IMMUNOASSAY METHODS IN SW-846:

RECOMMENDED FORMAT AND CONTENT FOR DOCUMENTATION SUPPORTING NEW SUBMITTALS

Rev 0; July, 1995

1. Background

The Methods Section of the Office of Solid Waste is responsible for the promulgation of rugged and reliable analytical techniques in support of the Resource Conservation and Recovery Act (RCRA) program. The methods published in Test Methods for Evaluating Solid Waste, SW-846, are used to measure the concentration of specific pollutants or to establish whether a waste stream demonstrates a hazardous characteristic (e.g. ignitability, corrosivity, reactivity or toxicity).

SW-846 currently provides reliable and sensitive laboratory methods for the analysis of Appendix VIII analytes. However, some of these methods may be too costly or require too much analysis time for some applications. The Methods Section also recognizes the savings that could be achieved by sending only contaminated samples to analytical laboratories for quantitative analysis. Therefore, the Methods Section has recognized the need for more rapid, less expensive screening procedures that can be used either in the field or in the laboratory.

The number of field or laboratory screening procedures with potential application to the RCRA program is increasing rapidly. The increasing number of supporting documentation packages to be reviewed, and the time required to interpret each developer's data format, has significantly increased the amount of time that it takes for a method to become "approved". As more and more methods are developed, this situation will become worse. Therefore, the Agency has prepared this guidance document to provide developers with a list of specific information which must be included in the documentation package submitted as demonstration of the efficacy of the test procedure. The format and information requested in this guidance document are based on the requirements of the Food and Drug Administration's 501(k) Premarket Notification. This guidance document does not supersede or replace the more rigorous requirements described in Test Method Equivalency Petitions, EPA/530-SW-87-008, OSWER Policy Directive No. 9433.00-2 (2/87). That document provides the requirements for a method equivalency petition which may be used to promulgate a method outside of the Work Group process.

2. The Evaluation Process

Specifically, the Agency plans to review three documents during the evaluation of a new immunoassay test product; 1) the package insert, 2) a documentation package that provides the information specified in this guidance document, and 3) a document (to be treated as confidential business information or CBI) delineating the developer's internal quality control criteria for insuring lot-to-lot consistency of performance and stability claims in product manufacture. The claims made in the package insert will be reviewed to determine if the test product is applicable to the RCRA program. If the test product is determined to be applicable, the information presented in the documentation package will be reviewed to verify that it fully and clearly supports each of the claims made in the package insert. If, however, the documentation package does not support the claims made in the package insert, the test product will not be accepted by the Agency. If the test product is accepted by the Agency, only the documents listed in items 1) and 2) will be included in the public

docket.

All documentation reviewed by the Agency in support of new immunoassay procedures will be reviewed with the expectation that all of the requested information is present. If this information is not readily apparent to the reviewer, the documentation will be returned to the submitter for revision. Therefore, submittal in the recommended format is strongly advised. Those methods with complete, acceptable submittals will be routed to the appropriate SW-846 Methods Workgroup for consideration for inclusion in SW-846. Completeness of a submittal will be evaluated using the elements defined in Section 4 of this document as criteria. Acceptability of a submittal will be evaluated using the following criteria:

2.1 Target Analytes

The analytes and sample matrices targeted by the method are regulated under RCRA, or are of interest to one or more of the RCRA program areas.

Cross-reactivity claims must indicate the recognition of other cross-reacting analytes relative to the target analyte(s) specified by the test product. The cross-reactivity data provided must demonstrate that the test product is most sensitive to targeted analytes. Section 3.2 provides guidance on the generation of cross-reactivity data.

2.2 Detection Limit

The detection limits targeted by the test product must have some utility in RCRA testing, *e.g.*, some regulatory action limit, or concentrations designed to be used as go/no go action indicators. The basis for the detection limit(s) selected must be clearly stated.

2.3 False Negative/False Positive Rate

False negatives are defined as a negative response for a sample that contains the target analyte(s) at the stated action level. Ideally, a candidate procedure should produce no false negatives. The maximum permissible false negative rate is 5% at the action level specified.

False positives are defined as a positive response for a sample that contains analytes below the claimed action level. The rate of false positives at the claimed action level will not be specified by the OSW, but must be represented by the developer in the package insert and supporting documentation package.

The SW-846 Methods Workgroups will be provided with the method in SW-846 format, a summary of the performance data (detection limit, false negative/positive rates, cross-reactivity data), and any comments from the reviewer. Any information submitted as part of the documentation package may be transmitted to the Workgroup, and will become part of the public record, unless the person/group submitting the package asserts a claim of confidentiality. Such claims must be made

on specific parts of the package rather than the entire submittal. For example, a developer's internal QA data are likely to be confidential, and as such, will be distributed on a limited basis and withheld from the public record. On the other hand, there is no reason for the information included in the package insert and/or field data to be considered confidential, and such an assertion will result in the package being returned to the submitter.

3. The Performance Data

3.1 False Negative/False Positives

While screening procedures need not be fully quantitative, they should measure the presence or absence of target analytes at or below regulatory action levels. Therefore, initial demonstration of method performance requires measurement of the percentage of false negatives and false positives generated using the procedure for the claimed sample matrices. These data must be generated by analyzing split samples using both the test product under evaluation and a reference method. The reference method should be an approved SW-846 quantitative method, including both preparation and determinative steps. In addition to comparing determinative data points, this comparison should demonstrate that the extraction efficiency of the test being evaluated correlates with (but does not have to be equivalent to) that of the standard method. For semivolatile analytes, Soxhlet extraction, either simple or automated, is considered to be the reference extraction procedure.

The percentage of false negatives and false positives should be measured using 20-50 samples of the claimed matrix(ces), spiked at the claimed action level, by determining the incidence of false negative results. A sufficient volume of each spiked sample should be prepared so that each test can be completed with one lot of material. In addition, a sufficient number of aliquots of each spiked matrix should be analyzed and the results compared to those of the SW-846 reference method in order to demonstrate correlation of results, and to characterize the sample in terms of target analytes and potential interferences. This demonstration must be made for each matrix for which the test product is claimed to be applicable.

3.2 Non-Target Interferences and Cross-Reactivity

A minimum of 20-50 negative samples, confirmed by an SW-846 reference method, must be analyzed to demonstrate that the test product is not susceptible to matrix interferences. A separate study should be conducted to establish the effect of non-target interferences. For example, the immunoassay may produce a positive response to non-target analytes similar to the targeted analytes, or to chemically dissimilar co-contaminants. EPA regards the selection, testing, number of samples analyzed, and reporting of relevant cross-reacting analytes to be the responsibility of the developers. The Agency expects that the issue of cross-reactivity will be addressed relative to the claimed target analyte(s), and be reported in the documentation package and package insert. It is also assumed that the developers will analyze and report on the effect of interfering analytes that may co-exist at a site, even if structurally unrelated to the target analyte(s). The Agency reserves the right to return these

test products for further evaluation if it believes that the issue of compromised performance from interfering analytes was not satisfactorily addressed, or effectively represented to the Agency or the intended users of the test product.

The package insert must present the concentration of a cross-reactant that will give a false positive response.

3.3 Matrix Applicability

In some cases, a single test product may be applicable to more than one matrix, utilizing extraction protocols to isolate the target analytes into a medium suitable for testing by immunoassay. In order to demonstrate applicability to the claimed matrices, test data should be submitted for three different types of samples of each matrix (*e.g.*, clay, sand and loamy soil). These samples should either be characterized reference materials or spiked matrices containing known amounts of target analytes. In either case, bulk samples should be carefully homogenized to reduce sub-sampling errors. The sample matrices should be selected to represent what is regulated under RCRA (*e.g.*, soil, oily waste or waste waters). OSW reserves the right to reject test products based upon non-representative analysis of matrices and analytes. Negative controls must be analyzed with each set of samples.

Matrix-specific performance data in support of claims, including detection limits, should be gathered by analyzing ten replicate aliquots of three different sample matrix types spiked at the claimed detection concentrations. The results of testing the low concentration samples should be reported as positive or negative response. The results of testing the high concentration samples should be reported as either quantitative screening results (above, below or within a numerical range) or as positive/negative response.

3.4 Field Trials

Data from at least one field trial (two to three are preferred) in support of product performance claims for a particular matrix are required. These data should demonstrate that the test product is applicable to analysis of the target analyte(s) and claimed matrices in at least 30 (preferably many more) real-world (un-spiked) samples 1) at or near the action levels specified in the test product, 2) well above the action levels, and 3) well below the action levels. Field data generated using an excessive proportion of samples that are not contaminated with the target analytes, or are only contaminated with high concentrations of the target analytes, are not useful. The field trials must provide a comparison between the test product results and results generated using a reference method. As with the generation of false negative/positive data, the reference method should be an approved SW-846 quantitative method, including both preparation and determinative steps. Furthermore, the results of these field trials should support the false negative/positive rate claims presented by the developer for the test product.

Field trials are not to be performed by the developer or personnel employed by or involved

in the development of the test product. Field trials studies are to be performed by a credible group that is not affiliated with the developer. The field trial plan, including the study objectives, methods, sample description, reference method employed, data and conclusions should be provided in the documentation supplied to OSW. Individuals performing field evaluations should be willing to discuss the study with EPA reviewers upon request, and such individuals should be identified in the documentation.

4. The Documentation Package

The documentation package should provide substantive documentation to support the claims being made about the test product, including each of the 24 elements described in this section.

4.1 General Information - The following information is required as background material. This will become part of the public docket that supports all method proposals and promulgations:

4.1.1 **Name and Address of Developer**

4.1.2 **Proprietary and Common Names of the test product(s)**

4.1.3 **Intended Use of the test product(s) -** This section should address both target analytes and applicable matrices, as well as detection limits.

4.1.4 **Summary of the Test** - This section should briefly explain the principle of the immunoassay and the quantitation system used in the test product. For example,

> *In general, the method is performed using a water sample or an extract of a water sample. The sample/extract and an enzyme conjugate reagent are added to immobilized antibody. The enzyme conjugate "competes" with the target analyte present in the sample for binding to immobilized antibody. The test is interpreted by comparing the colorimetric (yellow) response produced by testing a sample to the colorimetric (yellow) response produced by simultaneous testing of standard(s).*

4.2. The Test Product

4.2.1 **Reagents -** Provide a list of all reagents included in the test product, and a separate list of all reagents that are necessary for performance of the test, but are not included in the test product. Specify reagent, number of containers provided, and the volume and concentration provided or necessary.

4.2.2 **Instrumentation** - Provide a list of all instrumentation included with the test product, and a separate list of all equipment/instrumentation that are necessary for performance of the test, but are not included in the test product.

4.2.3 **Storage Conditions** - Provide the recommended range of storage temperatures, as well as any other storage recommendations (*i.e.*, humidity specifications, protection from light). Also provide the design of storage stability testing (*i.e.*, actual vs. accelerated) and summary tables of the results of this testing.

4.2.4 **Physical, Biological or Chemical Indications of Instability or Deterioration** - This section should provide specific indicators that may be used as evidence that one or more parts of the test product are unstable and should not be used.

4.3. The Test

4.3.1 **Warning or Precautions for Users** - This section should address any specific safety concerns presented by the test product material or performance of the test. General safety precautions (*i.e.*, wear eye protection) need not be addressed.

4.3.2 **Limitations of the Procedure** - Describe any limitations of the test when used as described in Section 4.3.4. For example, it may be critical that some test steps be performed exactly as written (*i.e.*, number or volume of washings, timing between steps). Describe and substantiate the following:

- o The acceptable temperature range across which the test product will exhibit the claims being made,
- o The storage stability of the test product,
- o The number of tests that may be performed simultaneously

4.3.3 **Specimen Collection and Preparation** - Provide any specific instructions for sample collection (*i.e.*, minimum sample volume or size) or preparation (*i.e.*, removal of particulate matter) that are necessary for successful performance of the test.

4.3.4 **Assay Procedure**

4.3.4.1 **Instructions for Preparation of Reagent and Substrate** - If the substrate or any of the reagents cannot be used as received in the test product, provide instructions for their preparation.

4.3.4.2 **Assay Procedure** - Provide step-by-step instructions for performance of the assay.

4.3.5 **Stability of the Final Reaction** - Provide data that describe the length of time that the final reaction (*i.e.*, color change) is stable. This information is critical in evaluating how many

tests may be run simultaneously, as well as determining when tests must be repeated if the analyst does not read test results promptly.

4.3.6 **Developer's Internal Quality Control** - Provide sufficient quality control data to support the assertion that intra- and inter-lot test product variability are controlled. Also discuss measures to ensure long-term production of the test product (*i.e.*, quantity of stored antibody, provisions for production of additional antibody). Internal QA data are confidential, and as such, will be distributed on a limited basis, with the consent of the developer, and withheld from the public record.

4.4. The Data

4.4.1 **The Dose-Response Curve** - A dose-response curve must be provided which provides a graphical representation of the signal generated by the test product vs. the concentration of target analyte required to generate that response.

4.4.2 **Performance Data**

4.4.2.1 **Reproducibility**

4.4.2.1.1 **Intra-Assay** - Provide data demonstrating the reproducibility of the test product when samples are analyzed repeatedly using test products from one manufacturing lot.

4.4.2.1.2 **Inter-Assay** - Provide data demonstrating the reproducibility of the test product when samples are analyzed repeatedly using test products from different manufacturing lots.

4.4.2.2 **Bias**

4.4.2.2.1 **Dilution Study** - Provide data demonstrating the bias introduced by serial dilution of samples (*i.e.*, is the recovery of target analyte a function of concentration?).

4.4.2.2.2 **Recovery Study** - Provide data demonstrating that the test being evaluated exhibits consistent recovery during any extraction step(s). Table 1 provides an example format for presentation of data from the recovery study.

4.4.2.2.3 **Correlation Study** - Provide data correlating immunoassay product test results with the results generated using an SW-846 reference method. These data must be used to calculate false negative/positive rates at or near the action level. Table 2 provides an example format for presentation of false negative/positive data. Example tables for presentation of the study data are

provided in Table 3 (Table 3a is for test products configured at a single action level, and Table 3b is for test products configured with multiple action levels).

4.4.2.3 **Cross Reactivity** - Provide data that illustrate the cross-reactivity of the test product for non-targeted analytes relative to the claimed target analyte(s). The cross-reactivity data provided must demonstrate that the test product is most sensitive to targeted analytes. All data are to be normalized to the response of the target analyte. Table 4 provides an example format for presentation of the cross-reactivity data.

4.5. Bibliography - Provide copies of published material relevant to the test product being evaluated.

4.6. Formatted Method - One copy of the method prepared in SW-846 format.

Table 1

Example Format for Data from the Recovery Study

Compound	Spike (ppm)	Soil	IA Test Results
Blank	0	Wake	<1
Blank	0	PAH-116	<1
Phenanthrene	1	Wake	1-10
Phenanthrene	1	PAH-116	1-10
Phenanthrene	1	PAH-141	1-10
Phenanthrene	10	Wake	>10
Phenanthrene	10	PAH-116	>10
Phenanthrene	10	PAH-141	>10
Benzo(a)anthracene	1.6	Wake	1-10
Benzo(a)anthracene	1.6	PAH-116	1-10
Benzo(a)anthracene	16	Wake	>10
Benzo(a)anthracene	16	PAH-116	>10
Benzo(a)pyrene	8.3	Wake	1-10
Benzo(a)pyrene	8.3	PAH-116	1-10
Benzo(a)pyrene	83	PAH-116	>10

Table 2

Example Data Format for False Negative/False Positive Data

Probability of False Negative and False Positive Results for PAHs at a 1 ppm Action Level

Spike Concentration Phenanthrene (ppm)	**Probability of False Positive (Mean ± SD)**	**Probability of False Negative (Mean ± SD)**
0	0% ± 0%	N/A
0.4	23% ± 17%	N/A
0.8	94% ± 13%	N/A
1.0	N/A	0% ± 0%

Results were obtained from spiking four different validation lots, using 3 operators, 12 matrices for a total of 201 determinations at each concentration of phenanthrene.

N/A = No false positive possible above action limit.
No false negative possible below action limit.

Table 3a

Example Data Format for Results of Correlation Study

SAMPLE NUMBER	SCREENING RESULT (units)	REFERENCE METHOD RESULT (units)	AGREEMENT[a] Y, FN, FP
001	>10	5.98	FP
002	>10	1.27	FP
003	<10	0.11	Y
004	>10	6.71	FP
005	>10	1.37	FP
006	>10	0.68	FP
007	>10	0.55	FP
008	>10	2.00	FP
009	>10	1.30	FP
010	>10	0.17	FP
011	>10	1.15	FP
012	<10	ND (>0.05)	Y
013	<10	1.13	Y
014	<10	0.18	Y
015	>10	9.13	FP
015D	>10	9.84	FP
016	>10	2110	Y
017	>10	2.55	FP
018	>10	45.4	Y
019	>10	6.70	FP
020	<10	0.07	Y
021	<10	0.06	Y
022	<10	0.54	Y
022D	<10	0.72	Y
023	>10	20.8	Y
024	<10	0.06	Y

[a] - Y = Acceptable agreement
FP = False Positive
FN = False Negative

Table 3b

Total PAH Content of Field Samples Using IA Test Product

	1 ppm Test		10 ppm Test			False +/-	
Sample ID	**<1**	**>1**	**<10**	**>10**	**GC/MS Lab Result (ppm)[1]**	**Eval @ 1 ppm**	**Eval @ 10 ppm**
PAH-1		*		*	0.2	+	+
PAH-2				*	12.2		
PAH-3				*	16.0		
PAH-4	*				0.0		
PAH-5	*				0.5		
PAH-6		*		*	8.7		+
PAH-7				*	148		
PAH-8				*	182		
PAH-9		*		*	4.4		+
PAH-10		*		*	0.2	+	+
PAH-11	*				0.0		
PAH-12				*	85.4		
PAH-12Dup				*	85.4		
PAH-13				*	28.5		
PAH-14	*		*		0.3		
PAH-15		*			0.6	+	
PAH-16	*		*		0.0		
PAH-17		*		*	1.8		+
PAH-18		*	*		3.4		
PAH-19		*	*		6.7		
PAH-20	*		*		0.9		
PAH-21				*	43.2		

[1]Sum of all PAHs detected.

Table 4

Example Format, Cross-Reactivity Data

Compound	Soil Equivalent Concentration (ppm) Required to Yield a Positive Result
Aroclor 1248	1
Bifenox	500
1-Chloronaphthalene	10,000
2,5-Dichloroaniline	>10,000
2,4-Dichlorophenyl-benzenesulfonate	1,000
Dichlorofenthion	10,000
2,4-Dichloro-1-naphthol	>10,000
Diesel fuel	>10,000
Gasoline	>10,000
Hexachlorobenzene	>10,000
Pentachlorobenzene	>10,000
Tetradifon	125
1,2,4-Trichlorobenzene	10,000