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COST AND PERFORMANCE REPORT FOR BIOAVAILABLE FERRIC IRON (BAFeIII) ASSAY

by

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This report describes the demonstration and validation of a novel analytical technology: a bioavailable ferric iron (BAFeIII) assay. Demonstration and validation of the BAFeIII assay was conducted at four Department of Defense (DoD) installations.

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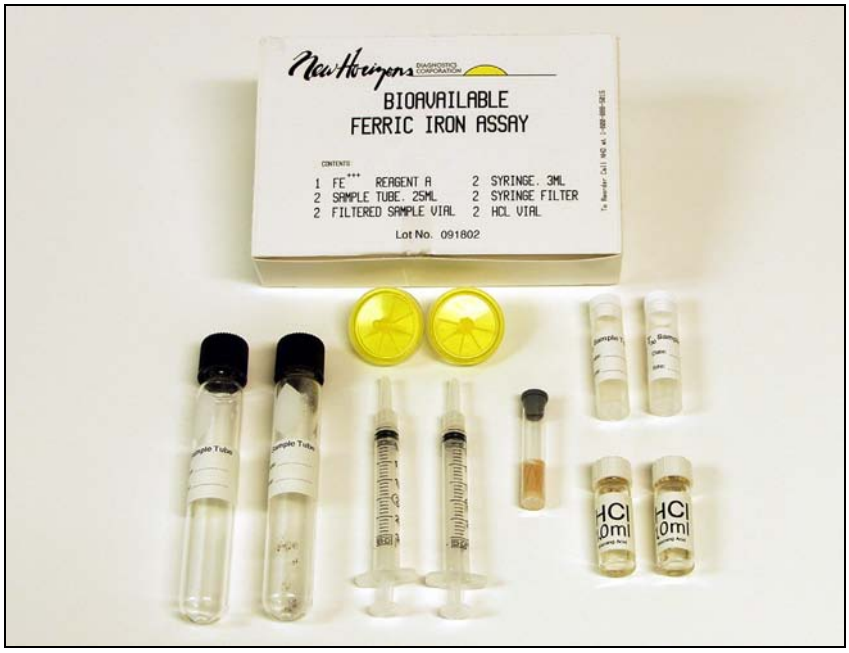

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Acronyms

AFB	Air Force Base
AHDS	Anthraquinol disulfonate
AO	Ammonium oxalate
AQDS	Anthraquinone disulfonate
ASTM	American Society for Testing Materials
AOFE	Ammonium oxalate extractable iron
BAFeIII	Bioavailable ferric iron
BET	Brunauer-Emmett-Teller
bgs	Below ground surface
BTEX	Benzene-toluene-ethylbenzene-xylenes
BrY	*Shewanella alga* BrY
CDB	Citrate dithionite bicarbonate
CDBFE	Citrate dithionite bicarbonate extractable iron
cDCE	*cis*-Dichloroethene
CDM	Camp Dresser & McKee Inc.
CDMBAFEIII	Bioavailable ferric iron measured by CDM
C_E	Extract concentration
CFR	Code of Federal Regulations
DCE	Dichloroethene
DoD	U.S. Department of Defense
EAB	Enhanced anaerobic biodegradation
EGDY	East Gate Disposal Yard
EPA/Ada	U.S. Environmental Protection Agency, Subsurface Remediation Division, National Risk Management Research Laboratory, Ada, Oklahoma
EPA/Athens	U.S. Environmental Protection Agency, Ecosystems Research Division, National Exposure Research Laboratory, Athens, Georgia
EPA	U.S. Environmental Protection Agency
EPABAFEIII	Bioavailable ferric iron measured by EPA/Ada
ESTCP	Environmental Security Technology Certification Program
Fe II	Ferrous iron
Fe III	Ferric iron
FeRB	Iron-reducing bacteria
FRTR	Federal Remediation Technologies Roundtable
F_S	Soil fraction
g/kg	Grams per kilogram
g/L	Grams per liter
GIT	Georgia Institute of Technology
HRC™	Hydrogen Release Compound™
lb	Pound
MFEBRY	Microcosm reducible ferric iron with BrY
MFEBRYFEOOH	Microcosm reducible ferric iron with BrY/FeOOH

mg/kg	Milligrams per kilogram
MNA	Monitored natural attenuation
msl	Mean sea level
MTBE	Methyl tertiary butyl ether
N	Number of samples
NAS	Naval Air Station
NAVFAC	Naval Facilities Engineering Command
NFESC	Naval Facilities Engineering Services Center
NHD	New Horizons Diagnostics Corporation
OU	Operable Unit
PCA	Principal components analysis
pH	pH – negative log of hydrogen ion concentration
PWIA	Public Works Industrial Area
RABITT	Reductive Anaerobic *In Situ* Treatment Technology
RMMAG	Electron microprobe analysis, relative mass percent magnetite
RPD	Relative percent difference
SBIR	Small Business Innovative Research
SCEC	Support Center, Elizabeth City
SPLP	Synthetic precipitation leaching procedure
SUBASE	Submarine Base
SVOC	Semivolatile organic compound
SW	Solid waste
T_0	Time 0 days
T_{30}	Time 30 days
TCE	Trichloroethene
TCLP	Toxicity characteristic leaching procedure
TEAPs	Terminal electron accepting processes
TFE	Total iron
UC	University of Colorado
USAF	U.S. Air Force
USCS	Unified Soil Classification System
USGS	U.S. Geological Survey
UST	Underground storage tank
V05FEII	0.5N HCl extractable ferrous iron
V05FEIII	0.5N HCl extractable ferric iron
V05FETOT	0.5N HCl extractable total iron
V6FEII	6N HCl extractable ferrous iron
V6FEIII	6N HCl extractable ferric iron
V6FETOT	6N HCl extractable total iron
VC	Vinyl chloride
VOC	Volatile organic compound
ZHE	Zero headspace extraction

Preface

This report describes the demonstration and validation of a novel analytical technology: a bioavailable ferric iron (BAFeIII) assay. Demonstration and validation of the BAFeIII assay was conducted at four Department of Defense (DoD) installations.

CDM in cooperation with the Naval Facilities Engineering Services Center (NFESC) was the principal investigator. Several organizations assisted in the validation of the BAFeIII assay, including the U.S. Environmental Protection Agency (EPA), U.S. Geological Survey (USGS), Georgia Institute of Technology (GIT), and University of Colorado (UC). Individuals contributing to completion of this project are listed below:

Carmen Lebron (PI)	NFESC
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Tom DiChristina, Ph.D.	GIT
John Drexler, Ph.D.	UC

This work also would not have been possible without the access to and help from the following DoD installations:

SUBASE Bangor, Washington
Ft. Lewis, Washington
NAS Pensacola, Florida
US Coast Guard Support Center, Elizabeth City, North Carolina

Points of contact for this project are provided in Section 8.

1.0 Executive Summary

1.1 Background

A bioavailable ferric iron (BAFeIII) assay was invented and developed by CDM with funding from the U.S. Air Force. This assay is a standardized bioassay that directly measures the concentration of BAFeIII in soil or sediment. A BAFeIII test kit based on the assay is manufactured by New Horizons Diagnostics Corporation (NHD) of Columbia, Maryland.

BAFeIII is defined as follows:

> *Ferric iron (Fe III) that is capable of being reduced by microorganisms that oxidize another chemical species and derive energy from the electron transfer.*

BAFeIII is an important terminal electron acceptor with significant assimilative capacity in many natural environments. Dissolved ferrous iron (Fe II) in groundwater is typically measured to assess Fe III reduction and calculate assimilative capacity, but this measurement underestimates this terminal electron accepting process because most Fe II remains bound to the soil. Dissolved Fe II also gives no indication of the amount of Fe III present in aquifer soil that is bioavailable. BAFeIII in the soil must be measured in order to quantify the true assimilative capacity of an aquifer.

Iron-reducing bacteria (FeRB) use and are dependent on BAFeIII. FeRB are known to oxidize or mineralize various organic compounds, such as benzene, toluene, vinyl chloride (VC), and methyl tertiary butyl ether (MTBE). Continued activity over a period of years is dependent on the presence of sufficient BAFeIII.

BAFeIII can also affect reductive dechlorination in MNA and EAB applications. BAFeIII can result in TCE being reductively dechlorinated to cDCE only and further reductive dechlorination can be inhibited (AFCEE, 2004). Thus knowledge of the BAFeIII concentration can indicate the potential for incomplete reductive dechlorination of TCE. It can also be used for planning EAB remedies. If the BAFeIII concentration is sufficient to inhibit cDCE reductive dechlorination, reductive dechlorination of TCE to cDCE and VC followed by oxidative biodegradation of VC and possibly cDCE under iron-reducing conditions may be a better approach.

1.2 Objectives of the Demonstration

The overall objective of this project was to demonstrate and validate the performance of the BAFeIII assay as an analytical technology for use in supporting bioremediation. Specific objectives were to:

- Validate the BAFeIII assay method using a combination of confirmatory analyses conducted by the U.S. EPA (EPA/Ada and EPA/Athens), Georgia Institute of Technology, and University of Colorado.
- Quantify costs associated with the technology.

1.3 Regulatory Drivers

Analysis of BAFeIII is not required at this time and is considered optional by regulatory agencies. Additionally, no method for BAFeIII has been approved by the EPA since it does not approve methods for unregulated compounds. The analyte (BAFeIII) of interest in this demonstration is discussed in the EPA technical guidance on monitored natural attenuation (MNA) and enhanced anaerobic bioremediation (EAB) of chlorinated solvents (U.S. EPA, 1998; AFCEE, 2004). These documents review the use of BAFeIII data to assess MNA of organic contaminants such as VC and consumption of injected electron donors during EAB.

1.4 Demonstration Results

Table 1-1 presents validation results and indicates that the BAFeIII assay is a precise analytical method for direct BAFeIII quantification.

Table 1-1. Performance Objectives and Results for the BAFeIII Assay

Type of Performance Objective	Primary Performance Criteria	Expected Performance (Metric)	Actual Performance Objective Met?
Qualitative	Relationship between BAFeIII assay and degree of iron oxide crystallinity/surface area.	Positive association	Yes
	Relationship between BAFeIII assay and confirmatory analyses.	Positive association	Yes
	Range of BAFeIII assay relative to other analytical techniques.	Similar or better range	Yes
	Sample throughput of BAFeIII assay.	Labor time ≤ similar methods	Yes
	Versatility of BAFeIII assay.	Consistent performance	Yes
Quantitative	Intra-laboratory precision of BAFeIII assay based on soil and laboratory replicates.	Absolute RPD ≤ 35	Yes
	Inter-laboratory precision of BAFeIII assay based on replicates analyzed by both CDM and EPA/Ada.	-35 ≥ RPD ≤ 35	Yes

1.5 Stakeholder/End-User Issues

The BAFeIII assay is an important tool that allows remedial project managers to obtain a more accurate and complete picture of site geochemistry and microbiology. This tool is useful in bioremediation projects involving natural attenuation and enhanced anaerobic bioremediation. Use of the direct BAFeIII assay is recommended as a replacement for indirect chemical extraction methods. Additionally, it is recommended that BAFeIII analysis of soil be conducted in addition to ferrous iron analysis in groundwater. The BAFe III assay purchase cost ranges from $50 to $75 each depending on the quantity purchased. Additional equipment, supplies, and labor are required and the estimated analysis cost was calculated to be $212 each based on analysis of 6 samples. BAFeIII analysis conducted by a commercial laboratory has been quoted at $250 per analysis.

2.0 Technology Description

2.1 Technology Development and Application

The BAFeIII test kit is pictured in **Figure 2-1**. The test kit is manufactured by New Horizons Diagnostics Corporation (NHD) of Columbia, Maryland. The BAFeIII assay involves addition of a soil sample to a test tube that contains the lyophilized iron-reducing bacterium *Shewanella alga* BrY, lactate as an electron donor, and a mineral salts medium supplemented with reagents that accelerate the assay.

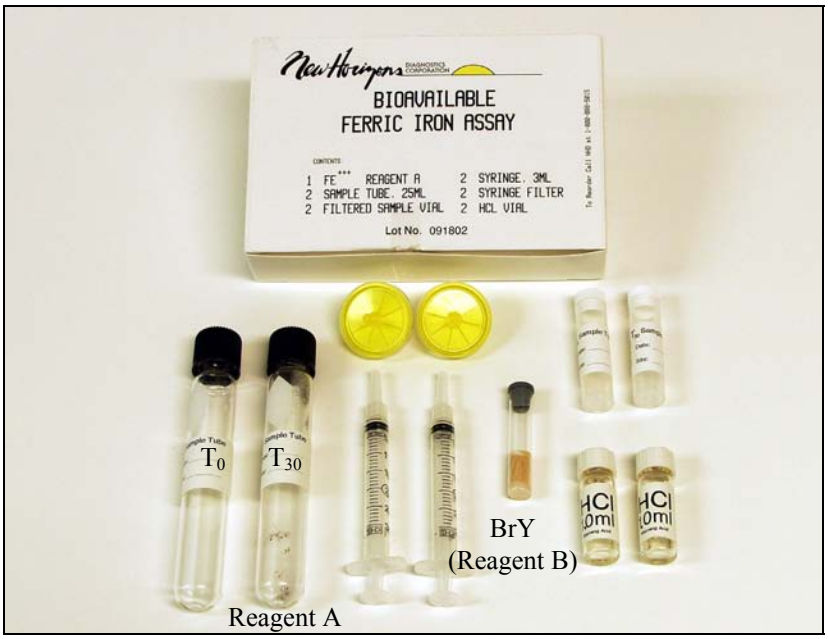


Figure 2-1. BAFeIII Assay Kit

The BAFeIII assay can be used for site characterization and monitoring in MNA and EAB applications. Natural attenuation of benzene-toluene-ethylbenzene-xylenes (BTEX) is one common example. Initial site characterization for MNA involves the calculation of assimilative capacity of an aquifer for biodegradation of BTEX. The BAFeIII assay can be used to estimate the assimilative capacity in the aquifer material for BTEX biodegradation. These results can be used to determine the mass of BTEX that has been degraded previously and the potential for future BTEX biodegradation.

BAFeIII can also affect reductive dechlorination in MNA and EAB applications. Reductive dechlorination is based on chlorinated compounds such as trichloroethene (TCE) serving as a terminal electron acceptor. Complete dechlorination of TCE to ethene requires that each dechlorination product (i.e., *cis*-dichloroethene [cDCE] and vinyl chloride [VC]) also serve as terminal electron acceptors. Terminal electron acceptors will be used preferentially according to thermodynamic and kinetic considerations. For example, VC may be dechlorinated to ethene

under methanogenic conditions (and correct microbial populations) but not under aerobic or denitrifying conditions in part because the free energies for reduction of oxygen and nitrate are greater (i.e., more negative) than for reduction of VC. The free energy for reduction of several BAFeIII oxides is greater than that for reductive dechlorination of cDCE to VC (Evans and Koenigsberg, 2001). BAFeIII can result in TCE being reductively dechlorinated to cDCE only and further reductive dechlorination can be inhibited (AFCEE, 2004). Thus knowledge of the BAFeIII concentration can indicate the potential for incomplete reductive dechlorination of TCE. It can also be used for planning EAB remedies. If the BAFeIII concentration is sufficient to inhibit cDCE reductive dechlorination, reductive dechlorination of TCE to cDCE and VC followed by oxidative biodegradation of VC and possibly cDCE under iron-reducing conditions may be a better approach.

A challenge in applying BAFeIII results is that insufficient experience exists to use the results in quantitative models at this time. Nevertheless, the results from the assay can be used in either a quantitative or qualitative manner. The BTEX example above represents a quantitative application of BAFeIII assay results. The enhanced anaerobic bioremediation application represents a qualitative application of the assay. Experience using the assay results in a qualitative fashion will lead to more quantitative applications as a database is developed. An example of a potential application is incorporation of BAFeIII as a variable in biodegradation computer modeling programs such as the EPA program BIOPLUME IV which is currently being beta-tested (John Wilson, personal communication). BIOPLUME is a two-dimensional, finite difference model for simulating the natural attenuation of organic contaminants in groundwater due to the processes of advection, dispersion, sorption, and biodegradation. The BIOPLUME program uses an USGS solute transport code and kinetic equations to determine the fate and transport of the organic contaminants and the electron acceptors (dissolve oxygen, nitrate, BAFeIII, sulfate, and carbon dioxide) and the reaction by-products (including dissolved Fe II). BioRedox-MT3DMS is another numerical fate and transport model that includes BAFeIII as an input parameter (Thompson et al. 2004).

2.2 Process Description

The procedure for the BAFeIII assay is graphically illustrated in **Figure 2-2** and includes the following steps following homogenization:

- Two 5-gram samples are placed into each of two 25-mL assay tubes labeled T_0 and T_{30}.
- The T_0 tube, which is used to determine the initial or ambient concentration of Fe II present in the soil immediately following sample collection, contains no reagents or BrY, is filled with distilled water and 1 mL concentrated HCl, capped, then placed on a tube rotator for 48 hours, during which time weakly associated Fe II is extracted from the soil.
- Following the extraction period, the T_0 extract liquid is filtered, if necessary, and analyzed for initial Fe II.
- The T_{30} tube is filled with distilled water plus the assay reagents, capped, mixed by hand, and then incubated in the dark at room temperature for 30 days. During the incubation period the iron-reducing bacteria (i.e., BrY) consume lactate and reduce BAFeIII to Fe II.

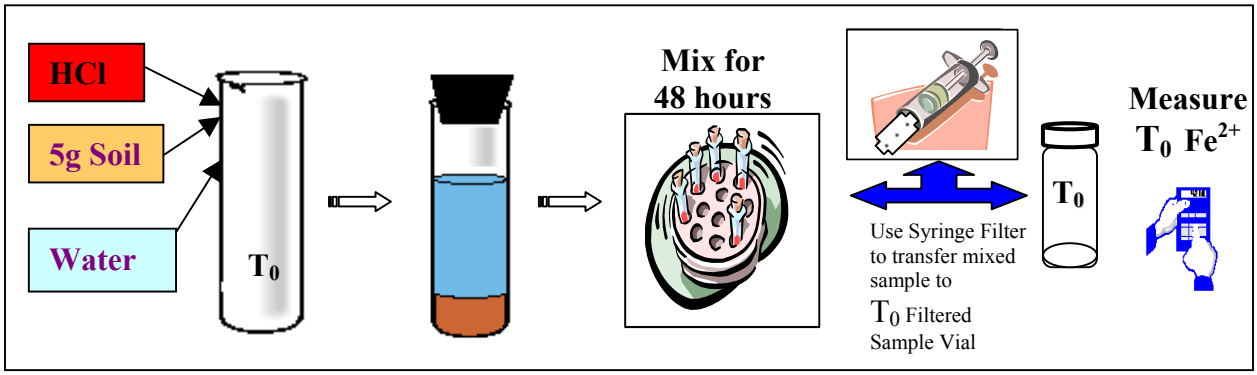
- Following the incubation period, 1 mL of liquid is withdrawn from the T_{30} tube, discarded, replaced with 1 mL concentrated HCl to create a 0.5N HCl solution, then the tube is placed on a tube rotator for 48 hours, during which time both initial Fe II and Fe II produced by BAFe III reduction are solubilized.
- Following the extraction period, the T_{30} liquid is filtered and analyzed for Fe II.
- The concentration of Fe II in the T_{30} extract liquid is the total Fe II – the sum of ambient Fe II (T_0 tube) and BAFeIII. The following formula is used to calculate BAFeIII:

$$\text{BAFeIII}(\text{mg/kg}) = \frac{(C_E \text{ in } T_{30}) - (C_E \text{ in } T_0)}{217\, F_S}$$

C_E is the measured concentration of Fe II in the extract liquid (mg/L) and F_S is the solids fraction (g dry soil/g wet soil).

- Extract Fe II concentrations (C_E) are measured using a Hach test kit (Hach Company, Method 8146) followed by dilution. Dilution requirements are determined using Quantofix® Iron 1000 test strips (VWR Part No. 60787-724) without the Iron 1 reagent.

STEP 1:



STEP 2:

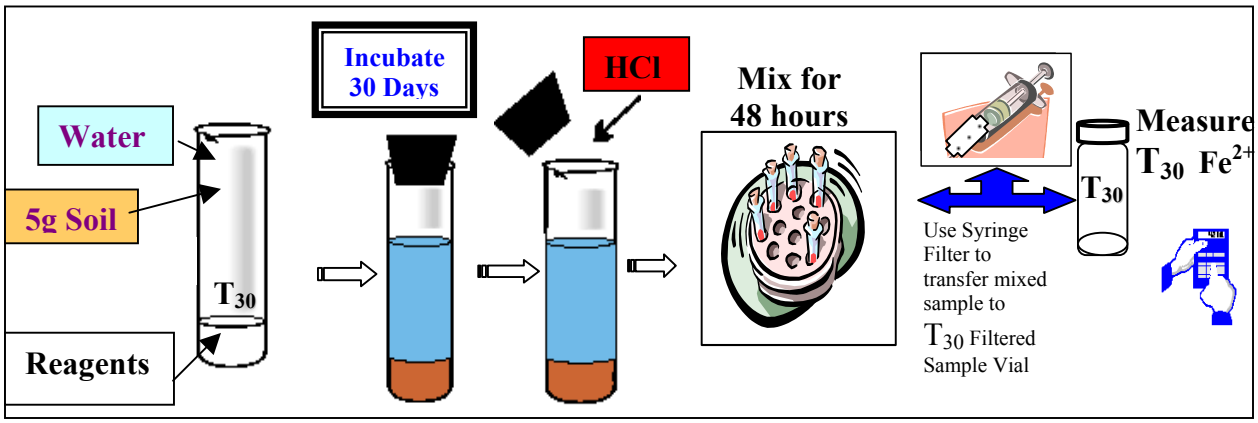


Figure 2-2. BAFeIII Assay Kit Procedure

2.3 Previous Testing of the Technology

Initial development and preliminary field-testing of the BAFeIII assay technology was conducted under a Phase I Small Business Innovative Research (SBIR) grant from the USAF (Evans, 1997; Evans, 2000). Further development and field-testing of the technology was conducted under Phase II of the SBIR, which led to development of a field test kit (Evans and Jones, 1999; Evans et al., 1999).

2.4 Advantages and Limitations of the Technology

Advantages and limitations of the BAFeIII assay are summarized in **Table 2-1**. These advantages are described in detail below.

Table 2-1. Advantages and Limitations of the BAFeIII Assay

Category	Advantages	Limitations
Analytical Methodology	Direct method that uses a bioassay rather than an indirect chemical extraction.	Bacteria must be stored frozen prior to use.
	Facultative bacterium is used that does not need to be stored anaerobically.	Uses *Shewanella alga* BrY which may not be representative of all sites.
	Assay composition and method has been standardized.	30-day incubation time.
Sampling Requirements	Frequent sampling is not necessary.	Requires soil or sediment samples and invasive sampling for their procurement.
	Requires 5 grams of soil per analysis.	
Technology Application	Indicates BAFeIII electron donor demand for MNA and EAB applications.	No reference method for BAFeIII analysis exists.
	Analysis is more robust than commonly used chemical extraction methods.	Can give maximum values for BAFeIII because of presence of electron shuttles in assay reagent.

BAFeIII data allow site managers and regulators to evaluate MNA and EAB at sites more completely and accurately than with dissolved Fe II data alone. In the case of BTEX natural attenuation, dissolved Fe II data allow calculation of the mass of BTEX that has been biodegraded historically and is being biodegraded currently. BAFeIII data allow calculation of the mass of BTEX that will be biodegraded in the future. It is impossible to calculate future potential for BTEX biodegradation using dissolved Fe II data alone. Furthermore, since most dissolved Fe II remains bound to the soil, the historical and current mass of biodegraded BTEX is underestimated using dissolved Fe II data for electron acceptor calculations. Completion of a mass balance and subsequent understanding of contaminant source fate is dependent on accurate electron acceptor calculations. In the case of EAB of TCE, BAFeIII data allow determination of the total electron donor demand. High electron donor demand can decrease the likelihood that TCE will be reductively dechlorinated beyond cDCE to VC and ethene upon addition of an electron donor such as molasses, lactic acid, vegetable oil, or HRC™ (Evans and Koenigsberg, 2001; AFCEE, 2004). High electron donor demand can also prevent complete reductive dechlorination under MNA conditions. Dissolved Fe II data alone give no indication of this electron donor demand.

While the BAFeIII assay provides these advantages over measurement of dissolved Fe II, it does depend on soil sampling in the saturated zone, which is costly and inconvenient for routine sampling. On the other hand, measurement of BAFeIII in soil likely does not require quarterly sampling of numerous locations. This decreased sampling frequency can minimize the additional cost associated with soil sample collection.

The BAFeIII assay evaluated in this report is in a sense a standardized bioassay. Besides being the first of its kind, the assay has many advantages that make it an easy-to-use and reliable analytical tool. Unlike laboratory-based microcosm studies, it is standardized, self-contained, portable, packaged for field or laboratory use, and includes lyophilized FeRB that are relatively stable. Care must be taken to store the lyophilized FeRB under freezing conditions for stability. The bioassay reagents other than FeRB are packaged separately from the FeRB and are stable at room temperature. These chemical components are present at optimal levels and are known to influence bioavailability. Their presence is intended to provide reproducible, standardized, and direct estimates of the maximum concentration of BAFeIII in a given soil sample. Recognition that the assay results are maximum values should be considered when using the data. For example, the amount of electron donor required to overcome iron reduction alone in an enhanced anaerobic bioremediation scenario may be less than predicted based on BAFeIII assay results.

A potential limitation of the BAFeIII assay is that the indigenous FeRB may be different in their iron-reducing capabilities when compared to the strain used in the assay (i.e., *Shewanella alga* BrY). Inclusion of BrY in the assay was intended to make the assay standardized and reproducible. Additionally, since BrY is a facultative microorganism, storage under anaerobic conditions is not necessary, further increasing the test kit's ease-of-use. BAFeIII is an operationally defined analyte (i.e., the measured value of the analyte is dependent on the method used for its analysis) and use of BrY is part of this operational definition. The BrY-based assay yields a reproducible maximum value of BAFeIII in a given sample. The decision whether or not to use BrY in the bioassay represents a trade-off of obtaining site-specific results versus standardization, reproducibility, and ease-of-use. If results using only indigenous bacteria are desired, the BrY culture can easily be left out of the assay since it is packaged separately. Iron reduction would then be accomplished via FeRB that are indigenous to the soil sample used in the assay. A new limitation would be introduced by conducting the assay in this manner, however, since the required incubation time would be unknown. Monitoring of the assay over time would be required which would decrease the ease-of-use of the assay. Direct comparison of BAFeIII results to results for other sites would also not be possible. In addition to BrY, electron shuttles (i.e., humic acids and AQDS) are included in the assay. The inclusion of electron shuttles is also part of the operational definition. Their inclusion increases the reliability and speed of the assay and also can result in determination of maximum BAFeIII values.

Another potential limitation of the BAFeIII assay involves the one-month incubation time. However, considering that standard turnaround time for most analytical laboratories is two weeks, this time requirement is acceptable in most cases.

Finally, no standardized technologies exist for directly measuring BAFeIII. Other methods that have been used or evaluated for BAFeIII measurement are presented in **Table 2-2** and discussed below.

Table 2-2. Other Methods for BAFeIII Measurement

Category	Method	Advantages	Limitations
Chemical Extraction	• 0.5 N HCl • 6 N HCl • Hydroxylamine HCl • Citrate dithionite bicarbonate • Ammonium oxalate	• Easy to use • Inexpensive	• Indirect • Indicates chemical extractability rather than bioavailability • Does not accurately represent true bioavailability of different crystalline phases
Redox Titration	• AHDS titration	• Data indicate good correlation with microcosms	• Not commercially available • Requires anaerobic conditions
Sophisticated Instrumentation	• Electron microscopy • Electron microprobe • X-ray diffraction • Near infrared spectrophotometry • Mössbauer spectroscopy	• Potential identification of specific crystalline phases	• Expensive • Some methods are insufficiently sensitive
Treatability Study	• Microcosm	• Potentially the best simulation of actual site geochemistry and microbiology	• Expensive • Not standardized

Chemical extraction, sophisticated instrument-dependent methods, and microcosm studies have been evaluated, but each has significant disadvantages. Selective extraction using a variety of extractants, including various concentrations of HCl, hydroxylamine-HCl, ammonium oxalate, citrate, citrate dithionite bicarbonate and other compounds has been used to attempt to quantify BAFeIII. However, these extractants do not provide direct measurements and do not necessarily correlate to the concentration of BAFeIII (Lovley and Phillips, 1987). Also, extraction methods do not take into account the effect of groundwater chemistry on bioavailability. A laboratory method for BAFeIII quantitation involving redox titration of soil with the reduced form of anthraquinone disulfonate (AQDS) also known as anthraquinol disulfonate (AHDS) has been evaluated (Hacherl et al., 2001). This method is not readily available. Sophisticated instrumentation, including electron microscopy, electron microprobe analysis, near infrared spectrophotometry, and Mössbauer spectroscopy have been evaluated but are not especially useful. Furthermore, these techniques are expensive and not readily available. Microcosm studies have been conducted in various laboratories but with different methods and media.

While microcosm studies are a direct approach to evaluation of BAFeIII, no standard method exists for conducting them; they are also time-consuming and expensive. Therefore, the major advantage of the BAFeIII assay over other methods is that it is a standardized and direct measurement of BAFeIII.

3.0 Demonstration Design

3.1 Performance Objectives

The BAFeIII assay is difficult to validate because no standard method exists to measure bioavailability of ferric iron. Nevertheless, performance criteria were developed *a priori* in order to be able to validate the BAFeIII assay. These criteria were based initially on the demonstrated relationship between Fe III bioavailability and Fe III oxide particle surface area (Roden and Zachara, 1996). Different Fe III oxides ranging from amorphous ferric oxyhydroxide to various crystalline forms have different specific surface areas. Oxides with greater specific surface area (amorphous oxides having the greatest) have been shown to be more bioavailable for iron reduction (Roden and Zachara, 1996). Thus the initial working hypothesis of the evaluation was that the BAFeIII concentration determined by the assay should correlate to the specific surface area of the oxide particles in a soil sample. In addition, other factors associated with groundwater may influence Fe III bioavailability (Evans, 2000; Roden and Urrutia, 2002) including pH, specific conductivity, divalent cations, electron shuttles such as humic acids, chelators, and adsorbed anions including ferrous iron. Performance objectives for the demonstration were based in part on these multiple factors and are presented in **Table 3-1**.

Table 3-1: Performance Objectives for BAFeIII Assay

Type of Performance Objective	Primary Performance Criteria	Expected Performance (Metric)	Actual Performance Objective Met?
Qualitative	Relationship between BAFeIII assay and degree of iron oxide crystallinity/surface area.	Positive association	Yes
	Relationship between BAFeIII assay and confirmatory analyses.	Positive association	Yes
	Range of BAFeIII assay relative to other analytical techniques.	Similar or better range	Yes
	Sample throughput of BAFeIII assay.	Labor time ≤ similar methods	Yes
	Versatility of BAFeIII assay.	Consistent performance	Yes
Quantitative	Intra-laboratory precision of BAFeIII assay based on soil and laboratory replicates.	Absolute RPD ≤ 35	Yes
	Inter-laboratory precision of BAFeIII assay based on replicates analyzed by both CDM and EPA/Ada.	-35 ≥ RPD ≤ 35	Yes

3.2 Selection of Test Sites

Selection of sites was based on the following criteria:

- Availability of an existing groundwater monitoring well network.
- Geological and hydrogeological characteristics.

- Terminal electron accepting processes (TEAPs) occurring in the aquifer.
- Concentrations of parent compounds and presence of daughter products.
- Groundwater chemistry.
- Ability to drill on site.
- Availability and quality of existing site characterization documentation.

The objective was to select sites that offered a range of iron concentrations, geochemical characteristics, and terminal electron accepting processes, to enable validation of the BAFeIII assay. Four test sites were used for the demonstration of the BAFeIII assay:

- Bangor Naval Submarine Base in Kitsap County, Washington (SUBASE Bangor) – dissolved petroleum hydrocarbons and chlorinated VOCs.

- Fort Lewis Logistics Center near Tillicum, Washington (Fort Lewis) – chlorinated VOCs.

- Naval Air Station (NAS) in Pensacola, Florida (NAS Pensacola) – chlorobenzene and TCE.

- U.S. Coast Guard Support Center in Elizabeth City, North Carolina – Fuel Farm site with petroleum hydrocarbons and MTBE and North Beach site with chlorinated hydrocarbons.

3.3 Test Site History/Characteristics

Summaries of the four demonstration site are provided in this section. Additional details are available in the Technology Demonstration Plan (CDM, 2001) and the Final Report (NAVFAC, 2005).

SUBASE Bangor

The study area for this demonstration is the vicinity of Operable Unit 8 (OU8), located in the Public Works Industrial Area (PWIA) of SUBASE Bangor. SUBASE Bangor is located near the town of Silverdale, Washington. An onsite UST is believed to be the source of a release of unleaded gasoline into the surrounding media between 1982 and 1986. In 1986, soil vapor extraction/air system and product recovery were implemented to clean up the site. To date, liquid petroleum hydrocarbons remain in several monitoring wells at the PWIA (EA, 2000). Chlorinated VOCs are also present in site groundwater (EA, 2000).

Geological conditions at OU8 at SUBASE Bangor have been highly characterized by drilling and monitoring well installation. The area consists of four stratigraphic units: construction fill, Vashon till (Qvt), Vashon Advance Outwash (Qva), and Lawton Clay. The construction fill can be found 2 to 3 feet bgs and consists of a sandy material. Underlying the construction fill and ranging to a depth of about 45 ft bgs is the Vashon till, which consists of silt, sand, gravel, and cobbles. This unit is 20 to 40 ft thick. The Vashon Advance Outwash (location of the shallow aquifer) is beneath the Vashon till and consists of sand, silt, and gravel. The thickness of the Vashon Advance Outwash is about 100 to 130 feet. Beneath the Vashon Advance Outwash is

the Lawton Clay aquitard. A silty transition zone in the bottom of the Vashon Advance Outwash separates the shallow aquifer from the lower aquitard.

Fort Lewis

The study area for this demonstration is the vicinity of the East Gate Disposal Yard (EGDY) of the Fort Lewis Logistics Center (Fort Lewis), located south of Tacoma, Washington. The EGDY, which is situated at the northwest corner of the base, originally was used for storage and disposal of various solid and liquid waste products. Since 1982, studies have been conducted at the EGDY to verify and delineate contamination at the site. Affected media were soil and groundwater, with the prominent contaminant being trichloroethene (TCE) (Battelle, 2000).

The upper portion of the EGDY at Fort Lewis consists of a brown to black alluvial sand and gravel matrix with local lenses of silts. The material gets coarse with depth. Underlying this formation at about 260 feet msl is the Vashon Till, which is a complex mixture of silt, sand, and clay. The Vashon Till has low permeability and serves as a barrier between the upper and deeper aquifers. At the source area the groundwater can be encountered between 8 and 15 feet bgs. Farther downgradient the groundwater is generally between 10 and 35 feet bgs. The upper aquifer is unconfined and mostly anaerobic. Groundwater flow is generally west to northwest. There are more than 80 monitoring wells and piezometers on site.

Battelle Memorial Institute (in cooperation with the Air Force Research Laboratory, USGS, EPA, and Cornell University) performed Reductive Anaerobic *In Situ* Treatment Technology (RABITT) at the EGDY of Fort Lewis, and further site characterization details can be found in their report (Battelle, 2000). The BAFeIII demonstration was done in the vicinity of the RABITT demonstration.

NAS Pensacola

The study area for this demonstration is the vicinity of the wastewater treatment plant at the NAS in Pensacola, Florida (NAS Pensacola), located near Pensacola Bay in the far northwest corner of the state (USGS, 1999).

The area predominantly consists of marine and fluvial terrace deposits ranging from fine- to medium-grained sands, silts, clays, and gravel. The site has two aquifers, a shallow aquifer and a deeper confined aquifer (referred to as the underlying main producing zone). There is a 20-foot-thick confining barrier of low-permeable silts and clays that separate the upper and lower aquifers. The upper aquifer is composed of fine- to medium-grained sands. The main producing zone is used locally as a water supply and consists of permeable sands and gravel. Two plumes have been identified at the site, one comprised of chlorinated ethenes and the other chlorinated benzenes. Most of the contaminants on site are located in the upper aquifer region. The depth of contamination ranges from 20 to 40 feet bgs.

Elizabeth City

The U.S. Coast Guard Support Center in Elizabeth City, North Carolina, is located on the southern bank of the Pasquotank River. Two separate areas at the site were used in this demonstration, the fuel farm area (petroleum hydrocarbon) and the North Beach area (chlorinated VOCs).

The following description of the fuel farm area was obtained from the report by Wilson, et al. (2000). The former fuel farm was located south of a concrete ramp used to recover seaplanes from the Pasquotank River. A plume of MTBE and fuel hydrocarbons in ground water emanates from a source area in the location of the former fuel farm, and flows under the concrete ramp toward the Pasquotank River to the north, and toward a drainage canal along the western side of the seaplane ramp. This source area corresponds to the former location of fuel storage tanks on the site. Fuel was stored at the site until December 31, 1991. The fuel farm had been in use since 1942, and originally consisted of a 50,000-gallon concrete underground storage tank and two steel underground storage tanks with a volume of 12,000-gallons and 15,000-gallons, respectively. The steel tanks were apparently removed in the mid-1980s. In addition to the underground storage tanks, two steel, aboveground storage tanks with a capacity of 50,000 gallons were installed in the mid-1980s. There was evidence of corrosion in the transfer lines from these tanks. They were taken out of service and removed from the site. No evidence of a release from the pipes was discovered. The U.S. Coast Guard began a free product recovery effort at the site in September 1990. Eight recovery wells were arranged around the source area in a circle. By March 1992, a total of 79,000 gallons of fuel was recovered.

The following description of the North Beach Disposal Area was provided by ARCADIS (2004). The North Beach Disposal Area occupies 4.8 acres in the northeast corner of the Support Center, Elizabeth City (SCEC). The site is bounded immediately north and west by the Pasquotank River and to the east by a drainage canal. The North Beach site is unpaved. Approximately half of the site is heavily wooded. The other half, where the majority of disposal activities may have occurred, consists of grass-covered open areas. Historical information and site investigation activities indicate that industrial wastes generated at the SCEC may have been buried at the North Beach Disposal Area. The exact quantity and nature of the wastes disposed of in the North Beach Disposal Area are unknown; however, it is suspected that the wastes may have included chlorinated solvents, batteries, petroleum wastes, scrap metals, paint sludges, and plating wastes. Disposal activities likely occurred from the 1940s to approximately 1975. Four separate areas of concern (i.e., Source Areas 1, 2, 3, and 4) were identified at the site and had elevated concentrations of metals, scrap-metal fragments, VOCs, and semivolatile organic compounds (SVOCs) in soil. Only PCE, TCE, cis-1,2-DCE, vinyl chloride and pentachlorophenol are present in groundwater at elevated concentrations.

Hydrogen Release Compound^{TM} (HRC^{TM}), a food-grade polylactate ester, was injected into the shallow aquifer zone at multiple points near Source Area 2 of the North Beach Disposal Area from January 21 to 25, 2003. The treatment area for HRC injection is a grid approximately 40 feet wide by 100 feet long, encompassing Monitor Wells GP20, GM315, GM330, and GM360. Within the grid area, Standard HRC was injected into 40 points while HRC Primer was injected

into 9 points. A total of 5,545 pounds of HRC was injected across the grid, with between 110 and 135 pounds of Standard HRC or primer injected at each point. The depths for these injections were 5 feet below ground surface (ft bgs) to 45 ft bls within the primary interval impacted by chlorinated VOCs. Quarterly monitoring has been conducted for one year since the HRC injections. Results indicate that HRC does not appear to have significant influence on groundwater geochemistry beyond the immediate vicinity of the injection points within the grid.

3.4 Physical Set-up and Operations

On-site operations involved collection and packaging of samples as described in Section 3.5. Site visits were conducted as follows:

Site 1 – SUBASE Bangor: January 22 to February 2, 2001
Site 2 – Fort Lewis: February 19 to March 2, 2001
Site 3 – NAS Pensacola: April 29 to May 3, 2002
Site 4 – Elizabeth City: October 23 to 25, 2002

Physical operation and set-up of the BAFeIII test kit was conducted as described in Section 2.2.

3.5 Sampling/Monitoring Procedures

Groundwater samples were collected from existing monitoring wells on each site using low flow techniques and a peristaltic or bladder pump system. Soil borings were completed for collection of soil samples using hollow-stem auger, direct push technology, or hand-auger. During drilling, a CDM engineer or scientist logged and sampled the borings. The soils were visually described and classified in accordance with the Unified Soil Classification System (USCS; ASTM D2488-84). Generally two sections of each boring were collected (i.e., the top and bottom portions). Specific sample locations and depths are presented in the Final Report (NAVFAC, 2005). Attempts were made to obtain different types of soil samples as defined by USCS. The soil from each section was homogenized by hand (mixing with stainless steel spoon in a bowl) and then placed in 4- or 8-ounce glass jars, capped with Teflon^{TM}-lined lids, and labeled prior to shipment to the labs. Samples were shipped in coolers with ice to maintain temperature between 2 and 6 ^{o}C. Soil samples were sent to the CDM laboratory in Bellevue, Washington for BAFeIII analysis. Soil samples were also sent to other organizations for analysis as detailed in **Figure 3-1**.

3.6 Analytical Procedures

Figure 3-1 illustrates the different analyses that were conducted to validate the BAFeIII assay. Appendices H and I of the Technology Demonstration Plan (CDM, 2001) and Appendix A of the Final Report (NAVFAC, 2005) include detailed analytical procedures that were conducted to demonstrate and validate the BAFeIII assay. The significance of each analysis relative to BAFeIII is provided, along with the method description and the organization that conducted the analysis.

Analyses were conducted immediately after sample collection with the following exceptions: 1) BAFeIII analysis of samples collected from SUBASE Bangor and Ft. Lewis were conducted on January 11, 2002 on archived samples, and 2) HCl extractions were repeated in March, 2002 using the ferrozine analysis method.

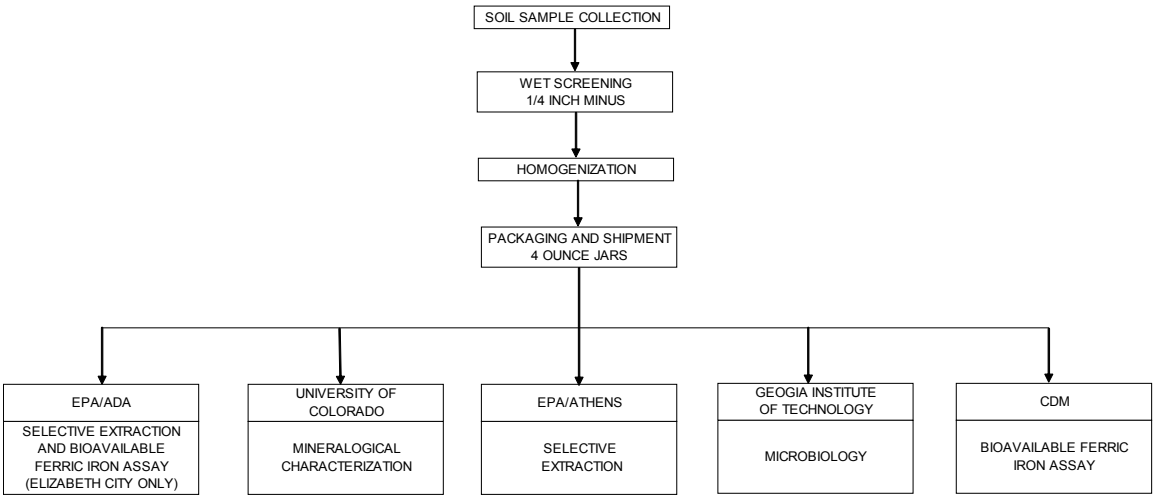


Figure 3-1. Soil Sample Allocation and Analysis

4.0 Performance Assessment

4.1 Performance Data

This section presents a brief summary of performance data for the BAFeIII assay. A more complete description of these data is presented in the Final Report (NAVFAC, 2005).

Replicate analyses were conducted to demonstrate the intra-laboratory precision of the BAFeIII assay. An absolute RPD of 35 was used to evaluate the replicate data. This value was selected as an approximate criterion for analyses of replicates of inherently non-homogeneous soils. Further discussion of the valid use of an RPD of 35 can be found in EPA guidance on analysis of solid matrices (U.S. EPA, 2002). The overall average absolute RPD for the 76 CDM intra-laboratory replicates was 29.7, which met the RPD ≤ 35 criterion, with absolute RPDs for most of the individual replicates (77.6%) also meeting the criterion (**Figure 4-1**).

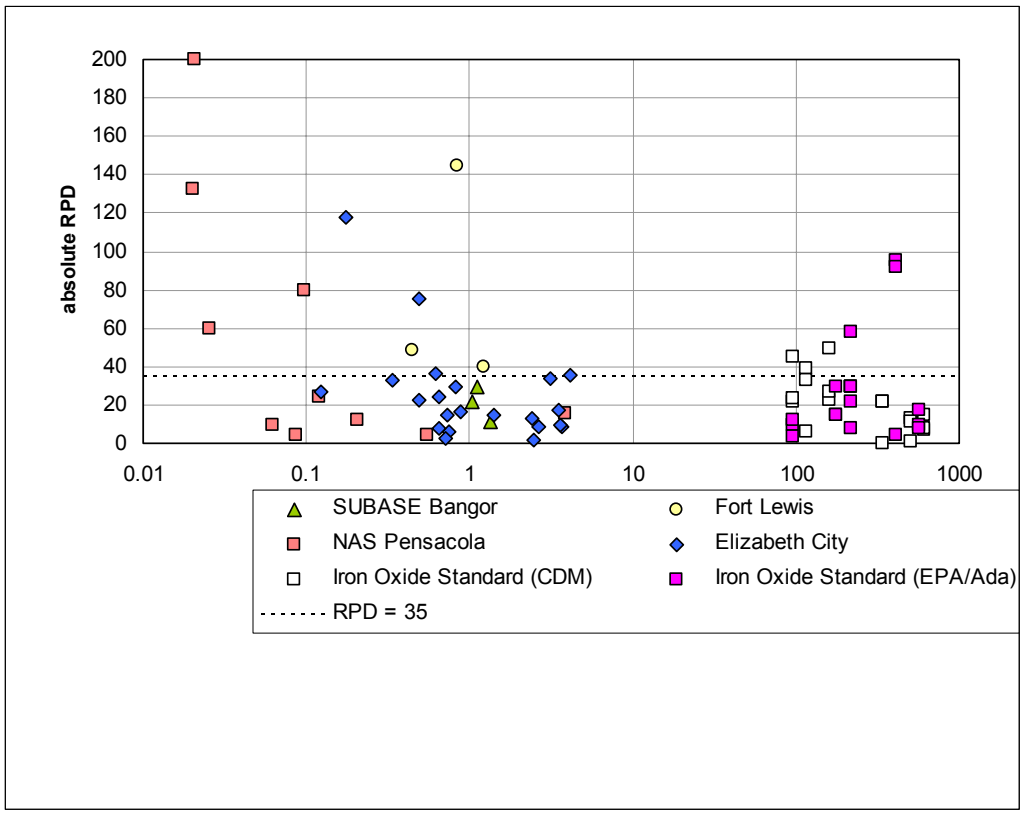


Figure 4-1. Intra-Laboratory Replicate Precision for BAFeIII Assay

Replicate analyses were also conducted to demonstrate the inter-laboratory precision of the BAFeIII assay. An RPD of 0 was used to evaluate the replicate data. This value was selected to represent no difference between the analyses as conducted by the two laboratories, i.e., a perfect 1:1 correlation and no inter-laboratory bias. The overall average RPD for the 40 inter-laboratory replicates was 12, which indicated that the CDM results were slightly higher, on average, than

the EPA results (**Figure 4-2)** but the difference was not statistically significant. The correlation coefficient for the log-transformed data was 0.98. For the aquifer samples, 25% of the samples agreed within a factor of 20%, 50% agreed within a factor of 37%, 75% agreed within a factor of 66%, and all of the samples agreed within a factor of 170%. For the iron oxide samples, 25% of the samples agreed within a factor of 8%, 50% agreed within a factor of 29%, 75% agreed within a factor of 63%, and all of the samples agreed within a factor of 160%.

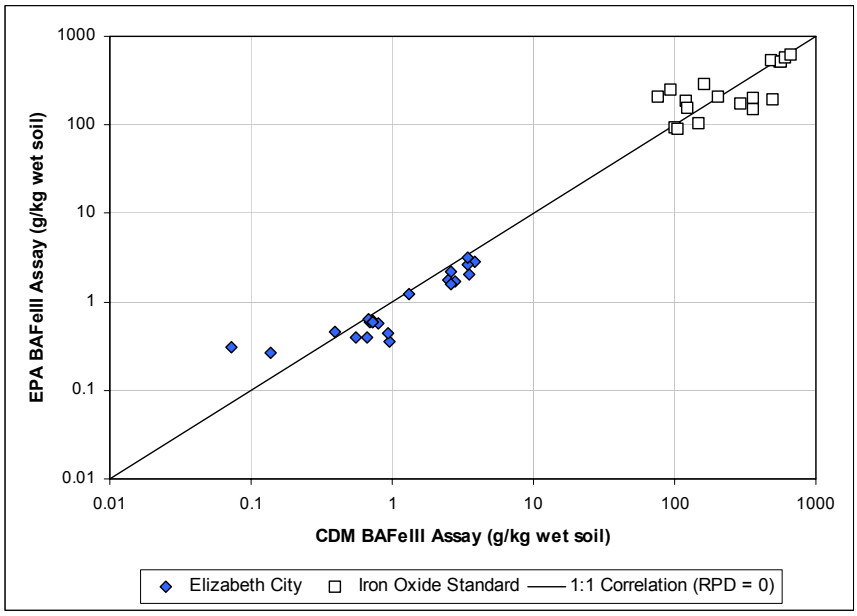


Figure 4-2. Inter-Laboratory Replicate Precision for BAFeIII Assay

Principal components analysis (PCA) was conducted to evaluate the relationships and associations among the various potential bioavailable Fe III factors. PCA is a statistical method of identifying correlations of a large number of variables by grouping inter-related variables into "components". Results indicated that approximately 43% of the total soil data set variance was explained by the first two components. The correlations between the original variables and the components are referred to as "loadings". Thus, variables with high loadings in a particular component are associated with each other (i.e., they are intra-correlated). A listing of the variables with loadings greater than 0.45 in the first 2 components is provided in **Table 4-1** (see the list of acronyms for definitions).

Table 4-1: Component Loadings

Component	Variables with Loadings > 0.45
1	V6FETOT, V6FEII, RMMAG, V6FEIII, V05FETOT, V05FEIII, MFEBRY, V05FEII
2	V6FEIII, V05FETOT, V05FEIII, MFEBRY, AOFE, EPABAFEIII, TFE, CDBFE, MFEBRYFEOOH, CDMBAFEIII

Component 1, which accounted for approximately 22% of the total variance in the data set, contained a number of factors that were associated with each other but not with the BAFeIII

assay. Component 1 was concluded to be associated with iron as opposed to BAFeIII. Component 2, which accounted for approximately 20% of the total variance in the data set, contained several of the same variables that loaded highly into Component 1. Component 2 also contained the CDM and EPA BAFeIII assay variables and the confirmatory analyses citrate dithionite bicarbonate and ammonium oxalate extractable Fe, total Fe, and iron oxide (FeOOH)-supplemented microcosm with BrY. Component 2 was concluded to be associated with BAFeIII. These results demonstrated that positive associations exist between the BAFeIII assay and confirmatory analyses that are related to and indicative of BAFeIII.

Figure 4-3 shows, for the synthetic iron oxides, iron concentrations measured using the BAFeIII assay, microcosms, and chemical extractions, al being expressed as fractions of total iron concentrations. The results for the BAFeIII assays conducted by CDM and EPA were qualitatively similar to results of microcosms conducted with *Shewanella alga* BrY. Results for the chemical extractions were qualitatively different from the BAFeIII assay results. These data demonstrate the BAFeIII assay yields a more representative estimate of iron oxide bioavailability than do chemical extractions.

CDM and EPA BAFeIII assay results for 6-line ferrihydrite, lepidocrocite, and magnetite were not significantly different (p values ranged from 0.28 to 0.48). CDM and EPA BAFeIII assay results for 2-line ferrihydrite and hematite were significantly different. The CDM value was 90% greater than the EPA value for 2-line ferrihydrite ($p = 0.0074$). The CDM value was 56% less than the EPA value for hematite ($p = 0.011$).

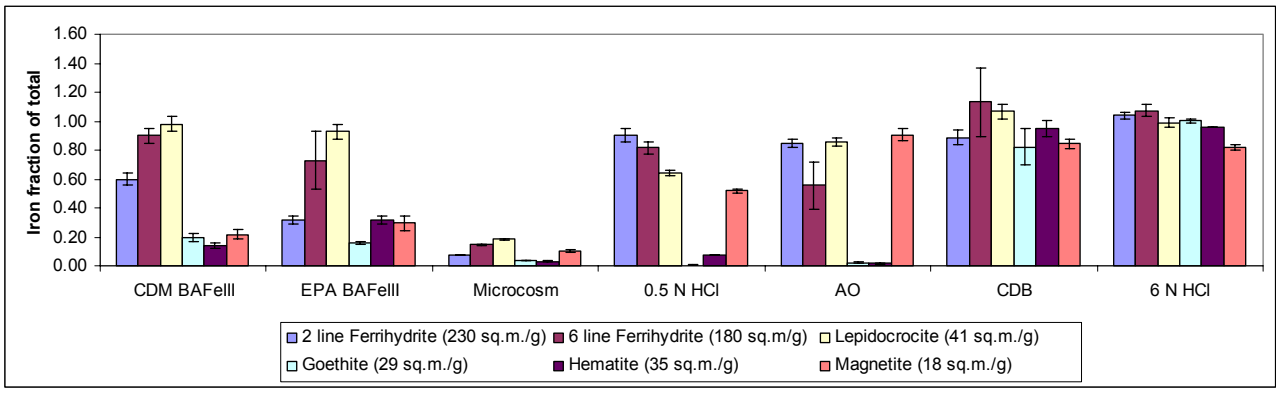


Figure 4-3. Summary of Iron Oxide Analytical Results

Figure 4-3 also shows the specific surface area for each oxide. Goethite, hematite, and magnetite had relatively low BET surface areas and correspondingly low BAFeIII fractions based on the BAFeIII assay. Poorly crystalline, high surface area iron oxides such as 2-line and 6-line ferrihydrite demonstrated greater BAFeIII fractions than highly crystalline, low surface area iron oxides such as magnetite, hematite, and goethite. Such a relationship between bioavailability and surface area has been demonstrated previously (Roden and Zachara, 1996). On the other hand, lepidocrocite had a relatively low surface area and yet the highest BAFeIII fraction. Previous investigations have demonstrated that lepidocrocite has a high bioavailability

even though it surface area is low (Roden, 2003; Schwertmann et al., 1986). The relatively high bioavailability of lepidocrocite appears to be related to its crystal structure (Cooper et al., 2000; Hersman et al., 2001). These data further indicate that factors other than surface area affect iron oxide bioavailability. Thus the direct BAFeIII bioassay yields results that are more representative of biological iron oxide reduction than are indirect chemical extractions.

A mass balance calculation on iron at the Elizabeth City Fuel Farm site was conducted to further validate the BAFeIII assay and illustrate the use of BAFeIII data. The mass balance was conducted by calculating the mass of BAFeIII originally present in the area impacted by hydrocarbons, calculating the mass of ferrous iron removed in soluble form via downgradient groundwater transport, and then comparing the two values. Comparisons of the calculated iron masses are presented in **Table 4-2.** These results indicate that the BAFeIII assay did not underestimate the amount of BAFeIII iron present in the soil and thus gave a more robust BAFeIII estimate. Estimates of BAFeIII obtained using 0.5 N HCl, ammonium oxalate, and citrate dithionite bicarbonate all underestimated the mass of BAFeIII.

Table 4-2: Iron Mass Balance for Elizabeth City Fuel Farm

Parameter	Estimated Mass (lb)
Minimum advectively removed Fe II	13,000
Maximum advectively removed Fe II	40,000
Minimum BAFeIII assay estimate	52,000
Maximum BAFeIII assay estimate	65,000
0.5 N HCl estimate	11,000
6 N HCl estimate	53,000
Ammonium oxalate estimate	16,000
Citrate dithionite bicarbonate estimate	15,000
Total iron estimate	96,000

4.2 Performance Criteria

Performance criteria and actual performance for the BAFeIII assay are summarized in **Table 4-3**.

Table 4-3: Expected Performance and Performance Confirmation Methods for BAFeIII Assay

Performance Criteria	Expected Performance Metric (pre-demonstration)	Performance Confirmation Method	Actual (post-demonstration)
Primary Criteria (Performance Objectives) (Qualitative)			
Relationship between BAFeIII assay and degree of iron oxide crystallinity/surface area.	Positive association	Measurement of both BAFeIII and BET surface area for iron oxide standards with varying degrees of crystallinity and surface areas.	Generally a positive association with the exception of lepidocrocite which was expected.

Table 4-3: Expected Performance and Performance Confirmation Methods for BAFeIII Assay (Cont'd)

Performance Criteria	Expected Performance Metric (pre-demonstration)	Performance Confirmation Method	Actual (post-demonstration)
Primary Criteria (Performance Objectives) (Qualitative)			
Relationship between BAFeIII assay and confirmatory analyses.	Positive association	Multivariate statistical analysis (principal components analysis). Loadings ≥0.45 for original variables within a principal component demonstrate positive association.	Most of the variance in the original variables (about 43%) accounted for by two principal components. Component 2 contained the BAFeIII variable and the BAFeIII-relevant confirmatory analysis variables with loadings greater than 0.45.
Range of BAFeIII assay relative to other analytical techniques.	Similar Range	Comparison of analytical range to citrate dithionite bicarbonate extractable Fe, ammonium oxalate extractable Fe, total Fe, 0.5N and 6.0N HCl extractable Fe III, and to microcosm reducible FeIII with BrY.	Range was similar to or greater than all comparable methods examined. Recommended minimum BAFeIII reporting limit is 0.1 g/kg.
Sample throughput of BAFeIII assay.	Labor time ≤ similar methods	Comparison with other methods used to characterize BAFeIII.	Labor time was less than or approximately the same as other methods.
Versatility of BAFeIII assay.	Consistent performance	BAFeIII assay conducted on a wide variety of soils and standards, at a wide variety of sites, and under a wide variety of environmental conditions.	Performance was consistent with other methods used to characterize BAFeIII.
Primary Criteria (Performance Objectives) (Quantitative)			
Intra-laboratory precision of BAFeIII assay based on soil and laboratory replicates.	Absolute RPD ≤ 35	Field and laboratory replicate sample collection and analyses.	Average absolute RPD = 29.7.
Inter-laboratory precision of BAFeIII assay based on replicates analyzed by both CDM and EPA.	-35 ≥ RPD ≤ 35	Field and laboratory replicate sample collection and analyses. Blind standard analysis.	Average RPD = 11.6 but difference between CDM and EPA results were not statistically significant.

4.3 Data Assessment

A general assessment of the data was included in Section 4.1 and a more detailed assessment is presented in the ESTCP Final Report (NAVFAC, 2005). In summary, the following conclusions were made based on the data:

- Intra-laboratory precision was better than the relative percent deviation (RPD) criterion of 35. This precision level is adequate for the intended use. Precision deteriorated at BAFeIII concentrations less than the recommended 0.1 g/kg minimum reporting limit.
- Inter-laboratory precision was excellent and no statistically significant difference between CDM and EPA results was observed.
- Positive associations between the BAFeIII assay and confirmatory analyses were observed using principal components analysis (PCA) of soil data. These results indicate the BAFeIII assay yields results that are representative of iron bioavailability.
- An iron balance conducted on data obtained from the U.S. Coast Guard Support Station fuel farm site indicated the BAFeIII assay yields a more robust estimate of BAFeIII compared to common chemical extraction methods.
- Data obtained using synthetic iron oxides of varying surface area indicated the direct BAFeIII bioassay yields results that are more representative of biological iron oxide reduction than are indirect chemical extractions.
- The BAFeIII assay is easy to use and involves addition of weighed soil samples to pre-prepared test tubes, incubation, extraction, and measurement of reduced Fe II with a Hach test kit.

4.4 Technology Comparison

No other standardized and direct method exists for measurement of BAFeIII. The data demonstrated that the BAFeIII assay yields a more robust estimate of BAFeIII and yields results that are more representative of biological iron reduction than chemical extraction methods.

5.0 Cost Assessment

ESTCP guidance states that costs should be reported in this section in the recommended Federal Remediation Technologies Roundtable (FRTR) format. However, this format is primarily suited for presenting costs associated with remedial process technologies where costs need to be broken down into categories such as capital, operational and maintenance, and life cycle costs. Costs associated with purchasing and using the bioavailable iron assays do not fall into these categories, and so the FRTR format has not been used. This alternative costing approach was used in the ESTCP Final Report (NAVFAC, 2005) that was approved by ESTCP. The subsections below have been prepared to describe all costs associated with obtaining the assays and using them to analyze soil samples that have already been collected from a given site.

5.1 Cost Reporting

Purchasing the Test Kit

The test kit is currently commercially available as the "Bioavailable Ferric Iron Assay" produced by New Horizons Diagnostics Inc. of Columbia, Maryland (NHD). Information about the kit and how to order can be found online at www.nhdiag.com . Orders can be placed at 800-888-5015 or 410-992-9357. As of the writing of this report, the costs of the kits were:

- 1 to 11 kits: $75 each
- 12 to 19 kits: $60 each
- ≥ 20 kits: $50 each

Since the kit includes a reagent that contains bacteria which are temperature-sensitive, overnight shipping (not included in the above costs) is required. The kits contain syringes, syringe filters, hydrochloric acid, incubation and sample vials, and the lyophilized BrY inoculum.

Additional Supplies/Equipment

To analyze ferrous iron before and after incubation with BrY, a Hach kit is typically used. The reagent needed to run the 1,10-phenanthroline ferrous iron method (Hach Method 8146) costs $15 for 100 Hach reagent powder pillows, or about $18 for 25 Hach AccuVac® ampules. The Hach method also requires colorimetric analysis to quantify the ferrous iron as shown below:

1. Using a high quality spectrophotometer (Hach models are about $2,000)
2. Using a Hach DR/800 series portable colorimeter (about $600 to $900)
3. Using a Hach color disc (about $30)

The choice of which of these methods to use will depend primarily on the number of samples that are to be analyzed in the long term, whether analyses are to be performed in the field, and on the availability of the required equipment. The color disc method is semi-quantitative, and is not recommended due to its low level of accuracy relative to the other two methods. Additionally, Quantofix® Iron 1000 test strips (VWR Part No. 60787-724) may be used (without the Iron 1 reagent) to bracket the Fe II range and thus the required dilution prior to conducting the Hach assay.

If only a few samples are to be analyzed, it may be most economical to have the T_0 and T_{30} extracts samples analyzed for ferrous iron by a commercial analytical laboratory. Typically, this analysis can be performed for approximately $30 per sample (i.e., $60/bioavailable iron sample since both the T_0 and T_{30} measurements must be conducted). A small tumbler or orbital shaker is needed for the HCl extraction steps of the assay to rotate the vials and provide mixing of the soil with the acid. This item can be purchased from most lab supply companies for approximately $250. Miscellaneous other supplies for performing the assay and ferrous iron analyses include pipettes, beakers, a small field balance (accuracy to 0.1 gram), and safety ware (gloves and glasses). An approximate cost for these supplies is $300.

Labor

The labor time required to perform the assay can be divided into three steps:

1. Vial T_0: Combine soil, HCl, and water. Vial T_{30}: Combine soil, water, and bioassay reagents.
2. Measure ferrous iron in Vial T_0 following mixing for 48 hours.
3. After a 4-week incubation period, add HCl and measure ferrous iron in Vial T_{30} following mixing for 48 hours.

The first step takes approximately one half hour, depending on the number of samples to be run. Running the Hach kit ferrous iron analysis for Vial T_0 (step 2) typically takes 1.5 hours for up to five samples – this includes time to run standards and prepare dilutions as necessary. Following the 4-week incubation period, another hour and a half would be needed for step 3 to add the HCl to Vial T_{30} and analyze for ferrous iron. If an analytical lab is used for ferrous iron analysis, the required labor would include labeling, packing, shipping the sample containers and filling out the chain of custody forms.

Cost Example

As a costing example, consider the following scenario:

- Six soil samples are to be analyzed for bioavailable iron at a given site.
- The samples have been collected (sample collection costs were not included).
- A field technician and bench space are available to perform the extraction steps.
- Neither a spectrophotometer nor a Hach color-measuring equipment is available for the ferrous iron analysis.

Costs under this scenario are shown in **Table 5-1**.

Table 5-1: BAFeIII Assay Costs

Item	Units	No. of Units	Unit Cost	Cost
Assay Kits	Each	6	$75	$450
Ferrous Fe Analysis (commercial lab)	Sample	12	$30	$360
Supplies	Lump Sum	1	$100	$100
Labor	Hour	6	$60	$360
Total				**$1,270**

The unit cost per sample is thus $212. As an alternative, commercial laboratories can also be contracted to conduct the BAFeIII analysis. Microseeps (www.microseeps.com) has quoted a price of $250 per sample for the BAFeIII analysis using the NHD test kit.

5.2 Cost Analysis

Cost Drivers

Primary drivers are the test kit procurement cost, Fe II analysis cost, and labor cost not including soil sampling costs. Soil sampling costs will be the primary driver in cases where soil sampling is conducted solely for the purpose of BAFeIII analysis.

While not directly related to the cost of performing the bioavailable iron kit method, the 4-week incubation period may in some circumstances result in higher indirect costs compared to a method that gives results over a 2-week period typically associated with analytical lab turnaround times. Such indirect costs need to be considered on a case-by-case basis.

If, based on the results of analyzing initial soil samples, it is determined that additional analysis is warranted, then additional costs associated with obtaining additional soil samples would be necessary. These costs would be highly site-specific and would depend on the depth of sample needed, number of samples to be collected, and site access issues.

Sensitivity Analysis

Incremental BAFeIII assay costs for soil sample collection are highly dependent on drilling method, depth, and sample collection frequency. For this sensitivity analysis both direct push and conventional (e.g., hollow-stem auger) methods were considered. For direct push it was conservatively assumed that 5 borings to a depth of 50 feet could be conducted per day and that 2 soil samples would be collected from each boring for BAFeIII analysis. At a daily drilling cost of $2,500 ($1,500 for the driller and $1,000 for engineering oversight) the incremental cost for drilling per sample is $250. For conventional drilling the incremental cost was based on a unit drilling cost of $50/foot and other parameters used for direct push. The incremental cost for drilling per sample is $1,350 ($1,560 - $212). **Table 5-2** summarized the results of this sensitivity analysis demonstrating the effect of drilling costs on the total assay cost. Often other analyses including total organic carbon, cation exchange capacity, metals, grain size distribution, and USCS classification may also be conducted on the soil samples. The incremental cost is then apportioned over the various analyses.

Table 5-2: BAFeIII Cost Scenarios

Scenario	Total BAFeIII Assay Cost per Sample
Drilling cost not included	$210
Direct push drilling included, no other analyses conducted	$460
Direct push drilling included, 4 other analyses conducted	$260
Conventional drilling included, no other analyses conducted.	$1,560
Conventional drilling included, 4 other analyses conducted.	$480

DoD-Wide Savings

Standardized and cost-effective analytical technologies to support MNA and EAB efforts are necessary. The BAFeIII assay is more costly than the current approach form BAFeIII measurement (i.e., it is infrequently measured at the present time). However, it is anticipated that use of this method will promote more widespread acceptance and more cost-effective implementation of MNA and EAB at DoD sites.

The DoD is responsible for approximately 2,093 characterized chlorinated solvent plumes (U.S. EPA, 1997). MNA is applicable to approximately 20% of chlorinated solvent sites, or 420 of the DoD plumes (U.S. EPA, 1998). Enhanced anaerobic biodegradation (EAB) may also be applicable to many of these sites. BAFeIII analysis has not been conducted in the past because of difficulty, lack of standardization, and cost. The BAFeIII assay and test kit is one of several tools that can now be used to support monitored natural attenuation and enhanced anaerobic bioremediation. This test kit will benefit the DoD by making this analysis available, which will promote application of MNA and EAB at these sites. However, estimation of the DoD savings attributable to the BAFeIII test kit alone is challenging. Nevertheless, the average cost for a pump and treat operation is $9.8 million per site (Quinton et al., 1997). If MNA is applied to 25% of the chlorinated plumes (~100 sites) at a cost of $1 million per site, the potential savings is significant.

5.3 Cost Comparison

Cost Comparison

For comparison purposes, the contract analytical lab costs for conducting synthetic precipitation leaching procedure (SPLP) or toxicity characteristic leaching procedure (TCLP) analyses with zero headspace extraction (ZHE) conducted on soil samples is on the order of $90. ZHE is required to prevent oxidation of Fe II to Fe III. The extractions would be modified to use a particular chemical extractant such as 6N HCl. However, it is important to note that extraction with 6N HCl overestimates the bioavailability of many iron oxides as shown in **Figure 4-3**. Analysis of extracts for total Fe and Fe II is on the order of $50. Thus the total cost is on the order of $140. There would be some labor required for labeling, packing, shipping the sample containers and filling out the chain of custody forms. This cost 30 percent less than the BAFe III assay cost. The cost of laboratory microcosms varies widely but typically is at least $10,000 and can be as high as $50,000. These costs are clearly greater than the BAFeIII assay.

Cost Basis

The analytical costs listed above are based on discussions with laboratories for performing an extraction procedure similar to the toxicity characteristic leaching procedure (TCLP) as described in 40 CFR 261/SW846 Method 1311 or synthetic precipitation leaching procedure (SPLP) as described in 40 CFR 261/SW846 Method 1312. Only the extraction acid would be modified from the standard TCLP or SPLP method. The extractant would be analyzed for ferrous and ferric iron using the phenanthroline method number 3500-Fe D (Greenberg et al., 1992) with appropriate controls for acidity of the extracts.

6.0 Implementation Issues

6.1 Cost Observations

The unit procurement cost for the BAFeIII test kit ranges from $50 to $75 depending on the number of kits purchased. The total BAFeIII analysis cost is about $210 not including soil collection costs. A BAFeIII site characterization may include anywhere from 10 to 100 analyses and thus the total analytical cost would range from $2,100 to $21,000. This cost is generally a small fraction of the total site characterization and remediation cost. Soil sample collection will comprise the majority of the cost and thus conducting multiple analyses on collected soil samples is clearly warranted.

6.2 Performance Observations

The BAFeIII test kit met all performance objectives and criteria. The fact that the relatively low surface area iron oxide lepidocrocite had a high BAFeIII concentration initially was an unexpected and interesting observation that supported the use of a direct bioassay approach in the test kit. This result deviated from the original hypothesis proposed for this demonstration/validation project, but was later determined to be consistent with current scientific data and theories on iron bioavailability. Therefore, the BAFeIII test kit was demonstrated to be a reliable, precise, easy-to-use, and cost-effective assay that yields realistic and relatively robust estimates of BAFeIII for a wide variety of soil types.

6.3 Scale-Up

The BAFeIII test kit can be used to analyze one or more samples. Scale-up is not especially relevant to this assay. However, processing of a large number of samples may warrant subcontracting the work to a commercial analytical laboratory. Larger federal laboratories or research institutions will likely be capable of easily conducting the assay in-house.

6.4 Other Significant Observations

The test kit Reagent B (i.e., lyophilized strain BrY) must be kept frozen until ready for use or it will lose viability. The test kit Reagent A is stable at room temperature.

Soil sampling should be conducted with care to minimize exposure to air and oxidation of reduced iron oxides. Use of an anaerobic glove box is not considered necessary, but saturated samples should be handled quickly and packed full into jars to minimize headspace. Preferably, the assay should be initiated as quickly as possible. Maximum sample holding times for this assay have not been determined.

The BAFeIII test kit can be procured from New Horizons Diagnostics Corporation in Columbia, Maryland. Their phone number is 1-800-888-5015 and their web address is www.nhdiag.com.

6.5 Lessons Learned

BAFeIII iron concentrations can vary laterally and vertically in impacted and background areas at a site. It is important to collect and analyze a sufficient number of samples to obtain useful results that are not obscured by heterogeneity. In general, samples should be collected from zones of greatest contaminant mass flux because it is in these zones where BAFeIII consumption will represent the most significant attenuation of contaminant mass. Samples should be collected at multiple depths along plume transects and should include several upgradient and/or cross-gradient background soil samples. Duplicate analysis of samples is recommended. While none of these recommendations are hard and fast, their intent is to dissuade the user from collecting just a few samples. Such a minimalistic approach is likely to result in less useful results.

The test kit can be used to estimate the maximum BAFeIII concentration in a soil or sediment sample. Groundwater chemistry also has a significant effect on iron bioavailability (Evans, 2000; Roden and Urrutia, 2002). Therefore, groundwater chemistry data should be considered in addition to BAFeIII assay results when making conclusions with respect to iron bioavailability.

6.6 End-User Issues

Education of regulators on the importance of BAFeIII is necessary because this parameter is not commonly measured or reported. Education of regulators on this test kit will also be necessary. End users will be able to refer regulators to this Cost and Performance Report and the ESTCP Final Report (NAVFAC, 2005) when establishing the validity of this BAFeIII test kit. Currently available models that include BAFeIII as an input parameter will also promote education about and acceptance of this assay as described further in Section 6.7.

6.7 Approach to Regulatory Compliance and Acceptance

Dr. John Wilson of the U.S. EPA has been a strong advocate of the need to quantify BAFeIII at contaminated sites. The EPA listed BAFeIII analysis as being under development in the Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water (U. S. EPA, 1998). Dr. Wilson identified the BAFeIII test kit as a possible solution to this need. He was thus an important partner in this ESTCP project. Measurement of BAFeIII at sites is being conducted at increasing frequency and is becoming a regular component natural attenuation and anaerobic bioremediation evaluations. An excellent example is the fate and transport model BIOPLUME IV which includes BAFeIII as an input parameter. This model is currently being beta-tested by EPA. The fate and transport model BioRedox-MT3DMS also includes BAFeIII as an input parameter (Thompson et al. 2004). The BAFeIII assay is also listed as an optional method to determine competition from iron reduction during EAB (AFCEE, 2004).

BAFeIII test kit results will be used to document natural attenuation processes and to design enhanced anaerobic bioremediation remedies. In both cases the data can be used to provide regulators with a more complete and accurate technical basis for the site remedial approach. This ESTCP Cost and Performance Report will be an instrumental component of this interaction with respect to technology validation.

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