REGION I, EPA-NEW ENGLAND

IMMUNOASSAY GUIDELINES FOR PLANNING ENVIRONMENTAL PROJECTS

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PREFACE

Region I, EPA-New England promotes the development and use of innovative technologies to improve environmental monitoring and remediation activities, while reducing both the time and expense involved. One such technology gaining increasing momentum in the field of environmental testing is immunoassay (IA). IA can provide real-time field analysis of a wide variety of environmental parameters at a fraction of the cost and time for conventional full protocol laboratory analyses. IA techniques can be used effectively in the hazardous waste remediation process to delineate the extent of contamination and to ascertain that cleanup activities have been successfully completed for a particular project.

However, for IA or any other field measurement technology to gain regulatory acceptance, the technology must be properly employed and must produce valid data that are usable for their intended purpose in project decision making. Use of this document, in conjunction with proper project planning, strict adherence to vendor procedural requirements, and good quality assurance/quality control practices; will result in the effective application of environmental IA techniques.

IA is an innovative technology that is in a fluid state of development. The project planner should be cognizant of product changes in the environmental IA market, and should rely heavily on vendor consultation and training to ensure proper IA kit selection and operation.

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1.0 INTRODUCTION

These guidelines provide the most current and comprehensive information available concerning environmental applications of immunochemical analysis and immunoassay (IA) techniques. Their purpose is to assist IA users in planning environmental sampling and analysis projects. Integration of immunoassay techniques into the project plan can cut costs and decrease time spent in the field. Using the information provided in these guidelines, the IA user should be able to successfully choose an IA kit to meet the project's Data Quality Objectives (DQOs), perform the IA analyses correctly, and produce usable IA data that meet the project objectives.

Proper planning, including establishing Data Quality Objectives (DQOs) is critical to project success. If project DQOs require fast turnaround field analyses at low costs to perform site activities such as hot spot screening, plume delineation, removal/remedial site clean-up, etc., then IA techniques may be applicable.

IA techniques are not applicable to sites with unknown site conditions and contaminants, or to sites that do not have established clean-up criteria. Sites with a single contaminant, or only one type or chemical class of contamination, are the most ideal sites for IA use. IA test kits may not be applicable to sites contaminated with complex mixtures of chemicals due to interferences arising from the contaminant sources.

This document is divided into nine sections. Section 1.0 provides an overview of the document's purpose, organization, and information sources. Section 2.0 contains a discussion of the basic principles of immunochemistry and the format of environmental IA kits. Section 3.0 provides information on characteristics and availability of environmental IA kits, and Sections 4.0 through 6.0 specify the physical and chemical constraints to IA use that must be considered for project success. Section 7.0 builds upon the information furnished in Sections 1.0 through 6.0 to walk the project planner through the process of selecting IA kits to meet project DQOs. The comparability and usability of IA data for making site decisions are discussed in Section 8.0. Finally, pertinent references are provided in Section 9.0.

These guidelines are not intended to endorse one product or vendor over another for any analytical parameter. They will be revised as needed to incorporate products that are currently in development and testing stages or that are otherwise unavailable at this time.

1.1 Information Sources

The information utilized to prepare this guidance document was obtained from the following sources:

Organization Name Address	Contact Name(s)	Telephone Numbers
D-Tech Strategic Diagnostics 128 Sandy Drive Newark, DE 19713	Joe Dautlick	(302) 456-6789
ENSYS PO Box 14063 Research Triangle Park, NC 27709	Dr. Kevin Carter Yli Vallejo Karen McKenzie	(919) 941-5509

Organization Name Address	Contact Name(s)	Telephone Numbers
Ohmicron 375 Pheasant Run Newtown, PA 18940	Mary Hayes Tim Lawruk	(800) 544-8881
Quantix/Idetek 1245 Reamwood Avenue Sunnyvale, CA 94089	Richard Lankow	(408) 745-0544
BioNebraska, Inc. 3820 NW 46th Street Lincoln, NB 68524	Craig Schweitzer	(402) 470-2345
Hach Company PO Box 389 Loveland, CO 80539-0389	Brett Poor Shirley Holmes	(970) 669-3050
California EPA DTSC/HML 2151 Berkley Way Berkley, CA 94704	Robert Hass Dr. G. Wolfgang Furs	(510) 540-2803 (510) 540-3076
U.S. Army Corp. Of Engineers	Kira-Pratt Lynch	(206) 764-6918
Sylvanus Environmental	Dr. Stephan B Friedman	(919) 545-0552

Additional IA information can also be found in the following Publications and Methods from Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846, 3rd Edition:

Publication	Recommended Format and Content for Documentation Supporting New Submittals
Method 4000	Immunoassay
Method 4010A	Screening for Pentachlorophenol by Immunoassay
Method 4015	Soil Screening for 2,4 Dichlorophenoxy Acetic Acid by Immunoassay
Method 4020	Soil Screening for Polychlorinated Biphenyls by Immunoassay
Method 4030	Soil Screening for Petroleum Hydrocarbons by Immunoassay
Method 4035	Soil Screening for Polynuclear Aromatic Hydrocarbons by Immunoassay
Method 4040	Soil Screening for Toxaphene By Immunoassay
Method 4041	Soil Screening for Chlordane by Immunoassay

Method 4042	Soil Screening for DDT by Immunoassay
Method 4050	TNT Explosives in Water and Soils by Immunoassay
Method 4051	Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX) in Soil and Water by Immunoassay

Section 9.0 of this guidance document contains other pertinent IA references.

2.0 ENVIRONMENTAL IMMUNOASSAY

2.1 Glossary of Terms

IA users must understand the scientific and non-scientific terms related to immunochemical processes and immunoassay techniques prior to considering its use for a project. IA users include personnel such as project planners, project chemists, and field samplers. The term "IA user" is employed in Sections 2.0 - 6.0 to denote a range of personnel that might encounter and/or utilize IA technology, while the term "project planner" is used in Sections 7.0 and 8.0 to denote actual project planning responsibilities.

Appendix A contains a comprehensive glossary which defines the terms used throughout these guidelines and the vendor literature. Basic IA principles are described in Section 2.2, while a more detailed technical discussion of immunoassay, which is suitable for more advanced IA users, is provided in Appendix B.

2.2 What is Immunoassay?

Clinical chemists have utilized immunoassay techniques to detect and quantify proteins, hormones, and drugs for decades. Currently, IAs are used in home pregnancy tests as well as in commercial laboratories to detect the presence of the HIV virus and the use of illicit drugs.

Immunoassay for environmental chemicals began in the 1970s with the analysis of selected pesticides. The most common version of environmental IA analysis is called ELISA (Enzyme Linked Immunosorbent Assay). ELISA is an immunoassay method that uses antibodies and enzyme conjugates to detect and quantify target compounds, otherwise known as compounds of interest (COIs), in field samples.

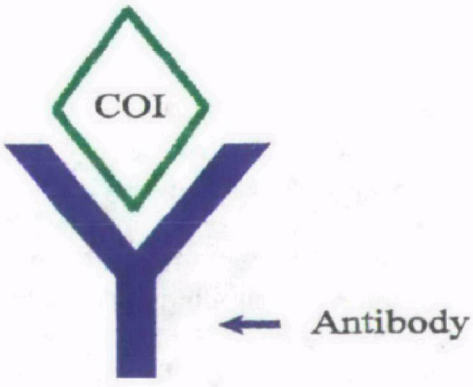


Figure 1

Antibody / COI
Lock and Key Mechanism

Antibodies are proteins produced by the mammalian immune system that can specifically bind with COIs. An antibody and its target chemical compound fit together like a lock and key as depicted in Figure 1. An enzyme conjugate is an enzyme to which a COI is bound as depicted in Figure 2. The COI portion of the enzyme conjugate can bind with the antibody as can the COI present in the samples (shown in Figure 3). ELISA tests are considered to be "competitive" assays because the sample-derived COI competes with the enzyme conjugate COI (which is

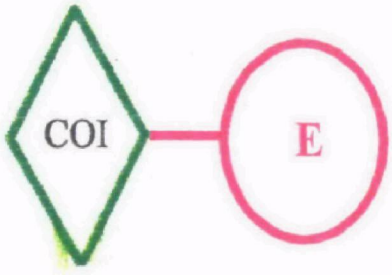


Figure 2

Enzyme Conjugate COI

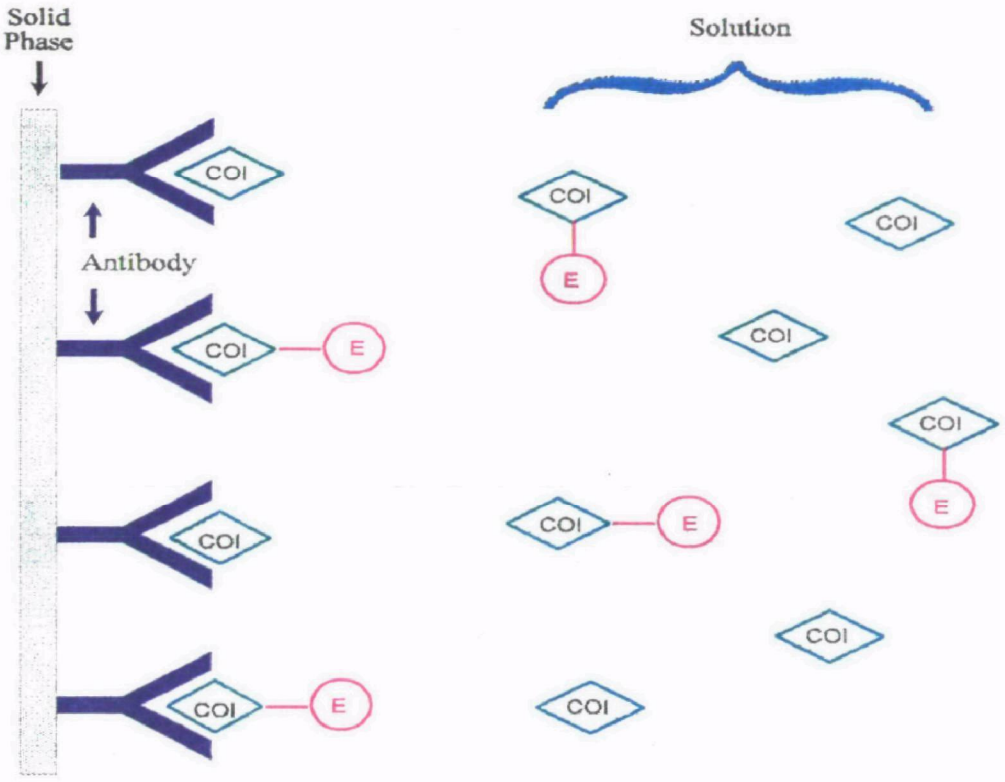


Figure 3 **Antibody Binding**

kept at a constant concentration) to bind to the finite number of antibody sites. The greater the concentration of sample-derived COI relative to the enzyme conjugate COI, the larger the proportion of antibody sites that are occupied by the sample-derived COI.

The enzyme portion of the enzyme conjugate serves as a catalyst to change a colorless compound to a measurable colored product that can be detected instrumentally. The amount of color produced is inversely proportional to the amount of sample-derived COI. More color equals less sample-derived COI. Less color equals more sample-derived COI because all the antibody sites are bound to sample-derived COI and there is less enzyme conjugate present to catalyze the color reaction.

2.3 Formats for Environmental IA Kits

Each vendor has developed an IA format that they believe to be the most applicable to environmental samples and the most easily used by project personnel. Almost all environmental IA kits presently available use the basic format that is described below. The predominant enzyme used in the enzyme conjugate is horseradish peroxidase.

BioNebraska's kit for the detection of mercury does not use the enzyme conjugate COI system. Instead, their kit uses a metal sensitive protein that is fixed to the wall of a test tube. The metal sensitive protein binds to sample-derived mercury. The protein/mercury complex then binds to an antibody/enzyme that catalyzes the release of a color agent. Therefore, the color developed is proportional to the mercury present, not inversely proportional as the other kits are designed.

Certain IA kits may have advantages over kits produced by other vendors for dealing with particular sample matrix or interference problems. However, each vendor's procedures have been engineered and validated for their specific kits. One vendor's procedures, therefore, cannot be used for another vendor's kit.

2.3.1 Basic Format

The basic format for an environmental IA kit is depicted in Figure 4 on the following page and can be described as follows:

1. The stationary antibody is engineered to react with the COI.

2. The sample-derived COI and the enzyme conjugate COI compete for sites on the fixed antibody. This is the incubation step that must be precisely controlled.

3. The antibody/COI complexes are fixed by design or fixed on a membrane by filtration or by magnetic forces. The complexes are washed to remove the sample matrices, excess enzyme conjugate, and reagents.

4. The enzyme portion of the enzyme conjugate COI remains complexed to certain antibody sites and catalyzes the reaction of the color agents to form a color that is inversely proportional to the amount of sample-derived COI.

5. The color reaction is stopped chemically.

6. The colored product is measured using UV/Visible spectrophotometry.

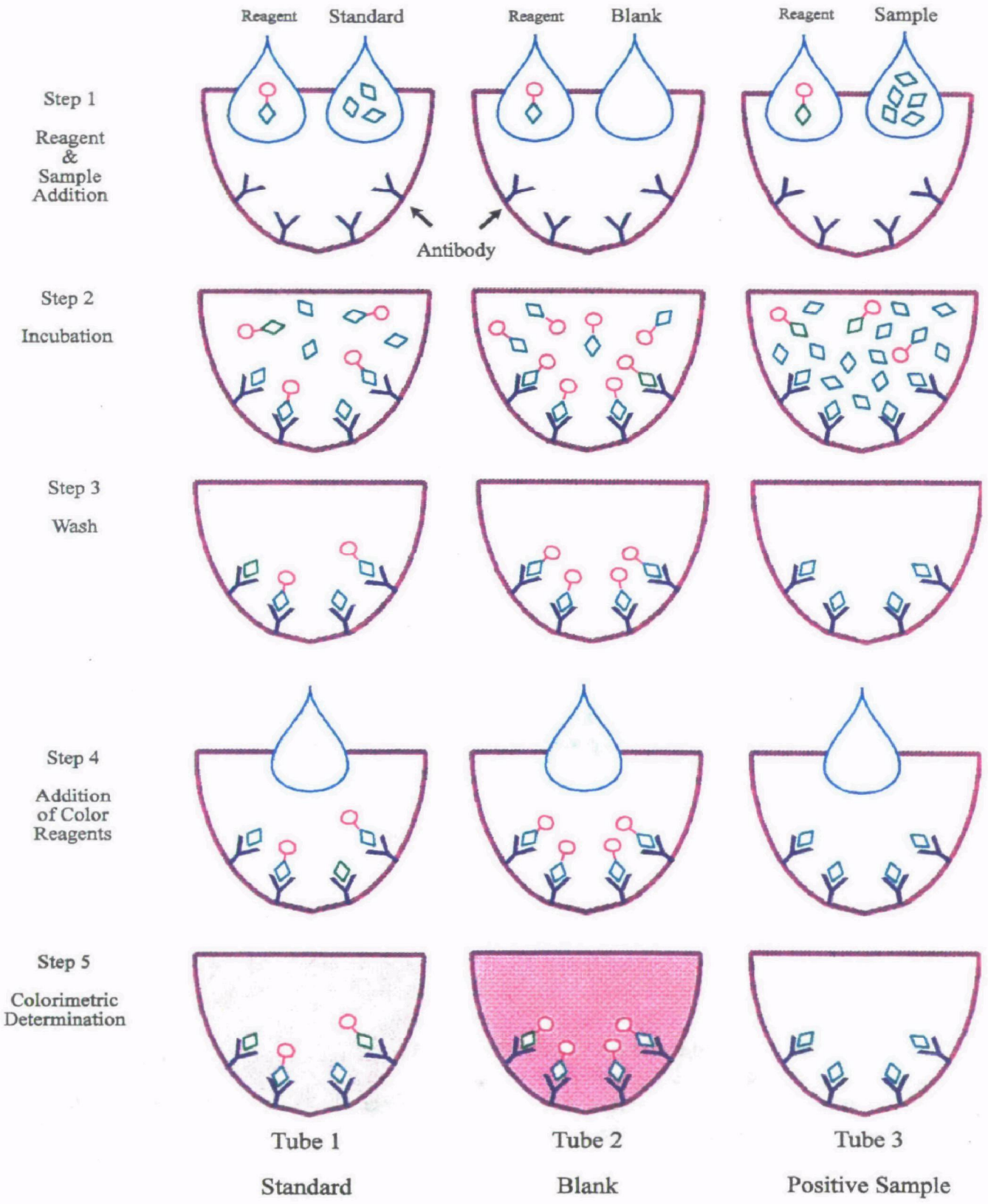
Each vendor has a variation of the general procedure described above. Detailed descriptions of each vendor's procedures can be found in their kit's literature. The formats are essentially the same except for how and where the engineered antibody is attached to the solid phase, how the sample and reagents are mixed and in what order, and how the analytical measurement (colorimetric determination) is performed (as discussed below). Regardless of a vendor's IA kit format, the solid phase, antibody, enzyme conjugate and color agents are manufactured in a manner that is highly reproducible.

2.3.2 Antibody Attachment to Solid Phase

The following types of fixed antibody formats are utilized in environmental IA kits:

- Antibody attached to the walls of a styrene polymer tube
- Antibody attached to the surface of a polymeric well
- Antibody attached to polymeric latex particles
- Antibody coated onto para-magnetic particles
- Antibody attached to a porous membrane
- BioNebraska uses a metal binding protein fixed to the wall of a test tube and the antibody is then added to bind to the immobilized mercury

Figure 4 Immunoassay Reaction Scheme - ELISA



2.3.3 Reagent and Sample Additions

The sequence and timing of reagent and sample additions is critical to the proper use of each IA kit. The timing of sample-derived COI and enzyme conjugate COI addition and exposure to the antibody greatly influences IA results. Each vendor requires that the IA user follow the exact sequence and timing for all samples, blanks, QC samples and standards. Any deviation from the prescribed procedure can affect IA results within and between batches.

The same warning applies to vendor washing procedures. The vendors using particles that get trapped on a membrane or particles attached to the reaction cell wall via magnetism rely on a very different separatory procedure than the vendors whose stationary antibody phase is attached to a tube wall.

2.3.4 Colorimetric Determination

The process that produces the analytical signal, or so called reporter system, is unique to each vendor and for each IA kit. Vendors use different color agents that produce different colored reactions. The different colors require specific wavelengths of UV/Visible light to measure the absorbance of the final solution. Other vendors use a visible light reflectance measurement of the trapped and plated enzyme conjugate/color agent complex. Later in this document, the practical pros and cons of these techniques will be discussed relative to sample matrix interferences.

2.4 Immunoassay and Environmental Chemicals

Certain compounds within a chemical class are more toxic than others and those are the compounds most tested because of their adverse effects on human health and the environment. Environmental IA kits have been engineered to detect a single target compound or one or more structurally similar target compounds within a chemical class, depending upon the compounds present in the chemical class, the molecular size of the target compound(s), and the specificity of the engineered antibody.

Individual contaminant compounds, such as pentachlorophenol (PCP), trinitrotoluene (TNT), 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD), and most of the pesticides, insecticides, and herbicides are analyzed as single target compounds in individual test kits.

However, IA is not as specific for some chemical classes of common site contaminants which originate from a commercial product or waste by-product. Some examples of these products and by-products are:

- Polychlorinated Biphenyls (PCBs): a chemical class comprised of 209 different individual congeners. PCBs were used in dielectric fluids for capacitors and transformers, hydraulic fluids, plasticizers, and carbonless papers.

- Polynuclear Aromatic Hydrocarbons (PAHs): a class of chemicals comprised of multi-cyclic unsaturated 6 ring compounds that may or may not be substituted with aliphatic (straight chain) branches. This class has been characterized traditionally by the analysis of 16 or 18 major compounds, but it contains many more compounds not normally identified and quantitated. PAH compounds are traditionally found in asphalts, creosotes, coal tars, coal gas residues and heavy grade fuel oils or crude oils.

- Total Petroleum Hydrocarbons (TPH): a class of chemicals that originate from man-made fuels and contain both aromatic and aliphatic compounds. There are many fuels being formulated today for various

energy producing purposes. The most commonly used are gasoline, diesel, and fuel oil #2. For this reason, vendors developed IA kits to: 1) analyze BTEX (benzene, toluene, ethyl benzene, and xylenes), which are compounds found in these fuels, or 2) analyze for the fuel mixtures (by using the fuel as a standard). Other fuels that are used in aircraft engines and jet turbines, such as aviation gas, JP-4, and JP-5, are also of interest in site remediation projects and are IA sensitive.

All three chemical classes can be found at many sites individually or together in mixtures and they can be analyzed as individual target compounds, product mixtures, or the sum of individual compounds within a particular class.

Vendors engineer IA kits to respond to one target compound, and that target compound may be chosen to represent a particular chemical class (such as PCBs) or a particular product (such as fuel oils).

IA kit engineers must decide what compound to target from each chemical class during the initial design phase. For instance, if all of the most prevalent PCB Aroclors found as site contaminants contain one of a specific penta, hexa, or hepta congener, then that congener would be chosen as the target compound for development of the antibody and enzyme conjugate used in the IA kit. Each Aroclor mixture containing the target compound would then have a certain level of sensitivity to the resultant IA kit.

Similarly, IA kit designers must determine what target compound is present in all petroleum hydrocarbon mixtures that are commonly spilled in the environment. Obviously, some custom manufactured hydrocarbon mixtures would be excluded from IA analysis if they did not contain that target compound. Products such as fuel oils, jet fuels, gasolines, and range oils contain similar compounds at measurable concentrations.

Environmental weathering of petroleum hydrocarbons must also be taken into account when engineering an IA kit. The petroleum hydrocarbon chosen as the target compound should not be susceptible to weathering, otherwise the kit will be less sensitive to the weathered fuels. If the IA kit designer chooses a target compound, such as m-xylene, that is present at measurable concentrations in manmade fuels and is less susceptible to environmental weathering, then the resultant IA kit would be sensitive to all fuel oil mixtures at some relative sensitivity. Petroleum hydrocarbon mixtures which do not contain m-xylene, such as lubricating oils, would thus not be sensitive to the kit.

The effectiveness of each IA kit for sample analysis will depend upon: 1) the various product mixtures present in the sample, 2) the kit's sensitivity to the target compound and structurally similar compounds, and 3) the presence of interferences in the sample. The availability and characteristics of environmental IA kits produced by various vendors will be discussed in Section 3.0.

3.0 ENVIRONMENTAL IA KITS

3.1 Currently Available Environmental IA Kits

Tables 1a and 1b, referred to as the vendor's matrix, contain a compilation of environmental IA kits produced by particular vendors as of July 1996. The information listed in these tables does not constitute an endorsement by EPA of any particular vendor or any specific IA kit. Information is provided solely for reference in identifying potential IA kit sources. One vendor that produces environmental IA kits failed to respond to EPA Region I's request for information. Those who did respond have complete information included in this document. Several vendor mergers were on-going during preparation of this document. The information presented here is as comprehensive as possible under the circumstances.

3.2 Field Kits Versus Laboratory Based Kits

Most vendors have designed their environmental IA kits for use in both field and laboratory settings. Laboratory kits are usually designed for better automation and sample through-put. All of the available field kits can be used by a fixed or field laboratory as a screening tool prior to sample preparation and/or instrumental analysis.

The one characteristic that differentiates field kits from laboratory based kits is the number of samples analyzed per batch. The sequence of standard, blank, samples and QC samples, then the standard and blank set again, constitute a batch sequence in both settings. Only the finite number of samples between the standard and blank sets changes. Field kits recommend performing less samples (4-6) between standard and blank sets, whereas laboratories will set up banks made up of several batches or sequences of up to ten samples each (possibly 40 samples at one time). The ratio of QC samples to field samples should be the same for both settings (approximately 10% duplicates and 5% spikes or PE samples). The exact number of QC samples will depend on the minimum number of samples run in a batch. Quality control requirements for environmental IA kits will be discussed in Section 3.3.3.

The 2,3,7,8-TCDD kit produced by ENSYS is designed only for fixed laboratory use due to the toxicity of the reagents, their ban from on-site use, the requirement for a solvent exchange step, and the disposal requirements for the derived waste.

3.3 Quantitative, Semi-Quantitative, and Qualitative IA Kits

Most vendors have designed their IA kits to be used in one or more of the following modes depending, in part, on the number of standards analyzed by the IA user:

- Kits that produce results that are analytically quantitative from a specified lower detection limit to a linear upper limit.

- Kits that produce semi-quantitative results one of two ways: 1) above or below a specified detection limit (Action Level or so called Go/No-Go test) or 2) between an upper and lower concentration range.

- Kits that produce qualitative results. These kits are designed to detect the presence or absence of a specific COI. The detection limit for this type of kit is usually conservatively set (based on field trials) by the manufacturer but may be uncertain due to the composition of the sample matrix and the presence of interferences.

Most environmental IA kits are utilized in the either the quantitative or semi-quantitative mode. Therefore, the use of IA kits that produce qualitative results will not be addressed in this document.

In general, environmental IA kits produced by D-Tech, Ohmicron, and Quantix/Idetek are designed to provide quantitative or semi-quantitative results while those produced by ENSYS and BioNebraska are designed to provide semi-quantitative results only.

Regardless of whether IA kits are used to produce quantitative or semi-quantitative results, there are two conditions which must be met for IA data to be considered usable.

The first condition is that quality control (QC) procedures must be performed at the correct frequency and must meet the criteria specified in the pre-approved project Quality Assurance Project Plan/Sampling and Analysis Plan (QAPjP/SAP). These key QC elements are discussed in Section 3.3.3.

The second condition is that IA results for a representative number of samples (10% minimum) must be confirmed through the use of split samples. Split samples should be collected throughout the entire sampling and analysis episode and should be prepared and analyzed using conventional full protocol analytical methods performed in a fixed laboratory or field laboratory (mobile or transportable) setting. The split sampling results obtained using both analytical methods must not deviate from the criteria specified in the pre-approved project QAPjP/SAP. Data comparability and usability will be discussed in Section 8.0.

3.3.1 Quantitative IA Results

Quantitative results can be obtained with IA technology as proven by the work performed daily in the medical diagnostic field. Environmental IA kits can be used to produce quantitative results when the two conditions listed above have been met along with the following requirements:

1. A multi-level linear calibration curve is generated which brackets the detection limit and the highest sample concentration. The calibration curve must meet the requirements specified in the pre-approved QAPjP/SAP. Deviations from the calibration curve at the upper and lower ends of the curve will yield inaccurate IA results. Also, calibration stability and accuracy must be periodically checked through the analysis of continuing calibration check samples (at a rate specified by the vendor) that meet the criteria specified in the pre-approved QAPjP/SAP.

2. A project-specific split sampling field study is performed prior to full scale implementation of IA techniques at a site, and the IA results correlate with the conventional full protocol fixed or field laboratory results. The split sampling results obtained using both analytical methods must not deviate from the criteria specified in the pre-approved project QAPjP/SAP.

Quantitative IA is enhanced if the source of the COI (i.e., product) is known and is used as a calibration standard for IA analysis.

3.3.2 Semi-Quantitative IA Results

The SW-846 methods that have been promulgated to date are performed in one of the semi-quantitative modes and are applicable for screening soil and water samples. IA kits for those methods are designed to estimate the concentration of a COI above or below a specified detection limit with only one standard as a reference point. The concentration of that standard should be less than, but in the same order of magnitude as, the project required Action Level. The concentration of the COI relative to the IA kit detection limit is engineered into the kit by the vendor, and is influenced by analysis precision, sample matrix interferences and other performance characteristics and limitations of the basic method.

Vendors also produce semi-quantitative IA kits that are designed to estimate the concentration of a COI above a lower limit (for example, >1 ppm) and below an upper limit (for example, <10 ppm). These kits require the analysis of two standards as reference points and are, therefore, generally more accurate than the single standard semi-quantitative kits discussed above. Future formats will follow this general scheme.

IA vendors have intentionally designed the possibility of false positives into their semi-quantitative kits so that IA users will make conservative site removal/remedial clean-up decisions. False positives are generally defined as a positive response for a sample that contains the COI below the claimed detection limit.

It follows, therefore, that semi-quantitative IA kits should yield virtually no false negatives. False negatives are defined as a negative response for a sample containing the COI at or above the stated detection limit. In a situation where immunoassay will be used to detect PCB contamination in soil at 1 ppm, the IA kit has been designed so that the 1 ppm PCB reference standard actually contains less than 1 ppm PCB. This reference standard is positioned to minimize the incidence of false negative results at the claimed detection limit.

The vendor measures the false negative rate of a semi-quantitative IA kit by analyzing split samples using the IA kit and a separate full protocol analytical method in a fixed laboratory. SW-846 allows semi-quantitative IA kits to produce a maximum of 5% false negatives at the specified detection limit. In general, SW-846 methods are designed for IA use in a field setting to delineate contamination or to confirm clean-up during site removal/remedial activities. False negatives cannot be tolerated when site clean-up and closure is the sampling objective.

Each vendor that produces a semi-quantitative IA kit generates method performance data for the percentage of false positives and false negatives. IA users should obtain and review vendor literature to determine the false positive and false negative rates for individual IA kits.

When quantitative IA results are produced in accordance with the requirements in Section 3.3.1, false positives and false negatives are not an issue because the detection limit and highest sample concentrations are bracketed by a linear calibration curve. (It should be noted that the concentration of a particular kit's detection limit must be chosen conservatively so that it is above the kit's finite limit of detection. Otherwise, false negatives may become an issue with samples that have COI concentrations at or near the kit's chosen detection limit.)

3.3.3 Kit Standardization and Quality Control

Every analytical process that determines the identification and/or concentration of target compounds in some media must have associated quality control (QC) elements. Key QC elements must be performed for all IA kits that are utilized in a field or laboratory setting, and the criteria specified in the pre-approved project QAPjP/SAP must be achieved for each key QC element. The key QC elements for IA analyses include process calibration, the analysis of continuing calibration checks, blanks, duplicates, and performance evaluation samples. Note, in developing the required QC elements for a particular project, the IA user should consult each vendor's IA kit instructions, which contain recommended QC requirements that have been tested and validated by the vendor.

Documentation that all key QC elements were performed and met project requirements is essential, regardless of intended data use. The preparation and analysis of each batch of samples, including related standards, QC samples and blanks, should be recorded in a field or laboratory notebook, run logs, and/or tabulated forms. Note, sample preparation should include kit lot numbers and expiration dates, and the ambient temperature at which the tests were performed.

Samples should not be analyzed until project QC criteria have been met. When pre-approved project QC criteria have not been met, appropriate and effective corrective actions must be immediately implemented and documented. Samples analyzed after the last in control QC sample should be re-prepared and/or reanalyzed after effective corrective action has been implemented. The project QAPjP/SAP should document the corrective actions that will be taken when each key QC element does not meet the project QC criteria. The content of QAPjPs/SAPs will be discussed in Section 7.6.

3.3.3.1 Calibration

IA calibration using standards of known concentrations is performed to determine the sensitivity and detection/calibration range for the IA kit.

Calibration of a semi-quantitative IA kit performed in the Action Level (Go/No-Go test) mode, uses one calibrator comprised of a standard that contains the target compound at the detection limit. A reagent blank should also be included to provide the reference color generated when no target compound is present. Calibration of semi-quantitative IA kits can also be performed in the detection range mode. Here, two calibrators are analyzed to delineate a detection range (i.e., a 1 ppm standard and a 10 ppm standard). Regardless of the semi-quantitative mode employed, the concentration of the single standard or lowest of two standards must be below the project Action Level.

Calibration of quantitative IA kits must be performed using multiple calibrators and a calibration (dose/response) curve must be developed. Usually, one of the calibrators is a zero point. In some situations, the product mixture specific to the site can be procured, prepared and diluted properly, and used as the calibrator for the IA analysis. For this to work effectively, the IA user must develop their own dose/response curve for that product mixture. That calibration curve must have a set correlation coefficient ("r" or goodness of fit) to be acceptable. The normal "r" would be 0.995, but an acceptable correlation coefficient may be as low as 0.990, depending upon the specific IA kit. The IA user must consult the vendor to obtain the proper "r" specification.

Continuing calibration checks must be performed to evaluate calibration accuracy and stability for each batch analyzed using semi-quantitative and quantitative IA kits. Multiple standard initial calibrations should be performed at the beginning of each batch of samples. If samples are from different areas of the site, or temperature or weather conditions change, then full calibrations should be performed before and after each batch. In the field setting, every 4 to 6 samples should be bracketed by a continuing calibration standard. The absorbance of the continuing calibration standard should not vary more than 20% from the absorbance of that standard in the initial calibration. Vendor IA kit instructions usually define how many samples can be successfully analyzed between standards. If the continuing calibration standard is not within 20%, then a full calibration should be performed and all samples run prior to the non-compliant standard should be rerun..

3.3.3.2 Blanks

Analysis of blanks must be performed to evaluate the presence of contaminants originating from sampling and analysis activities. Equipment blanks should be collected and analyzed to evaluate the effectiveness of equipment decontamination procedures performed in the field. Reagent blanks should be prepared and analyzed with every batch, or chosen sequence of samples, to evaluate the purity and reactivity of reagents used in the IA kits and assist the IA user in ascertaining the kit's response when no target contaminants are present. Blanks represent the highest absorbance of color and indicate the lack of the COI. They are also used to compare the color generated by the blank with the color generated at the detection limit concentration. Blanks should be run with each batch of samples and should not show contamination above the kit's detection limit. If contamination is found in the reagents or the equipment rinsates, then steps must be taken to determine the cause and eliminate the contamination. Samples should not be analyzed until the blanks meet the vendor's recommended acceptance criteria.

3.3.3.3 Duplicates

Duplicates must be analyzed to evaluate sampling and analysis precision. Field duplicates measure the precision of the IA test as well as the sample homogeneity. Field duplicate precision should fall within 30% D for waters and 50% D for soils. Duplicate precision should be tighter for IA kits that are utilized in a laboratory setting. Duplicates should be prepared and analyzed at a frequency of 1 per 10 samples, or 1 per batch of samples prepared, whichever is greater. Duplicates should be performed at a greater frequency where samples are known to be less homogeneous.

3.3.3.4 Performance Evaluation Samples

Performance evaluation (PE) samples (or some form of independent control sample) must be analyzed to evaluate qualitative and quantitative accuracy for each IA kit batch following the requirements contained in the EPA Region I Performance Evaluation Program Guidance, dated July 1996 or most recent revision. PE samples should contain the target compound at or near the project Action Level. Depending on the batch size of the individual analysis episode, a PE sample should be run at least once in 20 samples or once per day, whichever is greater. The PE sample must be analyzed under the identical conditions that the calibrations, blanks, field samples and PE samples are analyzed. If many sets or batches are analyzed under the same conditions during one day, then one PE per day is recommended. If there are changes in field conditions (temperature and relative humidity) during the sampling episode, then a PE sample should be analyzed more frequently. Poor PE sample score results may indicate incomplete sample extraction, operation of the IA kit outside its required operating temperature ranges, or inconsistent timing of reagent additions and performance of batch processes.

3.4 Kit Detection Ranges

Each IA kit is designed to function within a particular detection and/or calibration range, depending on whether the kit produces quantitative, semi-quantitative, or qualitative data. The IA user must ensure that IA kit detection limits are lower than project Action Levels.

IA kits are usually more sensitive than is needed for most environmental studies. This sensitivity generally requires the IA user to dilute soil sample extracts to bring the COI concentrations into the IA kit's detection/calibration range. Therefore, the IA user must determine the COI concentrations expected in the soil samples prior to kit selection. Historical site information and previously generated site data are the best sources for obtaining those details. The IA user then must ascertain sample homogeneity, sample weight/volume to be extracted, and required dilution factors to be utilized on project samples.

Vendor instruction guides usually detail step-by-step procedures for performing their specific assay on soil matrices. Several vendors have simplified this process by developing a formula to calculate the required dilution factor. Other vendors have ready-to-use dilution kits available to simplify IA use.

Vendor consultation by the IA user to determine the proper kit and to obtain the correct operating instructions for the project-specific sample matrix is critical to project success.

4.0 KIT SENSITIVITY

IA kit sensitivity is expressed in a manner similar to other analytical techniques, i.e., detection limit or quantitation limit. Sensitivity is a function of the operational consistency and the signal-to-noise characteristics of the dose-response curve.

Each vendor expresses their detection limits differently. Some vendors use the $\%B/B_0$ convention. Here, B_0 is the absorbance of the zero standard (i.e., blank) and represents the most highly colored standard (because no sample-derived COI is present and, therefore, enzyme conjugate COI is bound to all antibody sites so maximum color is produced and measured). %B is the relative absorbance of samples or standards in relation to B_0, the highest absorbance. Samples and standards must be less colored than the blank absorbance (B_0). The lowest detectable concentration of pure analyte (sometimes referred to as dose) that can be differentiated from the highly colored zero standard is defined as the detection limit. Some vendors set this detection limit as a numerical $90\%B/B_0$. $90\%B/B_0$ means that at values below 90% of the blank absorbance (B_0), the analyst can begin to differentiate a change in color. Likewise, at values between 90% of the blank absorbance and the blank absorbance value, the analyst is unable to differentiate a difference in color with any certainty.

Other vendors define detection limit as the concentration necessary to result in a positive detect measured at some set confidence limit. The confidence limit and the definition of the detection limit must be known if the IA user is to compare vendor kits and, ultimately, choose the correct kit.

The following describes each vendor's approach to establishing the detection limit or limiting detectable dose (LDD) as the measure of sensitivity for their IA kits.

Ohmicron:

Ohmicron defines limiting detectable dose as $90\%\ B/B_0$. This means that a compound can be reliably detected at 90% of the most colored zero standard.

D-Tech:

D-Tech defines the minimum detection limit as the lowest concentration of a compound that yields a positive test. Each kit has a specific confidence limit that has been set by the vendor for the indicated lowest concentration (+/-18% BTEX, +/-20% PCB, and +/-25% PAH). A 96% level of confidence occurs within the vendor-specified range for each kit. This means that 96% of the time, the analyst will get a positive test result.

Quantix/Idetek:

The minimum detection limit is defined as the compound concentration required to produce 20% inhibition in the immunoassay. This is essentially $80\%\ B/B_0$. The confidence limits for these IA kits are unknown.

ENSYS:

ENSYS defines detection limit as the concentration necessary to result in a positive detect greater than 95% of the time when tested at the stated concentration level. This approach is less limiting and allows for the determination of the level at which the user can see a positive result compared to the zero result.

BioNebraska:

BioNebraska defines their detection limit for mercury as a mean of three replicate values and the corresponding standard deviation. They do not define a method detection limit, only a detection limit for water and soil.

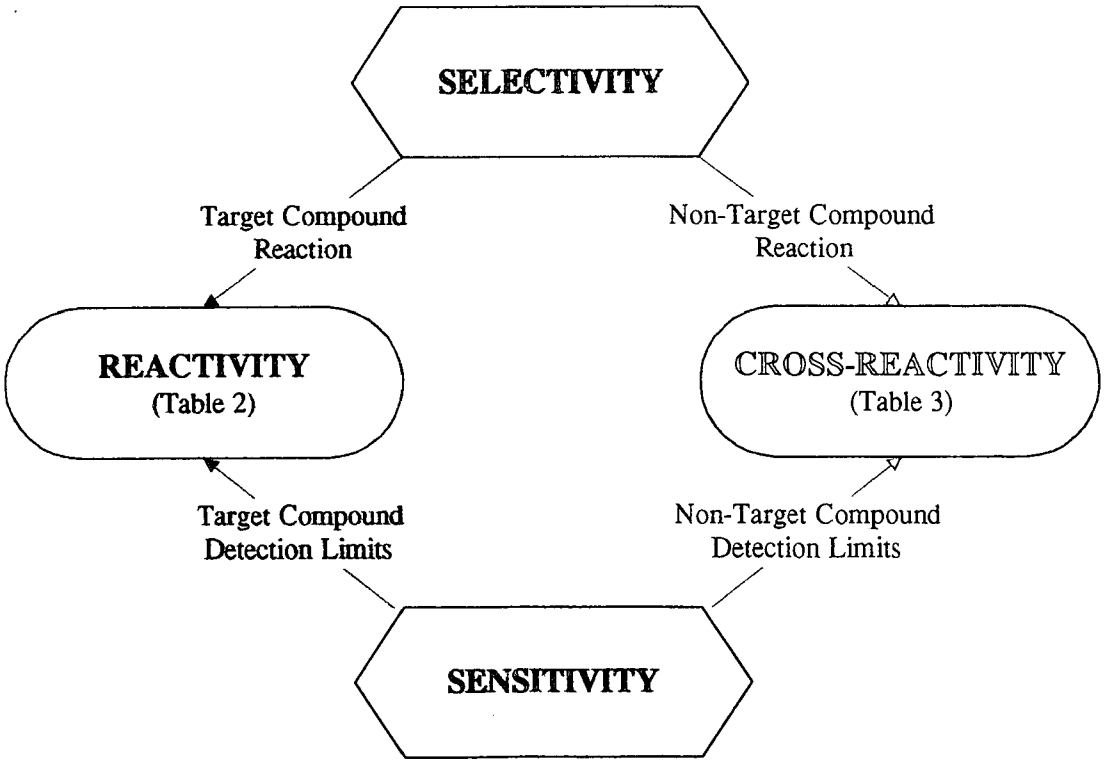
5.0 KIT SELECTIVITY

IA kits are designed to react to a single target compound or to one or more structurally similar target compounds within a chemical class. This is termed reactivity. The concept of reactivity was introduced in Section 2.4 and it will be discussed in greater detail in Section 5.1.

Non-target compounds that are structurally similar to the target compounds may also react with the antibody. Those non-target compounds are considered to be "cross-reactive" because they will compete for the finite number of antibody binding sites, which will impact the color development and yield false positive sample results. During IA kit development, designers characterize cross-reactivity by adding increasing amounts of potential cross-reactive substances to a sample and measuring their IA response. Cross-reactivity will be discussed in greater detail in Section 5.2.

The engineering of the antibody/COI in tandem with the enzyme conjugate controls the selectivity of the IA kit to particular target compounds and non-target compounds. Figure 5 depicts the relationship of selectivity and sensitivity to both reactivity and cross-reactivity. Reactive and cross-reactive compounds have finite sensitivities to individual IA kits.

Figure 5 IA Selectivity



5.1 Kit Reactivity

The reactivity and sensitivity of each vendor's kit to specific target compounds and product mixtures is detailed in the following tables:

Table 2a: TPH/BTEX Reactivity
Table 2b: PAH and c-PAH Reactivity
Table 2c: PCB Reactivity
Table 2d: TNT/RDX Explosives Reactivity
Table 2e: Individual Analyte Reactivity (mercury, pentachlorophenol, trichloroethylene, perchloroethylene, 2,3,7,8-TCDD, and benzene)
Table 2f: Individual Pesticide, Insecticide, and Herbicide Reactivity

These tables are designed to show the most sensitive compounds/mixtures within a chemical class that will react to a particular vendor's IA kit. The lower the detection limit number present in the tables, the more sensitive the IA kit is to that compound/mixture. The sensitivities were established by each vendor after testing their IA kits with the individual compounds or product mixtures.

TPH kits are normally designed to be sensitive to gasoline, kerosene, stoddard solvent, and the lighter fuel oils that contain o-xylene, m-xylene, and p-xylene. Some IA vendors use m-xylene as the indicator compound because it naturally occurs in all these fuel mixtures. BTEX kits are also normally designed to be sensitive to one of the xylenes and its quantitation represents that of BTEX (as per the design of the kit). The type of standards used to calibrate both types of kits differentiate the use of the kit. Fuels or BTEX that are weathered and have lost the benzene, toluene, ethylbenzene, and xylenes have little sensitivity to IA kits for the quantitation of TPH or BTEX.

All of the TPH and BTEX kits that are contained on Table 2a will react (to some degree) with toluene, ethyl benzene, and other xylenes, and will cross-react with naphthalene and styrene. These kits will generally not react with benzene.

Because similar reactive compounds are found in common fuel products and because these IA kits have some relative sensitivity to each of these reactive compounds (with the exception of benzene), then each common fuel product has its own finite sensitivity to a particular vendor's IA kit. For some kits, that sensitivity is directly related to the concentration of the most reactive compound(s) contained within the fuel. Fuels that contain more m-xylene, such as blended fuel oils, are overall more sensitive to IA kits.

Most PAH kits, on the other hand, are designed to be reactive to a single representative PAH compound. This compound defines the type of ring structure (2, 3, or more rings) for which the IA kit will be sensitive. Phenanthrene is the representative PAH compound most commonly used for general PAH kits so that those kits are most reactive to 2, 3, and 4-ring PAH compounds (i.e., PAHs present in fuel oil #2, diesel, and kerosene). Phenanthrene, however, is not a good choice as a representative PAH compound if the 4, 5, and 6-ring PAHs are required to be measured (i.e., PAHs present in creosote, coal tar, and fuel oil #6).

For this reason, one vendor offers a carcinogenic-PAH kit which keys in on the 3, 4, 5, and 6-ring PAHs and can be used to measure JP-4, mineral spirits, and mixtures of fuel oil, coal tar, and creosote. The reactivity of PAH kits is based more on each vendor's marketing philosophy relative to their niche in the environmental market (chemical products/mixtures to be measured that are sources of environmental contaminants). Later in this document there will be a discussion of IA kit selection when the project planner is faced with a site that contains mixtures of PAHs originating from multiple chemical products.

5.1.1 Compounds with Little Sensitivity

Because IA kits are engineered to have reactivity to a limited set of compounds within a compound class, some compounds of that class will have no sensitivity or very limited sensitivity. An example is benzene in the BTEX class. Benzene, by itself, has little sensitivity to the TPH/BTEX kits because it is structurally different than the indicator compound, m-xylene (or o-xylene, p-xylene), to which the antibodies were engineered to bind.

Benzene kits have been produced, but the kits require derivatization of the benzene molecule to a nitro-substituted benzene, which is then amenable to analysis by an IA kit specific to nitrobenzene.

Benzene's insensitivity to TPH/BTEX kits would only inhibit a project where benzene is the only COI for a project. If gasoline is the product/mixture of concern for a project, then a TPH/BTEX kit can be utilized because it is sensitive to gasoline, even though it has little sensitivity to the benzene that is present in the gasoline mixture. If the exact concentration of benzene is needed for risk assessment purposes, then another analytical method must be utilized.

Most PAH kits have little sensitivity to naphthalene. PAH kits are nominally engineered around phenanthrene or larger ring compounds, such as fluoranthene or pyrene, depending upon the vendor. If naphthalene is the prime COI for a project, then the TPH/BTEX kit should be used because most TPH/BTEX kits have excellent sensitivity to naphthalene. If PAHs or c-PAHs are of concern for a project, then a TPH/BTEX kit can be used to measure naphthalene and a PAH or c-PAH kit can be used to measure other PAHs, depending on the project DQOs.

Some vendor PAHs kits have little sensitivity to benzo(g,h,i)perylene and dibenzo(a,h)anthracene. If those PAH compounds need to be measured, then another vendor's IA kit or a conventional full protocol analytical method must be utilized.

5.2 Cross-Reactivity

Cross-reactivity is defined in SW-846 as: "The relative concentration of a non-target substance that would produce a response equivalent to a specified concentration of the targeted compound." In quantitative or semi-quantitative immunoassay, it provides an indication of the concentration of cross-reactant that would produce a positive response.

Cross-reactants react with the antibody because they have similar three dimensional structures as compared to the COI. To prevent cross-reactivity the IA engineer must produce a highly selective antibody. Many times this is not economically possible and may be less advantageous for environmental pollutants. In many cases, chemicals or groups of chemicals may have degradation products or biological metabolites that will also have sensitivity to the antibody. This type of cross-reactivity may work to the advantage to the project because the test is more comprehensive.

Cross-reactivity can yield more robust analyses allowing the IA user broader selectivity when entire groups of compounds such as PCBs, PAHs, TPHs, and Dioxins are being investigated. The down side to broader selectivity is that the IA user cannot measure the reactivity of individual compounds and has no measure of what substances contributed to the IA response. This can be a problem when the IA user is determining the comparability of IA data and data generated for split samples in the fixed laboratory using conventional full protocol methods. Vendors can only test the antibody response to individual cross-reacting compounds. No vendor has had the research time to determine the additive effects of various combinations of cross-reactants.

Cross-reactivity for individual compounds is often calculated as the ratio of target substance concentration to the cross-reacting substance concentration at 50% inhibition of the immunoassay's maximum signal X 100%. The 50% B/B_0 tables that are available in the vendor literature are very important resources when the project planner is faced with determining the effect of cross-reactive substances. Some vendors measure the amount of cross-reactant that gives the lowest response (similar to determining the detection limit of the COI). This number allows the user to determine what effect the individual cross-reactants will have on analysis of the sample-derived COIs.

The antibody response that occurs in a field sample, however, is the composite of all possible reactants and cross-reactants. This fact requires the IA user to test representative site samples by the chosen IA technique and by conventional full protocol analytical methods prior to initiating a full scale field sampling program. Full protocol analytical methods produce instrument responses that are comprised of the target compounds quantitated individually (and summed to get a total), whereas IA kits produce results that are composites of all reacting and cross-reacting compounds. Cross-reactivity of unknown interferences may result in the IA user eventually developing a valid correlation factor between IA results and results generated using full protocol analytical methods.

In many situations, the IA kit can react with far more substances than can be measured by full protocol methods, thus biasing the IA results on the high side. This can be the case with PAHs, c-PAHs, and PCB mixtures. IA kits will also over-estimate the total compound concentrations if dry, non-organic soil conditions are present on site and if the extraction efficiency of the sample matrix is good. Later in this document, this situation will be discussed in detail. Many of the IA kits have built in the bias factor because the kits have been standardized with complex mixtures of fuels and other naturally occurring compound mixtures.

It is very important that the IA user know the substances that may be encountered on site and the effect those substances will have on a particular IA kit's performance. The cross-reactivity and sensitivity of each vendor's kit to specific target compounds and product mixtures is detailed in the following tables:

Table 3a: TPH/BTEX Cross-Reactivity
Table 3b: PAH and c-PAH Cross-Reactivity
Table 3c: PCB Cross-Reactivity
Table 3d: TNT/RDX Explosives Cross-Reactivity
Table 3e: Individual Analyte Cross-Reactivity (mercury, pentachlorophenol, trichloroethylene, perchloroethylene, and 2,3,7,8-TCDD)

These tables are designed to show the most sensitive compounds/mixtures within a chemical class that will cross-react with a particular vendor's IA kit. The lower the detection limit number present in the tables, the more sensitive the IA kit is to that compound/mixture. Each vendor's pesticide application sheets should be reviewed to ascertain what compounds/mixtures will cross-react with their particular IA kits. Using Tables 3a through 3e, the vendor literature, and the site information, the correct IA kit can be selected and tested on field samples, in conjunction with split samples analyzed using a full protocol analytical method, prior to initiation of the full scale field sampling program, to facilitate interpretation of sample data generated in the presence of reactive and cross-reactive substances during the full scale field sampling program.

6.0 KIT OPERATIONAL CONCERNS

Analysis of environmental chemicals using conventional full protocol analytical methods has many pitfalls and nuances. The same is true for performing IA analyses. IA analyses are impacted by kit storage and operating circumstances, field conditions, and sample matrix characteristics. These factors, in conjunction with cost and time savings considerations, can influence IA kit selection and operation, and will be discussed in this section. In certain

situations, the combination of physical, chemical, and ambient sampling conditions could make the use of IA less feasible.

6.1 Temperature Range Considerations and Shelf Life

There are three basic storage and operating considerations that are critical to the effective use of IA kits:

1. Proper storage of the kits prior to use and when not in use.
2. The optimum operating temperature range of the kits.
3. Shelf life of the kits.

Each vendor, during IA kit development, investigates the proper kit storage conditions, the ideal operating temperature range, and the shelf life for each IA kit as discussed below. Table 4 details the storage temperature, operating temperature, and the shelf life of the IA kits available at this time.

6.1.1 Storage Conditions

Most IA kits should be stored at less than ambient temperatures (2°C to 8°C) prior to use. Kits should not be used beyond their shelf lives and/or expiration dates. IA kits must always be brought to ambient temperature just prior to use.

Some vendors have studied the degradation of kits held at elevated temperatures (up to 37°C, Ohmicron Quality Control of Immunoassays for Pesticide Residues, Mary Hayes et. al.) and found that the kits are susceptible to degradation at elevated temperatures. The IA user is urged to adhere to the kit storage and operational temperature ranges recommended in Table 4 and in the vendor's product literature.

6.1.2 Operating Temperature Range

The operating temperature range of an IA kit is one of the most important criteria for generating precise and accurate data. Use of IA kits in the field must be at temperatures that do not inhibit or advance the kit's recommended processing time sequences. Each vendor has specific criteria for the sample extraction, incubation, washing, color development and stop procedure. These critical timing sequences must be adhered to as much as possible. It should be noted that D-Tech's PCB literature contains a graph that depicts the time/temperature relationship for that kit. That graph should not be used to determine sample incubation time; it should be used solely as a guide or estimate to complete the color development step.

If large rises or drops in operating temperature occur during a field episode, then the IA user must ensure that standards, blanks, QC samples, and field samples are all analyzed at the same relative temperature conditions. In order to obtain comparable data within and between batches as well as from one sampling episode to another, the operating temperature for all analyses must be within the required range.

In Northern climates, where sampling may take place at temperatures below 50°F or even below 40°F, all IA kits will exhibit problems in reaction times for incubation and color development. The sluggishness of the kits at low operating temperatures will usually result in false negatives. This can be avoided if the IA user moves IA kit operation into a heated enclosure or field trailer.

Another problem arises when IA kits are utilized to analyze field samples during the day under normal ambient conditions (60-80°F) and later in the day when the temperature drops close to or below the lower limit of the operating temperature range (40°F). The data generated at 60°F will not be comparable to the data generated at 40°F using the calibration curves and QC samples analyzed at 60°F. IA testing should be stopped if a full calibration sequence at 40°F cannot be analyzed.

There are few instances when the ambient temperature exceeds the upper limit of an IA kit's recommended operating temperature range. In those circumstances, the entire sequence of standards, blanks, QC samples, and field samples should also be analyzed at the same relative temperature.

6.1.3 Shelf Life

Since IA kit reagents are biological media, the vendor and the IA user must be concerned with the length of time that the reagents can produce usable results. The vendor must identify the maximum length of time that the antibody, enzyme conjugate, and color reagents will last in order to determine the IA kit's shelf life and, therefore, the number of kits that can be kept in stock. Many of the vendors date each kit so that the IA user can tell when the kit will expire.

The IA user must be attentive to IA kit shelf life to maintain a current inventory of usable kits.

6.2 Water Characteristics

Water analyses using IA have far less matrix-related problems than do soil analyses because filtration is the only sample preparation step (i.e., extraction/dilution is not required). However, the analysis of water does warrant precautions.

IA analyses of water will be affected by sample pH, high concentrations of metals or salts, high ionic strength, and the presence of other soluble natural organic components. Insoluble organics that form emulsions, colloids, floating films and DNAPL may create problems with sample extraction, antibody complexation and/or the color development step. Each vendor has designed their kit to be used under specified conditions and most vendors have designed their reagent systems to buffer the water to the correct pH and ionic strength.

Vendors have also tested their kits under non-ideal conditions and have developed recommendations for the user when these conditions are encountered on site. In some situations, vendors can supply conditioning reagents to mitigate matrix interferences. Generally, vendors will only provide recommendations for possible sample adjustments. A representative water sample should always be tested prior to initiation of the full scale sampling episode to determine if a condition exists that will cause the IA kit to incorrectly quantitate the target compound(s). This is similar to pre-testing soils prior to initiation of full scale field sampling episodes.

6.3 Soil Characteristics

The analysis of soils by IA is much more problematic than waters due to the magnitude and frequency of matrix-dependent interferences and limitations. The type of soil greatly influences water retention and adsorption of organic compounds. The physical characteristics of the soil, mainly particle size and organic content, play a large role in affecting the adsorption and retention of organic compounds, especially chlorinated organics. The smaller the particle size of the soil, the larger the available surface area. The greater the surface area and organic content of the soil, the more sites there will be for the target compounds to adsorb onto and remain attached during the extraction process. Together, these characteristics may greatly inhibit the quantitative extraction of target compounds from soil samples.

Sandy soils are easier to extract because they have less available surface area and less organic content. Soils containing increasing amounts of silt, clay and organic content are much more difficult to quantitatively extract.

Other factors that may effect the extraction of soils include pH and the cation exchange capacity. Some of the organic target compounds, such as acid herbicides, may be in the salt form and, therefore, they will have poor extraction efficiencies.

Soils that are highly colored, or that cause highly colored solutions upon extraction, can be problematic for IA. The high color may adhere to the antibody/COI complex and interfere with the color development stage. For the D-Tech kits that concentrate the antibody/COI complex onto a membrane or a plastic well and then use a reflectance measurement to measure the color, the highly colored soils/solutions may increase the color reading, yielding false negatives or low measurements. IA kits produced by all the other vendors wash away the non-complexed sample and form the color reaction in solution. Those kits, therefore, are less prone to this problem since the excess sample matrix is removed prior to color development.

When PCB oils are encountered in soils or sediments, the presence of the oil will interfere with the reaction of the antibody with the COI. Although methanol extraction will normally dissolve the oil and put the oil in solution, when the extract is introduced into the water-based buffer and conjugate solution, the oil may precipitate or form an emulsion that coats the antibody and prevents the enzyme conjugate COI, and sample-derived COI from reacting with the antibody. This action will yield results that are biased high. It is recommended that samples contaminated with PCB oils be considered as pure oil samples, and an appropriate mineral oil waste kit be used to approximate the PCB concentration.

6.4 Extraction Solvents

In most cases, methanol was chosen by IA kit designers as the extraction solvent for soil/solid and wipe matrices because it is infinitely soluble in water, doesn't denature (decompose) the antibody or enzyme conjugate, and doesn't inhibit reactions between the antibody and the COI. Isopropanol (2-propanol) is used by Quantix/Idetek for the same reasons as methanol, and has the added bonus that isopropanol is less toxic to the user. Many attempts have been made by IA kit designers to use more efficient extraction solvents such as methylene chloride, hexane, acetone, and ethyl acetate, but when those solvents were carried through IA analysis they caused unacceptable changes in the slope of the IA calibration curve and its sensitivity.

IA analysis of waterborne or soluble contaminants is not a problem since the IA reagents are water-based and no dilutions are needed. Methanol (or isopropanol) extracts of soils/solids and wipes must be diluted into water prior to incubation with the antibody and the enzyme conjugate. The eventual dilution of the methanol also helps to preserve the aqueous antibody/COI reaction.

Although methanol is the solvent used by most IA kit vendors for extraction of soil/solid and wipe samples, it has limitations that must be addressed by the IA user during project planning. Methanol's extraction efficiency for soil and solid matrices can be diminished in the presence of large quantities of water (soil/solid matrices containing greater than 30% water). Water dilutes the methanol and reduces (limits) methanol's solubilizing properties, especially for higher molecular weight organic compounds. With high moisture soil/solid samples, the water is also weighed with the soil/solid material, thus less soil/solid material is present to be extracted. These two factors limit the IA kit's ability to extract and quantitate COIs at the recommended detection level.

The IA user must also be aware of the limited extraction efficiency of methanol and isopropanol for certain organic substances that have no moisture, such as grease, tar, asphalts and some forms of gummy or dried creosotes. Methanol

and isopropanol will not dissolve the higher molecular weight organic compounds that are present in those substances and use of a high efficiency extraction solvent in conjunction with a solvent exchange procedure should be considered by the IA user. Solvent exchange involves using a high efficiency extraction solvent to put the COI in solution, removing the high efficiency solvent using a gentle stream of nitrogen gas, and then reconstituting the sample extract with methanol/isopropanol. Dimethyl sulfoxide (DMSO) is the final solvent used in dioxin kits to dissolve the extract residue because, in its diluted form, it will not denature the antibody.

Solvent exchange procedures are not recommended unless the IA user is willing to determine that the solvent exchange procedure can achieve the project detection limits and can quantitatively recover all COIs. This determination should be performed prior to initiation of the full scale field sampling program. It should be noted, however, that extraction/solvent exchange procedures may not be feasible for field use due to the need for additional equipment and the use of toxic solvents on site.

Additionally, in situations where the COI is less soluble in methanol/isopropanol, the extraction step can be enhanced with gentle heat, shaking over a longer period of time, or the use of sonication. If the field samples require any of these enhancements, then all the standards, blanks, and QC samples must also be run in an identical manner.

6.5 Soil Moisture

As discussed in Section 6.4, the moisture content of soil/solid samples can affect extraction efficiency. Moisture contents over 30% will impact the extraction efficiency for normal sand/loam soils and will exacerbate extraction problems for highly organic soils. The incremental contribution of the water present in the soil/solid sample matrix (up to a maximum of 30% water) can be adjusted by the dilution factor used with each kit's specific procedure or through dry weight calculation.

Correcting for the loss of extraction efficiency due to the water content of soil/solid samples, however, is a more complex task. Soils or sediments with > 30% moisture may require further water removal techniques, such as decanting, filtration, air drying, or oven drying (if the soils/solids do not contain easily volatilized target compounds). Air drying of the moisture laden samples may be the IA user's only recourse for IA analyses that are performed in a field setting. Soil/solid samples to be analyzed for volatile organics cannot be dried to remove water since the drying procedure would cause volatilization of those compounds and would result in false negatives. Likewise, drying soil/solid samples for petroleum hydrocarbon analyses is not recommended due to loss of the more volatile low molecular weight components. Soil/solid samples to be analyzed for metals, PCBs, and selected non-volatile pesticides can be gently dried (<60°C) prior to sample extraction. For PAH analysis, gentle drying of the soil/solid samples will cause loss of the more volatile PAH compounds such as naphthalene, acenaphthylene, phenanthrene, anthracene, fluorene, acenaphthene, etc. If these PAH compounds are important to project DQOs, then other analytical methods should be considered.

Each vendor provides suggestions on lowering moisture content in soil/solid samples. These suggestions are compiled in Table 5. When soil/solid samples have moisture contents greater than 70%, the use of immunoassay techniques may not be applicable or advisable. Some matrices such as sands, sandy soils, or low organic content loams may dewater rapidly. In this case, the vendor-suggested use of coffee filters, paper napkins, diapers, or cheese cloth may be applicable to dewater samples. To do this, the soil is placed in the filter or cloth, which is then folded into a tight ball and squeezed to remove water through the porous surface. Although these procedures have some success with sandy materials, they do not work well with silt, organic sediments, or peat. For soil/solid samples containing greater than 90% moisture, the IA user may want to consider the sample matrix to be aqueous and proceed with an aqueous IA analysis. Project DQOs should drive this decision and such contingencies should be delineated in the pre-approved project QAPjP/SAP.

6.6 Operational Consistency

IA methods must be performed in a very consistent manner. Having the key QC elements, referenced in Section 3.3.3, in place will not ensure the production of usable data if the IA user does not perform the assays consistently, under the same operational conditions, and time each step precisely.

The operational conditions for performing IA must adhere to vendor requirements. As discussed in Section 6.1, IA kits must be brought to ambient temperature prior to use and IA analyses must be performed within the vendor-recommended operating temperature range. IA users must adhere to the shelf life and storage conditions recommended by IA kit vendors to eliminate variability in reagent performance.

In addition, individual steps in the assay must be consistently performed and precisely timed. If they are not precisely timed, then assay drift occurs. IA drift can be caused by inconsistent timing of the antibody/COI reaction and/or the color development and stop reactions. Immunoassays are commonly performed in batches where standards, blanks QC samples and field samples are assayed simultaneously in a group. When the protocol calls for precise sequential pipetting steps, there can be significant timing differences between when the immunochemical reaction begins with the first standard versus the last field sample. Reagent additions between samples, etc. must be performed rapidly, precisely, and consistently once the immunochemical reaction is started so that each sample, etc. will incubate with the same reagent volume for the same time period. The longer the period of incubation and/or reaction, the less propensity for IA drift. In designing the quality control program, each vendor has developed an understanding of the critical timing issues and has addressed them by defining batch sizes and the placement of standards, blanks, and QC samples.

The batch size is a key factor in producing consist IA results. For this reason, most field kits are limited to 4 to 6 samples, two calibrators and possibly a QC sample. Laboratory kits may use much larger batch sizes and even perform multiple batches simultaneously in racks. Multiple batches can only be performed using automatic multiple pipetters and an automated rack washing system.

IA user training is another key factor in producing consistently accurate and precise IA results and will be discussed in Section 7.0.

6.7 Cost and Time Considerations

The time required to collect environmental samples depends on the sampling technique, the physical conditions of the environment, and the sample matrix. Samples of water, which are taken from the shore of a surface water body using the sample bottle itself as a collection device, probably take the shortest time to collect with the least effort. Samples of soil, that are collected from a deep soil boring using an auger and that require compositing, probably take the most time. This document will not attempt to estimate the time associated with sample collection efforts.

The preparation and analysis time for use of IA in a laboratory setting will depend on the extent of laboratory automation and the experience of the chemist. IA vendors should be contacted directly to obtain this information. This section will focus solely on the time required to prepare and analyze a single sample using IA techniques in a field setting

Each vendor has estimated the number of samples that can be successfully analyzed per batch, depending on the sample matrix, and the time required for preparation and analysis of a sample batch. This information in detailed in Table 6a so that the IA user can compare the efficiency of each vendor's kit. The time estimates contained therein assume that the user is trained and practiced in the utilization of each IA kit.

In general, IA sample preparation includes the following steps:

- Sample measurement by volume or by weight
- Adjustment of the pH and/or buffering of waters
- Filtration of the water
- Introduction of the extractant for soils
- Extraction of the sample for soils
- Filtration of the extract for soils
- Pipetting the water sample or soil sample extract into the IA container

Sample preparation for waters takes very little time. The time required for preparation of soil samples will depend upon:

- The physical characteristics of the soil and the need to segregate large chunks of gravel or organic wood chips and twigs,
- The amount of quartering and compositing required to get a representative homogeneous sample,
- The need to remove excess moisture from the sample, and
- The difficulty in quantitatively extracting the soil depending on the organic content of the soil matrix (possibly increasing the extraction times).

The IA user must factor site information into the vendor time estimates to obtain realistic times for site-specific IA use. Analysis times for prepared water and soil samples do not vary by much because the same analytical steps take place during the IA process. Water samples, however, usually take slightly less time to analyze than soil extracts, since water samples require fewer dilutions. The time required to perform site-specific dilutions can be estimated by the vendor when the IA user contacts the vendor to determine the correct kit and dilution factor to use for the project.

The analysis step, which includes the time required for pipetting, incubation, and color development, usually takes 25 - 45 minutes per sample batch (or per sample if only one sample is being analyzed). The exact analysis time will depend upon the specific requirements of each vendor's kit and the COIs that are analyzed.

The timing sequences for each vendor's kit control the number of samples that can be accurately and precisely analyzed in a single batch. Approximately 35 to 200 samples/person/day can be processed using IA kits, depending on the COI tested and the extent of sample preparation. That estimate assumes that the IA user performing the sample preparation and analysis has experience with those particular IA kits.

Tables 6b and 6c contain the per sample costs for individual vendor IA kits. The IA user can use the information in Tables 6a - 6c to compare the time and cost required to prepare and analyze samples using IA kits versus conventional full protocol analytical methods. IA has the advantage of immediate turn-around time for obtaining sample results, whereas sample results obtained using conventional full protocol analytical methods performed in a fixed laboratory can take over a month.

The choice of IA techniques versus conventional full protocol analytical methods is usually driven by two factors: the project costs accrued while waiting for sample data and the quality of the sample data necessary to meet project DQOs. When excavation or drilling equipment are present on site, the equipment idle time can be significantly more expensive than the fixed laboratory analysis costs. The generation of quick reliable sample results to keep heavy equipment moving can be the critical factor in lowering project costs as long as the comparability of IA data and split sample confirmatory data meet project objectives.

The costs and time required for preparation and analysis of project samples using IA kits versus field laboratory analytical methods should be compared as well. The sample throughput of field laboratory methods may be equivalent to IA, but the personnel costs associated with staffing a field laboratory with experienced chemists that can operate the complex instrumentation competently may far outweigh the cost of personnel to perform IA analyses. The costs associated with providing an external power source for field laboratories also negatively impacts their use.

6.7.1 Time Savings

Each vendor provides helpful hints to save time when performing IA analyses. However, the major factor in saving time on site is practical and thorough planning as well as proper training of the site personnel. Batching of the samples into like matrices and similar dilution groups can save processing time. Practice with the kits of choice prior to the field episode using site-specific samples is recommended. Prior knowledge of the sample matrix and its physical characteristics is crucial to successful IA use and will speed sample processing for the extraction and dilution steps.

6.7.2 Cost Considerations

The cost of IA kits can be separated from the ancillary equipment cost for the weighing, pipetting and colorimetric measurement equipment because that equipment is now available from the vendors on a rental basis. IA users must check with each vendor to ascertain the availability of ancillary equipment. The purchase price for ancillary equipment may range up to $2000 and each manufacturer uses slightly different equipment depending on the format of their IA technique. Rental of the ancillary equipment may be more cost effective for the occasional IA user or the IA user that travels between sites. In contrast, purchase of the ancillary equipment may be more cost effective if the IA user is planning to process many samples at a single site over a long period of time. The power requirement for IA ancillary equipment is minimal and some vendors' equipment is even powered by battery. Ancillary equipment costs, power requirements, and site accessibility should be considered when the IA user compares use of field laboratory instrumentation to IA kit use for a particular project. Utilizing a gasoline powered generator on site to power analytical equipment may jeopardize the site investigation by introducing another source of BTEX and PAHs.

IA kit costs are dependent on the number of samples in a batch. The more samples that can be processed at one time between the proper standards, the less the ultimate per sample cost. Standards, blanks, and PE samples must also be included in the cost per sample. Vendors of IA kits offer discounts for volume sales and equipment rentals.

Fixed laboratories also offer volume discounts for performing confirmatory split sample analyses using conventional full protocol analytical methods. Again, the more sample analyses that can be batched into 20 sample lots, the less each sample will cost.

With proper planning, personnel training, QA/QC, and sufficient comparable split sample confirmatory data, IA users will find that the less costly and faster generated IA data can be as usable as conventional full protocol analytical data in meeting project objectives and making valid site decisions.

6.8 Disposal of IA-Related Waste

The IA user must address the disposal of project-related wastes originating from all field sampling, decontamination, and analysis processes that are performed on site. As is true for all site-derived wastes, wastes generated during the IA analysis process must be disposed of in a responsible manner.

IA waste disposal decisions are linked to the disposal decisions that are made for other site materials, such as drill cuttings, purge water, and excess composite samples. All of these materials can be containerized or buried on the site depending on the nature of the site activity and the Action Levels set by the regulatory body controlling the site.

IA analysis process wastes can be separated into two groups: the hazardous chemicals; and the non-hazardous, non-contaminated disposables. QC materials (calibration standards and PE samples) and the field samples that contain COI(s) must be considered to be contaminated and must be controlled. The packaging, pipette tips, and the reagents that do not contain COI(s) may not need to be controlled. IA wastes that are considered non-toxic and have not come in contact with the COI(s) usually can be disposed of in municipal trash. Some regulatory bodies will require that all site-related waste, regardless of toxicity and weight/volume, be disposed of in a regulated manner just because these items were on the site at some time. The IA user must ascertain the waste disposal requirements for the site from the subject regulatory body.

Disposal costs should also be calculated and added into the cost of on-site IA analyses. Disposal of IA-related wastes must be addressed during the project planning stage.

7.0 PRACTICAL PLANNING FOR PROJECTS USING IMMUNOASSAY

Figure 6 depicts a decision tree, which outlines the process that project planners should follow to plan environmental projects utilizing IA. There are six steps which must be followed when planning projects that will utilize IA techniques:

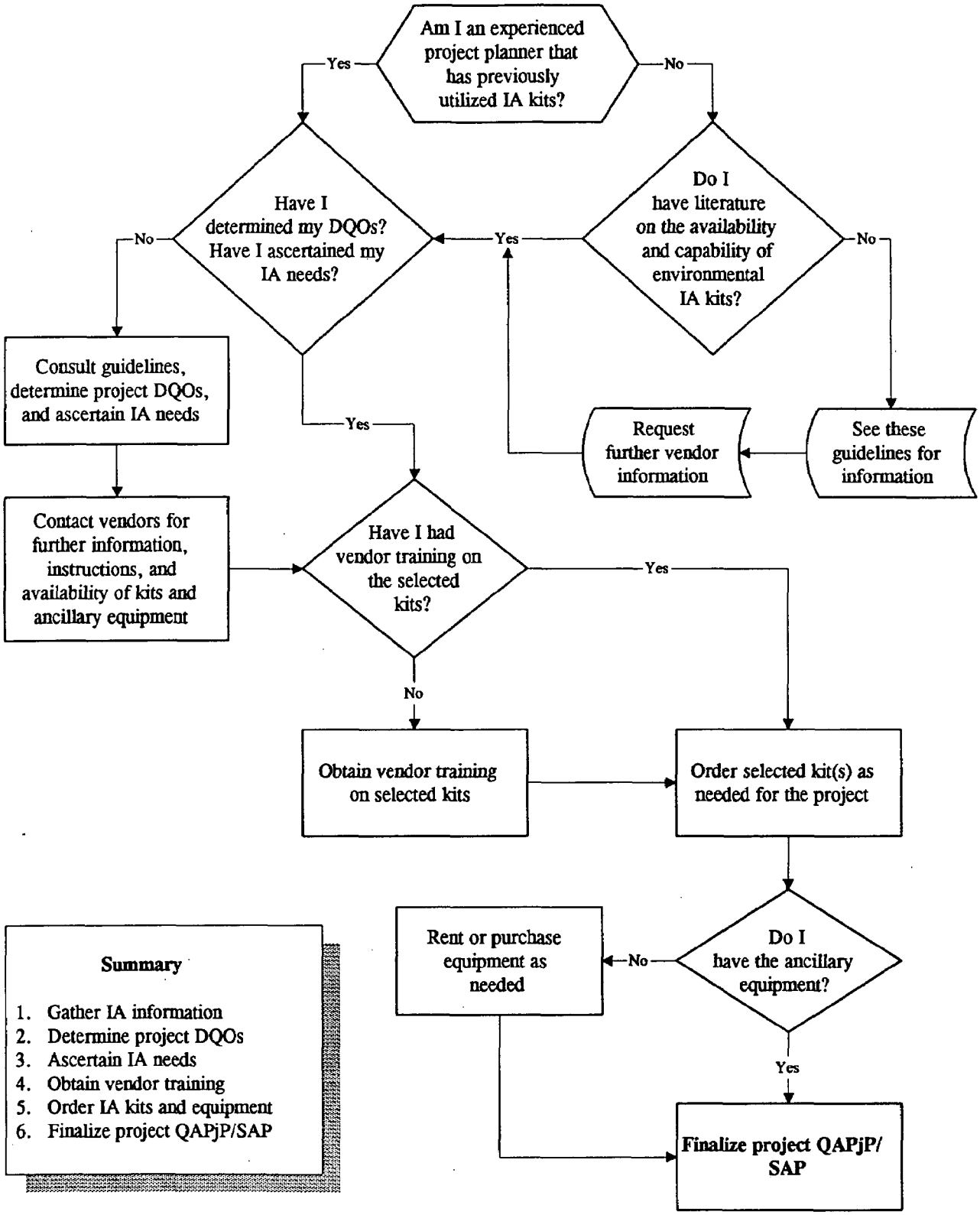
1. Gather IA information
2. Determine project DQOs
3. Ascertain IA needs
4. Obtain vendor training
5. Order IA kits and ancillary equipment
6. Finalize the project QAPjP/SAP

These six steps are the subject of this section, which will attempt to compile the informational needs and to outline the critical decision processes so that the project planner can properly choose an IA kit for a specific use. The process discussed in this section will also enable the project planner to help ensure that IA analyses will be performed correctly on site and that the resultant IA data are suitable for their intended use in site decision making.

7.1 Gathering IA Information

Section 2.0 discussed the basic principles of immunochemistry so that project planners would be familiar with environmental IA technology. Using the information provided in Section 2.0, project planners can review the characteristics and availability of specific IA kits from the various vendors outlined in Section 3.0. If any additional information (i.e., vendor literature) is needed in order to properly plan a project utilizing IA, then project planners should contact the vendor directly to obtain that information.

Figure 6 IA Project Planning Decision Tree



7.2 Determining Project Data Quality Objectives (DQOs)

Before the project planner can integrate immunoassay techniques into the project's sampling and analysis scheme, the project planner must establish the project DQOs. The project planner should obtain and evaluate as much historical information as possible about the site conditions and history, including the results of any previous investigations at the site, prior to DQO development so that the following questions can be answered during DQO development:

- What is the contamination on the site?
- Where is the contamination on the site?
- What is the source of contamination on the site?
- What is the purpose of sampling and analysis (data collection)?
- What sample matrices may be contaminated?
- What compounds or chemical classes (COIs) must be analyzed?
- What are the Action Level concentrations for the COIs?
- What non-target compounds or chemical classes may be present on the site?
- What is the intended use of the data?

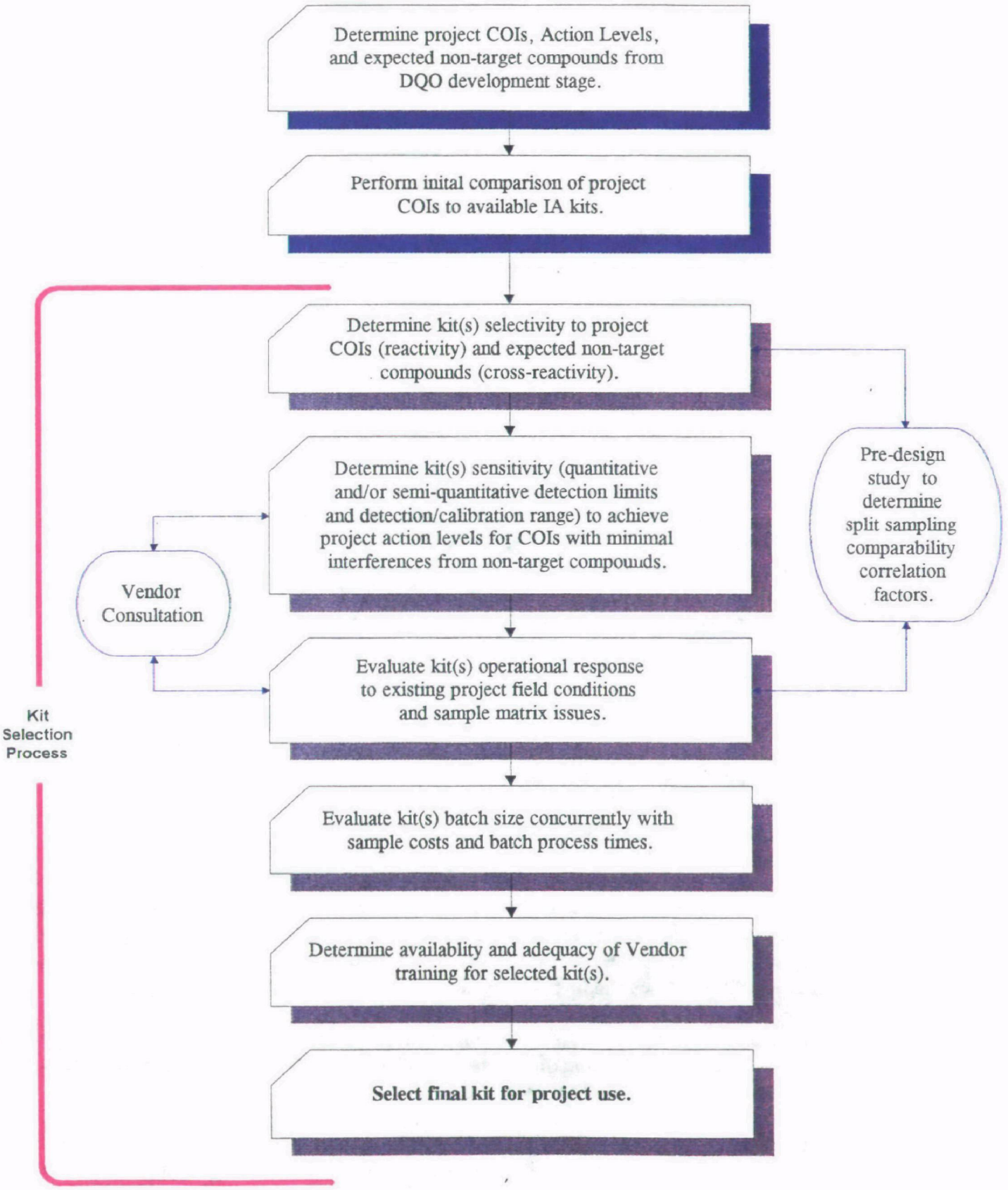
If the project planner, with input from the project team (risk assessors, hydrogeologists, chemists, etc.) can answer all of these questions completely, then the project DQOs have been developed properly and are understood. If the planner cannot answer these questions, then the process for DQO development must be undertaken.

DQO development will not be addressed in these guidelines. It is described, however, in the Agency document, Guidance for the Data Quality Objective Process, EPA QA/G-4, which describes the informational needs and critical decision processes that must be addressed during DQO development.

7.3 Ascertaining IA Needs

The COI(s) and Action Levels must be known prior to ascertaining IA needs because IA is not a technology that lends itself to initial investigations of sites that have unknown contaminants at unknown concentrations. The presence and estimated concentrations of non-target compounds or chemical classes on site must also be identified during the DQO development stage. Once the project COI(s), Action Levels, non-target compounds, and intended data uses have been established during DQO development, the project planner can proceed with ascertaining IA needs for the project as depicted in Figure 7.

Figure 7 Ascertaining IA Needs



The project planner should compare the COI(s) to the available IA kits (Tables 1a and 1b) and determine whether the project DQOs can theoretically be met using IA techniques. If the project planner decides that IA techniques may be applicable to project DQOs, then the planner must answer the following questions in order to select an appropriate IA kit:

- Which kit is best to use for analysis of project COI(s) at their required Action Level(s)?
- What type of IA data are required: quantitative, semi-quantitative, or qualitative?
- What sample matrices need to be tested?
- What is the concentration level or range for which COIs need to be tested?
- What reference method for confirmation will be used for the project?
- Will the resultant IA data meet project objectives?

The process for selecting IA kits is discussed in Section 7.3.1, while data comparability and usability in meeting project objectives is addressed in Section 8.0.

7.3.1 IA Kit Selection Factors

Now that the project planner has determined that IA kits are available to potentially meet project DQOs, the project planner must select the kit that best meets the project objectives. Numerous factors impact final kit selection, including kit selectivity (reactivity and cross-reactivity) and sensitivity, the need for quantitative vs. semi-quantitative results, expected field conditions and complexity of sample matrices, per sample costs and batch process times, and the availability and comprehensiveness of vendor training.

Reactivity

The planner must first evaluate which vendor kits are reactive to the project COI(s) as discussed in Section 5.1. Tables 2a - 2f should be consulted to perform this evaluation. A review of the relative sensitivity of each reactive compound/mixture is then required as discussed below.

Sensitivity and Quantitative versus Semi-Quantitative Kits

The planner must determine whether those selective vendor kits produce quantitative or semi-quantitative results that are sensitive enough to meet project Action Levels, especially since detection limits and detection/calibration ranges are specific to each kit's particular quantitative or semi-quantitative mode. This determination is critical to project success, and the information provided in Sections 3.3, 3.4, and 4.0 as well as Tables 2a - 2f should be used to make this determination.

There is no easy way to equate different vendor detection limits to one another. The project planner should understand each vendor's definition of detection limit and how detection limits are utilized in each vendor's IA kit to establish detection/calibration ranges.

As stated in Section 4.0, some vendors define the detection limit as the pre-determined 90% B/B_0 value while other vendors define the detection limit as the lowest concentration that gives a positive result at a specific

confidence limit (usually 95% confidence limit). Generally, quantitative kits use the 90%B/B_0 detection limit designation and semi-quantitative kits use the lowest detectable amount at 95% confidence limit. It should be noted that vendor kits may have very different detection limits for the same compounds/mixtures. This is due, in part, to vendor use of different antibodies that may be more or less sensitive to a specific compound/mixture. Tables 2a - 2f list the sensitivities of specific compounds/mixtures to various vendor IA kits. The lower the detection limit number presented in those tables, the greater the sensitivity of that IA kit to a specific compound/mixture. The compound/mixture that has the lowest detection limit for a particular kit is usually (but not always) the indicator compound/mixture that was used to engineer that kit.

To facilitate kit selection, the project planner should perform an initial comparison of vendor detection limits as if they were all calculated in the same manner. This comparison can narrow the number of kits that can meet the Action Levels for the project COIs from many to a few.

Next, the project planner must define which of those few kits is most applicable to the project by examining how close each kit's detection limit is to the project Action Level and where that Action Level falls within each kit's detection/calibration range. If the kit's detection limit is very close to the Action Level then detection uncertainty increases and it will be difficult, if not impossible, to make site decisions using data generated with that kit. In addition, the expected concentration of project COI(s) should fall into the middle of the kit detection/calibration range, where the most accurate identification and quantitation of COIs occurs. Identification and quantitation at the extreme ends of the kit's detection/calibration range must be avoided. This is generally not a problem for quantitative kits. Vendors of semi-quantitative kits have designed the kits so that the Action Level will fall into the middle of the kits' detection/calibration range. This type of design helps to eliminate false negatives, but does promote some false positives. Finally, the chosen kit(s) range of detection/calibration for project COI analysis should result in the least amount of kit manipulation, including changes to sample weights and dilution volumes (as discussed in Sections 3.3 and 3.4). Vendor consultation may be needed to help resolve potential detection range and sample dilution issues.

It should be noted that some kits are not sensitive to individual compounds within a chemical class such as benzene in the BTEX group, naphthalene and dibenzo[a,h]anthracene in the PAH group, and Aroclor 1221 in the PCB group. Benzene alone can be measured using a specially designed kit made by ENSYS. Naphthalene can be measured very well using the cross-reactivity of the BTEX/TPH kits. Dibenzo[a,h]anthracene and Aroclor 1221 are not measured well by any IA kit.

During the kit selection process, the project planner must also ensure that project objectives for generating quantitative data for particular compounds/mixtures will be met using the kit that is ultimately chosen. For example, a project's DQOs require quantitation of Aroclor 1242. From the historical site information, it was determined that Aroclor 1242 was the only PCB mixture ever used on site. Aroclor 1242 is a moderately chlorinated PCB mixture. IA kits for PCBs, however, are most sensitive to Aroclor 1254 (which is a more highly chlorinated PCB mixture). For each applicable vendor PCB kit, one must assume, therefore, that the minimum detection limit for Aroclor 1242 will probably be higher than the detection limit for Aroclor 1254 by some factor. The exact factor can be calculated from the sensitivity values provided in Table 2c. The results obtained for Aroclor 1242 using a particular PCB kit could then be multiplied by the calculation factor to obtain quantitative sample data for Aroclor 1242.

Use of a quantitation factor can not be applied to PAH and c-PAH kits. The molecular weight and number of 6 membered rings of the specific COI (PAH or c-PAH compound) should be matched to the kit most sensitive to that range of PAH rings. It will be difficult, if not impossible, to determine the number of rings present in the PAH compounds on-site, unless previous site data has been generated and indicates the specific PAH

compounds that were detected. If the project objective is to collect data for human health risk assessment, then the c-PAH kits are probably most applicable.

Cross-Reactivity

Next, the planner must factor the cross-reactivity of that select group of kits into the process. If non-target compounds/mixtures may be present at the site, then their cross-reactivity must be evaluated using the information provided in Section 5.2 and Tables 3a - 3e.

For individual pesticides, insecticides and herbicides, vendor literature must be obtained and examined to determine the cross-reactivity of the various COI metabolites since it was too voluminous to include in this document. In general, there are few cross-reactants that will affect the quantitation of pesticides, insecticides, and herbicides in field samples, unless the concentration of cross-reactant(s) is very high.

For sites that have known products containing mixtures of PAHs, petroleum hydrocarbons and BTEX, it is important to check that a specific kit has no major cross-reactants to other site-related compounds/mixtures such as PCBs or pesticides. The planner must always consider the difficulty in determining the additive effect of the cross-reactants in relation to whether the kit will have the required specificity to the COI.

The planner may also use cross-reactivity to their advantage when project DQOs require the analysis of total contaminant classes (total PAHs, total PCBs, or total TPH) rather than individual compounds. TPH kits that measure fuel products are also sensitive to naphthalene and substituted single ring aromatic compounds. Kits that measure PAHs and c-PAHs are also sensitive to many aliphatic substituted PAH analogs.

The following complex planning scenario illustrates use of cross-reactivity to measure site contaminants:

Coal tar has been discharged and is the major contaminant source. Fuel oil #2 was spilled on site and may have mixed with the coal tar discharge and fuel oil #6 that was spilled in a different area of the site. The project planner must do the following to satisfy the project objectives:

1) Delineate the extent of Total PAH contamination on the site.

2) Determine the human health risk of the 8 carcinogenic PAHs.

3) Determine the volume of soil, which is contaminated with the coal tar and fuel oil related PAHs, that must be remediated.

The project COIs include the range of high molecular weight to low molecular weight PAHs, and the carcinogenic PAHs that are components of all of the products found on site.

If the project planner is to use immunoassay kits, he/she must be cognizant of the specific PAH compounds that are potentially present in each product, and the ring structure and molecular weight of those PAH compounds.

- The #2 fuel oil has naphthalene and a small amount of low molecular weight PAHs.
- The coal tar has a wide range of PAHs, including naphthalene.
- The #6 fuel oil has medium to high molecular weight PAHs.

In order to ascertain the extent of contamination, the volume of soil to be remediated, and the human health risk from the carcinogenic PAHs, the planner may have to use more than 2 IA kits to satisfy the project objectives.

First, all the contaminated areas would need to be characterized using a c-PAH kit to assess the human health carcinogenic risks.

Next, the project planner would have to decide which additional kit(s) to use to satisfy objectives #1 and #3 listed above. Several different approaches could be taken.

The project planner could choose to use a PAH kit that is sensitive to a broad range of molecular weight PAHs to assess the extent of contamination and the soil volume to be remediated. The project planner must be aware, however, that naphthalene, which is present in measurable quantities in #2 fuel oil and coal tar, will not be detected using such a kit.

On the other hand, the project planner could use a TPH/BTEX kit, in conjunction with the c-PAH kit, to assess the extent of contamination and the soil volume to be remediated. The TPH/BTEX kit is sensitive to naphthalene in the #2 fuel oil and coal tar. The project planner must be aware, however, that the TPH/BTEX kit would overestimate the amount of naphthalene present in the samples, since that kit would also measure all of the single aromatic compounds (non-PAHs) that are present in the samples. The extent of contamination and the soil volume to be remediated for PAHs originating from the #6 fuel oil would then have to be determined using the results of samples analyzed using the c-PAH kit.

Other combinations of kits that analyze low to medium molecular weight PAHs and medium to high molecular weight PAHs could also be used to determine the extent of contamination and the soil volume to be remediated.

Although this is a complex example, it illustrates the need for the planner to know what target and non-target compounds/mixtures are present on site and what IA kits are available to analyze specific target compounds/mixtures.

Field Conditions and Sample Matrix Factors

The project planner has now considered selectivity (reactivity and cross-reactivity), sensitivity, and quantitative vs semi-quantitative use of the available kits. At this point, the project planner should be able to match appropriate kit(s) to their intended site use, as long as those kit(s) can be used under the expected field conditions and complexity of sample matrices that may be encountered on site.

Sections 6.1 - 6.5 discuss many of the field conditions and sample matrix factors that must be considered by the planner for use of IA techniques to achieve project DQOs. The pertinent factors include characteristics of water and soil matrices, matrix extraction efficiencies, soil moisture content, and ambient conditions

(temperature and sampling). Vendor consultation may be needed to help resolve selected field conditions and/or sample matrix issues prior to final kit selection.

It is critical to factor soil characteristics into the extraction and IA analysis of project soils. Prior to final kit selection, the project planner must evaluate potential moisture problems and the extraction efficiency of methanol/isopropanol for separating the COI from the sample matrix. Contingency steps for each of these circumstances should be detailed in the approved QAPjP/SAP and any pre-design studies must be performed prior to initiation of the full scale field sampling episode.

The project planner must always consider the ambient temperature and sampling conditions for the site. The project planner must evaluate the ideal ambient temperature operating range of the prime kits in conjunction with expected field conditions (ambient site temperature and location of IA testing). The planner must also ensure that IA analyses will not be impacted by airborne site contaminants. For sampling and IA analysis of target compounds (i.e., volatile organics, mercury, etc.) that can be affected by ambient site conditions (such as temperature, oxidation, and moisture), appropriate contingency steps for sampling, preparation, and/or IA analysis must be detailed by the project planner in the approved QAPjP/SAP.

Sample Costs and Batch Process Times

After the project planner has evaluated the response of the prime kit(s) to expected field conditions and sample matrix complexity, then the planner should examine sample costs and batch process times in conjunction with batch size, ease of kit use, and disposal of IA-related wastes. Sections 6.7 and 6.8 contain a discussion of those topics and Tables 6a - 6c contain information on batch processing times and per sample costs for specific IA kits. Final selection of an IA kit will depend, in part, on the number of samples that can be analyzed per batch, the per sample costs, and the batch processing times.

Availability of Vendor Training

The final factor pertaining to kit selection that must be considered by the project planner is the availability and comprehensiveness of vendor training courses. The training of personnel to use IA kits properly is an important part of a project's QA program. Each vendor's product has a specified format and protocol that must be adhered to much as possible. IA technologies require consistent process techniques as described in Section 6.6. Proficiency in sampling, weighing, pipetting, sample dilution, and colorimetric measurement is critical to resultant project data quality.

Each vendor offers a training program which describes their immunoassay format and equipment operation. Each training session usually culminates in a test of the proficiency of the IA user. Before a particular vendor's IA kit is utilized in the field for project work, the personnel performing IA should have attended that particular vendor's training course and have practiced with that kit prior to initiation of the full scale field sampling episode.

Final Kit Selection

Once all kit selection factors have been evaluated and any required vendor contact has been made to resolve potential site-specific issues/problems pertaining to the use of particular IA kits, the project planner can proceed to select the most appropriate kit for achieving project DQOs.

7.4 Obtaining Vendor Training

Once the final kit has been selected for project use, vendor training pertaining to the specific IA format and operation of that vendor's equipment must be obtained. All personnel that will perform IA on site must pass the vendor proficiency test that occurs at the end of the training session. Training should be obtained prior to finalizing the QAPjP/SAP so that the project planner can be notified of any project issues that may result from use of a particular IA kit.

7.5 Ordering IA Kits and Ancillary Equipment

Project planners should contact the individual vendors at the telephone numbers listed in Section 1.1 as needed to obtain current pricing and availability information, purchase IA kits, and purchase or rent the required ancillary equipment.

7.6 Finalizing the QAPjP/SAP

The IA project planner must develop a QAPjP/SAP that details the project description and DQOs; project personnel and their responsibilities; sampling protocols; sampling locations and numbers of samples to be collected per matrix for each compound and/or analysis parameter; IA and confirmational analytical protocols with frequency requirements, QC acceptance criteria, and corrective action measures; split sampling comparability acceptance criteria; and how the QC and field sample data will be used to determine whether project DQOs have been met (Data Quality Assessment).

EPA QA/R-5, EPA Requirements for Quality Assurance Project Plans (August 1994), details the requirements used by Region I, EPA-NE for the development of a QAPjP/SAP. The QAPjP/SAP need not be extensive for an IA episode, but it must sufficiently address all of the elements detailed in EPA QA/R-5.

DQO's must be developed in accordance with EPA QA/G-4, Guidance for the Data Quality Objective Process. How IA data will be used in project decision making is critical to document in the QAPjP/SAP. Justification must also be provided in the QAPjP/SAP for the final IA kit selected for use in the project. Expectations for IA data use must be realistic in relation to the nature of the site contamination.

The QAPjP/SAP must describe, in explicit detail, the sampling protocols that will be used for the project. The sampling protocols must demonstrate that proper procedures will be used consistently and will result in sufficient volume of representative sample to enable multiple IA analyses (in cases where duplicates are required) as well as split sample confirmation analysis. Samples of water must be taken where the water is in equilibrium with the environment. Well waters must be sampled using methods that do not disturb the aquifer such as the EPA Region I low flow procedure. Soils must be representative of the site conditions, and samples taken as composites must be well mixed prior to IA analysis. Soil samples that are high in moisture must be decanted, centrifuged, filtered, or gently dried to ensure that there is at least 70% solids prior to IA analysis. Samples of free product materials (pure oils or pure chemical solids) must be diluted with the vendor-recommended solvent prior to analysis. Most of all, sampling must be performed in a consistent manner for all samples collected during the project episode.

The QAPjP/SAP must also describe, in explicit detail, the analytical procedures that will be performed for sample preparation and analysis by IA techniques as well as by conventional full protocol analytical methods that are used for split sample confirmation analyses. The QAPjP/SAP must compile all the key QC elements that were discussed in Section 3.3.3 along with their frequency requirements, QC acceptance criteria, and corrective action measures when QC acceptance criteria have not been met. IA analysis must be performed in strict accordance with vendor-specified

procedures, with exact timing sequences and reagent additions. Inconsistencies in sampling and analysis procedures will make data comparability difficult, if not impossible.

Detailed standard operating procedures (SOPs) for the sampling and analysis protocols can be referenced in the QAPjP/SAP as long as the applicable SOPs are appended to that QAPjP/SAP. All SOPs that are pertinent to project operations must be developed in accordance with EPA QA/G-6, Guidance for the Preparation of Standard Operating Procedures for Quality-related Operations.

The QAPjP/SAP must also describe documentation requirements for the resultant IA and split sample confirmation data. Documentation that all key QC elements were performed and met project requirements is essential, regardless of intended data use. For IA techniques, the preparation and analysis of each batch of samples, including related standards, QC samples and blanks, should be recorded in a field or laboratory notebook, run logs, and/or tabulated forms. Note, sample preparation should include kit lot numbers and expiration dates, and the ambient temperature at which the tests were performed. For conventional full protocol analytical methods, complete data packages should be produced in accordance with the Region I, EPA-NE specifications contained in the Training Manual for Reviewing Laboratory Data Package Completeness, dated June 1994.

The procedure and requirements for determining data comparability and usability in meeting project objectives must also be described in the project QAPjP/SAP. Data comparability and usability are discussed in Section 8.0. Documentation of IA use for a project, prior to field sampling initiation, is critical to data interpretation after the field sampling has been completed. Many project planners have used IA as a panacea to reduce project analytical costs and speed up the remediation activities at contaminated sites. Used properly, with the appropriate QC procedures and a definitive QA program, IA can be a very effective tool. Used without regard to QC requirements and QA process controls, the resultant IA data may be unusable or uninterpretable.

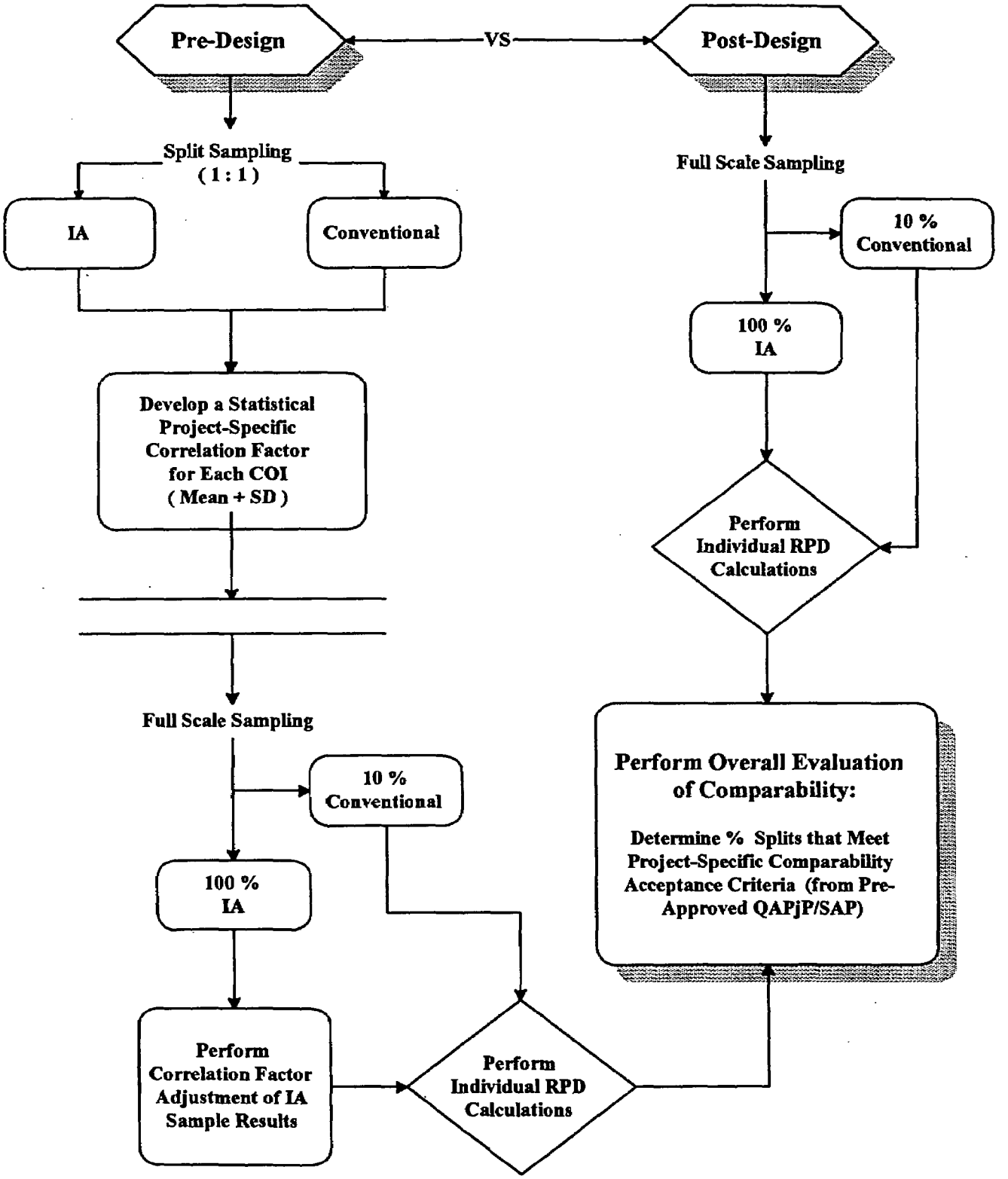
8.0 DATA COMPARABILITY AND USABILITY

8.1 Data Comparability

The comparability of IA data generated on site and split sample confirmation data generated in a fixed or field laboratory using conventional full protocol analytical methods is the most important factor for determining whether IA data will meet the project objectives and be usable for project decision making. The conventional full protocol analytical methods that are used to confirm the IA results must be scientifically valid and well documented protocols that have been routinely accepted by regulators, since data comparability decisions are based upon a limited number of samples analyzed by those conventional full protocol methods.

Figure 8 illustrates two approaches that can be used for determining data comparability. One involves the generation and application of pre-design correlation factors to adjust IA sample results prior to performing data comparability calculations. The other approach does not utilize correlation factor adjustment of IA sample results prior to performing data comparability calculations. Both approaches require that data comparability acceptance requirements be developed and documented in an approved project QAPjP/SAP prior to initiation of the full scale field sampling program. Both approaches also require that split samples be collected and analyzed at a 10% frequency throughout the duration of the full scale field sampling program to assess data comparability.

Figure 8 Comparability Determination



Generally, split sampling data generated using IA techniques and conventional full protocol analytical methods should meet comparability acceptance criteria of 30% relative percent difference (RPD) for water matrices and 50% RPD for soil matrices (assuming a one-to-one correlation). These recommended comparability acceptance criteria can be modified to meet project objectives, as long as the project-specific comparability acceptance criteria are documented in the pre-approved project QAPjP/SAP.

Since IA techniques and conventional full protocol analytical methods measure target compounds using different principles, a one-to-one correlation between data sets cannot always be expected. IA kits measure the sum of the reactive target compounds and cross-reactive non-target compounds present in the sample matrix. Conventional full protocol analytical methods, on the other hand, measure the concentrations of individual target compounds present in the sample matrix. Extraction efficiency differences between the full protocol method and the IA method may also account for correlation factors that are not one-to-one. The alcoholic solvent and extraction procedure used in IA kits may decrease the extraction efficiency of IA for certain chemical mixtures/products when compared to the more rigorous extraction solvents and procedures used in conventional full protocol analytical methods. The combination of less effective extraction and broader range of detection/quantitation will make the exact project-specific correlation factor hard to predict. It is likely, however, that a near one-to-one correlation will be achieved for the individual compounds listed in Tables 2e and 2f, since IA kits for those compounds are minimally impacted by the cross-reactants that are common to other environmental parameters. IA's cross-reactive properties could also potentially result in a greater than one-to-one correlation (IA to conventional analysis) for the kits that characterize chemical classes such as PCBs, PAHs, and TPH. During the project planning phase, the project planner must evaluate the impact that a greater than one-to-one correlation factor may have on achievement of project objectives through review of historical information concerning site conditions, history, and the results of any previous site investigations. That is one of the reasons why it is strongly recommended that a pre-design project-specific correlation factor for each COI be determined prior to initiation of the full scale field sampling program.

Ideally, during the project planning phase, a project-specific correlation factor for each COI is established based upon actual testing of representative split field samples using both the selected IA kit and the full protocol analytical method that will be used during the full scale field sampling program. The project planner should utilize previously generated IA case studies, vendor literature, and past experience to focus these pre-design studies. Once a valid correlation factor has been established, it can be used to calculate adjusted IA sample results prior to performing comparability calculations. Note, however, that all IA sample results must be reported without correlation factor adjustment, and the applicable correlation factor must be reported separately. Comparability calculations performed with IA sample results for which correlation factors have not been generated and/or applied may cause split sampling results to exceed the data comparability acceptance criteria contained in the approved project QAPjP/SAP (due to the increased impact of sampling and analysis variability) and, therefore, may potentially render entire IA data sets unusable for project decision making.

For large remediation projects requiring confirmation of site cleanup, generation and application of an accurate and stable correlation factor for each COI in conjunction with on-going 10% split sampling comparability checks will provide the remediator and the regulator with a comfort zone and some assurance that site cleanup is proceeding to the required action level.

8.2 Data Usability

The usability of IA data is directly related to proper project planning (realistic project expectations and explicitly delineated decision objectives), implementation (proper sample collection, preparation and analysis procedures) and assessment (data validation and split sampling data comparability evaluation) activities. Semi-quantitative IA analysis is suggested by SW-846. Quantitative IA analysis can be performed successfully when the project planner uses all

the available site information to design the sampling and analysis protocols. How well IA data compares to the split sample data generated using conventional full protocol analytical methods in a fixed or field laboratory is the key to determining data usability. Evaluation of PE sample score results should also be incorporated into the usability determination. If the conventional full protocol analytical data are valid and usable, then IA data which meet comparability acceptance criteria can also be considered valid and usable to make project decisions.

Validation of the split sampling data generated using conventional full protocol analytical methods is required, since those conventional full protocol analytical data will be the benchmark to which the IA data will be compared. Validation of the conventional full protocol analytical data must be performed and documented in accordance with the procedures and requirements contained in the Region I, EPA-New England Data Validation Functional Guidelines for Evaluating Environmental Analyses, dated July 1996 or most recent revision. Validation of IA data should also be performed by reviewing the results of calibration, continuing calibration checks, blanks, duplicates and PE samples. IA data validation should be performed and documented in accordance with the requirements contained in the pre-approved project QAPjP/SAP.

IA data that are produced for a project will be usable for their intended purpose in site decision making if the QC and confirmation data indicate that there were no false negatives and few false positives for samples with concentrations around the project Action Level. As stated previously, the IA kit's detection limit should be below the project Action Level to ensure that there are no false negatives. Likewise, the Action Level should fall in the middle of the detection/calibration range of the IA kit to facilitate accurate quantitation.

If IA data are used strictly for semi-quantitative screening purposes (to delineate the extent of contamination or to confirm site cleanup), then IA data can be considered usable when 90% of the split samples achieve the pre-established comparability acceptance criteria. Quantitative IA analyses should follow similarly stringent criteria. The project planner should identify the limiting factor for obtaining quantitatively accurate and precise project-specific IA sample results and develop a project-specific correlation factor for each COI prior to initiation of the full scale field sampling episode.

Using the information that has been provided in this document, the project planner should now be able to successfully choose an IA kit to meet the project's DQOs, successfully perform the IA analyses, and produce usable IA data that can be employed in project decision making.

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APPENDIX A

GLOSSARY

Accuracy - degree to which the value obtained in an assay corresponds to the "true" value.

Adjuvant - a substance, usually injected with an antigen, which improves the immune response to the antigen.

Affinity - The strength of the binding between binding reagent and a ligand.

Analog - a member of a family of molecules whose major structural characteristics are identical, with only minor structural differences.

Analyte - a compound or family of compounds in a sample to be analyzed in an assay.

Antibody (Ab) - an immunoglobulin produced in an animal in response to an antigen (or hapten combined with its macromolecular carrier) which can react specifically to form an antigen-antibody complex.

Antibody-Binding Site - Sites on the antibody that react with the determinant sites on antigens.

Antigen (Ag) - a substance which can elicit the formation of antibodies and react specifically with the antibodies formed.

Antisera - antibody containing sera.

Avidity - the strength of the bond between a binding reagent (antibody) and a ligand (antigen or hapten).

Bound - antigen which is present as a complex, attached to antibody; that fraction of the reaction mixture of an immunoassay which contains the antigen-antibody complex.

Carrier - An immunogenic substance that, when coupled to a hapten, renders the hapten immunogenic.

Chromogen Substrate - a substrate which produces a color when it reacts with its specific enzyme, the intensity of the color produced being directly proportional to the amount of enzyme available to react with the chromogen substrate.

Competitive Assay - an immunoassay based on the principle of competition between the test (unknown) antigen and antigen labeled with an isotope or an enzyme for a limited number of antibody binding sites.

Competitive Immunoassay - an immunoassay method involving an in-vitro competitive binding reaction.

Conjugates - enzymes linked to antigens or antibodies in such a manner that each retains the maximum amount of their reactivity.

Control - a sample-like preparation containing a known amount of analyte or devoid of analyte that is treated in the assay as an unknown sample.

Cross-reaction - reaction of an antibody with more than one antigenic structure.

Determinant - unique small three-dimensional surface sites on antigen molecules that react (combine) with antibody-binding on antibodies.

ELISA (Enzyme-Linked Immunosorbent Assay) - a heterogeneous immunoassay utilizing enzyme-labeled antigens or antibodies.

Enzyme - a protein capable of catalyzing a reaction of a substrate molecule to form a product. Enzymes can be highly specific for a given substrate.

Enzyme Conjugate - a molecule produced by the coupling of an enzyme molecule to an immunoassay component that is responsible for acting upon a substrate to produce a detectable signal.

Enzyme Immunoassay (EIA) - an immunoassay utilizing enzyme labeled antigens, antibodies, or haptens. There are two main types of EIA procedures; homogeneous assays and heterogenous (ELISA) assays.

False Negatives - a negative interpretation of the method containing the target analytes at or above the detection level. Ideally, an immunoassay test product included in an SW-846 method should produce no false negatives. The maximum permissible false negative rate is 5%, as measured by analyzing split samples using both the test product and a reference method.

False Positives - a positive interpretation for a sample is defined as a positive response for a sample that contains analytes below the action level.

Free - antigen which is not attached to antibody; that fraction of the reaction mixture in an immunoassay which contains the free antigen.

Hapten - a small molecule which is not antigenic in itself but when attached to a large molecule (macromolecular carrier) can stimulate the formation of antibodies. A hapten, like an antigen, can react with its specific antibodies once they have been produced.

Hapten-Carrier Conjugate - The coupling of a non-immunogenic molecule (e.g., targeted analyte) to an immunogenic substance (e.g., bovine serum albumin, keyhole limpet hemocyanin) for the purpose of stimulating an immune response.

Heterogeneous Immunoassay - a type of immunoassay that requires a separation of bound and free phases. Generally, accompanied by incubation and washing steps (ELISA Assays).

Homogeneous Immunoassay - a type of immunoassay that does not require washing steps i.e. it requires no physical separation between the bound and free phases.

Immunoassay - an analytical technique that uses an antibody molecule as a binding agent in the detection and quantitation of substances in a sample. (see Enzyme Immunoassay and ELISA)

Immunogen - a synonym for antigen, particularly when used to describe a substance used to elicit an immunologic response in an animal.

Immunity - a state, natural or acquired, in which the body is resistant to disease.

Immunoglobulin - the class of globular proteins (gamma globulins) which are antibodies. There are five classes of immunoglobulin (IgM, IgA, IgD, IgG and IgE).

Immunology - the science that deals with study of immunity to diseases.

Ligand - a member of a binding pair, generally the smaller member. For example, in an enzyme-substrate reaction the substrate is frequently referred to as a ligand. The antigen or hapten may be called a ligand in an antibody reaction.

Lymphocytes - One of the five classes of white blood cells found in the circulatory system of vertebrates.

Monoclonal Antibodies - a homogeneous preparation of antibodies directed at a single antigenic determinant produced from a single clone of an antibody producing lymphocyte hybridized with a "tumor" cell line to form a hybridoma which continuously secretes a single antibody molecule.

Polyclonal Antibodies - a group of antibody molecules that differ in amino acid composition and sequences, and that exhibit binding characteristics. Polyclonal antibodies are produced from a simulation of multiple clones of lymphocytes.

Polyclonal antiserum - an antibody containing serum which is made up of antibodies from more than one clone of lymphocyte. Usually produced in vivo by immunizing animals with an antigen.

Precision - extent to which the obtained measurements of a defined substance agree with one another, usually stated as coefficients of variation, relative standard deviations, or confidence limits.

Quality Control - a planned system of activities whose purpose is to provide a quality product.

Radioimmunoassay (RIA) - an immunologic test utilizing a radiolabeled antigen, antibody, or other reactants.

Replicates - repeated but independent determinations on the same sample by the same analyst at essentially the same time under the same conditions.

Sandwich Assay - an immunoassay technique for measuring antigen in a test sample that "sandwiches" the antigen being measured between two antibodies.

Sensitivity - sensitivity of a laboratory procedure refers to the ability of the test to detect or respond to small changes in concentration; the more sensitive the test, the more likely it will detect minute quantities.

Serology - generally considered a subdivision of immunology, it is the study of blood serum reactions such as antigen-antibody interactions and complement.

Solid Phase - separation method in which the binding reagent is immobilized by coupling to an insoluble material (magnetic particles, coated tubes, polymers, etc.).

Specificity - the characteristic of a laboratory test that distinguishes between true (specific) and inaccurate (non-specific) results. Non-specific reactions may be caused by cross-reactions or interferences from matrix factors.

Standard Curve - the dose-response curve generated in a quantitative immunoassay by running a series of reference standards containing known concentrations of analyte. It is used to calculate the concentration of unknown samples from their response data.

Standard Deviation - often the measure of precision. Mathematically defined as the square root of the sum of the squares of the difference between the individual values of a set and the arithmetic mean of the set, divided by one less than the number of values.

Stopping Reaction - stopping reaction refers to the addition of a solution to prevent further increases of color from occurring due to conversion of a chromogenic substrate. For example, the end result in ELISA assays is the intensity of color produced over a given period of time from conversion of a substrate caused by reaction with an enzyme conjugate. The stopping solution is used to prevent further increases in intensity of color after the given period of time.

Substrate - substances chosen to react with enzymes that provide a sensitive method for detection of the antigen or antibody being measured. Generally, chromogenic substrates are chosen which are colorless initially, but which form colored products as they are converted by the enzyme reaction.

Titer - the greatest dilution of a substance used in a serologic reaction that will produce the desired result.

APPENDIX B

TECHNICAL IMMUNOASSAY DESCRIPTION

Immunoassays are divided into two distinct classes that are referred to as heterogeneous and homogeneous. The present state-of-the-art in environmental IA analyses use a heterogeneous assay where an antigen/antibody complex, containing the Compound of Interest, is bound to a solid substrate and a separation step is required to remove excess free sample and reagents. Homogeneous assays do not require separation of the bound and free substances and the antibody can directly modulate the signal produced.

IA can rely on a single antibody (monoclonal) or mixtures of antibodies (polyclonal) to trap or collect the antigen(s), otherwise known as the Compound Of Interest (COI). Most environmental IA analyses rely on monoclonal antibodies. Antibodies are a class of proteins known as immunoglobulins which are produced in animals in response to a foreign substance (antigen). The small molecular weight antigen (COI) may not cause an immune response in the animal, so it must be coupled to a "carrier" molecule which will present the small molecule (haptan) to the immune system as a foreign substance. The antibody is capable of reacting specifically with the antigen to form an antigen/antibody complex (commonly referred to as the lock and key approach).

In immunochemical methods, the unreacted antigen and antibody are referred to as the free phase, while the antigen/antibody complex is referred to as the bound phase. This highly sensitive three dimensional stereo-chemical reaction between antigens and antibodies is the basis for immunoassay technology.

Because individual chemical compounds of low molecular weight have specific three dimensional stereo-chemical structures (molecular geometries), there are few other compounds of exact or similar three dimensional structures that will react with the antibody. Compounds having similar three dimensional stereo-chemical structures that react with the antibody are said to be cross-reactive.

The development of an appropriate antibody that will bind to an antigen (COI) or a COI attached to an enzyme (otherwise known as an "enzyme conjugate") is the most important phase of designing an immunochemical specific test kit. Monoclonal antibodies are produced by a single cell and generally have a high degree of specificity and low cross-reactivity. This makes them ideal for designing IA kits that are specific for one compound. Polyclonal antibodies contain a mixture of antibodies that detect a range of compounds having similar structures. Because they are less specific, polyclonal antibodies are better suited to the detection of classes of compounds and they are rarely used in environmental analyses.

Enzyme Linked Immunosorbant Assay (ELISA) is an enzyme immunoassay method that uses an immobilized antibody absorbed onto a plastic well, tube, polymer particle, or magnetic particle to facilitate the separation of the targeted analytes from the untargeted substances (free reaction components) using a washing step and an enzyme conjugate to generate the signal. The enzyme conjugate is the COI bound to an enzyme such as horseradish peroxidase. The signal, or reporter system, is a colorimetric determination of the chromogenic (color) response used for the interpretation or quantitation of results. Chromogenic responses are analyzed photometrically, and use the principles of Beer's Law to determine the concentration of analyte in a sample.

ELISA tests are competitive assays utilizing immobilized antibodies that bind to pure contaminants (COIs), enzyme conjugates, or sometimes both in proportion to the relative concentration of the sample. The greater the concentration of the sample-derived COI relative to the enzyme conjugate, the larger the proportion of antibody sites that are occupied by the COI molecules. The antibody in most IA tests cannot bind to both at the same time. The bound

antibody/COI/enzyme conjugate is then washed to remove sample solution and excess reagents leaving the bound COI and enzyme conjugate antibody complex. A chromogen (for example, hydrogen peroxide and tetramethylbenzidine or hydrogen peroxide and orthophenylenediamine) is added to produce a color when it reacts in the presence of the IA specific enzyme conjugate. Specifically, the horseradish peroxidase reacts with the hydrogen peroxide to release a proton, which in turn reduces the tetramethylbenzidine to form the colored product. The amount of response (color) in solution is directly proportional to the amount of enzyme conjugate available to catalyze the reaction of the chromogen and hydrogen peroxide. The presence of the enzyme conjugate bound to the antibody is proportional to the amount of color formed. The chromogen/hydrogen peroxide (sometimes called a substrate) only reacts in the presence of the enzyme conjugate and does not react in the presence of the COI. This process results in a color formation in solution that is inversely proportional to the amount of chemical contaminant. More color equals less contaminant. Less color equals more contaminant. In the presence of high concentrations of contaminants, all the antibody sites are taken up by the chemical contaminant, thus, no color is developed because there is no enzyme conjugate present to catalyze the chromogen color reaction.

APPENDIX C

IMMUNOASSAY TABLES

Table values are reported from vendor literature. For consistency, the following general guidelines were used: values < 1.0 are reported to two decimal places, if appropriate; values ≥ 1.0 are reported to two significant figures.

Table 1a - Vendor's Matrix

Parameter/Analyte	D-Tech		ENSYS		Ohmicron		Quantix/Idetek		BioNebraska		Hach	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*
TPH			x	x	x	x	x	x			x	x
BTEX	x	x	x	x	x	x	x	x				
Benzene			x								x	
PAH	x	x	x	x	x	x		x				
C-PAH					x	x						
PCB		x		x	x	x						x
PCB (Wipe)		x		x		x						
PCB (Oils)				x								
TNT	x	x		x	x	x						
RDX	x	x	x	x								
Dioxin (2,3,7,8-TCDD)				x								
Mercury (Hg)			x	x					x	x		
Trihalomethanes (THMs)			x									
Pentachlorophenol (PCP)	x	x	x	x	x	x						
Trichloroethylene (TCE)	x	x										

Table 1b - Vendor's Pesticide, Insecticide and Herbicide Matrix

Parameter/Analyte	ENSYS		Ohmicron		Quantix/Idetek	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*
2,4-D	x, p	x	x	x		
Acetanilide	p					
Alachlor	x, p		x		x	
Aldicarb	x, p		x			
Atrazine	x, p		x		x	
Benomyl	x, p		x	x	x	x
Bioresmethrin	p					
Captan			x	x		
Carbaryl			x	x		
Carbofuran	x, p		x	x		
Chlordane		x				
Chlorothalonil			x		x	x
Chlorpyrifos	p		x			
Chlorpyrifos-methyl	x (g)		x			
Chlorsulfuron	p					
Cyanazine	x, p		x			
Cyclodienes	x, p		x			
DDT		x				
Diazinon	p					
Endosulfan	p					
Fenitrothion	x, p					
Hexazinone	p					
Imazaquin					x	
Imazapyr	p					
Isoproturon	p					
Lindane		x				
Metsulfuron	p					
Metalaxyl	p					
Methomyl			x			
Methoprene	x, p					
Metolachlor	x, p		x		x	
Metribuzin			x			
Molinate	p					
Nicotine	p (t)					
Paraquat	p		x			
Parathion	p					
Pirimiphos-methyl	x, p					
Procymidone	x		x			
Silvex	x	x				
Thiabendazole			x			
Toxaphene		x				
Triasulfuran	p					
Triazine	x, p					
Trichloropyridinol			x			
Triclopyr	p					
Urea Herbicides	p				x	

x = tube kit
p = plate kit
(g) = for analysis in grain only
(t) = for analysis in tobacco only

Table 2a - TPH/BTEX Reactivity

Parameter /Analyte	D-Tech		ENSYS		Ohmicron		Quantix/Idetek	
	Water ppm*	*Soil* ppm*	*Water* ppm*	*Soil* ppm*	*Water* ppm	*Soil* ppm	*Water* ppm	*Soil* ppm
TPH								
Gasoline	–	–	0.16	10	0 43	4 3	6.0	60
Diesel	–	–	0.24	15	1.3	13	1.0	25
Jet A Fuel	–	–	0.28	15	2.7	27	10	100
JP-4	–	–	0.18	15	0.5	20	1.0	25
Kerosene	–	–	0 22	15	1 5	15	5 0	50
Fuel oil #2	–	–	0.21	15	0.4	13	1.0	25
Fuel oil #6	–	–	–	25	0.2	13	–	–
Mineral Spirits	–	–	0.49	40	1.1	11	NR	NR
BTEX								
Benzene	1.2	5.0	5.0	400	0.59	5.9	3.9	49
Toluene	0.6	2.5	0.74	40	0.44	4 4	0.6	4.9
Ethylbenzene	0 6	2.5	0.06	7.0	0.24	2.4	0.7	4.9
o-Xylene	0.6	2.5	0.10	8.5	0.22	2 2	8.0	31
m-Xylene	1.4	5.8	0.10	8.0	0.03	0 3	5.0	18
p-Xylene	1.3	5.4	0.59	45	0.13	1.3	0.6	1.7
Total BTEX	0.6	2.5	0.20	10	0 02	0 2	0.25	3.5
Benzene (only)	NA	NA	0 005 #	NA	NA	NA	NA	NA

* Lowest concentration that yields a positive test result
– = not tested
# = test can give presence/absence indication at two detection levels (0.005 and 0 05 ppm)
NR = Not Responsive
NA = Not Available

Table 2b - PAH and C-PAH Reactivity

Parameter/Analyte	(rings)	D-Tech		ENSYS		Ohmicron		Ohmicron C-PAH		Quantix/Idetek	
		Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*
		ppb*	ppm	ppb	ppm	ppb	ppm	ppb	ppm	ppb	ppm**
Acenaphthene	(3)	310	–	–	8 1	13	1.0	1100	>100	600	6.0
Acenaphthylene	(3)	310	–	–	7.5	10	1.3	150	22	20	0.2
Anthracene	(3)	10	–	–	0 81	0.54	0.05	0.44	0.58	–	>50
Benzo[a]anthracene	(4)	42	–	–	1.6	0.77	0.08	0.02	0.002	–	>50
Benzo[a]pyrene	(5)	10	–	–	8 3	0.50	0.05	0.08	0.01	–	>50
Benzo[b]fluoranthene	(5)	53	–	–	4.6	0.91	0.09	0.04	0.005	–	>50
Benzo[g,h,i]perylene	(6)	42	–	–	>200	15	1.5	0.30	0.12	–	>10
Benzo[k]fluoranthene	(5)	–	–	–	9 4	0.77	0.08	0 02	0.003	–	>50
Chrysene	(4)	8	–	–	1.2	0.40	0.04	0 04	0 005	–	>50
Dibenzo[a,h]anthracene	(5)	1100	–	–	>200	26	2.6	0 14	0 015	–	>50
Fluoranthene	(4)	5	–	–	1.4	0.32	0.03	2 0	0 22	–	0.40
Fluorene	(3)	110	–	–	1.5	1.6	0.16	37	3.5	200	1.6
Indeno[1,2,3-cd]pyrene	(6)	8	–	–	11	0.78	0.08	0 02	0.008	–	>10
Naphthalene	(2)	1800	–	–	200	65	6.5	380	36	100	1.2
Phenanthrene	(3)	420	–	–	1.0	0 70	0.07	2.7	0.43	30	0.3
Pyrene	(4)	10	–	–	3.5	0.20	0.02	2.0	0.09	400	4.0
Total PAH		8	0 6	–	–	–	–	–	–	50	0.7

* Sensitivity is defined by lowest concentration of compound that yields a positive detection.

** Lower Limit of Detection (LLD)

– = not tested

Table 2c - PCB Reactivity

Parameter/Analyte	D-Tech			ENSYS			Ohmicron		
	Wipe *		*Soil*	*Wipe*	*Oil/Liq.*	*Soil*	*Wipe*	*Water*	*Soil*
	Surf A	Surf B	ppm	ug/100cm²	ppm	ppm	ug/100cm²	ppm	ppm
Aroclor 1016	100	51	5.7	40	9	4	36	0 94	2.7
Aroclor 1221	450	220	25	500	75	50	–	14	27
Aroclor 1232	160	82	9.0	40	10	4	26	0 84	2.2
Aroclor 1242	27	14	1.5	20	3 5	2	12	0.34	0.80
Aroclor 1248	14	7.2	0.8	10	2.5	1	8	0.22	0.42
Aroclor 1254	9 0	4 5	0 5	5	1	0.5	5	0.20	0.50
Aroclor 1260	9.0	4.5	0.5	5	1	0.5	3	0.20	0.30
Aroclor 1262	9.0	4.5	0.5	–	1	–	6	0.36	0.64
Aroclor 1268	69	34	3.8	–	9	–	31	0.92	2.3

* wipe concentration = ug/100cm²

Surface A is used to interpret test results from non-porous surfaces such as smooth metal or glazed tile-like surfaces.

Surface B is used to interpret test results from painted surfaces, rusted metals, or concrete-like surfaces.

– = not tested

Table 2d - TNT/RDX Explosives Reactivity

Parameter/Analyte	D-Tech		ENSYS		Ohmicron	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*
	ppb	ppm	ppm	ppm	ppb	ppm
TNT	5	0.5	–	0.7	0.07	0.25
RDX	5	0 5	–	0.8	–	–

TNT = Trinitrotoluene

RDX = Hexahydro-1,3,5-trinitro-1,3,5-triazine

– = not tested

Table 2e - Individual Analyte Reactivity

Parameter/Analyte	D-Tech		ENSYS		Ohmicron		BioNebraska	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*
	ppm	ppm	ppb	ppm	ppb	ppm	ppb	ppm
Mercury	–	–	250**	0 5**	–	–	250	0.5
Pentachlorophenol	(1)	(1)	5	0 5	0.06	0.1	–	–
Trichloroethylene (TCE)	1.5	5	–	–	–	–	–	–
Tetrachloroethylene (PCE)	0.3	1	–	–	–	–	–	–
Dioxin (2,3,7,8-TCDD)	–	–	0.4	(2)	–	–	–	–
Benzene (see also BTEX)	–	–	5	–	–	–	–	–

** ENSYS Markets the BioNebraska kits

(1) Kit scheduled for release, sensitivity unknown.

(2) Laboratory analysis only MDL equal to parts per trillion (ng/L) or parts per quadrillion (pg/L) depending on sample concentration factor.

– = not tested

Table 2f - Individual Pesticide, Insecticide, and Herbicide Reactivity

	ENSYS		Ohmicron		Quantix/Idetek	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*
Parameter/Analyte	ppb	ppm	ppb	ppm	ppb	ppm
2,4-D @	0 5	0.2	0.70	–	–	–
2,4-DNT	–	0.5	–	–	–	–
2,4-D Butyric butyl ester	0 02**	1.8	–	–	–	–
Acetanilide	0.1	–	–	–	–	–
Acetochlor	0 1	–	–	–	–	–
Alachlor @	0 1	–	0.05	–	100	–
Alachlor Sulfonic acid	0.1**	–	–	–	–	–
Aldicarb @	1	–	0.25	–	–	–
Aldicarb Sulfone	5	–	0.27	–	–	–
Aldicarb Sulfoxide	10**	–	1.8	–	–	–
Aldrin	0.32**	–	0.29*	–	–	–
Ametryn	0.01**	–	–	–	–	–
Atrazine @	0 01	10**	0 05	–	–	–
Azinphos	10**	–	–	–	–	–
Benomyl @	0.1**	–	0 38	–	50	–
BHC, alpha	–	2	–	–	–	–
BHC, delta	–	2	–	–	–	–
BHC, gamma (lindane)	–	1	20*	–	–	–
Bioresmethrin	100	–	–	–	–	–
Captan @	–	–	0 01	–	–	–
Carbaryl @	–	–	0 25	–	–	–
Carbendazim	–	–	0.10	–	–	–
Carbofuran @	0 1**	–	0.06	–	–	–
Chlordane	10	0.02	–	–	–	–
Chlorpyrifos	0.05	–	0.10	–	–	–
Chlorsulfuron	0.04	–	–	–	–	–
Cholorthalonil		–	0 07	–	500	–
Cyanazine @	0.25	–	0 04	–	–	–
Cyclodienes	5.0	–	–	–	–	–
DDD.	–	0.01	–	–	–	–
DDE	–	0.18	–	–	–	–
DDT	–	0.2	–	–	–	–
Diazinon	0.03	–	–	–	–	–
Dicamba	98**	–	–	–	–	–
Dichlorprop	17**	12	–	–	–	–
Dieldrin	0.1	0 006	0.55**	–	–	–
Diquat	30**	–	–	–	–	–
Dursban	0.05	–	–	–	–	–

* Determined in Cyclodiene Ohmicron kit - see vendor literature

** Lower Level of Detection (LLD) under laboratory conditions

@ = available as PE samples

– = not tested

Table 2f - Individual Pesticide, Insecticide, and Herbicide Reactivity

Parameter/Analyte	ENSYS		Ohmicron		Quantix/Idetek	
	Water ppb	*Soil* ppm	*Water* ppb	*Soil* ppm	*Water* ppb	*Soil* ppm
Endosulfan	0.08	–	1.2*	–	–	–
Endosulfan I	–	0.006**	–	–	–	–
Endosulfan II	–	0.006**	–	–	–	–
Endrin	0.15**	0.006**	–	–	–	–
Ethylated Atrazines	0.05	–	–	–	–	–
Fenitrothion	100	–	–	–	–	–
Heptachlor	0.69**	0.006**	0.66	–	–	–
Hexazinone	0.1	–	–	–	–	–
Hydroxy atrazine		–	–	–	–	–
Imazapyr	0.30	–	–	–	–	–
Imazaquin	5.0	–	–	–	–	–
Isoproturon	0.05	–	–	–	–	–
Metalaxyl	0.1	–	–	–	–	–
Methomyl	–	–	0.45	–	–	–
Methoprene	1000	–	–	–	–	–
Metolachlor	0 1	–	0.05	–	100	–
Metribuzin	–	–	0.04	–	–	–
Metsulfuron	0 25	–	–	–	–	–
Molinate	0.5	–	–	–	–	–
Nicotine (in tobacco)	10	–	–	–	–	–
Paraquat	0.02	–	0.02	–	–	–
Parathion	0 04	–	–	–	–	–
Picloram	1000**	–	–	–	–	–
Pirimiphos-methyl	–	–	–	–	–	–
Procymidone @	–	–	0.80	–	–	–
Prometon		–	0.05	–	–	–
Prometryn		–	0.05*	–	–	–
Reldan	0.02	–	–	–	–	–
Silvex 2,4,5-TP	0.50	0.05	1.4	–	–	–
Simazene	0.04**	–	0.03	–	–	–
Thiabendazole	0.25	–	–	–	–	–
Toxaphene	3.8	0.005**	2.6**	–	–	–
Triasulfuron	0.05	–	–	–	–	–
Triazine	0.1	–	–	–	50	–
Trichloropyridinol	–	–	0.25	–	–	–
Trichopyr	–	–	0.03	–	–	–
Trifluralin	–	–	–	–	1.0	–
Urea Herbicides	0.05	–	–	–	–	–

* Determined in Cyclodiene Ohmicron kit - see vendor literature

** Lower Level of Detection (LLD) under laboratory conditions

@ = available as PE samples

– = not tested

Table 3a - TPH / BTEX Cross-Reactivity

	D-Tech		ENSYS		Ohmicron		Quantix/Idetek	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*
Parameter /Analyte	ppm*	ppm	ppm*	ppm*	ppm	ppm	ppm	ppm
Acenaphthene			0.01	0.5	0.17	1.7		
Anthracene					0.06	0.6		
Benzo(a)pyrene	6							
Benzoic acid	>500							
Biphenyl				10				31
Chrysene	6							
Creosote				1.5	0.10	1.0		1.0
1,2-Dichlorobenzene	5		0.02	2.5				
2,4-Dinitrotoluene	>500							
n-Decane					14	140		
2-Ethyltoluene	5							
4-Ethyltoluene	5							
n-Heptane			1.6	130	2.4	24		
Hexachlorobenzene			0.1	10	0.08	0.8		
Hexane (mixed)				65				
n-Hexane	>500				6 3	63		
Isooctane	72		0.11	8.5				
MTBE			>1200	>1000				
Methylcyclohexane	>500							
2-Methylpentane			0.45	35				
Naphthalene	11		0.008	0.8	0.03	0 3	0 08	2.2
Nitrobenzene	5							
2-Nitrophenol	>500							
n-Nonane					4 4	44		
n-Octane					3 4	34		
o-Cresol	5							
16 PAHs	>500							
Pentachlorophenol	>500							
Phenanthrene					0.08	0.8		
n-Propylbenzene					0.27	2.7		
Styrene			0.07	7.0	0.07	0 7		
1,2,4-Trimethylbenzene					0.04	0.4		
1,3,5-Trimethylbenzene					0.14	1 4		
Undecane			>12	>1000				

* lowest concentration that yields a positive test result.

Table 3b - PAH and C-PAH Cross-Reactivity

	D-Tech		ENSYS		Ohmicron		Ohmicron C-PAH		Quantix/Idetek	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*
Parameter/Analyte	ppm	ppm	ppm	ppm	ppb	ppm	ppb	ppm	ppm	ppm
Creosote				5 4	1 5	0.11	1.2	0.12	0.1	1.0
Fuel oil #1					[280]					
Fuel oil #2									4	30
Fuel oil #4					[16]			2.1		
Fuel oil #6					[5 0]	0.5	6.6	1.1	6	63
Heating fuel					17	1.3	20	3 2		
Diesel fuel					36	2.0	240	18	1	8
Turbine fuel					[20]					8
Fuel oil # 5					17		0 3	1 3		
JP-4					[610]				2	16
JP-5					[340]				10	100
Jet A Fuel					>10,000	>1000	>10,000	>1000		
Gasoline					13	100	200	160	4	40
Kerosene					1700	120	2800	830	6	60
BTEX	>100								3	32
Benzene				>200						>500
Phenol				>200						
Toluene				>200						>500
2,4,6-Trichlorobenzene				>20						
2,3,5,6-Tetrachloro-				>200						
benzene										
Pentachlorobenzene				>200						
Pentachlorophenol	>200			>200						
BEHP				>200						
1-methyl-Naphthalene				54						0.6
2-methyl-Naphthalene				58						1.9
1-Chloro-Naphthalene				59						
Halowax 1051				18						
Dibenzofuran				14						
Aroclor 1254	>100									
Biphenyl										31

[ ] = LLD x 50% B/B_0

Table 3c - PCB Cross-Reactivity

	D-Tech			ENSYS		Ohmicron	
	Wipe *		*Soil*	*Water*	*Soil*	*Water*	*Soil*
Parameter/Analyte	Surf A	Surf B	ppm	ppm	ppm	ppb	ppm
Biphenyl						>10,000	
Brfenox	450	220	25		1000		
Halowax 1000	18,000	9000	1000				
Halowax 1051					1000		
Halowax 1099	4500	2200	250				
2,5-Dichlorophenol						>10,000	
2,3,5-Trichlorophenol						>10,000	
Di-n-octylphthalate						>10,000	
Tetradifon					100		
2,4-Dichloro-1-naphthol					50		
See fact sheets for others							

* wipe concentration = ug/100cm²
Surface A is used to interpret test results from non-porous surfaces, smooth metal or glazed tile-like surfaces
Surface B is used to interpret test results from painted surfaces, rusted metals, or concrete-like surfaces

Table 3d - TNT/RDX Explosives Cross-Reactivity

Parameter/Analyte	D-Tech Water ppb	D-Tech Soil ppm	ENSYS Water ppb	ENSYS Soil ppm	Ohmicron Water ppb	Ohmicron Soil ppm
TNT						
2-Amino-4,6-dinitrotoluene	>500	>50				
4-Amino-2,6-dinitrotoluene	>500	>50		>100	0.10	5.9
2,6-Diaminonitrotoluene	>500	>50				
2,4-Dinitroaniline					.0.10	0.99
1,2-Dinitrobenzene					1000	3300
1,3- Dinitrobenzene					2.4	11
2,4-Dinitrophenol	>500	>50			8 0	240
2,4-Dinitrotoluene	120	12		1 1	1 0	3 3
2,6-Dinitrotoluene	>500	>50		2	100	360
HMX (3)	>500	>50				
Picric Acid					0 25	4 5
Nitrobenzene				>100	3400	>10,000
2-Nitrophenol				>100	2300	>10,000
2-Nitrotoluene	30	3.0			0 25	2.4
3-Nitrotoluene				>100	160	1400
4-Nitrophenol	>500	>50				
4-Nitrotoluene				>100	1200	5600
RDX (2)	>500	>50			700	2900
Tetryl (1)	15	1.5		0.9	0 10	1.9
1,3,5-Trinitrobenzene	20	2.0		1 0	0.04	0.15
RDX						
HMX (3)	150	15		2 4		
Nitroglycerine				8.9		
Nitroguanadine				10		
PETN (4)	>500	>50		1.0		
Tetryl (1)	>500	>50				
TNT (5)	>500	>50				
All others listed above	>500	>50				

(1) Tetryl = Methyl-2,4,6-trinitrophenylnitramine
(2) RDX = Hexahydro-1,3,5-trinitro-1,3,5-triazine
(3) HMX = Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
(4) PETN = Pentaerythritol tetranitrate
(5) TNT = Trinitrotoluene

Table 3e - Individual Analyte Cross-Reactivity

Parameter/Analyte	D-Tech		ENSYS		Ohmicron		BioNebraska	
	Water ppb	*Soil* ppm	*Water* ppb	*Soil* ppm	*Water* ppb	*Soil* ppm	*Water* ppm	*Soil* ppm
Mercury Test								
Gold trichloride							300	
Chromium nitrate							240	
Silver nitrate							17	
Pentachlorophenol	*	*						
CCA (1)			>10,000	>1000				
4-Chlorophenol			>800	>1000				
Creosote			>1000	>1000				
2,3-Dichlorophenol					610	>1000		
2,4-Dichlorophenol			>1000	>1000	880	>1000		
2,5-Dichlorophenol					63	210		
2,6-Dichlorophenol			600	700	270	420		
3,5-Dichlorophenol					1700	>1000		
Diesel fuel			>10,000	>10,000				
Hexachlorobenzene					1600	>1000		
Hexachlorocyclohexane					5800	>1000		
PCB (Aroclor 1254)			>1000	>1000				
Pentachlorobenzene			>1400	>1000				
Phenol			>600	>1000				
2,3,4-Trichlorophenol			600	400	53	120		
2,3,5-Trichlorophenol					1.5	3.7		
2,3,6-Trichlorophenol					2.4	4.4		
2,4,5-Trichlorophenol			500	100	22	38		
2,4,6-Trichlorophenol			100	16	15	22		
Tetrachlorohydroquinone			>1500	500	8.7	14		
2,3,4,6-Tetrachlorophenol					0 91	1.2		
2,3,5,6-Tetrachlorophenol			7		0 21			
TCE/PCE	*	*						
Dioxin (2,3,7,8-TCDD)			#	#				

* Contact D-Tech, literature unavailable at time of publication

(1) CCA = Chromated copper arsenate

2,3,7,8-TCDF only analyte to show cross reactivity (approx. 20 times less sensitive than 2,3,7,8-TCDD)

Table 4 - Assay Kit Storage and Operating Conditions

Parameter/Analyte	D-Tech		ENSYS		Ohmicron		Quantix/Idetek		BioNebraska	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*
Storage Temp.										
PCP			40°F (4°C)	Room temp	36-46°F (2-8°C)					
PCB	40-100°F (4-38°C)		Room temp.		36-46°F (2-8°C)					
PAH	40-100°F (4-38°C)		< 80°F (27°C)		36-46°F (2-8°C)			refrigerate		
BTEX/TPH	40-100°F (4-38°C)		Room temp.		36-46°F (2-8°C)		refrigerate	refrigerate		
TNT/RDX	refrigerate		Room temp		36-46°F (2-8°C)					
Pesticides			Room temp.		36-46°F (2-8°C)					
Benzene			Room temp.							
Dioxin			Room temp.							
Mercury			Room temp.						40°F (4°C) **	
TCE/PCE	40-100°F (4-38°C)									
THMs			Room temp.							
Operating Temp.										
PCP			55-90°F (13-32°C)		59-86°F (15-30°C)					
PCB	45-100°F (7-38°C)		40-90°F (4-32°C)		59-86°F (15-30°C)					
PAH	45-100°F (7-38°C)		48-90°F (9-32°C)		59-86°F (15-30°C)			50-85°F		
BTEX/TPH	45-100°F (7-38°C)		60-100°F (16-38°C)		59-86°F (15-30°C)		50-85°F	50-85°F		
TNT/RDX	Room temp		40-100°F (4-38°C)		59-86°F (15-30°C)					
Pesticides			64-81°F (18-27°C)		59-86°F (15-30°C)					
Benzene			55-90°F (13-32°C)							
Mercury			50-98°F (10-37°C)						50-98°F (10-37°C)	
TCE/PCE	45-100°F (7-38°C)									
Dioxin				Room temp.						
THMs			Room temp.							
Shelf Life										
PCP			3 mo. room/4 mo. refrig		1 Year					
PCB	expiration date		6 mo. <80°F (27°C)		1 Year					
PAH	expiration date		6 mo. <80°F (27°C)		1 Year			6 mo.		
BTEX/TPH	expiration date		12 mo		1 Year		6 mo.	6 mo.		
TNT/RDX	expiration date		24 mo. <80°F (27°C)		1 Year					
Pesticides			6 mo. <80°F (27°C)		1 Year					
Benzene			6 mo. <80°F (27°C)							
Dioxin			6 mo. <80°F (27°C)							
Mercury			6 mo 40°F (4°C)						6 mo. 40°F (4°C)	
TCE/PCE										
THMs			6 mo. <80°F (27°C)							

*All Kits are validated for 4°-37°C (39°-100°F)

** Mercury test kits stored at 4°C; extracts can be stored at room temp.

Table 5 - Moisture Removal Guidelines

Parameter/Analyte	ENSYS		Ohmicron		BioNebraska	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*
All parameters	Place sample on an absorbant material, (filter paper or coffee filter) and ring out. Reweigh sample. pH should be between 3-11 See Vendor's literature for special cases.		Measure sample into a coffee filter. Wrap filter with paper towels and squeeze out water. Remove the soil and weigh pH should be between 3-11 See Vendor's literature for special cases		No need to remove water, but more sample will be needed with high moisture samples Highly alkaline soils may need more acid to digest sample.	

Table 6a - Kit Process Times (Samples per batch / Batch process time)

Parameter/Analyte	D-Tech		ENSYS		Ohmicron		Quantix/Idetek		BioNebraska	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*
Total BTEX					50/60m	20/120m	3/30m	5/30m		
TPH			10/50m	10/70m	50/60m	20/120m	5/15m	5/30m		
C-PAHs					50/60m	20/120m				
PAHs		4/25m	10/70m	10/70m	50/60m	20/120m	5/30m	5/30m		
PCB		4/25m		10/75m	50/60m	20/120m				
PCB wipe		4/25m								
PCB oil				4/45m						
Pentachlorophenol				10/70m	50/60m	20/120m				
TNT		4/25m		10/40m	50/60m	20/120m				
RDX		4/25m		10/40m						
Mercury				16/90m						16/90m
Dioxin (2,3,7,8-TCDD)			5/70m							
THMs										
TCE/PCE	4/25m									
Pesticides			44/90m	12/30m	60/60m	60/120m				
Benzene			5/60m							

m = minutes
P = Plate kit

Table 6b - Cost per Sample

Parameter/Analyte	D-Tech		ENSYS		Ohmicron		Quantix/Idetek		BioNebraska	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*	*Water*	*Soil*
Total BTEX		$26	$25	$25	$7 / $11	$13 / $20	$32	$32		
TPH			$25	$25			$32	$32		
C-PAHs					$15 / $19	$20 / $27				
PAHs		$26	$40	$40	$13 / $18	$19 / $25	$36	$36		
PCB		$31	$30	$30	$13 / $18	$19 / $25				
PCB Wipe		$31		$30						
PCB Oil				$25						
Pentachlorophenol	$26	$26	$40	$40	$8 / $11	$14 / $15				
TNT	$26	$26		$21	$7 / $11	$13 / $20				
RDX	$26	$26		$25						
Mercury										$21
Dioxin (2,3,7,8-TCDD)				$50						
THM's				$15						
TCE/PCE		$26								
Extraction Kits		$25								
Comments:	Kits usually contain materials for 4 tests. Meter $299.00 Computer program $ 150.00		Sold as 12 tests per kit, with 1, 2 or more calibrators		Water kits sold in 100 or 30 tube kits. With standards and blanks, the 30 tube kit equates to 22 samples per kit. Soil kits sold in 80 or 20 samples/ kit. Includes sample collection and extraction kits Prices: Large kit / Small kit		Kit includes soil collector, sample preparation supplies, and extraction tubes Meter not included		May be purchased as the total kit, or just the assay kits, or extraction kits.	

Table 6c - Individual Pesticide, Insecticide, and Herbicide Cost per Sample

Parameter/Analyte	ENSYS		Ohmicron		Quantix/Idetek	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*
2,4-D	$8	$8	$12			
Alachlor	$8		$10		$8	
Aldicarb	$8		$10			
Atrazine			$10		$8	
Benomyl	$9		$12		$10	
Bioresmethrin	$8					
Captan			$12			
Carbaryl			$11			
Carbofuran	$8		$10			
Chlordane		$8				
Chlorpyrifos	$8		$13			
Chlorosulfuron	$8					
Chlorothalonil			$11		$10	
Cyanazine	$9		$11			
Cyclodienes	$8		$12			
DDT		$8				
Diazinon	$9					
Fenitrothion	$14					
Isoproturon	$8					
Imazaquin					$10	
Imazapyr	$9					
Lindane		$14				
Metsulfuron	$8					
Metalaxyl	$9					
Methomyl			$13			
Methoprene	$9					
Metolachlor	$14		$10		$8	
Metribuzin			$13			

Table 6c - Individual Pesticide, Insecticide, and Herbicide Cost per Sample

Parameter/Analyte	ENSYS		Ohmicron		Quantix/Idetek	
	Water	*Soil*	*Water*	*Soil*	*Water*	*Soil*
Nicotine (in tobacco)	$9					
Paraquat	$9		$12			
Procymidone	$8					
Silvex			$13			
Toxaphene		$8				
Triasulfuron	$7					
Triazine	$8					
Trichloropyndinol			$16			
Triclopyr			$13			
Trifluralin					$8	
Urea Herbicides	$8					
Comments:	Tube kits consist of 40 tubes, which equates to 24 field samples analyzed Plate kits contain 96 test wells per kit, which equates to 44 fields samples analyzed		Available in 30 and 100 tube kits. Prices based on average of 20 field samples analyzed in a 30 tube kit (remainder needed for standards).		48 assays per kit Microwell kits used	