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Environmental Technology Verification Report

INDUSTRIAL TEST SYSTEMS, INC.
CYANIDE REAGENTSTRIP™ TEST KIT

Prepared by
Battelle

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Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

INDUSTRIAL TEST SYSTEMS, INC. CYANIDE REAGENTSTRIP™ TEST KIT

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Notice

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technology across all media and to report this objective information to permittees, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of six verification centers. Information about each of these centers can be found on the Internet at <http://www.epa.gov/etv/>.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. Under a cooperative agreement, Battelle has received EPA funding to plan, coordinate, and conduct such verification tests for "Advanced Monitoring Systems for Air, Water, and Soil" and report the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at <http://www.epa.gov/etv/centers/center1.html>.

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List of Abbreviations

AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
ATEL	Aqua Tech Environmental Laboratories
DI	deionized
DPD	N,N-diethyl- <i>p</i> -phenylenediamine
DW	drinking water
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
ID	identification
KCN	potassium cyanide
L	liter
LFM	laboratory-fortified matrix
MDL	method detection limit
mg	milligram
mL	milliliter
NaOH	sodium hydroxide
NIST	National Institute of Standards and Technology
PE	performance evaluation
PT	performance test
QA	quality assurance
QC	quality control
QCS	quality control standard
QMP	quality management plan
RB	reagent blank
RPD	relative percent difference
RSD	relative standard deviation
TSA	technical systems audit

Chapter 1 Background

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by substantially accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permittees; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The EPA's National Exposure Research Laboratory and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of the Industrial Test Systems, Inc., Cyanide ReagentStrip™ test kit in detecting the presence of cyanide in water. Portable cyanide analyzers were identified as a priority technology verification category through the AMS Center stakeholder process.

Chapter 2 Technology Description

The objective of the ETV AMS Center is to verify the performance characteristics of environmental monitoring technologies for air, water, and soil. This verification report provides results for the verification testing of the Cyanide ReagentStrip™ test kit. Following is a description of the Cyanide ReagentStrip™ test kit, based on information provided by the vendor. The information provided below was not verified in this test.

The Industrial Test Systems, Inc., Cyanide ReagentStrip™ test kit is designed to detect free cyanide in water. This is done by converting cyanide in water to cyanogen chloride, which, in the presence of isonicotinic and barbituric acids, produces a color change that can be detected visually or with a colorimeter. Results can be determined by three methods, and the method selected is dependent upon the data needs of the user: (1) A semi-quantitative result in increments ranging from <0.1 milligram per liter (mg/L) to >10 mg/L can be obtained in approximately 1 minute by comparing the color change on ReagentStrip™ #2 to a color chart; (2) a semi-quantitative result for an expanded range of 0 mg/L to >200 mg/L can be obtained in 10 minutes by visually comparing the color of the water sample in a microcuvette with a separate



Figure 2-1. Industrial Test Systems, Inc., Cyanide ReagentStrip™ Test Kit

color chart designed for use with microcuvettes; and (3) a quantitative determination can be obtained in 10 minutes when the microcuvette is inserted into the optional ReagentStrip™ CO7500 colorimeter (also identified as the ReagentStrip™ Reader), and the intensity of the color is measured quantitatively. The ReagentStrip™ Reader generates a result in absorbance units that are converted to concentration units using the reference table provided by Industrial Test Systems, Inc. The absorbance units on the reference table convert to concentrations ranging from <0.01 mg/L to >60 mg/L.

The Cyanide ReagentStrip™ test kit includes one bottle each of Cyanide ReagentStrip™ #1 and #2, one graduated pipette, 20 microcuvettes, one microcuvette holder, one ReagentStrip™ Reader, two semi-quantitative visual color charts, one colorimeter absorbance reference chart, one instruction sheet, and a material safety data sheet. The list price of the Cyanide ReagentStrip™ test kit, including the optional ReagentStrip™ Reader, is \$559.99 for 50 tests. ReagentStrips™ #1 and #2 for additional tests can be purchased separately at an approximate cost of \$40 for 50 additional tests.

Chapter 3 Test Design and Procedures

3.1 Introduction

Cyanide can be present in various forms in water. This verification test focused on detecting the free cyanide ion prepared using potassium cyanide (KCN) and is referred to as simply “cyanide” in this report. At high doses, this form of cyanide inhibits cellular respiration and, in some cases, can result in death. Because of the toxicity of cyanide to humans, the EPA has set 0.2 mg/L as the maximum concentration of cyanide that can be present in drinking water (DW). In DW and surface water under ambient conditions, cyanide evolves from aqueous hydrogen cyanide, sodium cyanide, potassium cyanide, and other metal or ionic salts where cyanide is released when dissolved in water. Heavier cyanide complexes (e.g., iron) are bound tightly, requiring an acid distillation to liberate the toxic free cyanide ion, a step not verified as part of this test since the distillation step would prevent these analyzers from being field portable. Because disassociation of the free cyanide ion is unlikely under ambient conditions, the heavier salts are considered much less toxic than simple cyanide salts such as potassium and sodium cyanide.

This verification test was conducted according to procedures specified in the *Test/QA Plan for Verification of Portable Analyzers for Detection of Cyanide in Water*.⁽¹⁾ The verification was based on comparing the cyanide concentrations of water samples analyzed using the Cyanide ReagentStrip™ test kit with cyanide concentrations analyzed using a laboratory-based reference method. The Cyanide ReagentStrip™ test kit was verified by analyzing performance test (PT), lethal/near-lethal concentration, surface, and DW samples. A comparison of the analytical results from the Cyanide ReagentStrip™ test kit and the reference method provided the basis for the quantitative results presented in this report.

The Cyanide ReagentStrip™ test kit performance was evaluated in terms of

- Accuracy
- Precision
- Matrix effects
- Linearity
- Method detection limit
- Inter-unit reproducibility
- Lethal or near-lethal dose response
- Operator bias
- Field portability
- Ease of use
- Sample throughput.

3.2 Reference Method

Aqua Tech Environmental Laboratories (ATEL) in Marion, OH, performed the reference analyses of all test samples. ATEL received the samples from Battelle labeled with an identification (ID) number meaningful only to Battelle, performed the analyses, and submitted to Battelle the results of the analyses without knowledge of the prepared or fortified concentration of the samples.

The analytical results for the Cyanide ReagentStrip™ test kit were compared with the results obtained from analysis using semi-automated colorimetry according to EPA Method 335.1, *Cyanides Amenable to Chlorination*.⁽²⁾ This method was selected because it measures the concentration of the cyanide ion in water samples under ambient conditions, which is the same form of cyanide that the participating technologies are designed to measure. For the reference method analyses, the concentration of free cyanide was determined by the difference of two measurements of total cyanide. One colorimetric determination was made after the free cyanide in the sample had been chlorinated to cyanogen chloride, which degrades quickly, and a second was made without chlorination. Typically, samples were sent to the reference laboratory for analysis each testing day. The reference analysis was performed within 14 days of sample collection.

3.3 Test Design

The verification test was conducted between September 22, 2004, and October 5, 2004. All analyses were performed according to the manufacturer's recommended procedures. The verification test involved challenging the Cyanide ReagentStrips™ test kit with a variety of test samples, including sets of DW and surface water samples representative of those likely to be analyzed by buyers and users of the Cyanide ReagentStrips™ test kit. The results from the Cyanide ReagentStrips™ test kits were compared with the reference method to qualitatively and quantitatively assess performance. Multiple aliquots of each test sample were analyzed separately to assess the precision of the Cyanide ReagentStrips™ test kit and the reference method.

Results were generated using the Cyanide ReagentStrips™ test kit by a technical and a non-technical operator to assess operator bias. The non-technical operator had no previous laboratory experience. Both operators watched a brief training video provided by the vendor to become acquainted with the basic operation of the test kit. Both operators analyzed all of the test samples. Each operator manipulated separate water samples and reagents to generate a solution in which cyanide could be detected photometrically. Then, the operators analyzed their respective solutions using two ReagentStrip™ Readers to evaluate inter-unit reproducibility.

Sample throughput was estimated based on the time required to prepare and analyze a sample. Ease of use was based on documented observations by the operator and the Battelle Verification Test Coordinator. The Cyanide ReagentStrip™ test kit was used in a field environment as well as in a laboratory setting to assess the impact of field conditions on performance.

3.4 Test Samples

Test samples used in the verification test are shown in Table 3-1 and include quality control (QC) samples, PT samples, lethal/near-lethal concentration samples, DW samples, and surface water samples. The QC, PT, and lethal/near-lethal samples were prepared from National Institute of Standards and Technology (NIST) traceable standards. The PT and QC sample concentrations were targeted to the EPA maximum contaminant level in DW, which for cyanide is 0.2 mg/L.⁽³⁾ The PT samples ranged from 0.03 mg/L to 25 mg/L. The performance of the Cyanide ReagentStrip™ test kit also was evaluated quantitatively with samples prepared with cyanide concentrations up to 250 mg/L that could be lethal if ingested. Two surface water sources (Olentangy River and Alum Creek Reservoir) were sampled and analyzed. In addition, five sources of DW from around the United States and two sources of Columbus, OH, DW were evaluated.

3.4.1 Quality Control Samples

QC samples included laboratory reagent blanks (RBs), quality control standards (QCSs), and laboratory-fortified matrix (LFM) samples (Table 3-1). The RBs consisted of American Society for Testing and Materials (ASTM) Type II deionized (DI) water and were exposed to handling and analysis procedures identical to other prepared samples, including the addition of all reagents. These samples were used to help ensure that no sources of contamination were introduced in the sample handling and analysis procedures. One RB sample was analyzed for every batch of about 10 water samples. In several instances, the RB samples produced results slightly above <0.01 mg/L. When that was the case, the RB result (background) was subtracted from each result in the sample batch. QCSs of 0.2 mg/L cyanide were prepared in ASTM Type II DI water and analyzed (without defined performance expectations) after approximately every 10th sample by the Cyanide ReagentStrip™ test kit to demonstrate proper functioning to the operator. The LFM samples were prepared as aliquots of DW and surface water samples spiked with KCN as free cyanide to make the cyanide concentration also 0.2 mg/L. Four LFM samples were analyzed for each source of DW and surface water. These samples were used to determine whether matrix effects had an influence on the analytical results from both the Cyanide ReagentStrip™ test kits and the reference method.

QCSs were analyzed approximately every 10th sample to ensure the proper calibration of the reference instrument. According to its standard operating procedure for this reference method, the reference laboratory prepared the QCSs for its use at 0.2 or 0.15 mg/L from a stock solution independent of the one used to prepare the QCS analyzed using the Cyanide ReagentStrip™ test kit. The reference method required that the concentration of each QCS be within 25% of the known concentration. If at any point the difference was larger than 25%, the data collected since the most recent QCS would have been flagged; and proper maintenance would have been performed to regain accurate cyanide measurement, according to ATEL protocols. Section 4.1 describes these samples in more detail.

Table 3-1. Test Samples^(a)

Type of Sample	Sample Characteristics	Cyanide Concentration (mg/L)	Number of Samples
QC	RB	0 mg/L	every 10th sample
	QCS	0.2 mg/L ^(b)	every 10th sample
PT	Detection limit determination	0.05 mg/L	7
	Spiked DI water	0.03 mg/L	4
	Spiked DI water	0.1 mg/L	4
	Spiked DI water	0.2 mg/L	4
	Spiked DI water	0.4 mg/L	4
	Spiked DI water	1 mg/L	4
	Spiked DI water	5 mg/L	4
	Spiked DI water	15 mg/L	4
	Spiked DI water	25 mg/L	4
Lethal/ Near-Lethal Samples	Spiked DI water	50 mg/L	4
	Spiked DI water	100 mg/L	4
	Spiked DI water	250 mg/L	4
Surface Water and DW	Alum Creek Reservoir	Unspiked background	4
		0.2 mg/L LFM	4
	Olentangy River	Unspiked background	4
		0.2 mg/L LFM	4
	Des Moines, IA	Unspiked background	4
		0.2 mg/L LFM	4
	Flagstaff, AZ	Unspiked background	4
		0.2 mg/L LFM	4
	Montpelier, VT	Unspiked background	4
		0.2 mg/L LFM	4
	Seattle, WA	Unspiked background	4
		0.2 mg/L LFM	4
	Tallahassee, FL	Unspiked background	4
		0.2 mg/L LFM	4
	Columbus, OH, city water	Unspiked background	12
		0.2 mg/L LFM	12
	Columbus, OH, well water	Unspiked background	12
		0.2 mg/L LFM	12

^(a) Samples were analyzed in random order.

^(b) Maximum contaminant level for cyanide.

3.4.2 Performance Test Samples

The PT samples (Table 3-1) were prepared in the laboratory using ASTM Type II DI water. The samples were used to determine the accuracy, precision, linearity, and detection limit of the Cyanide ReagentStrip™ test kit. Seven non-consecutive replicate analyses of a 0.05-mg/L solution were made to obtain precision data with which to determine the method detection limit (MDL).⁽⁴⁾ Eight other solutions were prepared to assess the linearity over a 0.03- to 25-mg/L

range of cyanide concentrations. Four aliquots of each of these solutions were analyzed separately to assess the precision of the Cyanide ReagentStrip™ test kit. The concentrations of the PT samples are listed in Table 3-1. The operator analyzed the PT samples blindly and in random order to minimize bias.

3.4.3 Lethal/Near-Lethal Concentrations of Cyanide in Water

To assess the response of the Cyanide ReagentStrip™ test kit when cyanide is present in DW at lethal and near-lethal concentrations, samples were prepared in ASTM Type II DI water at concentrations of 50, 100, and 250 mg/L. The quantitative and semi-quantitative results generated by the Cyanide ReagentStrip™ test kit were compared to results from the reference method.

3.4.4 Surface Water; Drinking Water from Around the U.S.; and Columbus, OH, Drinking Water

Water samples, including fresh surface water and DW (well and local distribution sources) were collected from a variety of sources and used to evaluate technology performance. Surface water was collected near the shoreline by submerging 10-L high-density polyethylene containers no more than one inch below the surface of the water. In a similar container, representatives of five city water treatment facilities provided Battelle with a sample of water that had completed the treatment process, but had not yet entered the water distribution system. Two Columbus, OH, water samples were collected from local residential homes, one from a home with city water and one from a home with well water. Surface water samples were collected from

- Alum Creek Reservoir (OH)
- Olentangy River (OH).

DW samples were collected from

- Local distribution source water (post-treatment) from five cities (Des Moines, IA; Flagstaff, AZ; Montpelier, VT; Seattle, WA; and Tallahassee, FL).
- Columbus, OH, city water
- Columbus, OH, well water.

The water samples collected as part of this verification test were not characterized in any way (i.e., hardness, alkalinity, etc.) other than for cyanide concentration. Each sample was tested for the presence of chlorine, dechlorinated if necessary, and split into two subsamples. Figure 3-1 is a diagram of the process leading from sampling to aliquot analysis. One subsample was spiked with 0.2 mg/L of cyanide to provide LFM aliquots, and the other subsample remained unspiked (background). Four aliquots were taken from each subsample and analyzed separately using the Cyanide ReagentStrip™ test kit. Also, eight aliquots were taken from the background subsample

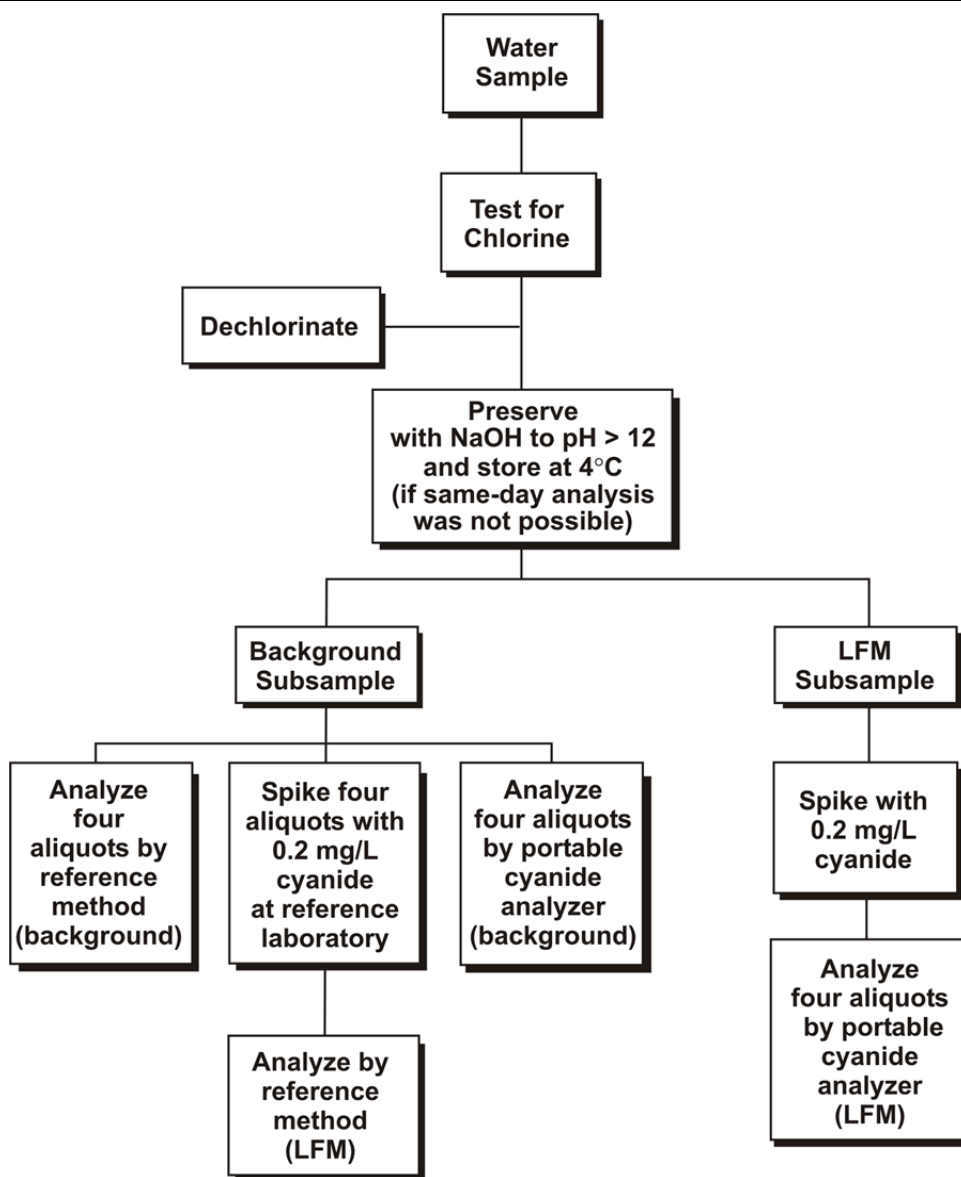


Figure 3-1. Sample Preparation and Analysis of Surface and Drinking Water Samples

and used for analysis by the reference method. Four of the aliquots were left unspiked and analyzed by the reference method, and four of the aliquots were fortified with 0.2 mg/L of KCN as free cyanide at the reference laboratory just before the reference analyses took place. This was done to closely mimic the time elapsed between when the LFM samples were fortified with 0.2 mg/L KCN as free cyanide and when they were analyzed during the testing of the Cyanide ReagentStrip™ test kit.

Columbus, OH, city and well water samples were used to verify the field portability of the Cyanide ReagentStrip™ test kit. Approximately 20 L of water were collected from an outside spigot at two participating residences, one with well water and one with Columbus, OH, city water. The sample aliquots prepared for analysis by the Cyanide ReagentStrip™ test kit were first analyzed in a Battelle laboratory. Then the samples were transported to the indoor field

location and analyzed there. Finally, the samples were taken to the outdoor field location for analysis. In both the laboratory and indoor field locations, the sample temperature was approximately 21°C at the time of analysis, while the outdoor temperature decreased the sample temperature at the time of analysis to approximately 17°C. Because the same sample aliquots were analyzed at the different locations on the same day, only one set of reference samples was sent to the reference laboratory for analysis. Each of the samples was treated as described above and as shown in Figure 3-1.

3.5 Test Procedure

3.5.1 Sample Preparation

QC and PT samples were prepared from a commercially available and NIST-traceable standard. The standard was dissolved and diluted to appropriate concentrations using ASTM Type II DI water in Class A volumetric glassware. The QC and PT samples were prepared within one day of testing. Samples sent to the reference laboratory were preserved with sodium hydroxide (NaOH) at a pH >12, and stored at 4°C until analysis.

Surface and DW samples were collected from the sources indicated in Section 3.4.4 and were stored in high-density polyethylene containers. Because free chlorine degrades cyanide during storage, at the time of sample receipt, before NaOH preservation, all of the samples were tested for free chlorine by adding one N,N-diethyl-*p*-phenylenediamine (DPD) chlorine indicator tablet (Orbeco Analytical Systems, Inc.) to 25 milliliters (mL) of the water sample and crushed with a glass stirring rod. If the water turned pink, the presence of chlorine was indicated. All the DW samples were tested in this manner; and, if the presence of chlorine was indicated, approximately 60 mg of ascorbic acid were added per L of bulk sample to dechlorinate the sample. A separate DPD indicator test (as described above) was done to confirm adequate dechlorination of the sample (indicated by no color change).

3.5.2 Sample Identification

Aliquots to be analyzed were drawn from the standard solutions or from source and DW samples and placed in uniquely identified sample containers for subsequent analysis. The sample containers were identified by a unique ID number. A master log of the samples and sample ID numbers for each unit being verified was kept by Battelle. The ID number, date, person collecting, sample location, and time of collection were recorded on a chain-of-custody form for all field samples.

3.5.3 Sample Analysis

Each day, test samples were prepared from the cyanide standard in either DI water, surface water, or DW matrix. Each sample was prepared in its own container and labeled only with a sample ID number that also was recorded in a laboratory record book, along with details of the sample preparation. Prior to the analysis of each sample, the verification staff recorded the sample ID number on a sample data sheet; then, after the analysis was complete, the result was

recorded on the sample data sheet. Four replicates of each test sample were analyzed. Method blank and QC standards were allowed to come to the same temperature as the samples prior to analysis.

The Cyanide ReagentStrip™ test kit testing procedure included the following steps for analyzing water samples for the presence of cyanide: (1) 2 mL of water sample (with pH adjusted to between 5 and 11) were added to a new disposable microcuvette using a disposable graduated pipette supplied in the kit. (2) ReagentStrip™ #1 was dipped repeatedly into the sample for 30 seconds (using a timer) with a constant up-and-down motion at a rate of about one up-and-down motion per second. The motion allowed the ReagentStrip™ to gently touch the bottom of the microcuvette. After 30 seconds, ReagentStrip™ #1 was removed and discarded. (3) ReagentStrip™ #2 was dipped into the sample for 30 seconds, with a constant up-and-down motion as in Step 2. After 30 seconds, ReagentStrip™ #2 was removed, shaken once to remove excess liquid, and immediately matched to the closest color on the color chart labeled “ReagentStrip™ Colors” (shown in Figure 3-2) to obtain a semi-quantitative cyanide result. Color matching was completed within 2 minutes, before the strip dried. If the color was between two blocks, the concentration was estimated to a concentration half-way between the two blocks. (4) The color of the solution in the microcuvette was then allowed to develop for a reaction time according to the sample temperature. The wait time guidelines were as follows: 10 minutes (but not more than 13 minutes) if the sample was 21°C to 28°C (all samples analyzed in the laboratory and at the indoor field location fit into this category), 20 minutes (but not more than 26 minutes) if the sample was 15°C to 19°C, and 40 minutes (but not more than 50 minutes) if the sample was 5°C to 14°C. (5) At the end of the microcuvette wait time, the microcuvette was placed on the color chart labeled “Microcuvette Colors” (as shown in Figure 3-2). Looking from above and down from the top, the microcuvette was moved within the various color boxes until the closest match to the dark band in the center of the microcuvette was found. Matching was completed within 1 to 2 minutes. If the color was between two blocks, the concentration was estimated to a concentration half-way between the two blocks. (6) Immediately after determining the semi-quantitative visual microcuvette result, 2 mL of unreacted water sample were added to a clean microcuvette marked “reference sample” and inserted into the ReagentStrip™ Reader so that the window faced front to back. It was pushed down fully so the microcuvette was locked into place. The ReagentStrip™ Reader was zeroed by pressing the gray button marked “R,” and the orange button marked “T” was pressed to verify that the blank read 0.00 absorbance units. The “reference sample” microcuvette was then set aside. (7) In a similar manner, the fully reacted sample microcuvette was inserted into the ReagentStrip™ Reader, and the orange button marked “T” was pressed to obtain an absorbance reading instantaneously. The absorbance value was converted to cyanide concentration by the operator, using the reference table provided with the kit. (8) The absorbance reading was obtained using both ReagentStrip™ Readers to obtain inter-unit comparability data.

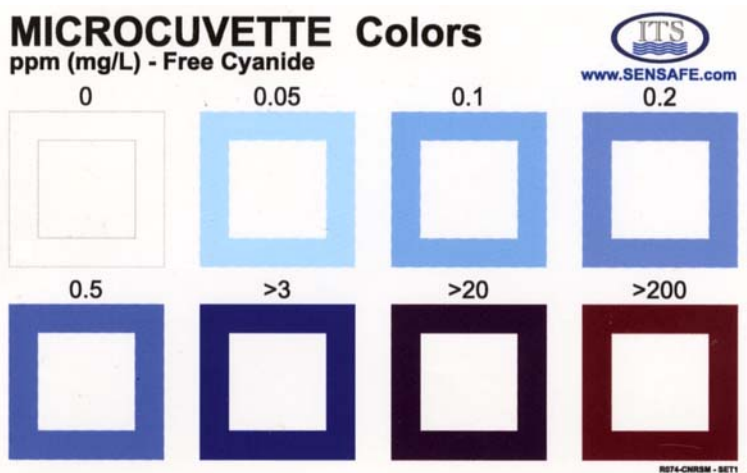
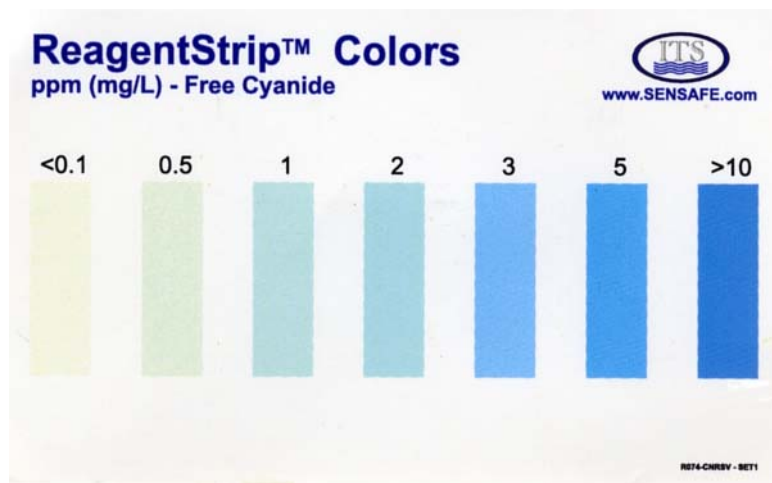


Figure 3-2. Cyanide ReagentStrip™ Test Kit Color Charts

Results were recorded manually on appropriate data sheets. In addition to the analytical results, the data sheets and corresponding laboratory notebooks included records of the time required for sample analysis and operator observations concerning the use of the Cyanide ReagentStrip™ test kit (i.e., ease of use, maintenance, etc.).

While the Cyanide ReagentStrip™ test kit was being tested, a replicate sample set was being analyzed by the reference laboratory. The reference instrument was operated according to the recommended procedures in the instruction manual, and samples were analyzed according to EPA Method 335.1⁽²⁾ and ATEL standard operating procedures. Results from the reference analyses were recorded electronically and compiled by ATEL into a report, including the sample ID and the analyte concentration for each sample.

Chapter 4

Quality Assurance/Quality Control

QA/QC procedures were performed in accordance with the quality management plan (QMP) for the AMS Center⁽³⁾ and the test/QA plan for this verification test.⁽¹⁾

4.1 Reference Method Quality Control Results

Analyses of QC samples were used to document the performance of the reference method. To ensure that no sources of contamination were present, RB samples were analyzed. The test/QA plan stated that if the analysis of an RB sample indicated a concentration above the reporting limit for the reference method, the contamination source was to be corrected and proper blank reading achieved before proceeding with the verification test. Fourteen RB samples were analyzed, and all of them were reported as below the 0.005-mg/L MDL for the reference method.

The reference instrument was calibrated initially according to the procedures specified in the reference method. The accuracy of the reference method was verified with QCS samples analyzed with each sample batch. One of two QCS samples, one with a concentration of 0.15 mg/L and the other with a concentration of 0.2 mg/L, were analyzed with each analytical batch (approximately every 10 water samples). The test/QA plan⁽¹⁾ required the QCS results to always be within the percent recovery range of 75 to 125%. As shown in Table 4-1, the percent recoveries were always between 95 and 107%.

Reference LFM samples were analyzed to confirm the proper functioning of the reference method and to assess whether matrix effects influenced the results of the reference method. The LFM percent recovery of the spiked solution was calculated from the following equation:

$$\% \text{ Recovery} = \frac{C_s - C}{s} \times 100 \quad (1)$$

where C_s is the reference concentration of the spiked sample, C is the reference concentration of the background sample which, in this case, was always zero (results were below the MDL for the reference method), and s is the fortified concentration of the cyanide spike. If the percent recovery of an LFM fell outside the range of 75 to 125%, a matrix effect or some other analytical problem was suspected. As shown in Table 4-2, there were no such instances during this verification test. To mimic the elapsed time between fortification and analysis by the ReagentStrip™ test kit, the reference LFM samples were spiked just minutes prior to analysis

Table 4-1. Reference Method Quality Control Standard Results

Date	Reference Method Result (mg/L)	Known QCS Concentration (mg/L)	% Recovery
9/27/2004	0.145	0.150	97
9/27/2004	0.189	0.200	95
9/29/2004	0.144	0.150	96
9/29/2004	0.199	0.200	100
9/30/2004	0.159	0.150	106
9/30/2004	0.207	0.200	104
10/1/2004	0.156	0.150	104
10/1/2004	0.213	0.200	107
10/4/2004	0.153	0.150	102
10/4/2004	0.206	0.200	103
10/4/2004	0.154	0.150	103
10/5/2004	0.156	0.150	104
10/5/2004	0.212	0.200	106
10/5/2004	0.150	0.150	100
10/5/2004	0.206	0.200	103

using the reference method. The precision of the reference method was evaluated for each set of samples analyzed by the reference method by calculating the relative standard deviation (RSD) (formula shown in Section 5.2). These results also are shown in Table 4-2. All sample sets resulted in RSDs <10%, indicating very reproducible results.

Table 4-2. Reference Method Laboratory-Fortified Matrix Analysis Results

Sample Description	Fortified Concentration (mg/L)	Average Reference Concentration (mg/L)	% Recovery	% RSD
Alum Creek LFM	0.200	0.219	110	2
Olentangy River LFM	0.200	0.203	102	5
Des Moines, IA, LFM	0.200	0.206	103	1
Flagstaff, AZ, LFM	0.200	0.206	103	7
Montpelier, VT, LFM	0.200	0.189	95	4
Seattle, WA, LFM	0.200	0.190	95	7
Tallahassee, FL, LFM	0.200	0.227	113	10
Columbus, OH, City Water LFM	0.200	0.196	98	4
Columbus, OH, Well Water LFM	0.200	0.190	95	6

4.2 Audits

4.2.1 Performance Evaluation Audit

A performance evaluation (PE) audit was conducted to assess the quality of the reference measurements in this verification test. For the PE audit, an independent standard was obtained from a different vendor than the one that supplied the QCSs. The relative percent difference (RPD) of the measured concentration and the known concentration was calculated using the following equation:

$$RPD = \frac{M}{A} \times 100 \quad (2)$$

where M is the absolute difference between the measured and known concentrations, and A is the mean of the same two concentrations. An RPD of <25% was required for the reference measurements to be considered acceptable. Failure to achieve this agreement would have triggered a repeat of the PE comparison. As shown in Table 4-3, all of the PE sample results ranged from 0.5 to 2%, well below the required range.

Table 4-3. Summary of Performance Evaluation Audit

Sample	Date of Analysis	Measured Concentration (mg/L)	Known Concentration (mg/L)	RPD (%)
PE-A	9/17/2004	0.199	0.200	0.50
PE-B	9/17/2004	0.199	0.200	0.50
PE-C	9/17/2004	0.196	0.200	2.02
PE-D	9/17/2004	0.199	0.200	0.50

4.2.2 Technical Systems Audit

Prior to using ATEL as the reference laboratory, the Battelle Quality Manager performed an audit to ensure that ATEL was proficient in the reference analyses. This audit entailed a review of the appropriate training records, state certification data, and the laboratory QMP. The Battelle Quality Manager also conducted a technical systems audit (TSA) to ensure that the verification test was performed in accordance with the test/QA plan⁽¹⁾ and the AMS Center QMP.⁽³⁾ As part of the audit, the Battelle Quality Manager compared the reference method used to the ATEL standard operating procedures, compared actual test procedures to those specified in the test/QA plan, and reviewed data acquisition and handling procedures. Observations and findings from this audit were documented and submitted to the Battelle Verification Test Coordinator for response. No findings were documented that required any corrective action. The records concerning the TSA are stored for at least seven years with the Battelle Quality Manager.

4.2.3 Audit of Data Quality

At least 10% of the data acquired during the verification test were audited. Battelle's Quality Manager traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting, to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

4.3 Quality Assurance/Quality Control Reporting

Each assessment and audit was documented in accordance with Sections 3.3.4 and 3.3.5 of the QMP for the ETV AMS Center.⁽³⁾ Once the assessment report was prepared, the Battelle Verification Test Coordinator ensured that a response was provided for each adverse finding or potential problem and implemented any necessary follow-up corrective action. The Battelle Quality Manager ensured that follow-up corrective action was taken. The results of the TSA were sent to the EPA.

4.4 Data Review

Records generated in the verification test were reviewed before these records were used to calculate, evaluate, or report verification results. Table 4-4 summarizes the types of data recorded. The review was performed by a technical staff member involved in the verification test, but not the staff member who originally generated the record. The person performing the review added his/her initials and the date to a hard copy of the record being reviewed.

Table 4-4. Summary of Data Recording Process

Data to be Recorded	Responsible Party	Where Recorded	How Often Recorded	Disposition of Data^(a)
Dates, times of test events	Battelle	Laboratory record books	Start/end of test; at each change of a test parameter	Used to organize/check test results; manually incorporated data into spreadsheets as necessary
Test parameters (sample temperature, analyte concentrations, location, etc.)	Battelle	Laboratory record books	When set or changed, or as needed to document stability	Used to organize/check test results; manually incorporated data into spreadsheets as necessary
Water sampling data	Battelle	Laboratory record books	At least at the time of sampling	Used to organize/check test results; manually incorporated data into spreadsheets as necessary
Reference method sample analysis, chain of custody, results	ATEL	Laboratory record book/data sheets or data acquisition system, as appropriate	Throughout sample handling and analysis process	Excel spreadsheets

^(a) All activities subsequent to data recording were carried out by Battelle.

Chapter 5

Statistical Methods and Reported Parameters

The statistical methods presented in this chapter were used to verify the performance parameters listed in Section 3.1.

5.1 Accuracy

Accuracy was assessed relative to the results obtained from the reference analyses. Samples were analyzed by both the reference method and the Cyanide ReagentStrip™ test kit. The results for each set of analyses were averaged, and the accuracy was expressed in terms of a relative average bias (B) as calculated from the following equation:

$$B = \frac{\bar{d}}{\bar{C}_R} \times 100 \quad (3)$$

where \bar{d} is the average difference between the Cyanide ReagentStrip™ test kit results and the result from the reference method, and \bar{C}_R is the average of the reference measurements. The semi-quantitative results were not evaluated using a bias calculation because of their subjective nature. Because the color charts have only discrete colors from which to choose, the bias is influenced by the number of possible results between one color and the next. To better summarize the semi-quantitative results, each test sample was assigned the color on each test strip representing the concentration closest to the concentration determined by the reference method. The frequency with which the test results matched that color exactly was evaluated, as well as how often the test results were within one color of the color closest to the reference concentration color.

5.2 Precision

The standard deviation (S) of the results for the replicate samples was calculated and used as a measure of Cyanide ReagentStrip™ test kit precision at each concentration.

$$S = \left[\frac{1}{n-1} \sum_{k=1}^n (c_k - \bar{c})^2 \right]^{1/2} \quad (4)$$

where n is the number of replicate samples, C_k is the concentration measured for the k^{th} sample, and \bar{C} is the average concentration of the replicate samples. The Cyanide ReagentStrip™ test kit precision at each concentration was reported in terms of the RSD, e.g.,

$$RSD = \left| \frac{S}{\bar{C}} \right| \times 100\% \quad (5)$$

For the semi-quantitative strip and microcuvette color chart results, the precision was evaluated by showing whether or not the same result was determined for each replicate analysis. A “yes” indicates that all four results were reported as the same color, and a “no” indicates that they were not.

5.3 Linearity

Linearity was assessed by linear regression, with the analyte concentration measured by the reference method as independent variable and the ReagentStrip™ Reader result from the Cyanide ReagentStrip™ test kit as the dependent variable. Linearity is expressed in terms of the slope, intercept, and the coefficient of determination (r^2). The semi-quantitative results were not conducive to an evaluation of linearity because of the relatively large concentration ranges encompassed by each individual color.

5.4 Method Detection Limit

The MDL⁽⁴⁾ for the Cyanide ReagentStrip™ test kit was assessed from the seven replicate analyses of a fortified sample with a cyanide concentration of approximately five times the manufacturer’s reported detection limit. The MDL was calculated from the following equation:

$$MDL = t \times S \quad (6)$$

where t is the Student’s value for a 99% confidence level, and S is the standard deviation of the replicate samples. The MDL for each ReagentStrip™ Reader was reported separately. Again, the semi-quantitative results were not conducive to a statistical evaluation of MDL. A qualitative evaluation of the concentration levels that produced detectable semi-quantitative results was reported.

5.5 Inter-Unit Reproducibility

The quantitative results obtained from two ReagentStrip™ Readers were compiled and compared to assess inter-unit reproducibility. The results were interpreted using a linear regression of the results for one ReagentStrip™ Reader plotted against the results produced by the other ReagentStrip™ Reader. If the ReagentStrip™ Readers function identically, the slope of such a regression will not differ significantly from unity.

5.6 Lethal or Near-Lethal Dose Response

The Cyanide ReagentStrip™ test kit was not designed to quantitatively measure near-lethal or lethal concentrations of cyanide in water. However, the Cyanide ReagentStrip™ test kit semi-quantitative strip and microcuvette analysis options give semi-quantitative information in addition to the indication that a sample has reached the top of the quantitative range. Additionally, the operators and the Battelle Verification Test Coordinator made qualitative observations of the Cyanide ReagentStrip™ test kit operation while analyzing such samples. Observations of unusual operational characteristics (rate of color change, unusually intense color, unique digital readout, etc.) were documented and reported.

5.7 Operator Bias

To assess operator bias for the Cyanide ReagentStrip™ test kit, the results obtained from a technical and non-technical operator were compiled independently and subsequently compared. The results were interpreted using a linear regression of the non-technical operator's results plotted against the results produced by the technical operator. If the operators obtained identical results, the slope of such a regression would not differ significantly from unity. The semi-quantitative strip and microcuvette results were evaluated in this manner by determining the frequency by which both operators produced results within one color gradient of one another.

5.8 Field Portability

The results obtained from the measurements made on DW samples in the laboratory and indoor and outdoor field settings were compared to assess the accuracy of the measurements under the different analysis conditions. The results were interpreted qualitatively because of the small number of samples.

5.9 Ease of Use

Ease of use was a qualitative measure of the user friendliness of the Cyanide ReagentStrip™ test kit, including how easy or hard the instruction manual was to use.

5.10 Sample Throughput

Sample throughput indicated the amount of time required to analyze a set of samples, including both sample preparation and analysis.

Chapter 6 Test Results

The results of the verification test of the Cyanide ReagentStrip™ test kit are presented in this section.

6.1 Accuracy

Tables 6-1a-d present the cyanide results from analysis of the PT samples; surface water; DW from various regions of the United States; and DW from Columbus, OH, respectively, for both the reference analyses and the Cyanide ReagentStrip™ test kit. The Cyanide ReagentStrip™ test kit results include the quantitative results from the ReagentStrip™ Reader and semi-quantitative results from the strips and microcuvettes. Results are shown for both ReagentStrip™ Readers that were tested (labeled as Unit #1 and #2), as well as for the technical and non-technical operators. On Tables 6-1 a-d, the individual results are shaded to indicate how the color matched with respect to the color that represented the reference concentration.

Tables 6-2a-d present the percent accuracy of the quantitative Cyanide ReagentStrip™ test kit results. The bias values were determined according to Equation (3), Section 5.1. Bias was not calculated for background samples with non-detectable concentrations of cyanide. The bias values shown in Tables 6-2a-d can be summarized by the range of bias observed with different sample sets. For example, for the quantitative results, biases ranged from -47 to 25% for the PT samples, -27 to 28% for the surface water samples, -41 to 3% for the DW samples from around the United States, and -91 to 30% for the Columbus, OH, DW samples. However, if the outdoor samples are removed, the range of biases changes to -42 to 30%.

The impact of the various surface water and DW sample matrices was not clear when evaluating the accuracy results. No particular source of water (DI water included) produced results with consistently low biases. In general, the bias results of the surface water and DW samples were in a range similar to the PT samples that were prepared in DI water; therefore, it seems that the matrix effect on these results was minimal.

The semi-quantitative results were not evaluated this way because of their subjective nature. Since the color charts have only discrete colors from which to choose, the bias could be greatly influenced because of the number of possible results between one color and the next. For example, for the strips, if the test concentration was 0.200 mg/L, the closest detectable result on

Table 6-1a. Cyanide Results from Performance Test Samples

Prepared Conc. (mg/L)	Ref. Conc. (mg/L)	Non-technical Operator				Technical Operator			
		Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)	Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)
0.03	0.038	0.03	0.03	<0.1	0.05	0.03	0.04	<0.1	0.05
0.03	0.035	0.04	0.02	<0.1	0.05	0.01	0.01	<0.1	0.05
0.03	0.024	0.03	0.04	<0.1	0.05	0.02	0.01	<0.1	0.05
0.03	0.035	0.02	0.03	<0.1	0.05	0.01	0.01	<0.1	0.05
0.1	0.108	0.08	0.08	<0.1	0.05	0.06	0.08	0.5	0.1
0.1	0.071	0.07	0.07	0.5	0.1	0.05	0.06	<0.1	0.075
0.1	0.093	0.06	0.08	<0.1	0.05	0.05	0.05	<0.1	0.075
0.1	0.091	0.05	0.05	<0.1	0.05	0.04	0.05	<0.1	0.075
0.2	0.211	0.16	0.17	0.5	0.2	0.14	0.14	0.25	0.1
0.2	0.195	0.12	0.14	0.5	0.2	0.14	0.14	0.25	0.2
0.2	0.209	0.11	0.13	0.5	0.2	0.13	0.13	0.25	0.15
0.2	0.174	0.10	0.10	0.5	0.2	0.13	0.14	0.25	0.15
0.4	0.392	0.43	0.36	0.5	>3	0.28	0.28	0.75	0.5
0.4	0.416	0.46	0.58	0.5	>3	0.30	0.31	0.75	0.5
0.4	0.366	0.32	0.35	1	>3	0.28	0.28	0.75	0.5
0.4	0.406	0.33	0.36	0.5	>3	0.24	0.26	0.75	0.2
1	0.955	1.1	1.2	2	>3	0.97	1.16	2.5	0.5
1	0.905	0.95	1.0	2	>3	0.85	0.92	2.5	0.5
1	1.01	0.96	1.1	2	>3	0.69	0.74	2.5	0.5
1	0.965	0.87	0.96	2	>3	0.67	0.72	1.5	0.5
5	4.90	5.0	4.9	5	>3	5	5	7.5	>3
5	4.88	6.9	6.9	5	>3	5	5.4	5	>3
5	4.79	5.8	5.4	5	>3	5.4	5.0	5	>3
5	4.60	6.2	6.2	5	>3	4.5	4.9	5	>3
15	14.0	17	16	>10	>20	14	14	10	>20
15	15.8	26	21	>10	>20	16	14	10	>20
15	13.2	15	14	>10	>20	17	16	10	>20
15	15.5	14	13	>10	>3	18	17	10	>20
25	22.8	21	19	>10	>3	20	19	10	>20
25	23.8	22	20	>10	>20	28	22	10	>20
25	23.1	31	24	>10	>3	19	16	10	>20
25	25.4	29	22	>10	>3	29	24	10	>20

exact match to the color that should represent the reference laboratory result
 within one color of the color that should represent the reference laboratory result

the strip color chart would be 0.5 mg/L, which would result in a bias of approximately 150%. The colors representing discrete concentration ranges cause a similar problem in evaluating the accuracy of the microcuvette result. To better summarize the semi-quantitative results, each test sample was assigned the color on each test strip representing the concentration closest to the concentration determined by the reference method. Then the frequency with which the test results (1) matched that color exactly, (2) were within one color of the “correct” color with respect to the reference concentration, or (3) were within two colors of the reference concentration was evaluated. These categories are shown with colored shading on the data tables.

Table 6-1b. Cyanide Results from Surface Water

Ref. Conc. (mg/L)	Non-technical Operator				Technical Operator			
	Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)	Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)
Alum Creek Background								
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	0.02	0.02	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
Alum Creek LFM								
0.216	0.19	0.19	0.5	0.5	0.18	0.18	0.25	0.2
0.215	0.21	0.19	0.5	0.5	0.18	0.18	0.5	0.5
0.221	0.12	0.09	0.5	0.5	0.15	0.16	0.5	0.5
0.224	0.16	0.17	0.5	0.5	0.2	0.22	0.5	0.5
Olentangy River Background								
<0.005	<0.01	<0.01	<0.1	0	0.03	0.03	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	0.02	0.02	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
Olentangy River LFM								
0.197	0.26	0.27	0.5	0.5	0.25	0.26	0.5	0.5
0.191	0.25	0.23	0.5	0.5	0.30	0.31	0.5	0.5
0.214	0.24	0.22	0.5	0.5	0.20	0.21	0.5	0.2
0.21	0.20	0.21	0.5	0.5	0.25	0.26	0.5	0.5

 exact match to the color that should represent the reference laboratory result
  within one color of the color that should represent the reference laboratory result

Table 6-3 summarizes this semi-quantitative accuracy evaluation for each type of test sample. For all strip results, 84% of the PT sample results matched the exact color and 16% were within one color on the color chart; 100% of the surface water and U.S. DW samples matched the exact color; and 83% of the Columbus, OH, DW samples matched exactly, with the remaining 17% within one color. For all microcuvette results, 64% of the PT samples matched exactly and 36% were within one color; 56% of the surface water samples matched exactly, with 44% being within one color. For U.S. DW, these numbers were 88% and 12%, respectively. For the Columbus, OH, DW samples, 66% matched exactly, 17% were within one color, and 17% were not within one color on the color chart.

6.2 Precision

Tables 6-4a-d show the RSD of the cyanide analysis results from PT samples; surface water; DW from around the U.S.; and DW from Columbus, OH, respectively, for the Cyanide ReagentStrip™ test kit and the reference method. Results are shown for both units that were tested. RSDs were not calculated for results reported as less than the reporting limit for the Cyanide ReagentStrip™ test kit. The RSD values shown in Tables 6-4a-d can be summarized by the range of RSDs observed with different sample sets. For example, the RSDs ranged from 4 to 86% for the PT samples

Table 6-1c. Cyanide Results from U.S. Drinking Water

Ref. Conc. (mg/L)	Non-technical Operator				Technical Operator			
	Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)	Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)
Des Moines, IA, Background								
<0.005	<0.01	<0.01	<0.1	0	0.01	<0.01	<0.1	0
<0.005	0.02	0.02	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
Des Moines, IA, LFM								
0.208	0.14	0.14	0.5	0.5	0.16	0.15	0.5	0.15
0.207	0.15	0.15	0.5	0.5	0.15	0.14	0.5	0.15
0.203	0.14	0.14	0.5	0.2	0.15	0.16	0.5	0.2
0.206	0.12	0.13	0.5	0.2	0.18	0.18	0.5	0.15
Flagstaff, AZ, Background								
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
Flagstaff, AZ, LFM								
0.22	0.11	0.11	0.5	0.5	0.12	0.12	0.5	0.2
0.193	0.12	0.14	0.5	0.5	0.11	0.12	0.5	0.2
0.206	0.11	0.11	0.5	0.5	0.14	0.14	0.5	0.2
0.206	0.16	0.18	0.5	0.5	0.12	0.12	0.5	0.15
Montpelier, VT, Background								
<0.005	0.01	0.01	<0.1	0	<0.01	<0.01	<0.1	0
0.018	0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
0.33	<0.01	<0.01	<0.1	0	0.01	<0.01	<0.1	0
<0.005	<0.01	0.01	<0.1	0	0.01	<0.01	<0.1	0
Montpelier, VT, Background								
0.187	0.15	0.15	0.5	0.2	0.18	0.18	0.5	0.2
0.194	0.17	0.17	0.5	0.2	0.21	0.21	0.5	0.2
0.197	0.15	0.15	0.5	0.2	0.18	0.18	0.5	0.2
0.198	0.17	0.18	0.5	0.2	0.21	0.21	0.5	0.2
Seattle, WA, Background								
<0.005	<0.01	<0.01	<0.1	0	0.01	0.01	<0.1	0
<0.005	0.02	0.02	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	0.01	0.02	<0.1	0	0.01	<0.01	<0.1	0
Seattle, WA, LFM								
0.199	0.15	0.16	0.5	0.2	0.17	0.18	0.5	0.2
0.171	0.14	0.14	0.5	0.2	0.19	0.20	0.5	0.2
0.202	0.15	0.15	0.5	0.2	0.20	0.21	0.5	0.2
0.188	0.15	0.17	0.5	0.2	0.15	0.16	0.5	0.2
Tallahassee, FL, Background								
<0.005	<0.01	<0.01	<0.1	0	0.02	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	0.02	0.02	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
Tallahassee, FL, Background								
0.208	0.15	0.16	0.5	0.5	0.17	0.16	0.5	0.2
0.21	0.16	0.16	0.5	0.5	0.16	0.14	0.5	0.15
0.231	0.16	0.17	0.5	0.5	0.16	0.17	0.5	0.15
0.257	0.15	0.15	0.5	0.5	0.18	0.17	0.5	0.2



exact match to the color that should represent the reference laboratory result



within one color of the color that should represent the reference laboratory result

Table 6-1d. Cyanide Results from Columbus, OH, Drinking Water

Ref. Conc. (mg/L)	Non-technical Operator ^(a)				Technical Operator			
	Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)	Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)
City Water Background - Outdoor Field Site								
<0.005	0.01	0.01	<0.1	0	0.01	0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	0.02	0.02	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
City Water Background - Indoor Field Site								
<0.005	<0.01	<0.01	<0.1	0	0.01	0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	0.01	0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	0.02	0.02	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	0.01	0.02	<0.1	0
City Water Background - Lab								
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
City LFM - Outdoor Field Site								
0.203	0.05	0.05	<0.1	0.05	0.04	0.06	<0.1	0.05
0.191	0.03	0.03	<0.1	0.05	<0.01	0.01	<0.1	0.05
0.187	0.02	0.03	<0.1	0.05	0.03	0.03	<0.1	0.05
0.201	0.05	0.06	<0.1	0.05	<0.01	<0.01	<0.1	0
City LFM - Indoor Field Site								
0.203	0.23	0.25	0.5	0.5	0.25	0.26	0.5	0.5
0.191	0.16	0.18	0.5	0.5	0.24	0.26	0.5	0.5
0.187	0.22	0.24	0.5	0.5	0.25	0.26	0.5	0.5
0.201	0.27	0.28	0.5	0.5	0.24	0.24	0.5	0.5
City LFM - Lab								
0.203	0.19	0.21	0.5	0.5	0.24	0.24	0.5	0.5
0.191	0.20	0.23	0.5	0.2	0.23	0.24	0.5	0.5
0.187	0.16	0.16	0.5	0.2	0.25	0.26	0.5	0.5
0.201	0.21	0.20	0.5	0.5	0.19	0.21	0.5	0.5
Well Water Background - Outdoor Field Site								
<0.005	<0.01	<0.01	<0.1	0	0.06	0.05	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	0.04	0.05	<0.1	0
<0.005	0.05	0.05	<0.1	0	<0.01	<0.01	<0.1	0
Well Water Background - Indoor Field Site								
<0.005	<0.01	<0.01	<0.1	0	0.01	0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	0.05	0.05	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	0.02	0.02	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	0.01	0.01	<0.1	0

Table 6-1d. Cyanide Results from Columbus, OH, Drinking Water (continued)

Ref. Conc. (mg/L)	Non-technical Operator ^(a)				Technical Operator			
	Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)	Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)
Well Water Background - Lab								
<0.005	<0.01	<0.01	<0.1	0	0.01	0.01	<0.1	0
<0.005	<0.01	<0.01	<0.1	0	0.01	0.01	<0.1	0
<0.005	0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
<0.005	0.01	<0.01	<0.1	0	<0.01	<0.01	<0.1	0
Well Water LFM - Outdoor Field Site								
0.188	0.01	0.01	<0.1	0	0.07	0.08	<0.1	0.05
0.186	0.05	0.06	<0.1	0.05	0.02	0.01	<0.1	0.05
0.205	0.02	0.03	<0.1	0	0.04	0.05	<0.1	0.05
0.18	0.02	0.02	<0.1	0	<0.01	<0.01	<0.1	0
Well Water LFM - Indoor Field Site								
0.188	0.12	0.13	0.5	0.5	0.20	0.21	0.5	0.15
0.186	0.08	0.09	0.5	0.2	0.21	0.21	0.5	0.2
0.205	0.12	0.12	0.5	0.5	0.18	0.19	0.5	0.15
0.18	0.12	0.12	0.5	0.2	0.15	0.16	0.5	0.2
Well Water LFM - Lab								
0.188	0.14	0.15	0.5	0.2	0.14	0.14	0.5	0.2
0.186	0.11	0.13	0.5	0.2	0.14	0.15	0.5	0.2
0.205	0.10	0.11	0.5	0.2	0.16	0.16	0.5	0.2
0.18	0.11	0.11	0.5	0.2	0.13	0.14	0.5	0.2

^(a) The non-technical operator's outdoor field results were recorded after a 40-minute reaction time.

exact match to the color that should represent the reference laboratory result
 within one color of the color that should represent the reference laboratory result
 within two colors of the color that should represent the reference laboratory result

Table 6-2a. Percent Accuracy of Performance Test Sample Measurements

Sample Concentration (mg/L)	Non-technical Operator		Technical Operator	
	Unit #1 (% bias)	Unit #2 (% bias)	Unit #1 (% bias)	Unit #2 (% bias)
0.03	-9	-9	-47	-47
0.1	-28	-23	-45	-34
0.2	-38	-32	-32	-30
0.4	-3	4	-30	-28
1	1	11	-17	-8
5	25	22	4	6
15	23	10	11	4
25	8	-11	1	-15

Table 6-2b. Percent Accuracy of Surface Water Measurements

Non-technical Operator		Technical Operator	
Unit #1 (% bias)	Unit #2 (% bias)	Unit #1 (% bias)	Unit #2 (% bias)
Alum Creek LFM			
-22	-27	-19	-16
Olentangy River LFM			
17	15	23	28

Table 6-2c. Percent Accuracy of U.S. Drinking Water Measurements

Non-technical Operator		Technical Operator	
Unit #1 (% bias)	Unit #2 (% bias)	Unit #1 (% bias)	Unit #2 (% bias)
Des Moines, IA, LFM			
-33	-32	-22	-24
Flagstaff, AZ, LFM			
-39	-35	-41	-39
Montpelier, VT, LFM			
-15	-14	3	3
Seattle, WA, LFM			
-22	-18	-7	-1
Tallahassee, FL, LFM			
-32	-29	-26	-29

Table 6-2d. Percent Accuracy of Columbus, OH, Drinking Water Measurements

Non-technical Operator ^(a)		Technical Operator	
Unit #1 (% bias)	Unit #2 (% bias)	Unit #1 (% bias)	Unit #2 (% bias)
City Water LFM - Outdoor Field Site			
-81	-78	-91	-87
City Water LFM - Indoor Field Site			
13	21	25	30
City Water LFM - Lab			
-3	2	16	21
Well Water LFM - Outdoor Field Site			
-87	-84	-83	-82
Well Water LFM - Indoor Field Site			
-42	-39	-3	1
Well Water LFM - Lab			
-39	-34	-25	-22

^(a) The non-technical operator's outdoor field results were recorded after a 40-minute reaction time.

Table 6-3. Semi-Quantitative Accuracy Evaluation

Sample Type (number of samples)	Qualitative Criteria	Strip Results			Microcuvette Results		
		Non-technical	Technical	Overall	Non-technical	Technical	Overall
PT (32)	Exact Color Match	81% (26)	88% (28)	84% (54)	47% (15)	81% (26)	64% (41)
	Within One Color	19% (6)	13% (4)	16% (6)	53% (17)	19% (6)	36% (23)
	Not Within One Color	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Surface (16)	Exact Color Match	100% (16)	100% (16)	100% (32)	50% (8)	62% (10)	56% (18)
	Within One Color	0% (0)	0% (0)	0% (0)	50% (8)	38% (6)	44% (14)
	Not Within One Color	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
U.S. DW (40)	Exact Color Match	100% (40)	100% (40)	100% (80)	75% (30)	100% (40)	88% (70)
	Within One Color	0%	0%	0% (0)	25% (10)	0% (0)	12% (10)
	Not Within One Color	0%	0%	0% (0)	0% (0)	0% (0)	0% (0)
Columbus, OH, DW (32)	Exact Color Match	83% (40)	83% (40)	83% (80)	66% (32)	66% (32)	66% (64)
	Within One Color	17% (8)	17% (8)	17% (16)	17% (8)	17% (8)	17% (16)
	Not Within One Color	0% (0)	0% (0)	0% (0)	17% (8)	17% (8)	17% (16)

Table 6-4a. Precision of Performance Test Measurements

Sample Conc. (mg/L)	Ref. Method (% RSD)	Non-technical Operator				Technical Operator			
		Unit #1 (% RSD)	Unit #2 (% RSD)	Strip	Microcuvette	Unit #1 (% RSD)	Unit #2 (% RSD)	Strip	Microcuvette
0.03	19	27	27	Yes	Yes	55	86	Yes	Yes
0.1	17	20	20	No	No	16	24	No	Yes
0.2	9	21	21	Yes	Yes	4	4	Yes	No
0.4	5	18	27	No	Yes	9	7	Yes	No
1	4	10	10	Yes	Yes	18	23	No	Yes
5	3	14	15	Yes	Yes	7	4	No	Yes
15	8	30	22	Yes	No	11	10	Yes	Yes
25	5	19	10	Yes	No	22	17	Yes	Yes

“Yes” or “no” indicates whether or not the four replicate samples generated the same results.

Table 6-4b. Precision of Surface Water Measurements

Ref. Method (% RSD)	Non-technical Operator				Technical Operator			
	Unit #1 (% RSD)	Unit #2 (% RSD)	Strip	Microcuvette	Unit #1 (% RSD)	Unit #2 (% RSD)	Strip	Microcuvette
Alum Creek LFM								
2	23	30	Yes	Yes	12	14	Yes	No
Olentangy River LFM								
5	11	11	Yes	Yes	16	16	Yes	No

“Yes” or “no” indicates whether or not the four replicate samples generated the same results.

Table 6-4c. Precision of U.S. Drinking Water Measurements

Ref. Method (% RSD)	Non-technical Operator				Technical Operator			
	Unit #1 (% RSD)	Unit #2 (% RSD)	Strip	Microcuvette	Unit #1 (% RSD)	Unit #2 (% RSD)	Strip	Microcuvette
Des Moines, IA, LFM								
1	9	6	Yes	No	9	11	Yes	Yes
Flagstaff, AZ, LFM								
5	19	25	Yes	Yes	10	8	Yes	Yes
Montpelier, VT, LFM								
3	7	9	Yes	Yes	9	9	Yes	Yes
Seattle, WA, LFM								
7	3	8	Yes	Yes	12	12	Yes	Yes
Tallahassee, FL, LFM								
10	4	5	Yes	Yes	6	9	Yes	Yes

“Yes” or “no” indicates whether or not the four replicate samples generated the same results.

Table 6-4d. Precision of Columbus, OH, Drinking Water Measurements

Ref. Method (% RSD)	Non-technical Operator				Technical Operator			
	Unit #1 (% RSD)	Unit #2 (% RSD)	Strip	Microcuvette	Unit #1 (% RSD)	Unit #2 (% RSD)	Strip	Microcuvette
City Water LFM - Outdoor Field Site^(a)								
4	40	35	Yes	Yes	118	106	Yes	No
City Water LFM - Indoor Field Site								
4	21	18	Yes	Yes	2	4	Yes	Yes
City Water LFM - Lab								
4	11	15	Yes	No	12	9	Yes	Yes
Well Water LFM - Outdoor Field Site^(a)								
6	69	72	No	No	92	106	Yes	No
Well Water LFM - Indoor Field Site								
6	18	15	Yes	No	14	12	Yes	Yes
Well Water LFM - Lab								
6	15	15	Yes	Yes	9	6	Yes	Yes

^(a) The non-technical operator’s outdoor field results were recorded after a 40-minute reaction time.

“Yes” or “no” indicates whether or not the four replicate samples generated the same results.

(if the 0.03 mg/L samples are removed, the upper end of that range was 27%); 11 to 30% for the surface water samples; 3 to 25% for the DW samples from around the United States; and, with the exception of the outdoor field site, 2 to 21% for the Columbus, OH, DW samples. The outdoor field site samples, as well as the 0.03 mg/L PT samples, resulted in concentrations extremely close to the detection limit; therefore, the RSDs were very high. Overall, the RSDs seemed similar regardless of the water matrix.

For the semi-quantitative results, precision was not represented by an RSD, but rather a “yes” or “no,” indicating whether the four replicate samples each generated the same result. For this

evaluation, if a result was extrapolated between two colors, the result was rounded up to the nearest color. For the strip results, the same result was obtained for all four replicates in 11 out of 16 PT sample sets, all four of the surface water sample sets, all 10 U.S. DW sample sets, and 11 out of 12 sample sets of Columbus, OH, DW. For the microcuvettes, consistent results were obtained for 11 of 16 PT sample sets, 2 out of 4 surface water sample sets, 9 out of 10 U.S. DW sample sets, and 7 out of 12 sample sets of Columbus, OH, DW.

6.3 Linearity

The linearity of the Cyanide ReagentStrip™ test kit was assessed by using a linear regression of the quantitative PT results (0.03 to 25 mg/L) against the reference method results (Table 6-1a). Figures 6-1 and 6-2 show scatter plots of the results from the non-technical and technical operators, respectively, versus the reference results. A dotted regression line with a slope of unity and intercept of zero also is shown in Figures 6-1 and 6-2. A linear regression of the data in Figure 6-1 for the non-technical operator gives the following regression equation.

$$y \text{ (non-technical operator results in mg/L)} = 1.03 (\pm 0.06)x \text{ (reference result in mg/L)} \\ + 0.19 (\pm 0.55) \text{ mg/L with } r^2 = 0.947 \text{ and } N = 72 \text{ (64 PT and 8 MDL).}$$

where the values in parentheses represent the 95% confidence interval (two times the standard error) of the slope and intercept, r^2 is the coefficient of determination, and N is the total number of results with corresponding reference analyses.

A linear regression of the data in Figure 6-2 for the technical operator gives the following regression equation:

$$y \text{ (technical operator results in mg/L)} = 0.97 (\pm 0.04)x \text{ (reference result in mg/L)} \\ + 0.07 (\pm 0.42) \text{ mg/L with } r^2 = 0.965 \text{ and } N = 72 \text{ (64 PT and 8 MDL).}$$

The slopes of these regressions are not significantly different from unity, neither intercept is significantly different from zero, and the r^2 values are both above 0.94.

Because of the 0.2-mg/L EPA maximum contaminant level for cyanide, this type of cyanide detection technology is often used to measure concentrations near the low end of the 0.03 to 25 mg/L concentration range. The linearity of the performance test samples ranging in concentration from 0.03 to 1 mg/L is shown in Figures 6-3 and 6-4. A linear regression of the data in Figure 6-3 for the non-technical operator gives the following regression equation:

$$y \text{ (non-technical operator results in mg/L)} = 1.08 (\pm 0.05)x \text{ (reference result in mg/L)} \\ - 0.03 (\pm 0.02) \text{ mg/L with } r^2 = 0.973 \text{ and } N = 48 \text{ (40 PT and 8 MDL).}$$

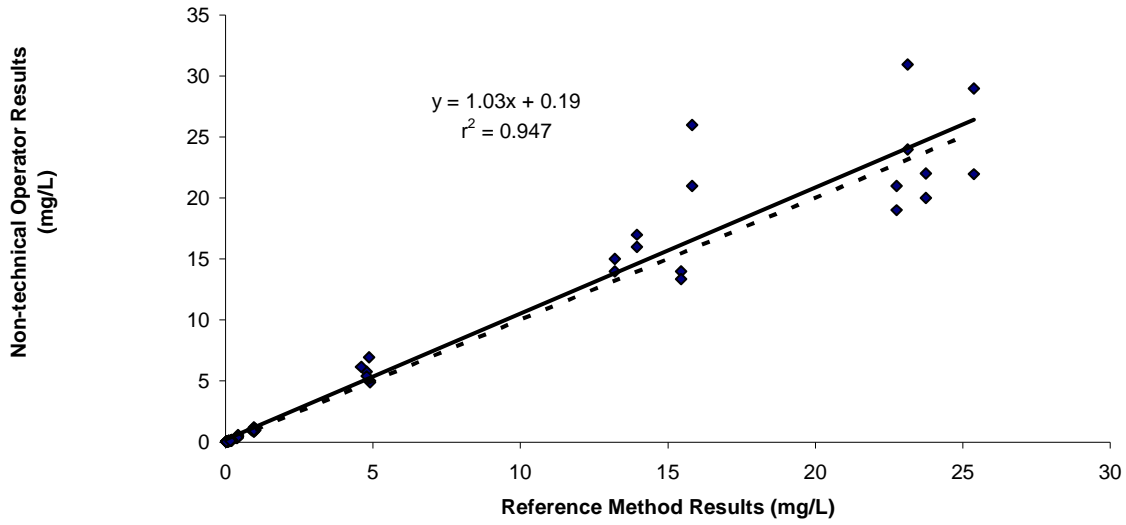


Figure 6-1. Non-technical Operator Linearity Results (0.03 to 25 mg/L)

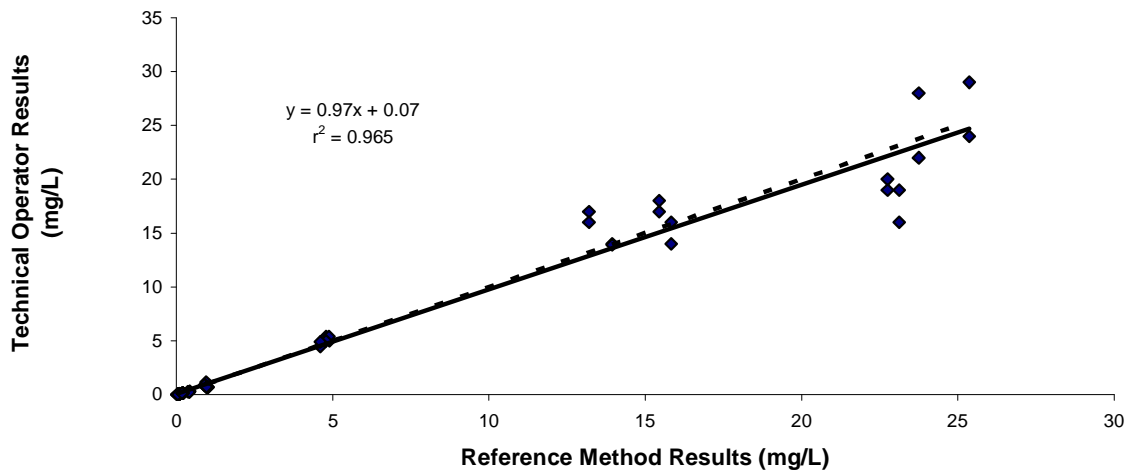


Figure 6-2. Technical Operator Linearity Results (0.03 to 25 mg/L)

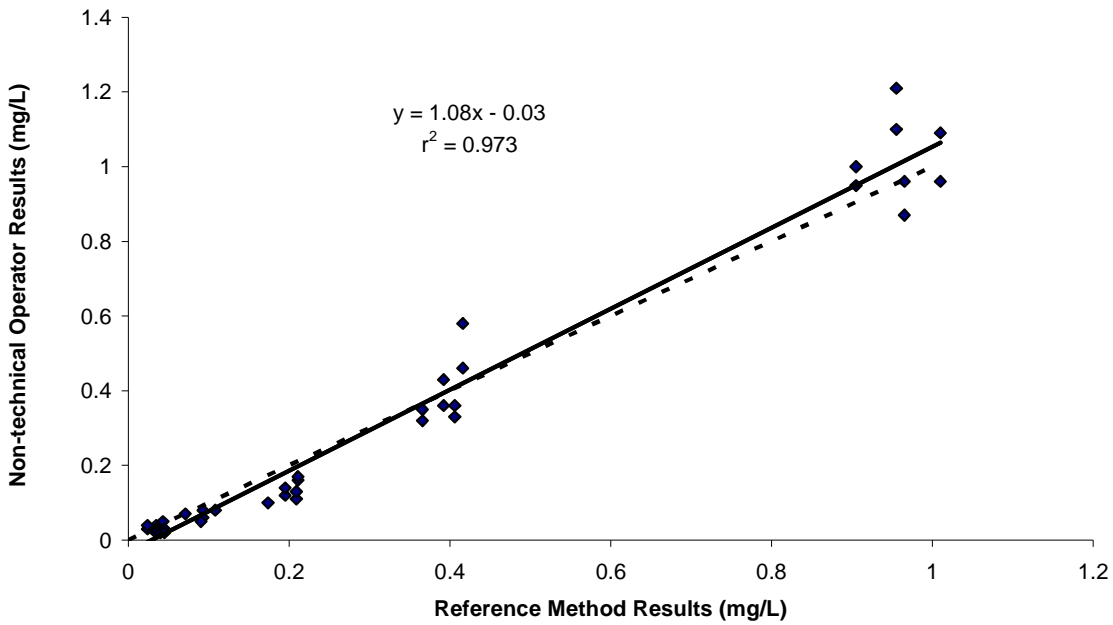


Figure 6-3. Non-technical Operator Linearity Results (0.03 to 1 mg/L)

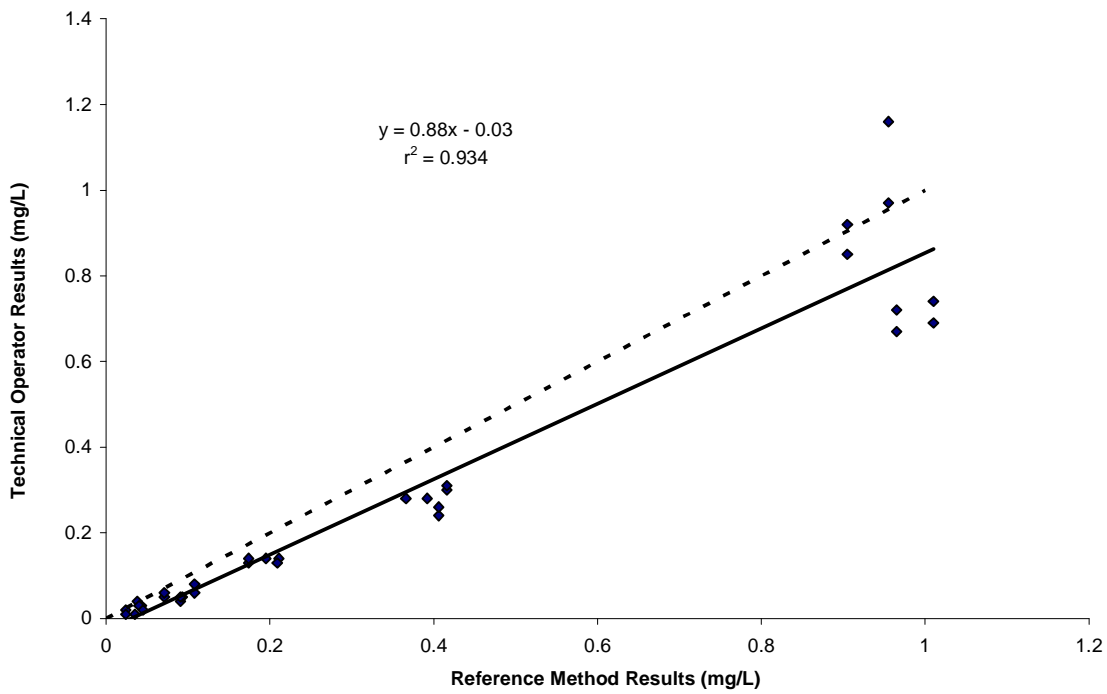


Figure 6-4. Technical Operator Linearity Results (0.03 to 1 mg/L)

A linear regression of the data in Figure 6-4 for the technical operator gives the following regression equation:

$$y \text{ (technical operator results in mg/L)} = 0.88 (\pm 0.07)x \text{ (reference result in mg/L)} \\ - 0.03 (\pm 0.03) \text{ mg/L with } r^2 = 0.934 \text{ and } N = 48 \text{ (40 PT and 8 MDL).}$$

Within this smaller concentration range, both slopes were significantly different from unity, while the intercepts were either not significantly different from zero or very close to it. This suggests that the non-technical operator's results have a slightly high bias, and the technical operator's results have a slightly low bias. This effect was not apparent with the wider concentration range. Linearity was not evaluated for the semi-quantitative results because the colors on the color charts encompassed discrete concentration ranges.

6.4 Method Detection Limit

The manufacturer's estimated detection limit for the Cyanide ReagentStrip™ test kit is 0.01 mg/L cyanide. The MDL⁽⁴⁾ was determined by analyzing seven replicate samples at a concentration of 0.05 mg/L. Table 6-5 shows the results of the MDL assessment. The MDLs determined as described in Equation (6) of Section 5.4 were 0.04 and 0.03 mg/L when used by the non-technical operator and 0.02 for both units when used by the technical operator. A quantitative evaluation of MDL was not performed for the semi-quantitative strips and microcuvettes. However, the results for the 0.05-mg/L samples are shown in Table 6-5. In all cases, the strips had non-detectable (i.e., <0.1 mg/L) results, and all of the microcuvette results were 0.05 mg/L. In both cases, the results were as expected for this concentration. An evaluation of the PT sample results reveals that the strips generated detectable results at all concentration levels 0.2 mg/L and above and the microcuvette at all concentration levels tested (0.03 mg/L and above).

6.5 Inter-Unit Reproducibility

The inter-unit reproducibility of the ReagentStrip™ Reader was assessed by using a linear regression of the results produced by one ReagentStrip™ Reader plotted against the results produced by the other ReagentStrip™ Reader. The results from all of the samples that had detectable amounts of cyanide (including the PT, surface, and DW samples) were included in this regression. Figure 6-5 shows a scatter plot of the results from both Cyanide ReagentStrip™ test kits. A dotted regression line with a slope of unity and intercept of zero also is shown in Figure 6-5.

Table 6-5. Results of Method Detection Limit Assessment

Sample Conc. (mg/L)	Non-technical Operator				Technical Operator			
	Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)	Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)
PT-0.05	0.05	0.05	<0.1	0.05	0.03	0.03	<0.1	0.05
PT-0.05	0.02	0.02	<0.1	0.05	0.02	0.03	<0.1	0.05
PT-0.05	0.03	0.03	<0.1	0.05	0.02	0.02	<0.1	0.05
PT-0.05	0.02	0.02	<0.1	0.05	0.02	0.02	<0.1	0.05
PT-0.05	0.03	0.03	<0.1	0.05	0.03	0.03	<0.1	0.05
PT-0.05	0.04	0.03	<0.1	0.05	0.02	0.02	<0.1	0.05
PT-0.05	0.04	0.03	<0.1	0.05	0.03	0.02	<0.1	0.05
Std. Dev.	1.11×10^{-2}	1.00×10^{-2}	NA ^(a)	NA	5.34×10^{-3}	5.35×10^{-3}	NA	NA
t (n=7)	3.14	3.14	NA	NA	3.14	3.14	NA	NA
MDL	0.04	0.03	NA	NA	0.02	0.02	NA	NA

^(a) NA = Not applicable because a statistical evaluation of MDL for the semi-quantitative results was not appropriate.

exact match to the color that should represent the reference laboratory results

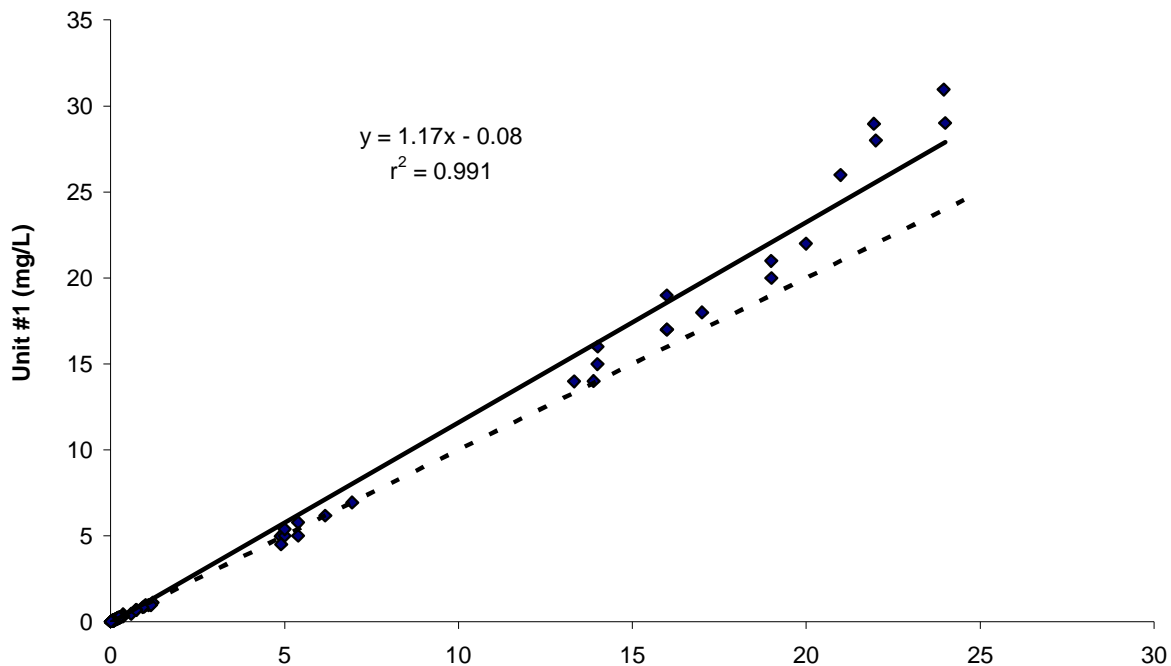


Figure 6-5. Inter-Unit Reproducibility Results

A linear regression of the data in Figure 6-5 for the inter-unit reproducibility assessment gives the following regression equation:

$$y \text{ (Unit \#1 result in mg/L)} = 1.17 (\pm 0.02)x \text{ (Unit \#2 in mg/L)} - 0.08 (\pm 0.08) \text{ mg/L}$$

with $r^2 = 0.991$ and $N = 213$ (includes all pairs of detectable results).

The intercept was not significantly different from zero; however, the slope was significantly larger than unity, suggesting that, in general, Unit #1 gave higher responses than Unit #2.

6.6 Lethal or Near-Lethal Dose Response

Samples at 50-, 100-, and 250-mg/L concentrations (close to what may be lethal if a volume the size of a typical glass of water were ingested) were analyzed by the Cyanide ReagentStrip™ test kit. Table 6-6 presents the measured cyanide results from analysis of the lethal/near-lethal concentration samples for both the reference analyses and the Cyanide ReagentStrip™ test kit. Results are shown in Table 6-6 for both ReagentStrip™ Readers. The vendor states in the instructions that cyanide concentrations above 40 mg/L exceed the linear range of the ReagentStrip™ Reader and need to be diluted to obtain quantitative results. Because of the various lower concentrations that were tested as PT samples, additional dilutions were not performed. The ReagentStrip™ Reader results for these concentrations ranged from 36 to >60 mg/L (the response to indicate that the top of the linear range of the ReagentStrip™ Reader has been reached). For the 50-mg/L samples, 11 out of 16 results produced a >60-mg/L result. For the 100- and 200-mg/L samples, 23 out of 32 results correctly produced a >60-mg/L result.

Table 6-6. Lethal/Near-Lethal Concentration Sample Results

Prepared Conc. (mg/L)	Ref. Conc. (mg/L)	Non-technical Operator				Technical Operator			
		Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)	Unit #1 (mg/L)	Unit #2 (mg/L)	Strip (mg/L)	Microcuvette (mg/L)
50	44.5	>60	>60	>10	>3	>60	>60	>10	>20
50	45.8	>60	>60	>10	>20	>60	41	>10	>20
50	45.5	>60	46	>10	>3	>60	38	>10	>20
50	49.8	>60	50	>10	>3	>60	55	>10	>20
100	102	>60	55	>10	>20	>60	>60	>10	>20
100	95.5	>60	>60	>10	>20	>60	>60	>10	>20
100	102	>60	60	>10	>3	>60	>60	>10	>20
100	94	>60	>60	>10	>3	>60	>60	>10	>20
250	265	>60	>60	>10	>20	>60	50	>10	>20
250	245	55	36	>10	>20	>60	36	>10	>20
250	239	>60	60	>10	>3	>60	41	>10	>20
250	253	>60	>60	>10	>3	>60	55	>10	>20

exact match to the color that should represent the reference laboratory result
 within one color of the color that should represent the reference laboratory result
 within two colors of the color that should represent the reference laboratory result

For the semi-quantitative strips, all 12 samples for both operators correctly resulted in a >10-mg/L (the highest concentration color) result. For the microcuvettes, all 12 samples tested by the technical operator resulted in >20-mg/L results. Eight of these (50 and 100 mg/L) were an exact color match and four were within one color. The microcuvettes analyzed by the non-technical operator generated results that included 3 exact color matches, 7 within one color, and 2 that were more than one color from the chart color that should have matched the reference result. For both operators, the 250-mg/L samples did not change to the color representing the >200-mg/L concentration. Considering only a visual observation of the lethal dose sample analysis process, the color changes on the strips and of the solution in the microcuvettes were quick and pronounced with respect to the much lower concentrations. In this manner, the presence of a high concentration of cyanide was easily ascertained through the use of all three detection mechanisms.

6.7 Operator Bias

The possible difference in results produced by the non-technical and technical operators was assessed by using a linear regression of the results produced by the non-technical operator plotted against the results produced by the technical operator. The results from all of the samples that had detectable amounts of cyanide (including the PT, surface, and DW samples) from both technologies were included in this regression. The total number of results with corresponding reference analyses (N) is smaller here than for the inter-unit comparability because of the large number of samples for which one unit or the other was non-detectable. Figure 6-6 shows a scatter plot of the results from both technologies. A dotted regression line with a slope of unity and intercept of zero also is shown in Figure 6-6. A linear regression of the data in Figure 6-6 for the inter-unit comparability assessment gives the following regression equation:

$$y \text{ (non-technical operator result in mg/L)} = 1.04 (\pm 0.04)x \text{ (technical operator result in mg/L)} + 0.08 (\pm 0.26) \text{ mg/L with } r^2 = 0.936 \text{ and } N = 167 \text{ (includes all pairs of detectable results).}$$

This regression, with a slope and intercept that are not different from unity and zero, respectively, suggests that the results generated by both operators were not different from one another. This is consistent with the wider concentration range linearity results. However, the linearity results over the concentration range of 0.03 to 1 mg/L suggested that the non-technical operator's results were biased slightly high, while the technical operator's results were biased slightly low. In this instance, because neither operator's results are necessarily closer to the reference method result (as the linearity data show), it is unlikely that the difference between the two operators was related to differences in training or experience. It is apparently the result of the normal variability of two different people performing the analyses at these lower concentrations.

For the semi-quantitative strip and microcuvette results, the operator-specific data were evaluated by evaluating the number of results in which one operator's results were more than one color chart color different from the other operator's result. For example, three of the colors on the microcuvette color chart represent, in order, 0.2, 0.5, and >3 mg/L; therefore, if one operator determined that the color of the microcuvette was most similar to the >3-mg/L color and the other

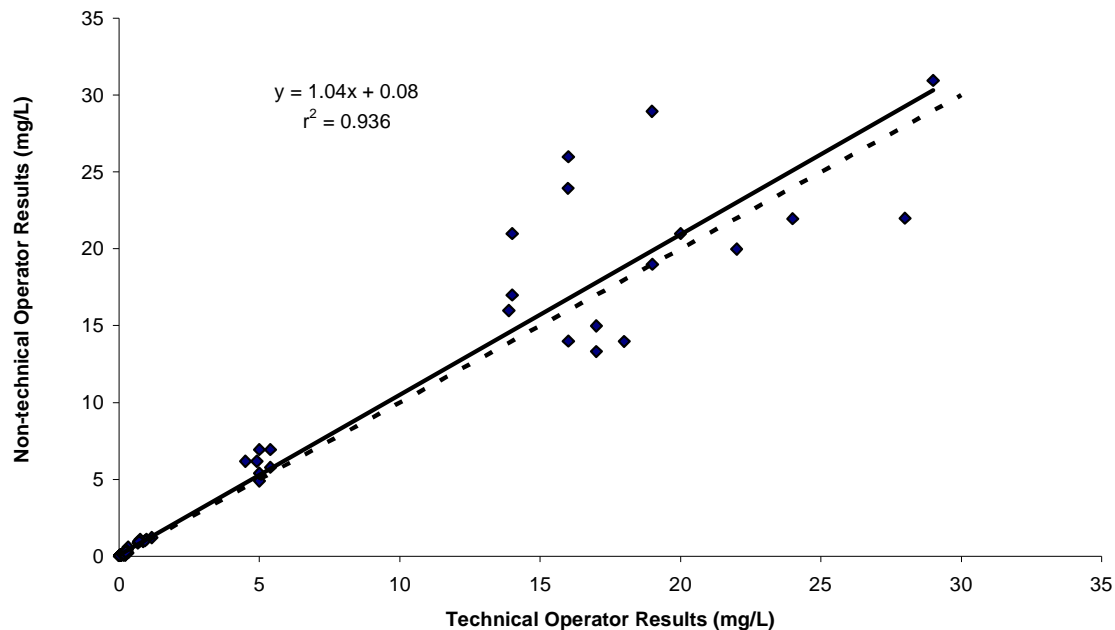


Figure 6-6. Operator Bias Results

operator determined that it was most similar to the 0.2-mg/L color, that would be considered more than one color different. For the strips, all of the results were within one color. For the microcuvettes, one out of 108 results was more than one color different from one another. This outlier had one result as the >3-mg/L color and one as 0.2 mg/L. The color closest to representing the reference concentration (0.4 mg/L) in this case would have been the 0.5 mg/L color. For this comparison, all extrapolations between two colors were rounded up.

For the lethal/near-lethal samples, the non-technical operator's result was a >3-mg/L result in 7 out of 12 instances when the seemingly correct result (according to the reference concentration) and the result of the technical operator was >20 mg/L. These colors are adjacent to one another on the color chart, but the results stood out from the rest because the concentrations are so different. Similarly, for the 0.4-mg/L and 1-mg/L PT samples, the non-technical operator's result was >3 mg/L, while the technical operator's was (seemingly more correctly) 0.5 mg/L. Overall, it seems that the non-technical operator had some difficulty distinguishing between the colors on the microcuvette color chart, especially between the 0.2 and 0.5, 0.5 and >3 mg/L, and >3 mg/L and >20 mg/L colors. The discrepancy between the results generated by the two operators may have more to do with the vision of the operators than their level of education and experience. While the non-technical operator was not previously aware of any color vision deficiency, this may have had some impact on the results.

6.8 Field Portability

The Cyanide ReagentStrip™ test kit was operated in laboratory and field settings during this verification test. Tables 6-1d, 6-2d, and 6-3d show the results of these measurements. From an operational standpoint, the Cyanide ReagentStrip™ test kit was easily transported to the field

setting, and the samples were analyzed in the same fashion as they were in the laboratory. No functional aspects of the Cyanide ReagentStrip™ test kit were compromised by performing the analyses in the field setting. However, performing analyses under cool conditions negatively affected the performance of the reagents. The low temperatures severely inhibited the chemical reaction rates, which decreased the color formation in the LFM samples spiked with cyanide.

Table 6-2d shows the bias of the samples analyzed in the field settings (indoors with sample temperatures of approximately 21°C and outdoors with sample temperatures of 17°C) and of the identical samples analyzed in the laboratory at approximately 21°C. The samples analyzed outdoors had extremely large negative biases ranging from -78 to -91%. According to the Cyanide ReagentStrip™ test kit instructions, a 20-minute reaction time was to be used because of the sample temperature of 17°C. Because of the lack of color development after 20 minutes, several samples were analyzed after a 40-minute reaction time to see if additional time would improve the results. When little or no additional color development took place, data again were collected after the 20-minute reaction time. The non-technical operator did not revert back to the 20-minute reaction time; therefore, the non-technical results collected outdoors represent a 40-minute reaction time.

No trend in the accuracy of samples analyzed in the laboratory and the indoor field location was apparent. For both operators, bias results from the laboratory ranged from -39 to +21% and from the indoor field location from -42 to +30%. Both operators had examples of very low biases for these samples. For example, at the laboratory using the city water sample, the non-technical operator had biases of -3 and +2%. Similarly, at the indoor field location using well water, the technical operator produced biases of -3 and 1%. However, as the above ranges indicate, there were examples with larger biases above and below the target concentration. With the exception of the low-temperature analyses, the Cyanide ReagentStrip™ test kit performance at the field location was similar to that in the laboratory.

6.9 Ease of Use

The Cyanide ReagentStrip™ test kit was easy to operate. The written instructions provided were clear, and the accompanying instructional video (lasting less than five minutes) was detailed and easy to understand. Because the required reagents were transferred into the test sample entirely by the repeated dipping of the two types of ReagentStrips, there was no measuring or mixing. The operators only had to hold strictly to the appropriate color development time as given in the instructions. The ReagentStrip™ Reader also was easy to use; the sample microcuvette was inserted and one button pushed to read the absorbance value. That absorbance value was compared to a table of absorbances to determine the corresponding cyanide concentration. Water samples within a pH range of 5 and 11 could be analyzed directly without pH adjustment. Samples with pHs outside that range required adjustment using NaOH or hydrochloric acid. This step required the availability of acid and/or base, pH paper or meter, and some knowledge of pH adjustment. Instructions for pH adjustment were not provided. Cleanup was simple and free of mess.

The semi-quantitative ReagentStrips and microcuvettes were also easy to use. For the strips, the color at the end of a paper strip was compared to a color chart immediately after dipping the strip into the test sample. For the microcuvettes, the color of the test solution after the color development time was compared to another color chart. Both color charts were conveniently designed to compare colors (see Figure 3-2). The color bar on the ReagentStrip color chart was in the shape of the end of a ReagentStrip so the strip could be held directly between two colors and compared. Similarly, the shape of the colors on the microcuvette color chart were outlines of squares of a size that allowed the microcuvettes to fit within the outline. The operators could look down at the sample in the microcuvette and compare the color of the solution to the color of the outlined squares.

6.10 Sample Throughput

Sample preparation, including accurate volume measurement and the addition of reagents (i.e., dipping the ReagentStrips) took only one to two minutes per sample. After preparing the sample, typically a 10-minute period of color development was required before sample analysis (because the sample temperature in the laboratory was approximately 21°C). Therefore, if only one sample is analyzed, it would take approximately 12 minutes. However, both operators were able to stagger the start of the color development period every two minutes for subsequent samples, so a typical sample set of 10 analyses took 30 to 40 minutes. The strip results were available within 1 minute of measuring the samples into the sample microcuvette.

Chapter 7 Performance Summary

The quantitative accuracy of the ReagentStrip™ Reader-derived results were evaluated by calculating biases with respect to the reference concentrations. Biases for the Cyanide ReagentStrip™ test kit ranged from -47 to 25% for the PT samples; -27 to 28% for the surface water samples; -41 to 3% for the DW samples from around the United States; and, with the exception of the samples analyzed outdoors, -42 to 30% for the Columbus, OH, DW samples. The matrix effect on these results was apparently minimal because the range of the bias for the surface water and DW results was similar to that of the PT samples that were prepared in DI water. For the semi-quantitative accuracy results, 84% of the PT sample results matched the exact color that should represent the reference concentration, and 16% were within one color on the color chart; 100% of the surface water and U.S. DW samples matched the exact color; and 83% of the Columbus, OH, DW samples matched exactly, with the remaining 17% within one color. For the microcuvette results, 64% of the PT samples matched exactly and 36% were within one color; 56% of the surface water samples matched exactly, with 44% being within one color; 88% of the U.S. DW samples matched exactly, and 12% were within one color; and 66% of the Columbus, OH, DW samples matched exactly, 17% were within one color, and 17% were within two colors on the color chart.

The quantitative precision of the Cyanide ReagentStrip™ test kit using the ReagentStrip™ Reader was evaluated by calculating RSDs for each sample set. The RSDs ranged from 4 to 86% for the PT samples (if the 0.03 mg/L samples are removed, the upper end of that range was 27%); 11 to 30% for the surface water samples; 3 to 25% for the DW samples from around the United States; and, except for the outdoor field site, 2 to 21% for the Columbus, OH, DW samples. The RSDs were similar regardless of the water matrix. For the semi-quantitative strip evaluation, the precision was evaluated by determining the frequency by which the qualitative result (color) was produced for each sample set. The same result was obtained for four replicates in 11 out of 16 PT sample sets, all of the surface water sample sets, all 10 U.S. DW sample sets, and 11 out of 12 sample sets of Columbus, OH, DW. For the microcuvettes, consistent results were obtained for 11 of 16 PT sample sets, 2 out of 4 surface water sample sets, 9 out of 10 U.S. DW sample sets, and 7 out of 12 sample sets of Columbus, OH, DW.

A linear regression of the PT sample results, ranging in concentration from 0.03 to 25 mg/L, gave the following regression equations:

$$y \text{ (non-technical operator results in mg/L)} = 1.03 (\pm 0.06)x \text{ (reference result in mg/L)} \\ + 0.19 (\pm 0.55) \text{ mg/L with } r^2 = 0.947 \text{ and } N = 72.$$

$$y \text{ (technical operator results in mg/L)} = 0.97 (\pm 0.04)x \text{ (reference result in mg/L)} \\ + 0.08 (\pm 0.42) \text{ mg/L with } r^2 = 0.965 \text{ and } N = 72.$$

where the values in parentheses represent the 95% confidence interval (two times the standard error) of the slope and intercept, r^2 is the coefficient of determination, and N is the total number of results with corresponding reference analyses. The slopes of these regressions are not significantly different from unity, neither intercept is significantly different from zero, and the r^2 values are both above 0.94. Linear regressions were also generated for a concentration range of 0.03 to 1 mg/L. Within this smaller concentration range, both slopes were significantly different from unity, while the intercepts were either not significantly different from zero or very close to it. This suggests that the non-technical operator's results have a slightly high bias, and the technical operator's results have a slightly low bias. This effect was not apparent with the wider concentration range.

The quantitative MDL of the Cyanide ReagentStrip™ test kit was determined by analyzing seven replicate samples at a concentration of 0.05 mg/L. The MDLs were 0.04 and 0.03 mg/L for the non-technical operator and 0.02 for both units when used by the technical operator. The strips generated detectable results at all concentration levels 0.2 mg/L and above, and the microcuvettes generated detectable results at all concentrations 0.03 mg/L and above.

A linear regression of the data for the inter-unit reproducibility assessment gave the following regression equation:

$$y \text{ (Unit \#1 result in mg/L)} = 1.17 (\pm 0.02)x \text{ (Unit \#2 in mg/L)} - 0.08 (\pm 0.08) \text{ mg/L} \\ \text{with } r^2 = 0.991 \text{ and } N = 213.$$

The intercept was not significantly different from zero; however, the slope was significantly larger than unity, suggesting that, on average, Unit #1 gave slightly higher responses than Unit #2.

The ReagentStrip™ Reader results for lethal to near-lethal concentrations ranged from 36 to >60 mg/L. According to the vendor, concentrations >40 mg/L exceed the linear range of the ReagentStrip™ Reader so these results would need to be clarified through dilution and reanalysis of samples. For the semi-quantitative strips, all 12 samples for both operators correctly resulted in a >10-mg/L (the highest concentration color) result. For the microcuvettes, all 12 samples tested by the technical operator resulted in >20-mg/L results, but the 250-mg/L samples did not appear to change to the >200 mg/L color. If the sample analysis procedure was only observed visually, the color changes on the strips and of the solution (for the lethal dose concentrations) in the microcuvettes were quick and pronounced with respect to the much lower concentrations. In this manner, the presence of a high concentration of cyanide was easily ascertained through use of all three detection mechanisms.

A linear regression of the data for the operator bias assessment gave the following regression equation:

$$y \text{ (non-technical operator result in mg/L)} = 1.04 (\pm 0.04)x \text{ (technical operator result in mg/L)} \\ + 0.08 (\pm 0.26) \text{ mg/L with } r^2 = 0.936 \text{ and } N = 167.$$

This regression, with a slope and intercept that are not different from unity and zero, respectively, suggests that the results generated by both operators were not different from one another. This is consistent with the wider concentration range linearity results. However, the linearity results over the concentration range of 0.03 to 1 mg/L suggested that the non-technical operator's results were biased slightly high, while the technical operator's results were biased slightly low. In this instance, because neither operator's results are necessarily closer to the reference method result (as the linearity data show), it is unlikely that the difference between the two operators was related to differences in training or experience. It is apparently the result of the normal variability of two different people performing the analyses at these lower concentrations. For the semi-quantitative microcuvette results, in only one out of 108 results was one operator's result more than one color different from the other operator's result. For the strips, the results from both operators were always within one color of one another.

No functional aspects of the Cyanide ReagentStrip™ test kit were compromised by performing the analyses in the field setting. However, performing analyses under cool conditions negatively affected the performance of the reagents as was evidenced by the large negative biases for the samples analyzed outdoors.

The Cyanide ReagentStrip™ test kit was easy to operate. The written instructions provided were clear, and the accompanying instructional video (lasting less than five minutes) was detailed and easy to understand. Because the required reagents were transferred into the test sample entirely by the repeated dipping of the two types of ReagentStrips™, there was no measuring or mixing. The strips, microvettes, and ReagentStrip™ Reader were easy to use; and cleanup was minimal.

The analysis of a set of approximately 10 samples, including sample preparation and reaction time, took 30 to 40 minutes.

Chapter 8 References

1. *Test/QA Plan for Verification of Portable Analyzers for Detection of Cyanide in Water*, Battelle, Columbus, Ohio, January 2003.
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3. *Quality Management Plan (QMP) for the ETV Advanced Monitoring Systems Center*, Version 5.0, U.S. EPA Environmental Technology Verification Program, Battelle, Columbus, Ohio, March 2004.
4. *Code of Federal Regulations*, Title 40, Part 136, Appendix B, “Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11.”