August 2001

Environmental Technology Verification Report

NECI F-NTK
NITRATE TEST KIT

Prepared by

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ETV ETV ETV

August 2001

Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

NECi F-NTK
Nitrate Test Kit

by

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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 permitters, 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 technology 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. In 1997, through a competitive cooperative agreement, Battelle was awarded EPA funding and support 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/07/07_main.htm.

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List of Abbreviations

AC	Alum Creek reservoir water
AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
BDL	below detection limit
CW	community water
DW	drinking water
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
HDPE	high-density polyethylene
IC	ion chromatograph
LFM	laboratory-fortified matrix
MB-B	Massachusetts Bay bottom water
MB-S	Massachusetts Bay surface water
MCL	maximum contaminant limit
MDL	method detection limit
NECi	Nitrate Elimination Co., Inc.
OR	Olentangy River water
ppb	parts per billion
ppm	parts per million
PE	performance evaluation
PT	performance test
QA	quality assurance
QC	quality control
QCS	quality control standard
QMP	Quality Management Plan
RB	reagent blank
RSD	relative standard deviation
SR	Scioto River water
TSA	technical systems audit
WW	well water

Chapter 1
Background

The U.S. Environmental Protection Agency (EPA) has created 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, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of regulators, buyers and vendor organizations; 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 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. This verification report presents the procedures and results of the verification test for the Nitrate Elimination Co., Inc. (NECi) F-NTK™ nitrate test kit.

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 F-NTK nitrate test kit. The following description of the F-NTK is based on information provided by the vendor, Nitrate Elimination Co., Inc. (NECi).

The NECi F-NTK provides the reagents and equipment necessary for analyzing for nitrate, nitrite, and total nitrate-nitrogen (nitrate-N) in environmental water samples and water-extracts of soil, plant tissue, and some foods. The F-NTK uses an enzyme-based (nitrate-N reductase) nitrate-N testing method and contains no toxic or hazardous chemicals. With the F-NTK, nitrate-N can be analyzed in two ranges of 0.5 to 10 parts per million (ppm) nitrate-N (1 ppm = 1 mg per liter) with a precision of ±1 ppm nitrate-N or 0.05 to 1.0 ppm nitrate-N with a precision of ±0.1 ppm nitrate-N.

Figure 2-1. NECi F-NTK Nitrate Test Kit

The sample results from the F-NTK nitrate test kit are evaluated using three nitrate-N standards, which are mixed and developed by the user, and a precision color chart for estimating nitrate-N content. All the necessary tools for conducting the nitrate-N tests are supplied in the F-NTK, including a test tube rack. The test kit provides semi-quantitative estimates of nitrate content. For more accurate quantitative data, the assay can be read using a colorimeter at 540 nm.

Chapter 3
Test Design and Procedures

3.1 Introduction

This verification test was conducted according to procedures specified in the *Test/QA Plan for Verification of Portable Analyzers*.[1] The verification was based on comparing results from the F-NTK test kit to those from a reference method. The reference method for nitrate-N analysis uses ion chromatography (IC) according to EPA Method 300.1[2] The F-NTK test kit was calibrated using standards supplied with the kit. The kit was tested by analyzing laboratory-prepared performance test samples and drinking water, residential tap water, reservoir, river, and ocean water with both the test kit and the reference method. The F-NTK test kit was evaluated for accuracy, precision, linearity, method detection limit, matrix interference effects, and operator bias, as well as ease of use, cost, and sample throughput.

3.2 Test Design

Two sets of the F-NTK test kit were tested independently by challenging them with test samples representative of those likely to be analyzed using the F-NTK. Each set of F-NTK test kits was used to analyze the full set of samples for nitrate-N. The preparation, calibration, and analyses were performed according to the manufacturer's recommended procedures. Results from the F-NTK test kit were recorded manually. In addition to the analytical results, the time required for sample analysis and operator observations concerning the use of the test kit (e.g., frequency of calibration, ease of use, maintenance) were recorded. The results from the F-NTK tests were compared to those from the reference method to quantitatively assess accuracy, linearity, and detection limit. Multiple aliquots of performance test samples and drinking water samples were analyzed to assess precision.

For each set of the test kit, identical sets of samples were analyzed independently by two separate operators (a technical and a non-technical Battelle staff member) to test for the existence of operator bias on the test kit performance. The technical operator was a research technician at Battelle with three years of laboratory experience and a B.S degree. The non-technical operator was a part-time laboratory helper at Battelle and a student at Ohio State University. Interferences and matrix effects were assessed by challenging the test kit with performance test samples of known nitrate-N concentrations containing both low-level and high-level interferences. Sample throughput was estimated based on the time required to analyze a sample set. Performance

parameters, such as ease of use and reliability, were evaluated based on documented observations of the operators.

3.3 Test Samples

Three types of samples were used in the verification test as shown in Table 3-1: quality control (QC) samples, performance test (PT) samples, and environmental water samples.

The QC and PT samples were prepared from purchased standards. The EPA has set the maximum contaminant limit (MCL) for nitrate at 10 ppm nitrate-N, under the Safe Drinking Water Act. However, the vendor suggested that nitrate concentrations in the natural environment are rarely above 2.0 ppm nitrate-N. For this reason, the QC sample concentrations for nitrate-N were targeted at a 2 ppm midpoint. The PT samples ranged from 10% to 1,000% of that level, i.e., from 0.2 to 20 ppm. The environmental water samples were collected from various drinking water and surface water sources. All samples were analyzed using the two F-NTK test kits and by a laboratory reference method. Every tenth sample was analyzed twice by the reference method, to document the reference method's precision.

3.3.1 QC Samples

As Table 3-1 indicates, prepared QC samples included both laboratory reagent blanks (RB) and laboratory-fortified matrix (LFM) samples. The RB samples consisted of American Society for Testing and Materials (ASTM) Type II deionized water, and were exposed to identical handling and analysis procedures as other prepared samples. These samples were used to help ensure that no sources of contamination were introduced in the sample handling and analysis procedures. Two types of LFMs were prepared. The LFM_F samples consisted of aliquots of environmental samples that were spiked in the field to increase the analyte concentration by 2 ppm nitrate-N. The spike solution used to prepare the LFM_F was prepared in the laboratory and brought to the field site. The LFM_L samples were aliquots of environmental samples that were spiked in the laboratory to increase the analyte concentration by 2.0 ppm nitrate-N. These samples were used to help identify whether matrix effects had an influence on the analytical results. At least 10% of all the prepared samples analyzed were RBs, and at least one sample taken from each sampling site was an LFM.

Quality control standards (QCS) were used as calibration checks to verify that the F-NTK and the reference instrument were properly calibrated and reading within defined control limits. These nitrate-N standards were purchased from a commercial supplier and were subject only to dilution as appropriate. Calibration of the test kit and the reference instrument was verified using a QCS before and after the testing period, as well as after every tenth sample. An additional independent QCS was used in a performance evaluation audit of the reference method.

Table 3-1. Test Samples[a] for Nitrate-N Used in Verification of the F-NTK Test Kit

Type of Sample	Sample Characteristics	Nitrate-N Concentration	No. of Samples/Analysis
Quality Control	RB[b]	~ 0	10% of all
	LFM[b]	2 ppm above native level	1 per site
	QCS[b]	2 ppm	10% of all
Performance Test	For the determination of detection limit for nitrate (PT6)	2.5 ppm (Five times the manufacturer's estimated detection limit)	1/7
	Nitrate (PT1)	0.2 ppm	1/4
	Nitrate (PT4)	0.6 ppm	1/4
	Nitrate (PT2)	2.0 ppm	1/4
	Nitrate (PT5)	6.0 ppm	1/4
	Nitrate (PT3)	20 ppm	1/4
	Analyte spiked with interference (LI)	3.0 ppm with low interference	1/8
	Analyte spiked with interference (HI)	3.0 ppm with high interference	1/8
Environmental	Drinking water (DW)	Unknown	1/4
	Community water (CW)	Unknown	1/4
	Well water (WW)	Unknown	1/4
	Alum Creek Reservoir (AC)	Unknown	4/1
	Olentangy River (OR)	Unknown	4/1
	Scioto River (SR)	Unknown	4/1
	Massachusetts Bay surface water (MB-S)	Unknown	4/1
	Massachusetts Bay water at sediment/water column interface (MB-B)	Unknown	4/1

[a] Listing is for clarity; samples were analyzed in randomized order for the verification testing.
[b] See Section 3.3.1 for descriptions of these samples.

3.3.2 PT Samples

The two types of PT samples used in this verification test (Table 3-1) were prepared in the laboratory using ASTM Type II water as the water source. One type of PT solution contained nitrate-N at various concentrations and was prepared specifically to determine F-NTK accuracy, linearity, and detection limit. To determine the detection limit of the test kit, a solution with a concentration five times the vendor's reported detection limit was used (i.e., 2.5 ppm nitrate-N). Seven nonconsecutive replicate analyses of this solution were made to obtain precision data with which to determine the method detection limit. Additionally, solutions were prepared to assess the linearity over a broad range of nitrate-N concentration. Four aliquots of each of these solutions were prepared and analyzed separately to assess the precision of the test kit.

The second type of PT sample helped establish the effects of potential matrix interferences on the performance of the F-NTK. These samples were prepared from solutions with known concentrations of nitrate-N and were spiked with potentially interfering species likely to be found in typical water samples. One sample contained low levels of interferences that consisted of 1 mg of iron, 3 mg of sodium chloride, and 0.1 mg of sulfate per liter at a pH of 6. The second sample contained high levels of interferences that consisted of 10 mg of iron, 30 mg of sodium chloride, and 1.0 mg of sulfate per liter at a pH of 3. Eight replicate samples of each of these solutions were analyzed.

3.3.3 Environmental Samples

Drinking water samples listed in Table 3-1 include drinking water from a Battelle drinking fountain (DW), and well water (WW) and community drinking water (CW) from two residential sites in Columbus, Ohio. These samples were collected directly from the tap into 2-L high-density polyethylene (HDPE) containers. Four (100-mL) aliquots of each sample were analyzed in the field at the time of collection by each set of the test kit being verified. One (100-mL) aliquot of each sample was returned to Battelle for reference analysis. The remaining collected sample was stored for later use, if necessary. These aliquots were stored at approximately 4°C and analyzed within appropriate holding times.

Freshwater samples from the Alum Creek Reservoir (AC), the Olentangy River (OR), and the Scioto River (SR) (in Columbus, Ohio) were collected in 500-mL HDPE containers. The samples were collected at the surface of the water near the shoreline by submerging the containers no more than one inch below the surface of the water. Each body of water was sampled at four distinct locations. The samples were split into three 100-mL aliquots. One aliquot of each sample was analyzed in the field at the time of collection by each set of the test kit being verified. The third aliquot of each sample was returned to Battelle for reference analysis. The remaining collected sample was stored for later use, if necessary. These aliquots were stored at approximately 4°C and analyzed within appropriate holding times.

Three 100-mL aliquots of salt water were collected at the surface of the Massachusetts Bay (MB-S) and from the sediment/water column interface (MB-B) at four distinct locations. One aliquot of each sample was analyzed at the time of collection by each set of the test kit being

verified. One aliquot of each sample was returned to Battelle for analysis by the reference method.

3.4 Reference Analysis

The reference nitrate-N analysis was performed using a Dionex DX-500 ion chromatograph (IC) according to EPA Method 300.1. The IC was equipped with a guard column, a separator column, an anion suppressor, and a conductivity detector. A cleanup step and a pretreatment cartridge were used for saltwater analysis. The instrument was calibrated with a minimum of three nitrate-N standards bracketing the range of samples to be analyzed. The samples were introduced into the instrument via an autosampler and swept through the columns using a 21 mM sodium hydroxide solution. The columns separated the nitrate-N from other anions and potential interferents. The nitrate-N was detected by the conductivity detector, providing a detection limit of 0.02 ppm for nitrate-N. The analytical results were converted from nitrate to nitrate-N.

3.5 Verification Schedule

Round 1 of the F-NTK verification test took place over a 10-day period in January 2001, at Battelle's laboratories in Columbus, Ohio, and in a subsequent three-day period in February 2001 at Battelle's Ocean Sciences Laboratory in Duxbury, Massachusetts. These two locations allowed collection of a variety of drinking water and surface water samples for use in the verification. Table 3-2 shows the daily testing activities that were conducted during these periods. During January 2001, separate days were devoted to laboratory analysis of samples at Battelle, and to collection and analysis of samples in the field. In all field locations, the collected samples were analyzed using the F-NTK test kits shortly after collection, by both the technical and the non-technical Battelle staff member. In February, the F-NTK kits were used on board a ship to analyze surface water collected from Massachusetts Bay.

As described in Section 6 of this report, the comparison between the F-NTK results and the reference results was poor for the freshwater field samples during Round 1. As a result, the vendor requested that the freshwater field sample comparison be repeated. That retesting with freshwater field samples was conducted at the vendor's expense, and is designated Round 2 testing in this verification. For this round of testing, the F-NTK kits incorporated new packaging developed by the vendor to better preserve the reagents and make the kits easier to use. Further comments on the changes in the kits are provided by the vendor in Appendix B of this report.

The Round 2 testing consisted of two phases. In the first phase, the freshwater field sampling and analysis portion of the verification (shown as days 5, 6, and 7 in Table 3-2), was repeated by Battelle technical and non-technical staff. Water samples were collected from Alum Creek Reservoir, the Olentangy River, and the Scioto River on June 19 and 20, 2001. In addition to the aliquots analyzed by the F-NTK and reference methods, additional aliquots of all field samples from this sampling were stored at Battelle. Those aliquots were then used in the second phase of

Table 3-2. Schedule of Round 1 Verification Test Days

Test Day	Testing Location	Activity
Day One 1/8-1/9/01	Battelle Columbus Laboratory	Analysis of PT samples and associated QC samples.
Day Two 1/10/01	Battelle Columbus Laboratory and a Columbus Field Location	Collection and analysis of drinking water samples and LFM samples within Battelle and at a residential site using well water.
Day Three 1/11/01	Columbus Field Location	Collection and analysis of environmental samples and LFM samples at a residential site using community water.
Day Four 1/12/01	Battelle Columbus Laboratory	Analysis of PT samples and associated QC samples.
Day Five 1/16/01	Columbus Field Location	Collection and analysis of environmental samples and LFM samples at four locations on the Olentangy River.
Day Six 1/17/01	Columbus Field Location	Collection and analysis of environmental samples and LFM samples at four locations on the Scioto River.
Day Seven 1/18/01	Columbus Field Location	Collection and analysis of environmental samples and LFM samples at four locations on the Alum Creek reservoir.
Day Eight 2/5/01	Transport to Battelle Duxbury, MA	Shipping and handling of analyzers undergoing verification to field test site.
Day Nine 2/7/01	Duxbury, MA, Field Location	Collection and analysis of environmental samples and LFM samples at salt water locations; shipping of environmental samples to Columbus for subsequent reference analysis.

Round 2, on July 11, 2001. For that phase, a representative of NECi came to Battelle, and used the F-NTK kits alongside a Battelle operator, to analyze the water samples collected in the field in the first phase of Round 1. All data from both Round 1 and Round 2 of testing are presented in this report, however, the data from Round 2 are more representative of the current F-NTK kits.

Chapter 4
Quality Assurance/Quality Control

Quality assurance/quality control (QA/QC) procedures were performed in accordance with the quality management plan (QMP) for the AMS Center[4] and the test/QA plan for this verification test.[1]

4.1 QC for Reference Method

Field RB and laboratory blank samples were analyzed to ensure that no sources of contamination were present. The test/QA plan stated that if the analysis of an RB sample indicated a concentration above the method detection limit (MDL) for the reference instrument, any contamination source was to be corrected and proper blank readings achieved before proceeding with the verification test. A total of 36 laboratory blanks and four field reagent blanks were analyzed, and all of the blanks analyzed were below the 0.02 ppm detection limit for nitrate-N.

The accuracy of the reference method was verified at the beginning and end of each day that reference analyses were performed. The instrument used for the reference method was initially calibrated according to the procedures specified in the reference method. Instrument calibration was then verified using an appropriate QCS. If the QCS analysis differed by more than ± 10% from the true value of the standard, the instrument was to be recalibrated before continuing the test. As shown in Table 4-1, all of the QCS analyzed in both Round 1 and Round 2 were within this required range, the maximum bias from the standard in any QCS analysis being 5.6%.

LFM samples were analyzed to assess whether matrix effects influenced the results of the reference method. The percent recovery (R) of the spiked samples was calculated from the following equation:

$$R = \frac{C_s - C}{s} \times 100 \qquad (1)$$

where C_s is the analyzed concentration of the spiked sample, C is the analyzed concentration of the unspiked sample, and s is the concentration equivalent of the analyte spike. If the percent recovery of an LFM fell outside the range from 85 to 115%, a matrix effect was suspected. As shown in Table 4-2, all of the LFM_L samples from both rounds of testing were within this range, and all but three of the LFM_F samples from both rounds of testing were within this range. One

Table 4-1. Reference Method QCS Analysis Results

Sample ID	Date of Analysis	Measured Nitrate-N (ppm)	Actual Nitrate-N (ppm)	Percent Bias
QCS-Initial	1/12/2001	4.96	5.00	0.8
QCS	1/12/2001	5.05	5.00	1.0
QCS	1/12/2001	5.04	5.00	0.8
QCS	1/12/2001	4.94	5.00	1.2
QCS-Final	1/12/2001	4.94	5.00	1.2
QCS-Initial	1/13/2001	4.98	5.00	0.4
QCS	1/13/2001	4.99	5.00	0.2
QCS-Final	1/13/2001	5.04	5.00	0.8
QCS-Initial	1/15/2001	5.11	5.00	2.2
QCS	1/15/2001	4.9	5.00	2.0
QCS-Final	1/15/2001	4.97	5.00	0.6
QCS-Initial	1/19/2001	4.89	5.00	2.2
QCS	1/19/2001	4.76	5.00	4.8
QCS	1/19/2001	4.79	5.00	4.2
QCS	1/19/2001	4.72	5.00	5.6
QCS-Final	1/19/2001	4.74	5.00	5.2
QCS-Initial	2/12/2001	4.8	5.00	4.0
QCS	2/12/2001	4.86	5.00	2.8
QCS-Final	2/12/2001	4.88	5.00	2.4
QCS-Initial	6/21/2001	4.96	5.00	0.8
QCS	6/21/2001	5.03	5.00	0.6
QCS	6/21/2001	5.01	5.00	0.2
QCS-Final	6/21/2001	5.10	5.00	2.0

river water sample from Round 1 showed a slightly high recovery, and one river sample from Round 2 had a low recovery. The salt water sample also showed a low recovery. For the LFM_F samples, however, a matrix effect cannot be confirmed because other spiked samples do not show a consistent pattern of recovery values.

Table 4-2. Reference Method LFM Analysis Results

Spiked Sample ID	Date of Analysis	Unspiked Sample Nitrate-N (ppm)	Spiked Sample Nitrate-N (ppm)	Spiked Amount Nitrate-N (ppm)	Percent Recovery
SPK-DW[a] LFM_F	01/12/2001	4.86	6.90	2.00	102.0
SPK-WW[a] LFM_F	01/12/2001	<0.02	1.72	2.00	86.0
SPK-CW[a] LFM_F	01/12/2001	1.64	3.38	2.00	87.0
SPK-OR LFM_F	01/12/2001	2.15	4.50	2.00	117.5
SPK-SR LFM_F	01/12/2001	6.78	8.91	2.00	106.5
WW1 LFM_L	01/12/2001	<0.02	0.61	0.68	90.7
CW1 LFM	01/12/2001	1.67	2.42	0.68	110.7
SPK-AC LFM_F	01/13/2001	2.01	3.73	2.00	86.0
SPK-MB LFM_F	01/13/2001	0.09	1.43	2.00	67.0
DW1 LFM_L	01/13/2001	4.64	5.36	0.68	105.0
OR1 LFM_L	01/19/2001	0.75	1.46	0.68	104.7
AC1 LFM_L	01/19/2001	1.77	2.38	0.68	89.3
MB1-B LFM_L	2/09/2001	0.07	0.66	0.68	86.7
SPK-OR LFM_F	06/21/2001	4.70	6.16	2.00	73.0
SPK-SR LFM_F	06/21/2001	6.40	8.19	2.00	89.5
SPK-AC LFM_F	06/21/2001	3.03	5.41	2.00	119.0
OR1 LFM_L	06/21/2001	4.17	4.93	0.68	111.8
SR1 LFM L	06/21/2001	6.54	6.82	0.68	41.2
AC1 LFM_L	06/21/2001	3.14	3.74	0.68	88.2

[a] Average of four duplicates.

[b] Results after samples were diluted 1:3 prior to spike.

[c] BDL = below detection limit.

4.2 Audits

4.2.1 Performance Evaluation Audit

A performance evaluation (PE) audit was conducted to assess the quality of the reference measurements made in this verification test. For the PE audit, an independent nitrate standard was obtained from a different vendor than the one that supplied the calibration standards. The performance evaluation standard was prepared and analyzed on January 19, 2001. Accuracy of the reference instrument was determined by comparing the measured nitrate-N concentration using the verification test standards to those obtained using the certified PE standard. Percent difference was used to quantify the accuracy of the results. The PE sample for the nitrate-N was a National Institute of Standards and Technology-traceable standard certified by THERMO Orion. Agreement of the standard within 10% was required for the measurements to be considered acceptable. Failure to achieve this agreement would have triggered recalibration of the

instruments with the original QC standards and a repeat of the performance evaluation comparison. As shown in Table 4-3, the PE sample analyzed was well within this required range.

Table 4-3. Reference Method PE Audit Results

Sample ID	Date of Analysis	Measured Nitrate-N (ppm)	Actual Concentration Nitrate-N (ppm)	Percent Agreement
PE-1	01/19/2001	2.91	3.00	-3.0

4.2.2 Technical Systems Audit

The Battelle Quality Manager conducted a technical systems audit from January 8 to 16, 2001, to ensure that the verification test was being performed in accordance with the test/QA plan and the AMS Center QMP. The standard/solution preparation was observed on January 8; on January 9 the performance test; on January 11 the environmental testing (drinking water); on January 12 the interference testing and reference method operations; and on January 16 environmental testing (Olentangy River). As part of the audit, the reference standards and method used were reviewed, actual test procedures were compared to those specified in the test/QA plan, and data acquisition and handling procedures were reviewed. Observations and findings from this audit were documented and submitted to the Verification Test Coordinator for response. No findings were documented that required any corrective action. The records concerning the technical systems audit (TSA) are permanently stored with the Battelle Quality Manager.

4.2.3 Audit of Data Quality

At least 10% of the data acquired during the verification test was 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 QA/QC 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.[4] Once the assessment report was prepared, the 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 and the audit of data quality were sent to the EPA.

4.4 Data Review

Records generated in the verification test received a one-over-one review within two weeks of generation, 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 Battelle technical staff member involved in the verification test, but not the staff member that 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 or ETV field data sheets	Start/end of test	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Test parameters (temperature, analyte/ interferant identities, and F-NTK results)	Battelle	Laboratory record books or ETV field data sheets	When set or changed, or as needed to document test	Used to organize/check test results, manually incorporated in data spreadsheets as necessary
Reference method sample analysis, chain of custody, and results	Battelle	Laboratory record books, data sheets, or data acquisition system, as appropriate	Throughout sample handling and analysis process	Transferred to spreadsheets

[a] All activities subsequent to data recording are carried out by Battelle.

Chapter 5
Statistical Methods

The statistical methods presented in this chapter were planned for verifying the performance factors listed in Section 3.1. However, in some cases the F-NTK test kits being verified yielded only semi-quantitative results that did not lend themselves well to statistical evaluation. In such cases qualitative comparisons are reported.

5.1 Accuracy

When possible, accuracy was assessed relative to the results obtained from the reference analyses. Samples were analyzed by both the reference method and the test kit being verified. The accuracy was expressed in terms of a relative average bias (*B*) as calculated from the following equation:

$$B = \frac{\bar{d}}{\bar{C}_R} \times 100 \qquad (2)$$

where $\bar{d}$ is the average difference between the readings from the F-NTK kit and those from the reference method, and $\overline{C_R}$ is the average of the reference measurements.

Because of the semi-quantitative nature of the test kit results, it was not possible to make this determination for much of the data. For this reason, all of the data were judged by a qualitative measure. If the result from the test kit agreed with the reference result within the test kit's stated precision capability of ± 1 ppm, the measurement was considered accurate; if it did not, the measurement was considered not to be accurate. The percentage of accurate measurements was determined for each of the four types of water samples as calculated from the following equation:

$$A = \frac{Y}{T} \times 100 \qquad (3)$$

where *A* is the percent of accurate measurements, *Y* is the number of measurements within the ±1 ppm criteria, and *T* is the total number of measurements.

The results were analyzed independently for the readings obtained from the two operators to determine if significant operator bias exists.

5.2 Precision

When possible, the standard deviation (*S*) of the results for the replicate samples was calculated and used as a measure of F-NTK precision at each concentration.

$$S = \left[\frac{1}{n-1} \sum_{k=1}^{n} \left(C_k - \overline{C} \right)^2 \right]^{1/2} \quad (4)$$

where *n* is the number of replicate samples, C_k is the concentration measured for the k^{th} sample, and $\overline{C}$ is the average concentration of the replicate samples. The instrumental precision at each concentration was reported in terms of the relative standard deviation (RSD), e.g.,

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

In some cases, F-NTK analysis results could only be quantified as within a range (e.g., 1 to 5 ppm). In those cases, both extremes of the range (i.e., 1 ppm and 5 ppm) were used as results to calculate a range of RSD values.

5.3 Linearity

Linearity was assessed by linear regression of F-NTK and reference results. However, the F-NTK test kit is semi-quantitative and was calibrated using 1-, 5-, and 10-ppm standards provided with the test kit. Because the color developed by the successive standards often was barely distinguishable, quantitative results were not often obtained with which to perform this assessment.

5.4 Method Detection Limit

The MDL for the test kit was assessed from the seven replicate analyses of a fortified sample with an analyte concentration of five times the vendor's estimated detection limit as described in 40 CFR part 136 Appendix B[3]. 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 with n = 7, and *S* is the standard deviation of the replicate samples. At times the operators could only report the results as a range

of values. Because of this, the MDL was calculated as a range, using the lowest value and the highest value of the range reported.

5.5 Matrix Interferences

The effect of interfering matrix species on the response of the test kit to a given analyte is typically calculated as the ratio of the difference in analytical response to the concentration of interfering species. For example, if the addition of 500 parts per billion (ppb) of an interfering species results in a difference of 10 ppb in the analytical result, the relative sensitivity of the test kit to that interferant would be calculated as 10 ppb/500 ppb = 2%. In this test, there were few quantitative results for use in assessing the matrix interferences; therefore, only qualitative observations could be made concerning matrix interferences.

5.6 Operator Bias

To assess operator bias for the test kit, the results obtained from each operator were compiled independently and subsequently compared. However, because of the qualitative nature of the test kit data, the existence of statistically significant operator bias could not be determined through a Student's *t*-test of the data as planned. Qualitative observations are made concerning the results from the two operators.

5.7 Rate of False Positives/False Negatives

The rate of false positives/false negatives of the test kit was assessed relative to the 2 ppm target nitrate-N level. Analytes reported as being above that level by the test kit, but below that level by the reference method, were considered false positives. Analytes reported as being below 2 ppm level by the test kit, but reported as above that level by the reference method, were considered false negatives. The rate of false positives/false negatives was expressed as a percentage of total samples analyzed for each matrix.

Chapter 6
Test Results

The results of the verification test of the F-NTK nitrate test kits are presented in this section. Results from Round 1 and Round 2 of testing are presented separately, because of the different test schedules, procedures, and test kit involved. Note that Appendix B of this report contains vendor comments on the kits and test results, along with Battelle responses on some items.

6.1 Round 1 Results

6.1.1 Accuracy

Tables 6-1a-d list the measured nitrate-N results from analysis of the four different types of water samples in Round 1. Both reference and F-NTK results are shown in the tables, and F-NTK results are shown for both the technical and non-technical operators. Sometimes F-NTK results could only be quantified in terms of a range of values (e.g., 1 to 5 ppm). This occurred when the difference in color between the 1-, 5-, and 10-ppm standards generated from the test kit was so slight that the operator could not make a precise determination. Some samples could not be distinguished from blank samples, and so were assigned a value of 0 ppm. No attempt was made to dilute and reanalyze samples with concentrations greater than 10 ppm.

Tables 6-2a-d show the results of evaluating the accuracy of the F-NTK Round 1 results listed in Tables 6-1a-d. Shown in the first two columns in each of Tables 6-2a-d are the percent bias values determined according to Equation 2, in Section 5.1. Clearly, relatively few of the F-NTK results from Round 1 were quantitative enough to allow calculation of percent bias. In fact, for the salt water field samples (Table 6-2d, respectively), no quantitative calculation of bias could be made. The percent bias values that are shown in Tables 6-2 a-c range from 10 to over 560%, indicating inconsistent behavior from the F-NTK kits in Round 1.

In the absence of quantitative bias results, the qualitative accuracy comparison using Equation 3 in Section 5.1 was used. The third and fourth columns in Tables 6-2 a-d show the assignment of each F-NTK result, in terms of whether that result fell within ±1 ppm of the reference value. Note that some of the F-NTK results were reported as a range of concentrations; those results may be assigned as both meeting and not meeting the ±1 ppm criterion in Tables 6-2a-d. The results of this qualitative evaluation of accuracy are shown in Table 6-3, which lists the overall percent of results meeting the ±1 ppm accuracy criterion, for each operator and sample type in

Table 6-1a. Results from Round 1 Laboratory Performance Test Sample Analyses

Sample	Non-Technical Staff Nitrate-N (ppm)	Technical Staff Nitrate-N (ppm)	Reference Test Nitrate-N (ppm)
PT1-1	< 1	< 1	0.19
PT1-2	< 1	< 1	
PT1-3	< 1	< 1	
PT1-4	< 1	< 1	
PT2-1	< 1	10	1.87
PT2-2	< 1	10	
PT2-3	< 1	10	
PT2-4	< 1	10	
PT3-1	> 10	> 10	18.9
PT3-2	> 10	> 10	
PT3-3	> 10	> 10	
PT3-4	> 10	> 10	
PT4-1	< 1	< 1	0.50
PT4-2	< 1	< 1	
PT4-3	< 1	< 1	
PT4-4	< 1	< 1	
PT5-1	5	1-5	5.54
PT5-2	5	1-5	
PT5-3	5	< 1	
PT5-4	5	< 1	

Table 6-1b. Results from Round 1 Drinking Water Analyses

Sample	Non-Technical Staff Nitrate-N (ppm)	Technical Staff Nitrate-N (ppm)	Reference Test Nitrate-N (ppm)
BF-1	5	5	4.71
BF-2	5	5	5.06
BF-3	5	5	4.62
BF-4	5	5	5.05
TH-1	< 1	< 5	< 0.02
TH-2	< 1	< 5	< 0.02
TH-3	< 1	< 5	< 0.02
TH-4	< 1	< 5	< 0.02
PH-1	1	< 1	1.67
PH-2	1	< 1	1.57
PH-3	1	< 1	1.66
PH-4	1	< 1	1.66

Table 6-1c. Results from Round 1 Freshwater Analyses

Sample	Non-Technical Staff Nitrate-N (ppm)	Technical Staff Nitrate-N (ppm)	Reference Test Nitrate-N (ppm)
OR-1	0[a]	< 1	0.75
OR-2	0	< 1	2.68
OR-3	0	< 1	2.15
OR-4	0	< 1	2.56
SR1	< 1	2	6.79
SR2	< 1	1	6.43
SR3	> 10	1	6.87
SR4	10	1	6.78
AC-1	0	< 1	1.77
AC-2	0	< 1	2.01
AC-3	0	< 1	1.99
AC-4	0	< 1	1.98

[a] Entry of zero indicates sample was indistinguishable from blank.

Table 6-1d. Results from Round 1 Salt Water Analyses

Sample	Non-Technical Staff Nitrate-N (ppm)	Technical Staff Nitrate-N (ppm)	Reference Test Nitrate-N (ppm)
MB1-S	< 1	< 1	0.07
MB1-B	< 1	< 1	0.07
MB2-S	< 1	< 1	0.05
MB2-B	< 1	< 1	0.07
MB3-S	< 1	< 1	< 0.02
MB3-B	< 1	< 1	0.06
MB4-S	< 1	< 1	< 0.02
MB4-B	< 1	< 1	0.09

Table 6-2a. Accuracy of the F-NTK Test Kit with Laboratory Performance Test Samples for Round 1

	Percent Bias[a] Non-Technical	Percent Bias[a] Technical	Within Range (Y/N)[b] Non-Technical Staff	Within Range (Y/N)[b] Technical Staff
PT1-1	--[c]	--	Y	Y
PT1-2	--	--	Y	Y
PT1-3	--	--	Y	Y
PT1-4	--	--	Y	Y
PT2-1	--	535	Y/N	N
PT2-2	--	535	Y/N	N
PT2-3	--	535	Y/N	N
PT2-4	--	535	Y/N	N
PT3-1	--	--	Y	Y
PT3-2	--	--	Y	Y
PT3-3	--	--	Y	Y
PT3-4	--	--	Y	Y
PT4-1	--	--	Y	Y
PT4-2	--	--	Y	Y
PT4-3	--	--	Y	Y
PT4-4	--	--	Y	Y
PT5-1	10	10-82	Y	Y/N
PT5-2	10	10-82	Y	Y/N
PT5-3	10	--	Y	N
PT5-4	10	--	Y	N

[a] Percent bias calculated according to Equation 2, Section 5-1. [b] Y = result within ±1 ppm of reference; N=result not within ±1 ppm of reference. [c] No calculation of bias can be made.

Table 6-2b. Accuracy of the F-NTK Test Kit with Drinking Water Samples for Round 1

	Percent Bias[a] Non-Technical	Percent Bias[a] Technical	Within Range (Y/N)[b] Non-Technical Staff	Within Range (Y/N)[b] Technical Staff
BF-1	6	6	Y	Y
BF-2	1	1	Y	Y
BF-3	8	8	Y	Y
BF-4	1	1	Y	Y
TH-1	--[c]	--	Y	Y
TH-2	--	--	Y	Y
TH-3	--	--	Y	Y
TH-4	--	--	Y	Y
PH-1	40	--	Y	Y/N
PH-2	36	--	Y	Y/N
PH-3	40	--	Y	Y/N
PH-4	40	--	Y	Y/N

[a] Percent bias calculated according to Equation 2, Section 5-1. [b] Y=result within ±1 ppm of reference; N=result not within ±1 ppm of reference. [c] No calculation of bias can be made.

Table 6-2c. Accuracy of the F-NTK Test Kit with Freshwater Samples for Round 1

	Percent Bias[a] Non-Technical	Percent Bias[a] Technical	Within Range (Y/N)[b] Non-Technical Staff	Within Range (Y/N)[b] Technical Staff
OR-1	100	565	Y	N
OR-2	100	86	N	N
OR-3	100	132	N	N
OR-4	100	95	N	N
SR-1	--[c]	--	N	N
SR-2	--	--	N	N
SR-3	--	--	N	N
SR-4	48	--	N	N
AC-1	100	--	N	Y/N
AC-2	100	--	N	N
AC-3	100	--	N	Y/N
AC-4	100	--	N	Y/N

[a] Percent bias calculated according to Equation 2, Section 5-1.
[b] Y = result within ±1 ppm of reference; N=result not within ±1 ppm of reference.
[c] No calculation of bias can be made.

Table 6-2d. Accuracy of the F-NTK Test Kit with Salt Water Samples for Round 1

	Percent Bias[a] Non-Technical	Percent Bias[a] Technical	Within Range (Y/N)[b] Non-Technical Staff	Within Range (Y/N)[b] Technical Staff
MB1-S	--[c]	--	Y	Y
MB1-B	--	--	Y	Y
MB2-S	--	--	Y	Y
MB2-B	--	--	Y	Y
MB3-S	--	--	Y	Y
MB3-B	--	--	Y	Y
MB4-S	--	--	Y	Y
MB4-B	--	--	Y	Y

[a] Percent bias calculated according to Equation 2, Section 5-1.
[b] Y = result within ±1 ppm of reference; N=result not within ±1 ppm of reference.
[c] No calculation of bias can be made.

Table 6-3. Summary of Qualitative Accuracy Results from Round 1 of Testing

	Percent Accuracy Non-Technical staff	Percent Accuracy Technical staff
Laboratory performance test samples	80-100	60-70
Drinking water samples	100	67-100
Freshwater samples	8	0-25
Salt water samples	100	100

Round 1 of testing. Table 6-3 shows that the qualitative accuracy of the F-NTK kits was relatively high for laboratory and drinking water samples, with both technical and non-technical operators. However, for freshwater field samples, the qualitative accuracy results were only 8% for the technical operator, and 0 to 25% for the non-technical operator. For the salt water samples, the qualitative accuracy was 100% with both operators. This result occurred because all reference results for the salt water samples were less than 0.1 ppm, and all F-NTK results for those samples were less than 1 ppm.

6.1.2 Precision

Tables 6-4a and b, respectively, show the data used to evaluate the relative standard deviation of the F-NTK results for the replicate laboratory performance test samples and drinking water samples in Round 1. Also shown in the tables is the percent RSD value for each set of replicate analyses. Calculation of precision was complicated by the qualitative nature of the F-NTK results. In most cases, percent RSD could not be calculated quantitatively, because all F-NTK results were "greater than" or "less than" values. However, these data sets do illustrate consistency in the F-NTK replicate analyses. In other cases, all F-NTK results for replicate samples were equal. These cases exhibit the reproducibility achievable with the kits, and result in percent RSD values of zero. Five of the 16 cases had this outcome. For the 5-ppm laboratory test samples, the percent RSD for the samples analyzed by the technical staff member was 115%. These results are based on assuming a value of zero for the "less than" F-NTK results.

6.1.3 Linearity

As is evident from the preceding sections, the F-NTK test kits yielded semi-quantitative results in Round 1 testing. The kits were calibrated using standards included in the test kit, at nominal concentrations of 1, 5, and 10 ppm. The color of the standards was compared with the color on the chart included in the sample kits. In all cases, the prepared standards were used to determine the results of the sample analyses. A new set of standards was analyzed each day by the non-technical and technical operators. The operators observed wide variation in the coloration of the standards, ranging from standards matching the color on the chart, to no color appearing in the 1-ppm standard, while the 5- and 10-ppm standard showed slight coloration, to absolutely no color being formed in any of the standards. At no time did a more concentrated standard appear lighter in color relative to a less concentrated standard. Overall, this variability in F-NTK-01 standards prevented any quantitative evaluation of linearity of response in Round 1.

Table 6-4a. Precision Results for F-NTK Kits from Laboratory Performance Test Samples for Round 1

Sample	Non-Technical Staff Nitrate-N (ppm)	Technical Staff Nitrate-N (ppm)
PT1-1	< 1	< 1
PT1-2	< 1	< 1
PT1-3	< 1	< 1
PT1-4	< 1	< 1
% RSD	**--[a]**	**--**
PT2-1	< 1	10
PT2-2	< 1	10
PT2-3	< 1	10
PT2-4	< 1	10
% RSD	**--**	**0%**
PT3-1	> 10	> 10
PT3-2	> 10	> 10
PT3-3	> 10	> 10
PT3-4	> 10	> 10
% RSD	**--**	**--**
PT4-1	< 1	< 1
PT4-2	< 1	< 1
PT4-3	< 1	< 1
PT4-4	< 1	< 1
%RSD	**--**	**--**
PT5-1	5	1-5
PT5-2	5	1-5
PT5-3	5	< 1
PT5-4	5	< 1
%RSD	**0%**	**115%[b]**

[a] No % RSD calculated.

[b] For the purpose of calculating standard deviation, "less than" values were considered to be zero.

Table 6-4b. Precision Results for F-NTK Kits from Drinking Water Samples for Round 1

Sample	Non-Technical Staff[a] Nitrate-N (ppm)	Technical Staff[a] Nitrate-N (ppm)
DW BF-1	5	5
DW BF-2	5	5
DW BF-3	5	5
DW BF-4	5	5
% RSD	**0%**	**0%**
WW TH-1	< 1	< 5
WW TH-2	< 1	< 5
WW TH-3	< 1	< 5
WW TH-4	< 1	< 5
% RSD	**--[a]**	**--**
CW PH-1	1	< 1
CW PH-2	1	< 1
CW PH-3	1	< 1
CW PH-4	1	< 1
%RSD	**0%**	**--**

[a] No % RSD calculated.

6.1.4 Method Detection Limit

The method detection limit of the F-NTK kits used in Round 1was determined based on seven replicate samples at a concentration of 2.5 ppm (five times the vendor's reported detection limit of 0.5 ppm). The data, and parameters needed for the calculation of MDL by Equation 6 in Section 5.4, are shown in Table 6-5. Shown are the values of S and t needed for the calculation, and the resulting values for the MDL. The calculated MDL for the non-technical operator was 3.0 to 5.4 ppm and for the technical operator 10.8 to 11.2 ppm (Table 6-5). Thus, the analyte level in the test sample was less than the determined MDL. These results are clearly influenced by the large variability in F-NTK results. For example, results with the technical operator ranged from <1 to 10 ppm in analysis of the 2.5 ppm sample. This variability is not considered characteristic of the F-NTK kits; further comments on detection capabilities in the absence of this variability are presented in Section 6.2.

6.1.5 Matrix Interferences

Tables 6-6a and b show the analytical results from Round 1 laboratory performance test samples with low and high levels of interferences, respectively. The non-technical operator did not detect any analyte in any of the low interference samples (Table 6-6a), and the technical operator did not detect any analyte in three of those samples. In five of the eight samples spiked with a low amount of interferences, the technical operator detected analyte at 167 to 333% of the true value of the analyte. For the samples spiked with a high level of interferences (Table 6-6b), both

Table 6-5. Method Detection Limit Results for the F-NTK Kits for Round 1 for Round 1

Sample	Non-Technical Staff Nitrate-N (ppm)[a]	Technical Staff Nitrate-N (ppm)[a]
PT6-1	< 1	1-5
PT6-2	< 1	1-5
PT6-3	2	10
PT6-4	1-5	1-5
PT6-5	2-3	1-5
PT6-6	2	< 1
PT6-7	2	< 1
Std. Dev'n. (S)	0.951 - 1.732	3.45–3.56
t at n=7[b]	3.14	3.14
MDL[c]	3.0 -5.4	10.8-11.2

[a] For the purpose of calculating standard deviation, all "less than" values are considered to be zero.
[b] *t* is the Student's value for a 99% confidence level.
[C] MDL = t × S (see Section 5.4).

Table 6-6a. Data from Laboratory Performance Test Samples with Low-Level Interferences for Round 1

Sample	Non-Technical Staff Nitrate-N (ppm)	Technical Staff Nitrate-N (ppm)	Reference Method Nitrate-N (ppm)
LI-1	< 1	< 5	3.02[a]
LI-2	< 1	< 5	3.02[a]
LI-3	< 1	< 5	3.02[a]
LI-4	< 1	5	3.02[a]
LI-5	< 1	5	3.02[a]
LI-6	< 1	10	3.02[a]
LI-7	< 1	10	3.02[a]
LI-8	< 1	10	3.02[a]

[a] LI solution only analyzed once by reference method. Eight aliquots of single solution were analyzed by F-NTK test kits.

Table 6-6b. Data from Laboratory Performance Test Samples with High-Level Interferences for Round 1

	Non-Technical Staff Nitrate-N (ppm)	Technical staff Nitrate-N (ppm)	Reference Method Nitrate-N (ppm)
HI-1	> 10	> 10	3.7[a]
HI-2	> 10	> 10	3.7[a]
HI-3	> 10	> 10	3.7[a]
HI-4	> 10	> 10	3.7[a]
HI-5	> 10	> 10	3.7[a]
HI-6	> 10	> 10	3.7[a]
HI-7	> 10	> 10	3.7[a]
HI-8	> 10	> 10	3.7[a]

[a] HI solution only analyzed once by reference method. Eight aliquots of single solution were analyzed by F-NTK test kits.

operators reported values greater than 333% of the true value in all samples. These results suggest that the higher level of interferences caused overestimation of the nitrate-N level to a much greater extent than did the lower interference levels. However, the variability in test kit behavior is likely to have affected these results, and no quantitation of interference effects can be made.

6.1.6 Operator Bias

No evaluation of the effect of operator skill level could be made in Round 1 of testing because of the variability and qualitative nature of the test results. The results of the Round 1 tests indicated that the uncertainty of the individual test kit used, especially due to the color development of its standards, was much greater than the variability due to the operator skill level.

6.1.7 Rate of False Positives/False Negatives

Tables 6-7 and 6-8, respectively, show the data and results for the rates of false positives and false negatives obtained from the test kit in Round 1 of testing. All performance test and environmental samples (Table 3-1) were considered for this evaluation. However, F-NTK values reported as ranges spanning the 2-ppm midpoint value, or as a "less than" value that was greater than the 2-ppm midpoint value, were deemed not applicable to this evaluation.

Table 6-7 shows that there were 32 samples with reference analyte concentrations less than the target midpoint of 2 ppm. The samples tested by the technical operator showed four samples that were not applicable to this analysis, and four others in which F-NTK results exceeded 2 ppm. The result was a false positive rate of 14% relative to the 2 ppm value. The samples tested by the non-technical operator had a false positive rate of 0%, with no F-NTK results above the 2-ppm midpoint value.

Table 6-8 shows that there were 43 samples with reference analyte concentrations greater than the target midpoint of 2 ppm. The samples tested by the technical operator had a false negative rate of 32%. In 11 of the samples, the analyte was detected at a level less than 2 ppm, and nine of the samples were not applicable. The samples tested by the non-technical operator had a false negative rate of 38%. In 16 of the samples, the analyte was detected at a level less than 2 ppm, and one of the samples was not applicable.

6.1.8 Other Factors

The operators felt the F-NTK test kit was easy to use and free of maintenance. The kit was easy to transport by car, boat, and airplane. The sample test tube rack included in the F-NTK-01 made it easy for the operators to perform the analysis in the field with rough terrain or bumpy seas. The test kit allowed analysis of three standards and five samples. The preparation of the reagents in each kit takes approximately 45 minutes, and the analysis of the standards and samples take approximately 45 minutes each.

Table 6-7. Rate of False Positives from F-NTK Test Kit for Round 1

Sample	Technical Result Nitrate-N (ppm)	Non-Technical Result Nitrate-N (ppm)	Reference Method Result Nitrate-N (ppm)	Technical False Positive (Y/N)	Non-Technical False Positive (Y/N)
PT1-1	< 1	< 1	0.19	N	N
PT1-2	< 1	< 1	0.19	N	N
PT1-3	< 1	< 1	0.19	N	N
PT1-4	< 1	< 1	0.19	N	N
PT2-1	10	< 1	1.87	Y	N
PT2-2	10	< 1	1.87	Y	N
PT2-3	10	< 1	1.87	Y	N
PT2-4	10	< 1	1.87	Y	N
PT4-1	< 1	< 1	0.50	N	N
PT4-2	< 1	< 1	0.50	N	N
PT4-3	< 1	< 1	0.50	N	N
PT4-4	< 1	< 1	0.50	N	N
WW-1	< 5	< 1	< 0.02	NA	N
WW-2	< 5	< 1	< 0.02	NA	N
WW-3	< 5	< 1	< 0.02	NA	N
WW-4	< 5	< 1	< 0.02	NA	N
CW-1	< 1	1	1.67	N	N
CW-2	< 1	1	1.57	N	N
CW-3	< 1	1	1.66	N	N
CW-4	< 1	1	1.66	N	N
OR-1	< 1	0	0.75	N	N
AC-1	< 1	0	1.77	N	N
AC-3	< 1	0	1.99	N	N
AC-4	< 1	0	1.98	N	N
MB1-S	< 1	< 1	0.07	N	N
MB1-B	< 1	< 1	0.07	N	N
MB2-S	< 1	< 1	0.05	N	N
MB2-B	< 1	< 1	0.07	N	N
MB3-S	< 1	< 1	< 0.02	N	N
MB3-B	< 1	< 1	0.06	N	N
MB4-S	< 1	< 1	< 0.02	N	N
MB4-B	< 1	< 1	0.09	N	N
Total number of applicable samples				28	32
Total false positives				4	0
Percent false positives				14%	0%

NA = Not applicable
Y = Yes
N = No

Table 6-8. Rate of False Negatives from F-NTK Test Kit for Round 1

Sample	Technical Result Nitrate-N (ppm)	Non-Technical Result Nitrate-N (ppm)	Reference Method Result Nitrate-N (ppm)	Technical False Negative (Y/N)	Non-Technical False Negative (Y/N)
PT3-1	> 10	> 10	18.87	N	N
PT3-2	> 10	> 10	18.87[a]	N	N
PT3-3	> 10	> 10	18.87[a]	N	N
PT3-4	> 10	> 10	18.87[a]	N	N
PT5-1	1-5	5	5.54	NA	N
PT5-2	1-5	5	5.54[a]	NA	N
PT5-3	< 1	5	5.54[a]	Y	N
PT5-4	< 1	5	5.54[a]	Y	N
PT6-1	1-5	< 1	2.38	NA	Y
PT6-2	1-5	< 1	2.38[a]	NA	Y
PT6-3	10	2	2.38[a]	N	N
PT6-4	1-5	1-5	2.38[a]	NA	NA
PT6-5	1-5	2-3	2.38[a]	NA	N
PT6-6	< 1	2	2.38[a]	Y	N
PT6-7	< 1	2	2.38[a]	Y	N
DW-1	5	5	4.71	N	N
DW-2	5	5	5.06	N	N
DW-3	5	5	4.62	N	N
DW-4	5	5	5.05	N	N
LI-1	< 5	< 1	3.02	NA	Y
LI-2	< 5	< 1	3.02[a]	NA	Y
LI-3	< 5	< 1	3.02[a]	NA	Y
LI-4	5	< 1	3.02[a]	N	Y
LI-5	5	< 1	3.02[a]	N	Y
LI-6	10	< 1	3.02[a]	N	Y
LI-7	10	< 1	3.02[a]	N	Y
LI-8	10	< 1	3.02[a]	N	Y
HI-1	> 10	> 10	3.69	N	N
HI-2	> 10	> 10	3.69[a]	N	N
HI-3	> 10	> 10	3.69[a]	N	N
HI-4	> 10	> 10	3.69[a]	N	N
HI-5	> 10	> 10	3.69[a]	N	N
HI-6	> 10	> 10	3.69[a]	N	N
HI-7	> 10	> 10	3.69[a]	N	N
HI-8	> 10	> 10	3.69[a]	N	N
OR-2	< 1	0	2.68a	Y	Y
OR-3	< 1	0	2.15	Y	Y
OR-4	< 1	0	2.56	Y	Y
SR-1	2	< 1	6.79	N	Y
SR-2	1	< 1	6.43	Y	Y
SR-3	1	>10	6.87	Y	N
SR-4	1	10	6.78	Y	N
AC-2	< 1	0	2.01	Y	Y
Total number of applicable samples				34	42
Total false negatives				11	16
Percent false negatives				32%	38%

NA = Not applicable
Y = Yes
N = No
[a] Only one sample analyzed by reference method. Multiple aliquots of sample were analyzed by F-NTK test kit.

Each F-NTK test kit includes five reagent packets. Each packet contains enough reagent to analyze three standards and five samples. When more than five samples were analyzed in a day, additional reagent packets were used without preparing additional standards.

The biggest difficulty in Round 1 was the large difference from one reagent pack to another in the development of the color in the nitrate-N standards. At times it was difficult to discern the faint gradations in the pink color in the standards and samples. There were times when the 1 ppm standard appeared as colorless as the blank. One set of standards had no color development at all.

The operators' impression was that the analysis of the samples was accurate when the color of the standards developed completely and was comparable to the color chart provided in the kit. However, they felt that when an additional reagent packet was required, the samples could not be compared accurately to the standards prepared from the previous packet.

6.1.8.1 Costs

The cost of one F-NTK test kit is $30. The kit contains five reagent packets, each of which is sufficient to conduct five sample analyses.

6.1.8.2 Data Completeness

All portions of the verification test were completed, and all data that were to be recorded were successfully acquired. Thus, data completeness was 100%.

6.2 Round 2 Results

Round 2 of this verification involved analysis of freshwater field samples, blanks, and spiked samples only. The first phase of Round 2 involved the same Battelle operators as in Round 1; the second phase of Round 2 involved the Battelle technical operator and a vendor representative.

The results of the second round of the verification test of the F-NTK are presented in this section.

6.2.1 Accuracy

Table 6-9 lists the F-NTK results and reference data obtained in the field in the first phase of Round 2 by the technical and non-technical Battelle operators. Table 6-10 lists the F-NTK results and reference data obtained in the laboratory in the second phase of Round 2 by the vendor representative and the Battelle technical operator.

Table 6-9. Field Results from Freshwater Samples, Round 2, First Phase

Sample	Non-Technical Staff Nitrate-N (ppm)	Technical Staff Nitrate-N (ppm)	Reference Test Nitrate-N (ppm)
OR-Blank	<1	< 1	<0.02
QCS	1.5	<1	1.89
OR-1	3	<1	4.17
OR-2	3	3	3.99
OR-3	4	< 1	3.91
OR-4	1.5	<1	4.70
LFM_F	1.5	1	6.16
SR-Blank	<1	<1	<0.02
QCS	3	2	1.84
SR1	1.5	<1	6.54
SR2	<1	<1	6.48
SR3	4	4	6.27
SR4	1.5	2	6.40
LFM_F	3	2	8.19
AC-Blank	<1	<1	<0.02
QCS	<1	<1	1.84
AC-1	2	< 1	3.14
AC-2	<1	< 1	3.09
AC-3	<1	<1	2.96
AC-4	<1	< 1	3.03
LFM_F	<1	< 1	5.41

Inspection of Table 6-9 shows that in the field testing that comprised the first phase of Round 2, neither the technical nor non-technical staff obtained high accuracy with the F-NTK test kits. Although the field and spiked samples contained approximately 3 to 8 ppm of nitrate-N, many of the F-NTK results were reported as < 1 ppm. Such non-detect values indicate reasonable accuracy when analyzing the three blank samples, given the 1 ppm resolution of the F-NTK kits. However, both operators reported < 1 ppm results even with samples containing as much as 6.5 ppm nitrate-N (sample SR2, Table 6-9). Of the 18 non-blank samples in Table 6-9, quantitative results (i.e., not less-than values) were reported for 12 samples by the non-technical operator, and for just six samples by the technical operator. When those subsets of the results are treated by Equation 2 (Section 5.1) to determine the relative average bias of the F-NTK kits, a relative average bias of 52.8% is found for the results with the non-technical operator, and 58.4% for the results with the technical operator.

The F-NTK vendor indicated that the erratic performance of the F-NTK kits in the first phase of Round 2 was unusual. As a result a second phase of Round 2 was conducted, consisting of a reanalysis of aliquots of the same freshwater field samples. This time the analysis was performed in the laboratory by the technical operator and a vendor representative. In this second phase of

Round 2, the performance of the F-NTK kits was greatly improved, in that all the 1, 5, and 10 ppm standards included in the kits gave definite and easily distinguishable color formation. The F-NTK and reference results from that second phase are shown in Table 6-10, which lists results from the F-NTK kits with both the vendor representative and the Battelle technical operator.

Table 6-10. Laboratory Results from Reanalysis of Freshwater Samples, Round 2, Second Phase

Sample	Vendor Representative Nitrate-N (ppm)	Battelle Technical Staff Nitrate-N (ppm)	Reference Test Nitrate-N (ppm)
OR-Blank	<1	<1	<0.02
QCS	2	2	1.89
OR-1	5	5	4.17
OR-2	3	4	3.99
OR-3	5	5	3.91
OR-4	2	4	4.70
LFM_F	3	5	6.16
SR-Blank	<1	<1	<0.02
QCS	2	2	1.84
SR1	3	5	6.54
SR2	5	3	6.48
SR3	4	3	6.27
SR4	5	3	6.40
LFM_F	5	5	8.19
AC-Blank	<1	<1	<0.02
QCS	1	1	1.84
AC-1	4	4	3.14
AC-2	2	2	3.09
AC-3	3	2	2.96
AC-4	4	3	3.03
LFM_F	5	5	5.41

Those data indicate much better accuracy relative to the reference results, compared to the first phase of Round 2. With both the vendor and Battelle operator, the F-NTK kits correctly gave < 1 ppm values only for the three blank samples, and gave quantitative results for all of the other 18 samples. When those 18 sample results are treated by Equation 2 (Section 5.1) to determine the relative average bias of the F-NTK kits, a relative average bias of 31.4% is found for the results with the vendor operator, and 28.9% for the results with the Battelle technical operator.

For consistency with the data evaluation in Section 6.1.1, the results of Round 2 have also been evaluated in terms of the frequency with which F-NTK results were within the expected 1 ppm precision relative to the reference results. Tables 6-11 and 6-12 show the results of this comparison for each sample in the first and second phases of Round 2 (Tables 6-9 and 6-10, respectively). Table 6-13 summarizes the results, listing for each operator in each phase of Round 2 the percentage of results within 1 ppm of the reference. The second phase results are clearly better than the first phase results. For the second phase, 57% of the results with the vendor operator and 62% of the results with the Battelle operator were within the stated 1-ppm precision of the kits. Furthermore, if the precision criterion is relaxed slightly, to 1.1 ppm, the resulting percentages increase substantially, i.e., to 67% with the vendor operator and 72% with the Battelle operator. These results show that the F-NTK kits are capable of approximately 1-ppm resolution at nitrate-N concentrations of less than 10 ppm.

The improved performance in the second phase of Round 2 appears related to the improved packaging of the F-NTK kits. The Battelle operators noted that the kits used in that phase of testing produced clear and consistent color changes for all standards and solutions, unlike the inconsistent to non-existent changes observed in some of the previous test activities. As a final evaluation of the F-NTK kits, the kit that did not produce color change in the first phase of Round 2 was used in conjunction with a kit from the second phase of Round 2. In this evaluation, 2 ppm and 5 ppm nitrate-N standards were analyzed using the new version of the kit, but with one reagent at a time from the old kit substituted for the corresponding reagent in the new kit. This evaluation showed that, whereas the buffer, color reagent, and NADH reagent in the old kit worked normally, the enzyme reagent in the old kit was inactive. This finding is consistent with the highly variable results seen in parts of this verification test, and suggests that the previous means of packaging the F-NTK test kits may have resulted in loss of enzyme activity. In any case, the results from the second phase of Round 2 clearly indicate that the current packaging approach of the F-NTK kits preserves the enzyme activity.

Table 6-11. Performance of the F-NTK Test Kit Relative to 1-ppm Precision on the Field Freshwater Samples, Round 2, First Phase

Sample	Within Range of 1 ppm (Y/N) Non-Technical Staff	Within Range of 1 ppm (Y/N) Technical Staff
OR-Blank	Y[a]	Y
QCS	Y	N
OR-1	N	N
OR-2	Y	Y
OR-3	Y	N
OR-4	N	N
LFM_F	N	N
SR-Blank	Y	Y
QCS	N	Y
SR1	N	N
SR2	N	N
SR3	N	Y
SR4	N	N
LFM_F	N	N
AC-Blank	Y	Y
QCS	N	N
AC-1	N	N
AC-2	N	N
AC-3	N	N
AC-4	N	N
LFM_F	N	N

[a] Y = result within 1 ppm of reference; N = result not within 1 ppm of reference.

Table 6-12. Performance of the F-NTK Test Kit Relative to 1 ppm Precision on the Laboratory Reanalysis of Freshwater Samples, Round 2, Second Phase

Sample	Within Range of 1 ppm (Y/N) Vendor Representative	Within Range of 1 ppm (Y/N) Technical Staff
OR-Blank	Y[a]	Y
QCS	Y	Y
OR-1	Y	Y
OR-2	Y	Y
OR-3	N	N
OR-4	N	Y
LFM_F	N	N
SR-Blank	Y	Y
QCS	Y	Y
SR1	N	N
SR2	N	N
SR3	N	N
SR4	N	N
LFM_F	N	N
AC-Blank	Y	Y
QCS	Y	Y
AC-1	Y	Y
AC-2	N	N
AC-3	Y	Y
AC-4	Y	Y
LFM_F	Y	Y

[a] Y = result within 1 ppm of reference; N = result not within 1 ppm of reference.

Table 6-13. Summary of F-NTK Results Relative to 1 ppm Precision for Round 2

Test Phase	F-NTK Operator	Percent of Results Within 1 ppm of Reference
First	Non-technical Battelle	29
	Technical Battelle	19
Second	Vendor Representative	57
	Technical Battelle	62

6.2.2 Linearity

The linearity of the F-NTK readings was assessed by means of a linear regression of the F-NTK results against the reference results, using the 21 data points from the second phase of Round 2 (Table 6-10). In this regression, results reported as below detection limit by either the F-NTK or the reference method were assigned a value of half the detection limit. For the data in Table 6-10, only the blank values showed non-detect results. Therefore the blank results were each assigned values of 0.01 ppm for the reference value and 0.5 ppm for the F-NTK value. Figure 6-1 shows a scatter plot of the F-NTK data from both the vendor and the Battelle technical operators, versus the reference nitrate-N results. The one-to-one line is also shown in Figure 6-1.

A linear regression of the data in Figure 6-1 gives the following regression equations:

for the F-NTK with the vendor operator,

F-NTK, ppm = 0.538 (±0.208) x (Reference, ppm) + 1.02 (±0.926) ppm,
with r = 0.779;

for the F-NTK with the Battelle technical operator,

F-NTK, ppm = 0.545 (±0.204) x (Reference, ppm) + 1.00 (±0.908) ppm,
with r = 0.789,

where the values in parentheses represent the 95% confidence interval of the slope and intercept. The slopes are significantly different from 1.0, and the intercepts are significantly different from zero.

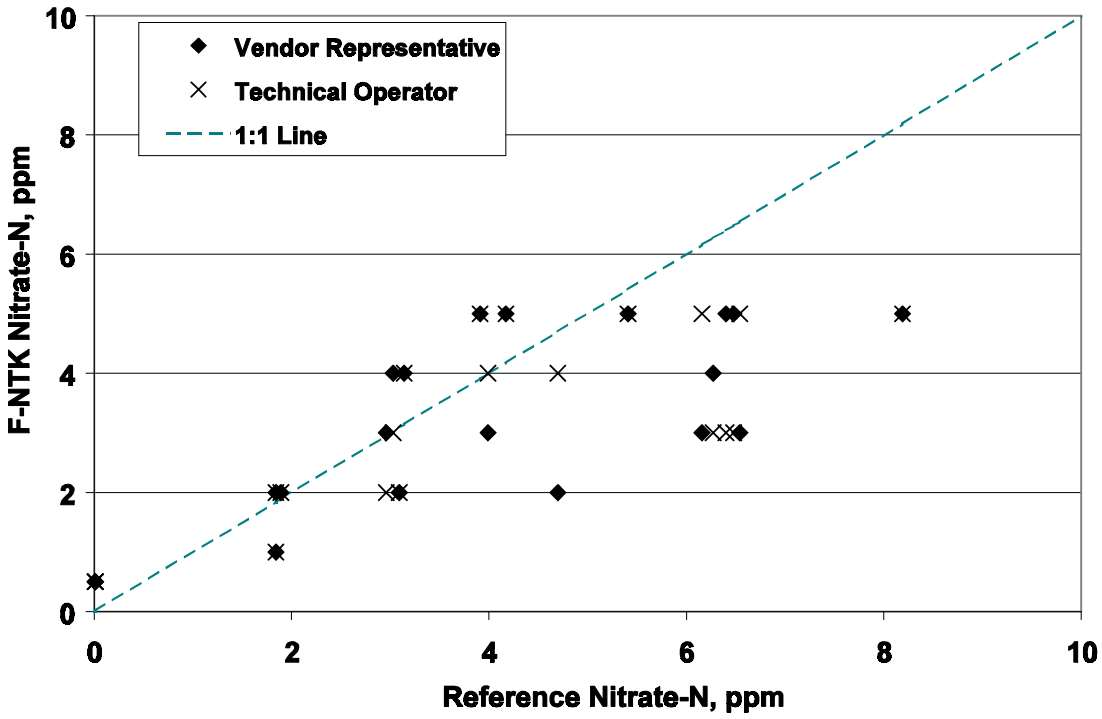

Figure 6-1. Comparison of F-NTK Results to Reference Results from Round 2, Phase 2

6.2.3 Detection Limit

In Round 2 of verification of the F-NTK kits, the method detection limit was not determined according to the procedure used in Section 6.1.4. However, there was clear color development in all the F-NTK 1 ppm standards, and in all the 2 ppm QCS samples shown in Table 6-10. These results indicate that the F-NTK kits with new packaging are capable of detecting nitrate-N at levels at least as low as 1 ppm.

6.2.4 Operator Bias

The regression results presented in Section 6.2.2 are closely similar for the F-NTK kits with two different operators. In fact, consideration of the 95% confidence intervals of the regression results shows that neither the two slope values nor the two intercepts are significantly different from one another. Thus, at least in this case, there is no evidence of any operator bias in the readings of the F-NTK test kits.

Chapter 7
Performance Summary

The F-NTK test kits demonstrated inconsistent performance in much of this verification test, in the form of widely different levels of color formation from nitrate-N standards when different test reagent kits were used. The current packaging for the F-NTK kits appears to have resolved that problem. A second round of testing was requested and funded by the vendor because of this inconsistent performance. Unfortunately, performance of some of the tested kits was affected by this variability, and only qualitative assessments could be made of some of the kit's performance characteristics, including precision, detection limit, effect of operator skill level, and effects of interferences.

Quantitative accuracy could not be assessed in Round 1 testing. In Round 2, the average percent bias of the current F-NTK test kit relative to the reference method was 28.9% with a technically trained Battelle operator, and 31.4% with a vendor operator, at nitrate-N levels of about 2 to 8 ppm in surface freshwater samples. An additional criterion for accuracy was the percentage of samples for which the F-NTK result was within 1 ppm of the reference result. By this criterion the F-NTK kits yielded accurate results for 80 to 100% of laboratory performance samples with a non-technical operator, and 60 to 70% with a technical operator. The corresponding percentages for drinking water samples were 100% with the non-technical operator and 67 to 100% with the technical operator. For surface freshwater samples, the corresponding averages were 57% with a vendor operator and 62% with a Battelle technical operator. For saltwater samples, the F-NTK kits with both technical and non-technical operators correctly indicated that nitrate-N levels were below 1 ppm in 100% of the samples.

In Round 1, no quantitative evaluation of linearity of response could be made due to variation in color of standards prepared from the F-NTK. In Round 2, the linearity of response of the current F-NTK kits was assessed using surface freshwater samples containing about 2 to 8 ppm nitrate-N. The linear regression equation for F-NTK results with the vendor representative as operator was: F-NTK ppm nitrate-N = 0.538 × (Reference ppm) + 1.02 ppm, with a correlation coefficient (r) of 0.779. The corresponding equation for results with the Battelle technical operator was: F-NTK ppm nitrate-N = 0.545 × (Reference ppm) + 1.00 ppm, with a correlation coefficient (r) of 0.789.

The precision of the F-NTK kits could not be evaluated quantitatively in Round 1, because of frequent non-detect results. However, when detectable results were obtained the F-NTK kits typically gave the same result for each replicate analysis of a single sample. The determination of

the F-NTK detection limit was similarly hampered by variability in the response of the kits in Round 1, but 1 ppm levels were readily measured with the current version of the kits that was used in the second phase of Round 2 testing.

In Round 1 testing, the presence of high levels of iron, NaCl, sulfate, and acid resulted in greater overestimation of nitrate-N levels than did low levels of these interferents. However, no quantitation of interference effects could be made.

The rates of false positives and false negatives of the F-NTK test kits were assessed relative to the reference method in Round 1, using 2 ppm nitrate-N as the decision level. The rate of false positives of the F-NTK test kit was 14% when used by the technical operator, and zero when used by the non-technical operator. The rate of false negatives was 32% with the technical operator and 38% with the non-technical operator.

Each F-NTK kit costs $30, and includes five packets of reagents, each of which is capable of analyzing three standards and five samples. The test kit allowed analysis of three standards and five samples. The preparation of the reagents in each kit takes approximately 45 minutes, and the analysis of the standards and samples take approximately 45 minutes each. The F-NTK test kit was easy to use, easy to transport, and required no maintenance. The test results show no strong effect of the operator skill level on F-NTK results. Data completeness in the test was 100%.

Chapter 8
References

1. *Test/QA Plan for Verification of Portable Analyzers*, Battelle, Columbus, Ohio, December 8, 2000.

2. Methods for the Determination of Inorganic Substances in Environmental Samples, EPA 16001R-93-100 - August 1993.

3. U.S. Code of Federal Regulations, Title 40, Part 136 Appendix B.

4. *Quality Management Plan (QMP) for the ETV Advanced Monitoring Systems Pilot*, Version 2.0, U.S. EPA Environmental Technology Verification Program, Battelle, Columbus, Ohio, October 2000.

Appendix A
Data Recording Sheet

ETV Field Data Sheet

Technician:_____ Sample Location:_____
Time:_____ Total Amount Collected:_____(L)
Date:_____ Water Temperature:____(F)____(C)

Air Temperature:_____(F)_____(C)
Humidity:_____
Barometric Pressure:_____(in Hg)
Weather Conditions:_____

Sample ID	Results	Notes

Comments:_____
_____
_____
_____
_____
_____

Technician's Signature:_____Date:_____
Technical Reviewer's Signature:_____Date:_____

Appendix B
Vendor Comments

Appendix B
Vendor Comments

(Battelle notes in parentheses and italics)

1. How the NECi Nitrate Assay Works

Enzymes are highly effective protein catalysts that accelerate specific chemical reactions in living systems. The enzyme used in the NECi test kits is nitrate reductase (NaR), which we purify from corn seedlings or yeast. The biological electron donor NADH (nicotinamide adenine dinucleotide, a B vitamin) provides the electrons required for the reduction. The catalytic rate of NaR is about 200 nitrate to nitrite conversions per second per molecule of NaR. The reaction is irreversible and goes to completion:

$$NADH + NITRATE \rightarrow NITRITE + NAD^{+} + OH^{-}$$

The resulting nitrite is then detected using standard Griess reaction chemistry. Nitrite in an acid solution will react with sulfanilamide and N-Naphthylethylenediamine (often called NED) to form a pink product. The intensity of the color is directly proportional to the quantity of nitrite in the solution. (The more color, the more nitrite is present.) Because all of the nitrate in the sample has been converted – reduced -- to nitrite by the nitrate reductase enzyme, the pink color is also directly proportional to the amount of nitrate that was present in the sample. In NECi's lab format nitrate test kits, the acid used is 3N HCl, and test results are read using a photometer or Microplate reader. A less corrosive, solid organic acid is used in our Field and Consumer kits to make them safer and more environmentally friendly. Nitrate content in these kits is determined based on visual comparison to nitrate standards and color charts when a photometer is not available.

NECi's nitrate assay is therefore comparable to EPA Standard Method 353.2 & 353.3, APHA 4500-NO_3 E & F, USGS I-2545-90, etc. The difference is that the nitrate reduction step is catalyzed by a rapid, selective, and highly specific enzyme rather than by cadmium, a toxic heavy metal. It is also comparable to the reagent system used in many conventional nitrate test kits and methods: again, the difference is that the toxic and less specific cadmium or zinc catalyst has been replaced by a protein.

Substances that can interfere with NaR activity have been fully studied and are limited to NaCl (NECi has saltwater kits that overcome this problem), and millimolar levels of chlorate and heavy metals.

NECi designed all test kits to give a strong color response to nitrate so that users can easily see the difference between 1 and 10 ppm nitrate-N in the Standard range kits, and between 0.05 – 1.0 ppm in our Low Range products. Color intensity of the 10 ppm standard yields an absorbance at 540nm (A_{540}) between 0.6 and 0.8 absorbance units (AU), i.e., a very dark pink solution. The kits are designed so that virtually 100% of the nitrate in a 50 μl sample of a 10 ppm nitrate-N

solution will be reduced to nitrite within 15 minutes. This is achieved by carefully controlling the amount of enzyme used per assay.

The Field kit reagent system was developed with funding from the Small Business Innovation Research program of the US Dept of Agriculture. A list of publications and abstracts describing the assay is included at the end of this document.

2. Standard Curves and Product Stability

The Field kits are designed so that they can be stored at 4°C or colder for at least six months; the stability of the NaR (enzyme) reagent is not guaranteed after that. The freeze-dried desiccant packets of NaR will retain sufficient activity to perform within NECi specifications after being exposed to up to six days of 85°F (typical summer weather); this permits NECi to ship at ambient temperature and allows users to bring kits to a field site. The NADH reagent also needs to be handled as specified. NADH is stable for long periods in the dry form, but loses viability (becomes oxidized to NAD^+) once it is in solution; NECi suggests a four hour window unless the reagent is stored below 5°C.

Once the NaR has been dissolved, it is stable for:

- Up to four hours once diluted in the Assay Buffer
- 24 - 36 hours at 85°F if reconstituted with NECi Enzyme Diluent
- 3 - 5 days at 4°C (standard refrigerator) in Enzyme Diluent
- Up to 12 months when stored in a freezer in Enzyme Diluent.

All NECi kits come with nitrate standards. Users are instructed to run at least one nitrate standard every time they run an assay (use the kit). We provide three concentrations of nitrate standard with each kit, and recommend using all three. This insures the user that 1) they have followed the instructions, and 2) all reagents are working. The color development of the standards also compensates for any degradation in reagent quality that may have occurred over time. Any data obtained without running at least one nitrate standard ***concurrently*** with samples is invalid. We also stress that the 10 ppm standard should yield a decidedly pink color (or dark gray when the user is colorblind).

NECi's QC procedures specify that a standard curve generated by the Standard Range Field Kits will yield a Blank of less than 0.025 AU at 540nm, and a line with a slope of at least 0.06 AU/ppm (meaning that the 10.0 ppm nitrate standard gives an absorbance at 540nm of at least 0.6 AU) with linearity of 0.95 or better.

Additional comments:

NECi has developed a Field Kit designed for analysis of low levels of nitrate in sea water, which has been used by Woods Hole and other demanding researchers for determination of nitrate levels between 0.05 – 1.0 ppm nitrate. Battelle adopted a literal interpretation of what constitutes a different product, which prevented us from providing our Low Range Seawater Kit format for testing.

(Battelle Note: ETV requirements are that a verification must address a single technology. The identification of the F-NTK kit as the subject of this verification was clearly specified in the ETV Vendor Agreement that NECi signed in order to participate in this test. That Vendor Agreement states that "ETV verification applies only to the single model of the subject technology submitted by the vendor for verification.")

NECi made changes in packaging format between Rounds 1 and 2 of the verification tests, because we are still refining the kits to be as user-friendly as possible without loss of accuracy or major increase in cost per test. Our customers had not liked the new format because they felt there were too many mixing steps. Since every step any user (novice or otherwise) makes increases the chance of introducing error in following any protocol, we decided to simplify the format. Any changes in kit formats have been limited to things such as number of assays per batch of reagent, or in how many premixed or premeasured reagents we can afford to provide.

The data from the second round of testing would have been unacceptable at NECi, falling far outside our QC/QA specifications. We are especially concerned with the Interference and Standard Addition studies data. The spiking studies, in which a known amount of nitrate was added to a solution containing no or little nitrate, failed to show any response to the added nitrate. NECi has performed hundreds of spiking studies, in deionized water, tap water, lake water, water from fish tanks, plant extracts, tissue culture media, etc. All yielded quantitative response to added nitrate. Some of this data has been published (see for example the ***Current Protocols*** chapter, reference number 4 in the NECi Publications list below). We do not understand why the data from the verification test show no response whatsoever. None of the labs to whom we have sent prototypes and products has ever reported such a result to us.

The Interference data is also puzzling. The data from the High Level study show *increased* nitrate values in the presence of moderate levels of iron, sulfate, and sodium chloride. These results cannot be supported in the literature. Sulfate has no affect, inhibitory or stimulating, on nitrate reductase activity. Metals can be inhibitors, but only at higher concentrations and when no EDTA is present in the buffer (we include this chelator in the Assay Buffer in all kits). Sodium chloride is a known **inhibitor** of the enzyme at 0.3%, its concentration in seawater, meaning that, if anything, the response to nitrate would have been decreased. See the review by WH Campbell (***Annual Reviews in Plant Physiology and Plant Molecular Biology***, 1999, reference number 1 in the Recent Academic Publications list below) for confirmation of these statements. And we cannot find any information in the literature that these concentrations of these chemicals can interfere in any way with the Griess reagents. We must conclude that there was a problem with this part of the work that had nothing to do with our reagent system.

(Battelle Note: All instructions for use of the F-NTK kits were followed in performing the verification test, and all test procedures were conducted and documented as specified in the test/QA plan for this verification. Technical Systems Audits confirmed that the procedures were performed properly. The literature on potential interferences notwithstanding, the observations from the matrix interference test were as presented in this report. Those results may well have been affected by the variability observed in test kit response in Round 1 of testing. In any case, the report makes no quantitative conclusions about the extent of interferences.)

We have never understood why the verification team rejected all suggestions to read the results of the testing in a photometer after the color observations had been recorded. We make very specific claims about test kit performance in our literature, and there is no way to prove or disprove our claims without use of a photometer. In addition, it would have been helpful for Battelle personnel to gauge the accuracy of their color judgment by comparing their observations with instrument data. We would be embarrassed to sell a product that could not differentiate between 5 and 10 ppm. I have made presentations at conferences, and we have written articles that have been published, asserting that our test kits provide semi-quantitative data for nonskilled users. We have user data that further confirms our results. However, it remains impossible to evaluate or troubleshoot because we have only subjective information regarding color development.

(Battelle Note: The F-NTK test kits were tested according to their intended use, i.e., as Field Nitrate Test Kits. For that reason, the test/QA plan for this verification called for visual reading and comparison of the color development of samples and standards. NECi approved that peer-reviewed test/QA plan before testing began. Furthermore, the primary observation of the Round 1 tests was that color development in F-NTK standards was weak to non-existent. Comparison to ion chromatographic reference results would not be improved by use of a colorimeter, when color development is absent to begin with. Finally, the comments above, that F-NTK performance claims can only be evaluated using a colorimeter, seem to suggest that the F-NTK kits cannot meet those claims in the field without the use of a colorimeter. This is contradictory to the stated use of the kits as field visual testing kits.)

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NECi PUBLICATIONS on Nitrate Reductase:

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3. Glazier, S.A., E.R. Campbell and W.H. Campbell (1998) Construction and characterization of nitrate reductase-based amperometric electrode and nitrate assay of fertilizers and drinking water. **Analytical Chemistry** 70/8:1511-1515.

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