 was inserted and removed from the cuvette at the same frequency rate. Cyanide ReagentStrip no. 2 was shaken off to remove any excess water and was compared to the ReagentStrip color chart within 2 min. Given the ambient temperature, the water samples in the cuvette were allowed to react for 10 min and were compared to the microcuvette color chart within 4 min of the allowed reaction time. On the ReagentStrip color chart, concentration ranged from 0.1 to 10 mg/L, and on the microcuvette color chart, concentration ranged from 0 to 200 mg/L. All results were reported based upon the closest color match on either chart. Any results observed to be out of the range were reported with a > symbol, and any results observed to be between color match standards were reported with the ranges of the closest matches.

For the 500 mg samples, all blanks were negative, and all spiked samples provided positive results that were outside the highest range of the test kit.

Table 11. eXact Cyanide Test Strips: 500 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.5000	0	2.00	0	0	0	0	0
	0.5000	1	2.00	250	>200	>200	>200	>200
	0.5000	10	2.00	2500	>200	>200	>200	>200
Powdered sugar	0.5000	0	2.00	0	0	0	0	0
	0.5000	1	2.00	250	>200	>200	>200	>200
	0.5000	10	2.00	2500	>200	>200	>200	>200
Talcum powder	0.5000	0	2.00	0	0	0	0	0
	0.5000	1	2.00	250	>200	>200	>200	>200
	0.5000	10	2.00	2500	>200	>200	>200	>200

For the 50 mg tests, all negative controls showed no false-positive results. Results were positive for all of the spiked samples, but values were consistently lower than expected.

Table 12. eXact Cyanide Test Strips: 50 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0500	0	2.00	0	0	0	0	0
	0.0500	1	2.00	25	>3	>3	>3	>3
	0.0500	10	2.00	250	>20	>20	>20	>20
Powdered sugar	0.0500	0	2.00	0	0	0	0	0
	0.0500	1	2.00	25	0.5–3	0.5–3	>20	NA
	0.0500	10	2.00	250	>20	>20	>20	20
Talcum powder	0.0500	0	2.00	0	0	0	0	0
	0.0500	1	2.00	25	>3	>3	>3	>3
	0.0500	10	2.00	250	>20	>20	>20	>20

Note: Yellow highlighting indicates results that were inconsistent with theoretical calculations.
NA, not applicable.

The negative controls for the 5 mg test showed no interferences. Results for the spiked samples were consistent with those anticipated, except that powdered sugar spiked with 0.1% w/w potassium cyanide.

Table 13. eXact Cyanide Test Strips: 5 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0050	0	2.00	0	0	0	0	0
	0.0050	1	2.00	2.5	3	>10	3	~5.3
	0.0050	10	2.00	25	>20	>20	>20	>20
Powdered sugar	0.0050	0	2.00	0	0	0	0	0
	0.0050	1	2.00	2.5	1	0.2	3	1.4
	0.0050	10	2.00	25	>20	>20	>10	NA
Talcum powder	0.0050	0	2.00	0	0	0	0	0
	0.0050	1	2.00	2.5	3	2	3	2.67
	0.0050	10	2.00	25	>20	>20	>20	>20

Note: Yellow highlighting indicates results that were inconsistent with theoretical calculations.
NA, not applicable.

Additionally, the eXact kit was evaluated against a known cyanide standard concentration to determine the lowest detectable concentration (Table 14). A 100 mg/L cyanide standard was prepared and then spiked into DI water to yield a final cyanide concentration solution at the lower limits of the test.

For eXact testing, samples were prepared by spiking 1.0 µL of 100 mg/L cyanide standard into DI water and then adding DI water to produce a final volume of 2 mL and yield a water sample at 0.05 mg/L cyanide. All samples produced a color change that was darker than expected; therefore, subsequent samples were prepared and tested until no color change was observed. On the ReagentStrip color chart, concentration ranged from 0.1 to 10 mg/L, and on the microcuvette color chart, concentration ranged was from 0 to 200 mg/L. All results were reported based upon the closest color match on either chart. Any results observed to be out of the range were reported with a > symbol, and any results between color match standards were reported with the ranges of the closest matches.

All samples yielded some color change until the cyanide in solution was decreased below 0.0125 mg/L.

Table 14. eXact Cyanide Test Strips: Spiked Test

Matrix	100 mg/L CN Spike Volume (µL)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
DI H_2O	1	2.00	0.05	0.05–0.01	0.05–0.01	0.05–0.01	0.05–0.01
	0.5	2.00	0.025	<0.05, >0	<0.05, >0	<0.05, >0	<0.05, >0
	0.25	2.00	0.0125	<0.05, >0	<0.05, >0	<0.05, >0	<0.05, >0
	0.125	2.00	0.00625	0	0	0	0

Due to the mechanics of the eXact kit test, water volumes of less than 2 mL were insufficient for the kit to work. Therefore, the eXact kit was not tested using less than 2 mL of water.

2.4 MQuant Cyanide Test Strips: Procedures and Results

The MQuant cyanide test strips were tested using enriched wheat flour, powdered sugar, and talcum powder with potassium cyanide at 0, 0.1, and 1.0% w/w, respectively. Each matrix was evaluated in triplicate at three different masses: 500 mg (Table 15), 50 mg (Table 16), and 5 mg (Table 17).

The spiked or unspiked samples were weighed in culture tubes, and 5 mL of DI water was added to each tube. The pH was measured to confirm the samples were between pH 6 and 7, in accordance with manufacturer's specifications. One level spoonful of cyanide-1 powder (using the spoon included in the test kit) was added to each tube, and the tubes were agitated to dissolve the reagent. Subsequently, 5 drops of cyanide-2 was added to each tube, and the tubes were again agitated to dissolve the reagent. After agitation, test strips were submerged in the solutions for 30 s and then removed. The test strips were visually compared with the reference chart within 10 s, and approximate concentrations were recorded. The MQuant reference chart range was from 0 to 30 mg/L. Any results observed to be outside the range were reported with a < or > symbol. Visual color changes observed to be between concentration ranges were reported with a ~ symbol based upon the closest color match.

For the 500 mg samples, all negative controls were free false positives, and all spiked samples resulted in detections that were above the highest range of the test.

Table 15. MQuant Cyanide Test Strips: 500 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.5000	0	5.00	0	0	0	0	0
	0.5000	1	5.00	100	>30	>30	>30	>30
	0.5000	10	5.00	1000	>30	>30	>30	>30
Powdered sugar	0.5000	0	5.00	0	0	0	0	0
	0.5000	1	5.00	100	>30	>30	>30	>30
	0.5000	10	5.00	1000	>30	>30	>30	>30
Talcum powder	0.5000	0	5.00	0	0	0	0	0
	0.5000	1	5.00	100	>30	>30	>30	>30
	0.5000	10	5.00	1000	>30	>30	>30	>30

For the 50 mg samples, all negative controls were free of interferences. All spiked samples resulted in positive detections, but at 10-fold or more below the expected results.

Table 16. MQuant Cyanide Test Strips: 50 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0500	0	5.00	0	0	0	0	0
	0.0500	1	5.00	10	~1	~1	~1	~1
	0.0500	10	5.00	100	~10	~10	~10	~10
Powdered sugar	0.0500	0	5.00	0	0	0	0	0
	0.0500	1	5.00	10	0	0	0	0
	0.0500	10	5.00	100	~3	~3	~3	~3
Talcum powder	0.0500	0	5.00	0	0	0	0	0
	0.0500	1	5.00	10	~1	~1	~1	~1
	0.0500	10	5.00	100	~10	~10	~10	~10

Note: Yellow highlighting indicates results that were inconsistent with theoretical calculations.

For the 5 mg samples, all negative controls were free of interferences. All spiked samples resulted in no positive detections.

Table 17. MQuant Cyanide Test Strips: 5 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0050	0	5.00	0	0	0	0	0
	0.0050	1	5.00	1	0	0	0	0
	0.0050	10	5.00	10	0	0	0	0
Powdered sugar	0.0050	0	5.00	0	0	0	0	0
	0.0050	1	5.00	1	0	0	0	0
	0.0050	10	5.00	10	0	0	0	0
Talcum powder	0.0050	0	5.00	0	0	0	0	0
	0.0050	1	5.00	1	0	0	0	0
	0.0050	10	5.00	10	0	0	0	0

Note: Yellow highlighting indicates results that were inconsistent with theoretical calculations.

Additionally, MQuant was evaluated against a known cyanide standard concentration to determine the lowest detectable concentration (Table 18). MQuant samples were prepared by spiking 50 µL of 100 mg/L cyanide standard into DI water and then adding DI water to a final volume of 5 mL to yield a water sample at 1 mg/L cyanide. Results for the test were positive but below the low test concentration of 1 mg/L. Subsequent samples were prepared at lower cyanide concentrations until no color change was observed.

Table 18. MQuant Cyanide Test Strips: Spiked Test

Matrix	100 mg/L CN Spike Volume (µL)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
DI H_2O	50	4.95	1	<1, >0	<1, >0	<1, >0	<1, >0
	25	4.98	0.5	0	0	0	0

Finally, the MQuant test kit was evaluated with 50 mg of a 0.1% w/w spiked matrix but using one-half and one-fourth of the required amount of DI water (Table 19). This effectively increased the concentration of the sample 2- or 4-fold. MQuant samples were prepared in the same manner that was used for the previous spiked matrix tests, with the exception that either 2.5 or 1.25 mL of water was used. None of the samples yielded a positive result.

Table 19. MQuant Cyanide Test Strips: Reduced-Volume Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0500	1	2.50	20	0	0	0	0
	0.0500	1	1.25	40	0	0	0	0
Powdered sugar	0.0500	1	2.50	20	0	0	0	0
	0.0500	1	1.25	40	0	0	0	0
Talcum powder	0.0500	1	2.50	20	0	0	0	0
	0.0500	1	1.25	40	0	0	0	0

Note: Yellow highlighting indicates results that were inconsistent with theoretical calculations.

2.5 Visocolor ECO Cyanide Test Kit: Procedures and Results

The Visocolor ECO cyanide test kit was tested using flour, powdered sugar, and talcum powder spiked with potassium cyanide at 0, 0.1, and 1.0% w/w, respectively. Each matrix was evaluated in triplicate at three different masses: 500 mg (Table 20), 50 mg (Table 21), and 5 mg (Table 22).

The spiked or unspiked samples were weighed in culture tubes, and 5 mL of DI water was added to each tube. Five drops of cyanide-1 was added to each tube, and the solution was thoroughly mixed. Afterward, 1 level measuring spoon of cyanide-2 (using the spoon included in the test kit) was added to each vessel and was dissolved by swirling. Then, 5 drops of cyanide-3 was added to each tube, and the tubes were closed and vortexed to dissolve the contents. After agitation and separation in a centrifuge and a 15 min reaction time, the supernatant of each sample was retained and transferred to a 5 mL vial that was provided with the test kit. Five milliliters of DI water was added to a vial. The vial was placed in the "A" position of the comparator, and the reacted sample was placed in the "B" position. The

comparator was moved along the concentration color chart until the colors matched (as observed through the top inspection viewport). The Visocolor ECO reference chart range was from 0 to 0.20 mg/L. Any results observed to be outside the range were reported with a < or > symbol. Visual color changes observed between concentration ranges were reported with a ~ symbol based upon the closest color match.

The 500 mg negative-control samples were free of false positives and yielded positive results that were above the maximum calibration level for all spiked matrices.

Table 20. Visocolor ECO Cyanide Test Kit: 500 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.5000	0	5.00	0	0	0	0	0
	0.5000	1	5.00	100	>0.2	>0.2	>0.2	>0.2
	0.5000	10	5.00	1000	>0.2	>0.2	>0.2	>0.2
Powdered sugar	0.5000	0	5.00	0	0	0	0	0
	0.5000	1	5.00	100	>0.2	>0.2	>0.2	>0.2
	0.5000	10	5.00	1000	>0.2	>0.2	>0.2	>0.2
Talcum powder	0.5000	0	5.00	0	0	0	0	0
	0.5000	1	5.00	100	>0.2	>0.2	>0.2	>0.2
	0.5000	10	5.00	1000	>0.2	>0.2	>0.2	>0.2

The 50 mg negative-control samples were free of interferences and yielded positive results that were above the maximum calibration level for all spiked matrices.

Table 21. Visocolor ECO Cyanide Test Kit: 50 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0500	0	5.00	0	0	0	0	0
	0.0500	1	5.00	10	>0.2	>0.2	>0.2	>0.2
	0.0500	10	5.00	100	>0.2	>0.2	>0.2	>0.2
Powdered sugar	0.0500	0	5.00	0	0	0	0	0
	0.0500	1	5.00	10	>0.2	>0.2	>0.2	>0.2
	0.0500	10	5.00	100	>0.2	>0.2	>0.2	>0.2
Talcum powder	0.0500	0	5.00	0	0	0	0	0
	0.0500	1	5.00	10	>0.2	>0.2	>0.2	>0.2
	0.0500	10	5.00	100	>0.2	>0.2	>0.2	>0.2

The 5 mg negative-control samples were free of interferences and yielded positive results above the maximum calibration level for all spiked matrices, except for one replicate with talcum powder at a 0.1% w/w.

Table 22. Visocolor ECO Cyanide Test Kit: 5 mg Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0050	0	5.00	0	0	0	0	0
	0.0050	1	5.00	1	>0.2	>0.2	>0.2	>0.2
	0.0050	10	5.00	10	>0.2	>0.2	>0.2	>0.2
Powdered sugar	0.0050	0	5.00	0	0	0	0	0
	0.0050	1	5.00	1	>0.2	>0.2	>0.2	>0.2
	0.0050	10	5.00	10	>0.2	>0.2	>0.2	>0.2
Talcum powder	0.0050	0	5.00	0	0	0	0	0
	0.0050	1	5.00	1	>0.2	>0.1	>0.2	>0.17
	0.0050	10	5.00	10	>0.2	>0.2	>0.2	>0.2

Note: Yellow highlighting indicates a result that was inconsistent with theoretical calculations.

In addition to testing different spiked matrices, Visocolor ECO was evaluated against a known cyanide standard concentration to determine the lowest detectable concentration (Table 23). A 100 mg/L cyanide standard was prepared and then spiked into DI water to yield a final cyanide concentration solution at the lower limits of the test.

Visocolor ECO samples were prepared by spiking 5 µL of 100 mg/L cyanide standard into DI water and then adding DI water to a final volume of 5 mL to yield a water sample with 0.01 mg/L of cyanide. Results for the 0.01 mg/L test were positive, and subsequent samples were prepared at lower concentrations until no color change was observed.

Table 23. Visocolor ECO Cyanide Test Kit: Spiked Test

Matrix	100 mg/L CN Spike Volume (µL)	DI H_2O Volume (mL)	CN Solution Conc (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
DI H_2O	0.5	5.000	0.01	0.01	0.01	0.01	0.01
	0.25	5.000	0.005	<0.01, >0	<0.01, >0	<0.01, >0	<0.01, >0
	0.125	5.000	0.0025	0	0	0	0

Finally, the Visocolor ECO kit was evaluated with 50 mg of a 0.1% w/w spiked matrix but using one-half and one-fourth of the required amount of DI water (Table 24). This effectively raised the concentration of the sample by 2- or 4-fold. Visocolor ECO samples were prepared in the same manner that was used for the previous spiked matrix tests, with the

exception that either 2.5 or 1.25 mL of water was used. All samples resulted in positive detections above the maximum calibration level except for one replicate of 50 mg of flour spiked at a 0.1% w/w using one-half the required volume of water.

Table 24. Visocolor ECO Cyanide Test Kit: Reduced-Volume Test

Matrix	Sample Mass (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	CN Solution Conc. (mg/L)	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Flour	0.0050	1	2.50	2.00	0.03	>0.2	>0.2	>0.2
	0.0050	1	1.25	4.00	>0.2	>0.2	>0.2	>0.2
Powdered sugar	0.0050	1	2.50	2.00	>0.2	>0.2	>0.2	>0.2
	0.0050	1	1.25	4.00	>0.2	>0.2	>0.2	>0.2
Talcum powder	0.0050	1	2.50	2.00	>0.2	>0.2	>0.2	>0.2
	0.0050	1	1.25	4.00	>0.2	>0.2	>0.2	>0.2

Note: Yellow highlighting indicates a result that was inconsistent with theoretical calculations.

3. PHASE II TESTING

3.1 Test Kit and Matrix Selection

After Phase I testing was completed, two test kits were recognized as suitable matches for further evaluation. The Cyantesmo paper and Visocolor ECO cyanide test kit proved to be suitable tests for further evaluation because they met the requirements of increased sensitivity, reduced sample mass or volume, and sample analysis time. Cyantesmo paper had an added advantage of being unaffected by highly turbid samples, given the reaction takes place in the gaseous space directly above the sample. For these reasons, Cyantesmo paper was the sole test method selected for further evaluation.

Further evaluation of Cyantesmo paper involved the 15 matrices spiked at 1 and 0.1% w/w with potassium cyanide. Those matrices were baking soda, boric acid, brewer's yeast, chalk dust, chitin, coffee powder (instant coffee), cornstarch, drywall dust, flour, kaolin, γ-aminobutyric acid, L-glutamic acid, powdered coffee creamer, powdered milk, and talcum powder. Additionally, all samples were evaluated without potassium cyanide to check for false positives and test interference.

Quality checks were performed using clean DI water as a method blank. Positive controls of 5, 20, 50, and 200 µg of total cyanide were prepared and tested using a 1000 µg/mL commercial cyanide standard. All Phase II testing was performed in accordance with the U.S. Department of Agriculture's Food Emergency Response Network (FERN) Standard Operating Procedure T005(004).[1]

[1] Flurer, C.L.; Barnes, B.S.; Urban, J.R.; Roberts, J.N.; Toomey, V.M. *Screen for the Presence of Cyanide*; SOP T005(004); Forensic Chemistry Center, U.S. Food and Drug Administration: Cincinnati, OH, 2006; UNCLASSIFIED Procedure.

3.2 Procedures and Results

Each of the powdered matrices was tested at 0, 0.1, and 1% w/w with potassium cyanide. Samples were prepared by weighing out 5 and 50 mg of spiked and unspiked samples in triplicate. Each sample was added to a 10 mL screwcap culture tube and diluted with 5 mL of DI water. The tubes were tightly capped and agitated by vortex until the samples were either dissolved or in a homogenous mixture if not soluble in water. After mixing, the pH was checked and recorded. Two drops of concentrated sulfuric acid was added, and the samples were lightly shaken to disperse the acid. Strips of Cyantesmo paper approximately 45 mm long were submerged in DI water (to wet the paper) and then placed in the culture tube while making sure the paper stayed in the headspace immediately above the liquid. The tubes were tightly capped and allowed to stand for 15 min. After 15 min had passed, the Cyantesmo paper was observed for signs of cyanide, as indicated by the appearance of a blue color in the middle of the paper strip.

For the 5 mg samples (Table 25), all unspiked powdered matrices were free of any test interference that could result in a false positive for cyanide. Spiked matrices yielded inconsistent results even between triplicate samples, and brewer's yeast, L-glutamic acid, and coffee creamer exhibited no positive results among any of the spiked samples.

All 5 mg spiked samples that did not yield positive results were retested using 50 mg of sample (Table 26). The unspiked samples showed no interference. All spiked samples returned positive results with the Cyantesmo paper.

Table 25. Cyantesmo Paper: 5 mg Supplemental Testing Results

Matrix	Sample Mass 1 (g)	Sample Mass 2 (g)	Sample Mass 3 (g)	KCN Conc. (mg/g)	DI H2O Volume (mL)	KCN Solution Conc. 1 (mg/L)	KCN Solution Conc. 2 (mg/L)	KCN Solution Conc. 3 (mg/L)	Measured pH 1	Measured pH 2	Measured pH 3	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Baking soda	0.0055	0.0052	0.0054	0	5.00	0	0	0	8	8	8	<0.2	<0.2	<0.2	<0.2
	0.0049	0.0054	0.0049	1	5.00	0.98	1.08	0.98	8	8	8	≥0.2	<0.2	<0.2	<0.2
	0.0053	0.0056	0.0050	10	5.00	10.6	11.2	10	8	8	8	<0.2	≥0.2	≥0.2	≥0.2
Boric acid	0.0055	0.0051	0.0055	0	5.00	0	0	0	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0055	0.0053	0.0051	1	5.00	1.1	1.06	1.02	5	4	5	<0.2	<0.2	<0.2	<0.2
	0.0051	0.0054	0.0055	10	5.00	10.2	10.8	11	5	4	5	<0.2	≥0.2	≥0.2	≥0.2
Brewer's yeast	0.0047	0.0050	0.0052	0	5.00	0	0	0	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0049	0.0049	0.0054	1	5.00	0.98	0.98	1.08	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0051	0.0051	0.0053	10	5.00	10.2	10.2	10.6	5	5	5	<0.2	<0.2	<0.2	<0.2
Chalk dust	0.0054	0.0051	0.0048	0	5.00	0	0	0	6	6	6	<0.2	<0.2	<0.2	<0.2
	0.0049	0.0050	0.0054	1	5.00	0.98	1	1.08	5	6	6	<0.2	<0.2	<0.2	<0.2
	0.0055	0.0055	0.0052	10	5.00	11	11	10.4	6	7	6	<0.2	≥0.2	≥0.2	≥0.2
Chitin	0.0053	0.0051	0.0049	0	5.00	0	0	0	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0052	0.0056	0.0051	1	5.00	1.04	1.12	1.02	5	5	5	<0.2	≥0.2	≥0.2	≥0.2
	0.0055	0.0048	0.0050	10	5.00	11	9.6	10	6	5	5	≥0.2	<0.2	<0.2	<0.2
Coffee powder	0.0050	0.0055	0.0048	0	5.00	0	0	0	4	4	4	<0.2	<0.2	<0.2	<0.2
	0.0055	0.0047	0.0054	1	5.00	1.1	0.94	1.08	5	5	5	≥0.2	≥0.2	≥0.2	≥0.2
	0.0047	0.0054	0.0054	10	5.00	9.4	10.8	10.8	7	7	7	≥0.2	≥0.2	≥0.2	≥0.2
Cornstarch	0.0050	0.0054	0.0053	0	5.00	0	0	0	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0056	0.0055	0.0051	1	5.00	1.12	1.1	1.02	5	5	5	<0.2	≥0.2	<0.2	<0.2
	0.0051	0.0054	0.0048	10	5.00	10.2	10.8	9.6	5	5	5	<0.2	<0.2	≥0.2	<0.2
Drywall dust	0.0051	0.0047	0.0055	0	5.00	0	0	0	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0052	0.0050	0.0048	1	5.00	1.04	1	0.96	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0053	0.0051	0.0053	10	5.00	10.6	10.2	10.6	6	7	6	≥0.2	≥0.2	≥0.2	≥0.2
Flour	0.0050	0.0054	0.0051	0	5.00	0	0	0	6	6	6	<0.2	<0.2	<0.2	<0.2
	0.0049	0.0048	0.0055	1	5.00	0.98	0.96	1.1	5	6	6	<0.2	<0.2	≥0.2	<0.2
	0.0050	0.0052	0.0054	10	5.00	10	10.4	10.8	6	6	6	≥0.2	≥0.2	≥0.2	≥0.2

(continued)

Table 25. Cyantesmo Paper: 5 mg Supplemental Testing Results (Continued)

Matrix	Sample Mass 1 (g)	Sample Mass 2 (g)	Sample Mass 3 (g)	KCN Conc. (mg/g)	DI H_2O Volume (mL)	KCN Solution Conc. 1 (mg/L)	KCN solution Conc. 2 (mg/L)	KCN solution Conc. 3 (mg/L)	Measured pH 1	Measured pH 2	Measured pH 3	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Kaolin	0.0048	0.0050	0.0053	0	5.00	0	0	0	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0052	0.0052	0.0054	1	5.00	1.04	1.04	1.08	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0052	0.0053	0.0049	10	5.00	10.4	10.6	9.8	5	7	5	<0.2	≥0.2	≥0.2	≥0.2
γ-aminobutyric acid	0.0053	0.0049	0.0051	0	5.00	0	0	0	4	4	4	<0.2	<0.2	<0.2	<0.2
	0.0050	0.0054	0.0052	1	5.00	1	1.08	1.04	4	4	4	<0.2	<0.2	<0.2	<0.2
	0.0049	0.0048	0.0051	10	5.00	9.8	9.6	10.2	4	4	4	≥0.2	≥0.2	<0.2	≥0.2
L-glutamic acid	0.0055	0.0050	0.0051	0	5.00	0	0	0	4	4	4	<0.2	<0.2	<0.2	<0.2
	0.0052	0.0047	0.0055	1	5.00	1.04	0.94	1.1	4	4	4	<0.2	<0.2	<0.2	<0.2
	0.0049	0.0048	0.0052	10	5.00	9.8	9.6	10.4	4	4	4	<0.2	<0.2	<0.2	<0.2
Powdered coffee creamer	0.0055	0.0054	0.0049	0	5.00	0	0	0	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0048	0.0050	0.0051	1	5.00	0.96	1	1.02	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0046	0.0049	0.0052	10	5.00	9.2	9.8	10.4	5	5	5	<0.2	<0.2	<0.2	<0.2
Powdered milk	0.0051	0.0054	0.0048	0	5.00	0	0	0	6	6	6	<0.2	<0.2	<0.2	<0.2
	0.0051	0.0054	0.0055	1	5.00	1.02	1.08	1.1	6	6	6	≥0.2	<0.2	≥0.2	≥0.2
	0.0051	0.0050	0.0049	10	5.00	10.2	10	9.8	6	6	6	<0.2	≥0.2	≥0.2	≥0.2
Talcum powder	0.0050	0.0054	0.0052	0	5.00	0	0	0	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0049	0.0051	0.0053	1	5.00	0.98	1.02	1.06	5	5	5	<0.2	≥0.2	<0.2	<0.2
	0.0051	0.0049	0.0048	10	5.00	10.2	9.8	9.6	5	5	5	<0.2	<0.2	<0.2	<0.2

Note: Yellow highlighting indicates results that were inconsistent with theoretical calculations.

Table 26. Cyantesmo Paper: 50 mg Supplemental Testing Results

Matrix	Sample Mass 1 (g)	Sample Mass 2 (g)	Sample Mass 3 (g)	KCN Conc. (mg/g)	DI H2O Volume (mL)	KCN Solution Conc. 1 (mg/L)	KCN Solution Conc. 2 (mg/L)	KCN Solution Conc. 3 (mg/L)	Measured pH 1	Measured pH 2	Measured pH 3	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Baking soda	0.0550	0.0550	0.0500	0	5.00	0	0	0	9	8	8	<0.2	<0.2	<0.2	<0.2
	0.0499	0.0497	0.0502	1	5.00	9.98	9.94	10.04	9	8	8	≥0.2	≥0.2	≥0.2	≥0.2
	0.0503	0.0533	0.0505	10	5.00	100.6	106.6	101	9	8	8	≥0.2	≥0.2	≥0.2	≥0.2
Boric acid	0.0550	0.0500	0.0507	0	5.00	0	0	0	6	5	5	<0.2	<0.2	<0.2	<0.2
	0.0501	0.0506	0.0507	1	5.00	10.02	10.12	10.14	6	5	6	≥0.2	≥0.2	≥0.2	≥0.2
	0.0503	0.0529	0.0498	10	5.00	100.6	105.8	99.6	6	6	6	≥0.2	≥0.2	≥0.2	≥0.2
Brewer's yeast	0.0499	0.0496	0.0503	0	5.00	0	0	0	6	6	6	<0.2	<0.2	<0.2	<0.2
	0.0517	0.0500	0.0507	1	5.00	10.34	10	10.14	6	6	6	≥0.2	≥0.2	≥0.2	≥0.2
	0.0503	0.0546	0.0504	10	5.00	100.6	109.2	100.8	6	6	6	≥0.2	≥0.2	≥0.2	≥0.2
Chalk dust	0.0540	0.0528	0.0509	0	5.00	0	0	0	7	5	6	<0.2	<0.2	<0.2	<0.2
	0.0497	0.0500	0.0498	1	5.00	9.94	10	9.96	7	5	6	≥0.2	≥0.2	≥0.2	≥0.2
	0.0503	0.0499	0.0506	10	5.00	100.6	99.8	101.2	7	8	7	≥0.2	≥0.2	≥0.2	≥0.2
Chitin	0.0521	0.0494	0.0506	0	5.00	0	0	0	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0498	0.0508	0.0504	1	5.00	9.96	10.16	10.08	5	5	5	≥0.2	≥0.2	≥0.2	≥0.2
	0.4970	0.0496	0.0503	10	5.00	994	99.2	100.6	5	5	5	≥0.2	≥0.2	≥0.2	≥0.2
Coffee powder	0.0501	0.0499	0.0516	0	5.00	0	0	0	4	4	4	<0.2	<0.2	<0.2	<0.2
	0.0508	0.0497	0.0509	1	5.00	10.16	9.94	10.18	4	4	4	≥0.2	≥0.2	≥0.2	≥0.2
	0.0501	0.0516	0.0502	10	5.00	100.2	103.2	100.4	4	4	4	≥0.2	≥0.2	≥0.2	≥0.2
Cornstarch	0.0500	0.0520	0.0501	0	5.00	0	0	0	6	5	5	<0.2	<0.2	<0.2	<0.2
	0.0497	0.0495	0.0510	1	5.00	9.94	9.9	10.2	6	6	6	≥0.2	≥0.2	≥0.2	≥0.2
	0.0498	0.0513	0.0499	10	5.00	99.6	102.6	99.8	6	6	6	≥0.2	≥0.2	≥0.2	≥0.2
Drywall dust	0.0509	0.0505	0.0500	0	5.00	0	0	0	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0499	0.0503	0.0506	1	5.00	9.98	10.06	10.12	5	5	5	≥0.2	≥0.2	≥0.2	≥0.2
	0.4990	0.0514	0.0529	10	5.00	998	102.8	105.8	6	6	6	≥0.2	≥0.2	≥0.2	≥0.2
Flour	0.0500	0.0501	0.0496	0	5.00	0	0	0	6	5	6	<0.2	<0.2	<0.2	<0.2
	0.0504	0.0510	0.0500	1	5.00	10.08	10.2	10	6	5	6	≥0.2	≥0.2	≥0.2	≥0.2
	0.0504	0.0499	0.0546	10	5.00	100.8	99.8	109.2	6	7	7	≥0.2	≥0.2	≥0.2	≥0.2

(continued)

Table 26. Cyantesmo Paper: 50 mg Supplemental Testing Results (Continued)

Matrix	Sample Mass 1 (g)	Sample Mass 2 (g)	Sample Mass 3 (g)	KCN Conc. (mg/g)	DI H2O Volume (mL)	KCN Solution conc 1 (mg/L)	KCN Solution conc 2 (mg/L)	KCN Solution conc 3 (mg/L)	Measured pH 1	Measured pH 2	Measured pH 3	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
Kaolin	0.0511	0.0516	0.0528	0	5.00	0	0	0	5	5	5	<0.2	<0.2	<0.2	<0.2
	0.0510	0.0509	0.0500	1	5.00	10.2	10.18	10	5	5	5	≥0.2	≥0.2	≥0.2	≥0.2
	0.0500	0.0502	0.0499	10	5.00	100	100.4	99.8	5	5	5	≥0.2	≥0.2	≥0.2	≥0.2
γ-Aminobutyric acid	0.0495	0.0506	0.0550	0	5.00	0	0	0	6	6	6	<0.2	<0.2	<0.2	<0.2
	0.5060	0.0504	0.0497	1	5.00	101.2	10.08	9.94	6	6	6	≥0.2	≥0.2	≥0.2	≥0.2
	0.0505	0.0503	0.0533	10	5.00	101	100.6	106.6	6	6	6	≥0.2	≥0.2	≥0.2	≥0.2
L-glutamic acid	0.0504	0.0509	0.0499	0	5.00	0	0	0	3	3	3	<0.2	<0.2	<0.2	<0.2
	0.0504	0.0498	0.0497	1	5.00	10.08	9.96	9.94	4	4	4	≥0.2	≥0.2	≥0.2	≥0.2
	0.0509	0.0506	0.0516	10	5.00	101.8	101.2	103.2	4	4	4	≥0.2	≥0.2	≥0.2	≥0.2
Powdered coffee creamer	0.0550	0.0503	0.0494	0	5.00	0	0	0	6	7	6	<0.2	<0.2	<0.2	<0.2
	0.0504	0.0507	0.0508	1	5.00	10.08	10.14	10.16	6	8	7	≥0.2	≥0.2	≥0.2	≥0.2
	0.0504	0.0504	0.0496	10	5.00	100.8	100.8	99.2	6	9	8	≥0.2	≥0.2	≥0.2	≥0.2
Powdered milk	0.0510	0.0507	0.5040	0	5.00	0	0	0	6	6	6	<0.2	<0.2	<0.2	<0.2
	0.0503	0.0507	0.5100	1	5.00	10.06	10.14	102	7	6	6	≥0.2	≥0.2	≥0.2	≥0.2
	0.0500	0.0498	0.5120	10	5.00	100	99.6	1024	6	6	6	≥0.2	≥0.2	≥0.2	≥0.2
Talcum powder	0.0500	0.0500	0.0520	0	5.00	0	0	0	7	5	6	<0.2	<0.2	<0.2	<0.2
	0.0502	0.0502	0.0495	1	5.00	10.04	10.04	9.9	7	5	6	≥0.2	≥0.2	≥0.2	≥0.2
	0.0502	0.0505	0.0513	10	5.00	100.4	101	102.6	7	7	7	≥0.2	≥0.2	≥0.2	≥0.2

Finally, positive-control samples that contained 5, 20, 50, and 200 µg of free cyanide were prepared by spiking a 200 mg/L standard into 5 mL of DI water (Table 27). The 200 mg/L cyanide standard was prepared by performing a 1:5 dilution of a 1000 mg/L standard with DI water. Additionally, a method blank was prepared using DI water that was not spiked with cyanide standard. All replicates of the method blank were negative for cyanide, and all spiked samples returned positive cyanide detections.

Table 27. Cyantesmo Paper: Positive- and Negative-Control Supplemental Testing Results

Matrix	200 mg/L KCN Spike Volume (µL)	DI H_2O Volume (mL)	KCN Solution Conc. (mg/L)	Measured pH	Result 1 (mg/L)	Result 2 (mg/L)	Result 3 (mg/L)	Result Average (mg/L)
DI H_2O	0	5.00	0	6	<0.2	<0.2	<0.2	<0.2
	25	4.98	1	6	≥0.2	≥0.2	≥0.2	≥0.2
	100	4.90	4	6	≥0.2	≥0.2	≥0.2	≥0.2
	250	4.75	10	6	≥0.2	≥0.2	≥0.2	≥0.2
	1000	4.00	40	6	≥0.2	≥0.2	≥0.2	≥0.2

4. DISCUSSION AND CONCLUSIONS

During Phase I testing, five cyanide test kits were evaluated against standard conditions to determine suitably for use in a production-oriented environment. The Quantofix cyanide test strips are currently being used; therefore, they were included in the evaluation as a baseline measure to determine equivalency to other test procedures. Current test procedures require 500 mg of sample and can only accurately measure down to 1 mg/mL of free cyanide in solution. Based upon the results from Phase I testing, both the Cyantesmo paper and the Visocolor ECO cyanide test kit proved to be more sensitive and require less sample mass as compared with the Quantofix cyanide test strips. Cyantesmo paper had an added advantage in that the cyanide detection occurred by measurement of hydrogen cyanide in the headspace above the sample matrix, which reduced the possibility of interference from highly turbid or colored samples. Additionally, Cyantesmo tests were typically performed in a 15 to 30 min timeframe, with no test extending beyond 90 min. This resulted in the Cyantesmo analysis period falling well within the 8 h production day. Based upon these observations and criteria, Cyantesmo paper was selected for further evaluation in Phase II testing.

During Phase II testing, the Cyantesmo paper was subjected to more sample matrices for analysis against any possible interference. Additionally, tests were conducted to determine the lowest sample mass that could reliably be used with consistent repeatability. When 5 mg of sample was used, the detection of hydrogen cyanide was not reliably repeatable in any samples at 0.1% and was only mildly repeatable in samples at 1.0%. All tests were then performed again using 50 mg of sample, and all results were reliably repeatable for both the positive- and negative-control samples. To determine the cause of the false positives that occurred with use of the 5 mg sample masses, positive- and negative-control samples were prepared using DI water spiked with a standardized potassium cyanide solution to obtain

solutions that were higher and lower in concentration than the final theoretical concentration of the spiked 5 mg samples. The results showed that Cyantesmo paper was able to detect cyanide at 1 mg/L, even though no cyanide was detected in the 5 mg samples that had the same theoretical concentration. Although no further tests were requested, it is possible that the lack of detection at such small masses was due to sampling error. In some samples, potassium cyanide crystals could be visually observed in the matrix. The samples were vigorously mixed in an attempt to equally disperse the potassium cyanide throughout the sample matrix. However, by collecting such a small sample mass, there was no way to be certain that each aliquot contained the analyte of interest.

Blank

APPENDIX A
TEST AND EVALUATION PLAN FOR COMMERCIALLY AVAILABLE CYANIDE TEST KITS AT 485

A.1 Purpose

The purpose of this test plan is to evaluate commercially available cyanide detection kits and test strips and, if possible, identify an alternative to the current method used in Building 485 Hit (unknown) sample characterization.

A.1.1 Background

The cyanide testing method was developed as a portion of the Hit sample characterization. The current method uses color-changing test strips that detect free cyanide (CN^-) in liquid samples. Solid/powder samples are dissolved or agitated in water prior to analysis. According to the manufacturer's claims, the strips can detect down to 1 mg/L CN^-, but the color change may be too faint to be observed by the untrained eye.

The test strips require 5 mL of liquid sample. To achieve a sufficient concentration for detection, 500 mg of solid/powder Hit sample or 500 µL of liquid Hit sample is added to water. However, given the paucity of material from most Hit samples, this amount can represent a substantial portion of the material that will be subjected to a destructive analysis. Therefore, a method that uses less material yet still allows for detection of low CN^- concentration would be beneficial for conserving Hit sample material for additional analyses.

In response to this need, a market survey was performed, and several potential commercially available methods were identified. From these, a subset was down-selected for further investigation (Table A-1). In addition, since the initial testing of the current method was not well documented, it will be re-evaluated to determine if it can be effective with less material.

Table A-1. Test Strips and Kits To Be Included in the Evaluation

Method	Manufacturer	Lowest Detectable Concentration
Quantofix cyanide test strips	Macherey-Nagel	1 mg/L CN^-
MQuant cyanide test strips	EMD Millipore	1 mg/L CN^-
Cyantesmo paper	Macherey-Nagel	0.2 mg/L HCN
eXact cyanide test strips	Industrial Test Systems	0.1 mg/L CN^-
MColortest system	EMD Millipore	0.03 mg/L CN^-
Visocolor ECO cyanide test kit	Macherey-Nagel	0.01 mg/L CN^-

A.1.1.1 Responsibilities

The Environmental Monitoring Laboratory will be responsible for purchasing all test strips, kits, standards, and required testing material and evaluating the methods. Testing will occur at Building 485 by chemical laboratory technicians. Data will be delivered to and analyzed by the science advisor, who will also write the final report with recommendations for further testing and/or method acceptance.

A.1.1.2 Test Setup

Samples will involve dry mixtures of potassium cyanide (KCN) spiked into various common powder matrices at 1 and 0.1% weight-to-weight ratio (w/w) and a blank (no KCN) powder sample to test for false positives. The first powder matrices to be tested will be flour, powdered sugar, and talcum powder. Depending on the results from the initial round, passing methods may be tested with additional matrices. A cyanide standard solution will be used as a positive control.

Kits will be tested first with the current amount of material used: 500 mg of spiked powders. Kits that are able to detect samples of this amount will then be subjected to decreasing amounts of material until detection is not possible, as denoted by a lack of a color change.

A.1.1.3 Data Reporting

Technicians will record and deliver the following data, at a minimum, to the science advisor:

- Results from testing with spiked samples, blank samples, and the standard;
- Lowest amounts of sample that can be detected for each concentration; and
- Any unusual occurrences (e.g., unexpected color changes or other reactions).

The data will be used to determine false-positive and false-negative rates and other potential issues.

APPENDIX B
EVALUATION OF COMMERCIALLY AVAILABLE CYANIDE TEST KITS
SUPPLEMENTAL TESTING—23 JUNE 2015

B.1 Background

In 2015, several commercially available cyanide detection kits were evaluated with different amounts of dry mixtures of potassium cyanide (KCN), spiked at 1% weight-to-weight ratio (w/w) and 0.1% w/w, in flour, powdered sugar, and talcum powder. Additionally, the kits were tested with a cyanide standard solution to determine sensitivity at the lower end of the reported calibration ranges and with less water than the manufacturers' recommendations to determine effectiveness with less sample volume. Of the kits tested, only two were able to detect cyanide regardless of the amount of sample, matrix, or water volume: Cyantesmo paper and the Visocolor ECO cyanide test kit (both from Macherey-Nagel; Düren, Germany). Of these, the Cyantesmo paper was chosen for further testing because it was the quicker of the two methods and would not have an issue with turbid samples.

In the initial testing, the kits were only tested against three powders. In this supplemental testing, the Cyantesmo paper will be evaluated with a broader panel of spiked matrices to increase confidence that their performance is not limited to the aforementioned matrices.

B.1.1 Test Setup

Reagents and equipment include the following;

- Cyantesmo paper (Macherey-Nagel);
- Potassium cyanide (KCN);
- Distilled water;
- pH paper (0–13 range);
- Concentrated H_2SO_4 (18 M);
- 1000 µg CN^-/mL standard solution;
- Common powders (Table B-1);
- Vessels that can be capped and accommodate 5 mL (e.g., 15 mL tubes, capped glass vials);
- Balance;
- Vortex mixer; and
- Transfer pipettes.

Table B-1. Additional Powder Matrices

Powder Matrices
Baking soda
Boric acid
Brewer's yeast
Chalk dust
Chitin
Coffee powder
Cornstarch
Dry wall dust
Flour
Kaolin
γ-Aminobutyric acid
L-Glutamic acid
Powdered coffee creamer
Powdered milk
Talcum powder

B.1.2 Test Protocol

The testing will involve the following:

- Powder matrices spiked at 1 and 0.1% w/w with KCN;
- Powdered matrices without KCN;
- Method blank (i.e., an aliquot of water treated in the same manner as a sample)/negative control; and
- Positive controls of 5, 20, 50, and 200 µg of CN^- in 5 mL, made with the 1000 µg/mL standard.

For the spiked powder matrices, 5 mg, which was the lowest amount used in the initial test, will be analyzed. If detection is not possible, the analysis will be repeated with 50 mg and, if necessary, 500 mg to characterize the detection capability in the presence of the matrix of concern. The unspiked powder matrices will be analyzed at 5 mg. Powder matrices, both spiked and unspiked, will be analyzed in triplicate.

The testing will use a protocol adapted from the Food Emergency Response Network (FERN) Cyantesmo protocol,[1] as outlined below:

1. Place an aliquot of the sample into a vessel that can be tightly capped.
2. Add 5 mL of water.
3. Check the pH of the solution.

[1] Flurer, C.L.; Barnes, B.S.; Urban, J.R.; Roberts, J.N.; Toomey, V.M. *Screen for the Presence of Cyanide*; SOP T005(004); Forensic Chemistry Center, U.S. Food and Drug Administration: Cincinnati, OH, 2006; UNCLASSIFIED Procedure.

4. Place an ~2-in. piece of Cyantesmo paper into distilled water to wet it. The length of the paper can be adjusted, depending on the vessel and sample volume.
5. Add ~100 µL of concentrated H_2SO_4 (two drops from a transfer pipet) to the mixture and shake or swirl to mix.
6. Remove the Cyantesmo paper from the distilled water and remove excess water. Suspend the paper in the headspace above the acidified sample. Cap the vessel.
7. Check the sample after 15 min. The Cyantesmo paper will turn blue in the presence of CN^-. If the paper has not turned blue after 15 min, the sample can be left to react overnight.

B.1.3 Data Reporting

Technicians will record and deliver the following data, at a minimum, to the science advisor:

- Results from testing with spiked matrices, blank samples, and the standard;
- Highest amount of sample detected, if the initial 5 mg testing failed; and
- Any unusual occurrences (e.g., unexpected color changes or other reactions).

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