METHOD 4030

SOIL SCREENING FOR PETROLEUM HYDROCARBONS BY IMMUNOASSAY

1.0 SCOPE AND APPLICATION

1.1 Method 4030 is a procedure for screening soils to determine whether total petroleum hydrocarbons (TPH) are likely to be present. Depending on the testing product selected, samples may be used to locate samples with low (<40-100 ppm), medium, and high (>1000 ppm) concentrations of contaminates, or to determine if TPH is present at concentrations above 5, 25, 100, or 500 mg/kg. Method 4030 provides an estimate for the concentration of TPH by comparison against standards, and can be used to produce multiple results within an hour of sampling.

1.2 Using the test kit from which this method was developed, 95 % of samples containing 25 ppm or less of TPH will produce a negative result in the 100 ppm test configuration.

1.3 The sensitivity of any immunoassay test depends on the binding of the target analyte to the antibodies used in the kit. The testing product used to develop this method is most sensitive to the small aromatic compounds (e.g., ethylbenzene, xylenes, and naphthalene) found in fuels. Refer to the package insert of the testing product selected for specific information about sensitivity.

1.4 The sensitivity of the test is influenced by the nature of the hydrocarbon contamination and any degradation processes operating at a site. Although the action level of the test may vary from site to site, the test should produce internally consistent results at a particular site.

1.5 In cases where a more exact measurement of TPH concentration is required, additional techniques (i.e., gas chromatography Method 8015 or infra-red spectroscopy Method 8440) should be used.

1.6 This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Test kits are commercially available for this method. The manufacturer's directions should be followed.

2.2 In general, the method is performed using an extract of a soil sample. Filtered extracts may be stored cold, in the dark. An aliquot of the extract and an enzyme-TPH conjugate reagent are added to immobilized TPH antibody. The enzyme-TPH conjugate "competes" with hydrocarbons present in the sample for binding to immobilized anti-TPH antibody. The test is interpreted by comparing the response produced by a sample to the response produced by a reference reaction.

3.0 INTERFERENCES

3.1 Compounds that are chemically similar to petroleum hydrocarbons may cause a positive test (false positive) for TPH. The data for the lower limit of detection of these compounds are provided in Tables 1A and 1B. Consult the information provided by the manufacturer of the kit used for additional information regarding cross reactivity with other compounds.

3.2 Storage and use temperatures may modify the method performance. Follow the manufacturer's directions for storage and use.

3.3 Appropriate standards must be used (i.e., diesel standards for diesel analysis, JP-4 for analysis of JP-4, etc.), or excessive false negative or false positive rates may result.

4.0 APPARATUS AND MATERIALS

Immunoassay test kit: PETRO RISc Soil Test (EnSys, Inc.), EnviroGard™ Petroleum Fuels in Soil, (Millipore, Inc.), or equivalent. Each commercially available test kit will supply or specify the apparatus and materials necessary for successful completion of the test.

5.0 REAGENTS

Each commercially available test kit will supply or specify the reagents necessary for successful completion of the test.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

6.2 Soil samples may be contaminated, and should therefore be considered hazardous and handled accordingly.

7.0 PROCEDURE

7.1 Follow the manufacturer's instructions for the test kit being used. Those test kits used must meet or exceed the performance specifications indicated in Tables 2-12.

7.2 Appropriate standards must be used to prevent excessive rates of false negative or false positive results.

8.0 QUALITY CONTROL

8.1 Follow the manufacturer's instructions for the test kit being used for quality control procedures specific to the test kit used. Additionally, guidance provided in Method 4000 and Chapter One should be followed.

8.2 Use of replicate analyses, particularly when results indicate concentrations near the action level, is recommended to refine information gathered with the kit.

8.3 Do not use test kits past their expiration date.

8.4 Do not use tubes or reagents designated for use with other test kits.

8.5 Use the test kits within their specified storage temperature and operating temperature limits.

8.6 Method 4030 is intended for field or laboratory use. The appropriate level of quality assurance should accompany the application of this method to document data quality.

9.0 METHOD PERFORMANCE

9.1 A single laboratory study was conducted with the PETRO RISc Soil Test, EnSys, Inc., using five contaminated soil samples. The samples were contaminated with oxygenated gasoline, oxygenated gasoline 24 hours after contamination, low aromatic diesel (purchased in California), normal diesel (purchased in Northern Virginia), and JP-4 jet fuel. Five replicate determinations were made using the kits, and the data compared with values obtained using GC/FID (Method 8015) and IR (Method 8440). Several different analysts ran the immunoassay analyses. Samples two- to five-fold below the action level generally gave readings less than the action level. Samples two fold above the action level gave readings greater than the action level. Samples at or near the action level give mixed results (e.g., both less than and greater than the action level). Tables 2 - 6 summarize these results.

9.2 Sensitivity of the EnviroGard Petroleum Fuels in Soil Test Kit was determined by establishing the "noise" level expected from matrix effects encountered in negative soil samples and determining the corresponding TPH concentration by comparison to the analyte-specific response curve. 8 different soils which did not contain TPH were assayed. Each of these soils was extracted in triplicate and each extract was assayed in three different assays. The mean and the standard deviation of the resulting %Bo's (%Bo = $[(OD_{sample}/OD_{negative\ control})x100]$) were calculated and the sensitivity was estimated at two standard deviations below the mean. The sensitivity for Method 4030 was determined to be 80% Bo at a 95% confidence interval. Based on the average assay response to home heating oil (HHO), this corresponds to 5.8 ppm. These data are shown in Table 7.

9.3 The effect of water content of the soil samples was determined by assaying three different soil samples which had been dried and subsequently had water added to 30% (w/w). Aliquots of these samples were then fortified with HHO. Each soil sample was assayed three times, with and without added water, and with and without HHO fortification. It was determined that water in soil up to 30% had no detectable effect on the method. These data are shown in Table 8.

9.4 The effect of the pH of the soil extract was determined by adjusting the soil pH of three soil samples. Soil samples were adjusted to pH 2 - 4 using 6N HCl and pH 10 - 12 using 6N NaOH. Aliquots of the pH adjusted soil samples were fortified with home heating oil. Each soil sample was assayed unadjusted and with pH adjusted to 2-4 and 10-12, both unfortified and fortified. These extracts were assayed three times. It was determined that soil samples with pH ranging from 2 to 12 had no detectable effect on the performance of the method. These data are shown in Table 9.

9.5 Two field studies were conducted at contaminated sites using the PETRO RISc Soil Test, EnSys, Inc.. In Field Trial 1, the method was used to locate soil contamination resulting from a leaking above ground gasoline tank. In Field Trial 2, the method was used to evaluate diesel fuel contamination in a railroad contaminated soil, sludge, and wastewater impound. Overall, a high degree of correlation was observed between the standard method and the immunoassay method. The application of the immunoassay method to 23 samples (46 analyses) resulted in eight conclusive false positive results (17%) and three conclusive false negative results (7%). Tables 10 and 11 summarize these results. There was agreement for 76% of the samples tested in the two trials for which data are presented.

9.6 Two field trials were undertaken to investigate the ability of the EnviroGard Petroleum Fuels in Soil Test Kit to identify soil samples which were contaminated with TPH. In trial 1 the method was used to identify soil which was contaminated with gasoline from leaking underground storage tanks. The immunoassay was compared to Method 8015. Twenty samples were analyzed by both methods. Interpreting the results at a cutoff of 100 ppm resulted in 1/20 (5%) false negatives and 0/20 (0%) false positives. In trial 2, the method was used to identify soil which was contaminated with JP-4 jet fuel from leaking semi-submerged storage tanks. The immunoassay was compared to Method 8015. Ten samples were analyzed by both methods. Interpreting the results at 1,000 ppm resulted in 0/10 (0%) false negatives and 1/10 (10%) false positives. Overall, for both field trials, there were 1/30 (3.3%) false negatives and 1/30 (3.3%) false positives. These data are summarized in Table 12.

10.0 REFERENCES

1. PETRO RISc™ Users Guide, Ensys Inc.

2. Marsden, P.J., S-F Tsang, and N. Chau, "Evaluation of the PETRO RISc™ kit Immunoassay Screen Test System", Science Applications International Corporation under contract to EnSys Inc., June 1992, unpublished

3. EnviroGard™ Petroleum Fuels in Soil Test Kit Guide, Millipore, Inc.

TABLE 1A

CROSS REACTIVITY[a]

Compound	Soil Equivalent Concentration (ppm) Required to Yield a Positive Result
Gasoline	100
Diesel fuel, regular #2	75
Jet A fuel	75
Kerosene	100
Fuel oil #2	100
Mineral Spirits	<30
Light lubricating oil	7,000
Lithium grease	10,000
Brake fluid	>10,000
Chain lubricant	>10,000
Toluene	200
o-Xylene	50
m-Xylene	100
p-Xylene	300
Ethylbenzene	50
Hexachlorobenzene	<30
Trichloroethylene	1,000
Acenaphthene	<30
Naphthalene	<30
Creosote	<30
2-Methylpentane	150
Hexanes, mixed	250
Heptane	300
iso-Octane	30
Undecane	>10,000

[a] PETRO RISc™ Soil Test, EnSys, Inc.

TABLE 1B

CROSS REACTIVITY[a]

Compound	Concentration Required for Positive Interpretation (ppm)
1,2,4 - Trimethylbenzene	0.1
m - Xylene	0.3
Acenaphthylene	0.3
Acenapthene	0.4
p - Xylene	0.5
Naphthalene	0.7
1,3,5 - Trimethylbenzene	2
Fluorene	2
Phenanthrene	2
o - Xylene	3
Ethylbenzene	5
Toluene	7
Propylbenzene	11
Chlordane	45
Benzene	70
Toxaphene	70

The following compounds were tested and found to yield negative results for concentrations up to 1000 ppm:

PCB (Aroclor 1248)	TNT
Pentachlorophenol	DDT

[a] EnviroGard™ Petroleum Fuels in Soil, Millipore, Inc.

TABLE 2

RESULTS FOR JP-4
(5 replicates/test)

Nominal concentration[a]	20 ppm	40 ppm	90 ppm	260 ppm	1000 ppm
PETRO RISc™ [b]	2/5, >40 ppm	5/5, >40 ppm	5/5, >40 ppm	1/5, >400 ppm	5/5, >400 ppm
Method 8015[c]	27 ± 2.1 ppm	38 ± 12 ppm	93 ± 30 ppm	260 ± 100 ppm	3000 ± 600 ppm
IR[d]	NA	2.8-5.3 ppm	52-95 ppm	380-620 ppm (1 outlier)	1370-2700 ppm

a. Samples were taken as cores at a contaminated Air Force Base. Nominal concentrations were determined by GC/FID analysis.

b. PETRO RISc™ test was run according to Method 4030 using the hydrocarbon supplied with the kit.

c. Method 8015 was run using a JP-4 standard, 20 ppm extract was not analyzed.

d. Method 418.1 was run using the mixture of solvents specified in the method. Because of the variability of the results, the range of values is reported. No analyses were conducted on the 20 ppm sample.

TABLE 3

RESULTS FOR LOW AROMATIC DIESEL
(5 replicates/test)

Nominal concentration[a]	12.5 ppm	75 ppm	105 ppm	150 ppm	1000 ppm
PETRO RISc™ [b]	4/4, <150 ppm	4/4, <150 ppm	5/5, <150 ppm	3/5, >150 ppm	5/5, >1500 ppm
Method 8015[c]	nd	54 ± 7 ppm	90 ± 15 ppm	125 ± 12 ppm	960 ± 105 ppm
IR[d]	30.5 -51.7 ppm	106.0 - 292.0 ppm	129.0 - 305.0 ppm	NA	810.0 - 1798.0 ppm

a. Samples were prepared by spiking sandy loam soil with known amounts of low aromatic diesel sold in California (Section 2256, CCR)

b. PETRO RISc™ test was run according to Method 4030 using the hydrocarbon supplied with the kit, 1/5 determinations at 35 and 75 ppm out of QC limits.

c. Method 8015 was run using a diesel standard purchased at a California station. nd - not detected.

d. Method 418.1 was run using the mixture of solvents specified in the method. Because of the variability of the results, the range of results is reported. NA - no IR determination made for the 150 ppm sample.

TABLE 4

RESULTS FOR REGULAR DIESEL
(4 replicates/test)

Nominal concentration[a]	25 ppm	75 ppm	150 ppm
PETRO RISc™ [b]	2/4, <75 ppm	2/3, >75 ppm	4/4, >75 ppm
Method 8015[c]	51.2 ± 6.4 ppm	75.9 ± 7.8 ppm	162 ± 10.4 ppm

a. Samples were prepared by spiking sandy loam soil with known amounts of regular number 2 diesel.

b. PETRO RISc™ test was run according to Method 4030 using the hydrocarbon supplied with the kit, one determination on 75 ppm sample out of QC limits.

c. Method 8015 was run using a diesel standard purchased at a Virginia station.

TABLE 5

RESULTS FOR OXYGENATED GASOLINE - FRESH SPIKE
(5 replicates/test)

Nominal concentration[a]	50 ppm	100 ppm	200 ppm	1000 ppm
PETRO RISc™ [b]	3/4, <100 ppm	4/5, >100 ppm	5/5, >100 ppm	5/5, >1000 ppm
Method 8015[c]	22.2 ± 1.6 ppm	39.4 ± 4.2 ppm	84.8 ± 10.9 ppm	434 ± 26 ppm

a. Samples were prepared by spiking sandy loam soil with known amounts of an oxygenated fuel, sample were maintained in closed jars until analyzed.

b. PETRO RISc™ test was run according to Method 4030 using the hydrocarbon supplied with the kit, one determination on 50 ppm sample out of QC limits.

c. Method 8015 was run using a gasoline standard purchased at a California station.

TABLE 6

RESULTS FOR OXYGENATED GASOLINE - HELD OPEN
(5 replicates/test)

Nominal concentration[a]	50 ppm	100 ppm	200 ppm
PETRO RISc™ [b]	3/4, <100 ppm	4/5, >100 ppm	2/4, >100 ppm
Method 8015[c]	nd	3.6 ± 0.4 ppm	7.3 ± 0.9 ppm

[a] Samples were prepared by spiking sandy loam soil with known amounts of an oxygenated fuel, analyses were conducted 24 hours after homogenizing the sample. Spiked samples were stored open to the atmosphere. Nominal concentrations are based on the spiking level.

[b] PETRO RISc™ test was run according to Method 4030 using the hydrocarbon supplied with the kit, 1/5 determinations at 50 and 200 ppm out of QC limits.

[c] Method 8015 was run using a gasoline standard purchased at a California station. Later eluting peaks were used for quantitation.

nd - not detected

TABLE 7

METHOD SENSITIVITY

Part 1 - Average Response with Negative Soils

Soil#	Soil Type	Average % Bo (n = 9)	Standard Deviation
SAND	91.4	4.1	
S2	LOAM	83.1	3.2
S3	CLAY	84.4	3.1
S4	LOAM	80.9	1.3
S5	CLAY	89.7	1.7
S6	LOAM/SAND	91.2	0.2
S7	SAND/LOAM	89.0	0.3
S8	LOAM	90.0	1.4
AVERAGE		87.5	4.0

Part 2 - Average Response with Calibrators

Calibrator Concentration (ppm)	Average Absorbance	Average %Bo
0	1.339	N/A
5	1.097	81.9
15	0.825	61.7
50	0.427	31.9
125	0.219	16.3

Part 3 - Method Sensitivity

Based on Part 1 and Part 2 Above:

Average %Bo - 2 SD = 79.6 which is equivalent to 5.8 ppm
Average %Bo - 3 SD = 75.6 which is equivalent to 7.0 ppm

(%Bo = [(OD_{sample}/$OD_{negative\ control}$)x100])

TABLE 8

EFFECT OF WATER CONTENT IN SOIL SAMPLES

Soil	% Water	Fortified?	Rep. 1*	Rep. 2	Rep. 3	Mean	Std. Dev.	± 2 SD Range
S1	0	No	101.3	99.1	111.8	104.1	6.8	90.4 - 117.7
S1	30	No	100.5	115.5	109.1	108.4	7.5	93.4 - 123.4
S1	0	Yes	59.2	65.8	69.6	64.9	5.3	49.9 - 75.5
S1	30	Yes	60	74.7	83.1	72.3	11.7	49.2 - 96.0
S2	0	No	57.9	53.9	72.3	61.4	9.7	42.0 - 80.8
S2	30	No	74.5	91.8	85.2	83.8	8.7	66.4 - 101.2
S2	0	Yes	40.3	40.9	45.6	42.3	2.9	36.5 - 48.1
S2	30	Yes	44.5	67.8	68.4	60.2	13.6	33.0 - 87.4
S3	0	No	70.1	85.6	76.7	77.5	7.8	61.9 - 93.1
S3	30	No	81.5	109.4	103.4	98.1	14.7	68.7 - 127.5
S3	0	Yes	41.1	46.6	60.7	49.5	10.1	29.3 - 69.7
S3	30	Yes	61.3	76.7	63.1	67.0	8.4	50.2 - 83.8

* All values shown are $\%Bo = [(OD_{sample}/OD_{negative\ control})x100]$

TABLE 9

EFFECT OF pH ON SOIL SAMPLES

Soil	pH Adj.	Fortified?	Rep.1*	Rep.2	Rep.3	Mean	Std. Dev.	± 2 SD Range
S1	None	No	88.9	93.2	92.8	91.6	2.4	86.8 - 96.4
S1	Acidic	No	108.9	66.0	88.1	87.7	21.5	44.7- 109.2
S1	Basic	No	101.2	90.3	90.6	94.0	6.2	81.6 - 106.4
S1	None	Yes	64.3	55.7	58.0	59.3	4.5	50.3 - 68.3
S1	Acidic	Yes	52.9	41.1	49.4	47.8	6.1	35.6 - 60.0
S1	Basic	Yes	69.3	61.7	57.5	62.8	6.0	50.8 - 74.8
S2	None	No	76.2	86.4	83.1	81.9	5.2	71.5 - 92.3
S2	Acidic	No	101.2	82.4	99.5	94.4	10.4	73.6 - 115.2
S2	Basic	No	89.9	72.1	77.7	79.9	9.1	61.7 - 98.1
S2	None	Yes	59.4	60.3	53.7	57.8	3.6	50.6 - 65.0
S2	Acidic	Yes	68.1	62.3	59.3	63.2	4.5	54.2 - 72.2
S2	Basic	Yes	47.8	51.7	39.4	46.3	6.3	33.7 - 58.9
S3	None	No	83.4	88.4	85.3	85.7	2.5	80.7 - 90.7
S3	Acidic	No	89.3	84.9	91.0	88.4	3.1	82.2 - 94.6
S3	Basic	No	80.6	84.2	90.3	85.0	4.9	75.2 - 94.8
S3	None	Yes	60.2	53.6	58.8	57.5	3.5	47.7 - 64.5
S3	Acidic	Yes	58.8	58.5	62.0	59.8	1.9	56.0 - 63.6
S3	Basic	Yes	53.4	41.8	59.9	51.7	9.2	33.3 - 70.1

* All values shown are $\%Bo = [(OD_{sample}/OD_{negative\ control})x100]$

TABLE 10

PETRO RISc™ SOIL TEST
FIELD TRIAL 1

Sample ID	IR Method (ppm)	100 ppm Test		1000 ppm Test	
		Result	Agreement Y, FP, FN	Result	Agreement Y, FP, FN
AST-01	<20	< 100	Y	< 1000	Y
AST-02	520	≥ 100	Y	≥ 1000	FP
AST-03	1700	≥ 100	Y	≥ 1000	Y
AST-04	130	≥ 100	Y	< 1000	Y
AST-05	20	≥ 100	FP	< 1000	Y
AST-06	40	≥ 100	FP	< 1000	FN
AST-07	400	≥ 100	Y	< 1000	FN
AST-08	640	≥ 100	Y	< 1000	FN
AST-09	1600	≥ 100	Y	≥ 1000	Y

Y = Immunoassay and GC or IR results agree
FP = False Positive
FN = False Negative

TABLE 11

PETRO RISc™ SOIL TEST
FIELD TRIAL 2

Sample ID	GC Extractables (ppm)	TRPH (ppm)	75 ppm Test			750 ppm Test		
			Result	Agreement Y, FP, FN		Result	Agreement Y, FP, FN	
				TRPH	GC		TRPH	GC
1-B	5720	20800	≥ 75	Y	Y	≥ 750	Y	Y
2-A	610	14700	≥ 75	Y	Y	≥ 750	FP	Y
2-B	370	6800	≥ 75	Y	Y	≥ 750	FP	Y
2-C	2270	1950	≥ 75	Y	Y	≥ 750	Y	Y
3-B	4870	18600	≥ 75	Y	Y	≥ 750	Y	Y
3-C	760	1180	≥ 75	Y	Y	< 750	FN*	FN
4-A	66	4100	≥ 75	FP*	Y	< 750	Y	FN
4-B	303	2100	≥ 75	Y	Y	< 750	Y	FN
5-A	20400	29600	≥ 75	Y	Y	≥ 750	Y	Y
5-B	26300	28600	≥ 75	Y	Y	≥ 750	Y	Y
5-C	267	330	≥ 75	Y	Y	≥ 750	FP	FP
6-B	550	22700	≥ 75	Y	Y	≥ 750	FP	Y
8	59300	64400	≥ 75	Y	Y	≥ 750	Y	Y
9	26500	12900	≥ 75	Y	Y	≥ 750	Y	Y

Y = Immunoassay and GC or IR results agree
FN = False Negative
FP = False Positive
FN* = False Negative, but within 25% of GC or IR results
FP* = False Positive, but within 25% of GC or IR results

TABLE 12

IMMUNOASSAY COMPARED TO METHOD 8015

Field Trial 1: Gasoline (Interpretation at 100 ppm)

Sample ID	Method 8015 (ppm)	Immunoassay	Concurrence?
MW-18-1	270	Negative	False Negative
MW-18-2	15	Negative	YES
MW-18-3	15	Negative	YES
MW-18-A1	20	Negative	YES
MW-18-A1 Duplicate	15	Negative	YES
MW-18-A2	1500	Positive	YES
DB3	300	Positive	YES
MW-12-3	250	Positive	YES
MW-13-1	40	Negative	YES
MW-13-3	50	Negative	YES
MW-13-4	20	Negative	YES
MW-17-3	250	Positive	YES
MW-17-4	180	Positive	YES
MW-17-5	180	Positive	YES
MW-16-2	11,500	Positive	YES
MW-16-2 Duplicate	11,500	Positive	YES
MW-19-2	10	Negative	YES
MW-19-3	70	Negative	YES
MW-14-1	280	Positive	YES
MW-17-A1	560	Positive	YES

Field Trial 2: JP-4 Jet Fuel (Interpretation at 1,000 ppm)

Sample ID	Method 8015 (ppm)	Immunoassay	Concurrence?
TB1 6.5-7.0	15,900	Positive	YES
TB2 6.5-7.0	16,800	Positive	YES
TB1 5.0-5.5	900	Negative	YES
TB2 5.0-5.5	100	Positive	False Positive
TB3 5.0-5.5	ND(<5)	Negative	YES
TB3 6.5-7.0	29,500	Positive	YES
TB5 5.0-5.5	5,000	Positive	YES
TB5 6.5-7.0	2,000	Positive	YES
TB4 6.5-7.0	19,000	Positive	YES
TB4 5.5-6.0	5,900	Positive	YES