Determination of 1-Naphthol in Well Water Samples by ELISA

This unit describes the determination of 1-naphthol in well water samples by an enzyme-linked immunosorbent assay (ELISA). 1-Naphthol is the major hydrolysis product of the insecticide carbaryl. Because of the metabolic and environmental instability of this insecticide, the analytical protocol described in this unit can be considered as complementary to the protocol for the determination of carbaryl described in *UNIT 2C.8*. The protocol is based on an indirect immunoassay format and uses a polyclonal antibody along with (5-hydroxy-2-naphthylenyl)oxy acetic acid covalently coupled to bovine serum albumin (2b-BSA) as a coating antigen. A mixture of the buffered samples and the antibody solution is added to antigen-coated ELISA plates and incubated 1 hr at room temperature. A second antibody reagent, goat anti-rabbit IgG conjugated to horseradish peroxidase (GAR-HRP), is then added and incubated for an additional 1 hr. Finally, the fraction of specific antibodies bound to the plate is made visible by adding an appropriate substrate which will yield a colored product under enzymatic catalysis. The absorbance of the product is measured at 450 nm and is inversely proportional to the concentration of 1-naphthol in the sample. The working range of this immunoassay is 3.2 to 118 μg/L, and the limit of detection is 1.7 μg/L. The assay shows minimal cross-reactivity for carbaryl (0.5%) and therefore is very suitable for selective detection of 1-naphthol.

QUALITY CONTROL

To verify method performance, negative and positive controls should be included on each plate. These controls are of two types—prepared in assay buffer or prepared in the sample matrix. The responses of the controls prepared in assay buffer indicate whether the immunoassay is under control. The responses of the controls prepared in the sample matrix indicate the relative magnitude of a matrix effect.

If the negative control, prepared in sample matrix, produces a positive response in the ELISA, a matrix effect or cross-contamination of the sample is presumed. This negative-control value should be subtracted from the value for the positive controls prepared in sample matrix. If the resulting recovery value is within the quality-control criteria, then the method of standard addition should be used to quantitate samples. If the values fall outside the quality-control criteria, the method is out of control and the cause should be determined. Typically the sample should be diluted or a cleanup step should be introduced to bring the method back into control.

Positive controls consist of assay buffer or sample matrix spiked with 1-naphthol at concentrations of 3.5, 20.1, and 115.2 ng/mL. Recovery of 1-naphthol from the positive controls prepared in assay buffer should not deviate by >15% from the expected value. Recovery of 1-naphthol from the positive controls prepared in sample matrix should be between 70% and 130%, with a relative percent standard deviation (% RSD) of <30%. If these criteria are met, method performance is considered acceptable and sample analysis may begin.

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Current Protocols in Field Analytical Chemistry (1998) 2C.9.1-2C.9.11

BASIC PROTOCOL

ELISA PROCEDURE FOR DETERMINING 1-NAPHTHOL IN WELL WATER

Materials

Coating antigen: 2b-BSA (see Reagents and Standards)
Coating buffer (see Reagents and Standards)
Anti-1-naphthol polyclonal antibody R3907 (see Reagents and Standards)
1× and 10× PBST (see Reagents and Standards)
Water samples for analysis
1-Naphthol standards (see Reagents and Standards)
Quality control samples (see Reagents and Standards)
GAR-HRP (see Reagents and Standards)
Substrate solution (see Reagents and Standards)
2 M sulfuric acid

96-well ELISA high-binding microtiter plates (e.g., Nunc Maxisorb)
Refrigerator
Acetate adhesive plate sealers
Mixing plates: polystyrene 96-well plates for mixing reagents and preparing dilutions
Microtiter plate reader with 450-nm filter
Software to analyze data using a four-parameter logistic curve-fitting equation (e.g., Genesis from Titertek or GraphPad Prism)

Additional reagents and equipment for determining optimal concentrations of coating antigen and primary antibody by two-dimensional titration experiments (*UNIT 2C.8*, Support Protocol)

Coat the ELISA plates (perform on day before assay)

1. Prepare a working solution of 2 µg/mL (or other optimal concentration as determined by two-dimensional titration experiment; see *UNIT 2C.8*, Support Protocol) of coating antigen (2b-BSA) in coating buffer.

   For one microtiter plate, add 22 µL of the coating antigen stock solution (concentration 1.1 mg/mL) to 12 mL of coating buffer to obtain the above concentration.

2. Add 100 µL of the coating antigen working solution to each well of the ELISA microtiter plate.

3. Cover the microtiter plate with an adhesive plate sealer and incubate overnight at 4°C.

Preincubate the standards, quality-control samples, and well water samples with the antibody (perform on day before assay)

4. Prepare the antibody solution by diluting the anti-1-naphthol antibody R3907 1:3200 (or to other optimal concentration as determined by two-dimensional titration experiment; see *UNIT 2C.8*, Support Protocol) with 1× PBST.

   For one microtiter plate, add 3.1 µL of antiserum to 10 mL of 1× PBST to obtain the above concentration.

5. Mix 450 µL of water sample with 50 µL of 10× PBST.

   In the absence of a matrix effect, buffered samples can be used directly for analysis (see Quality Control in this unit and in UNIT 2C.8 and the Support Protocol in UNIT 2C.8 for information on determination of matrix effects).

6. Prepare standards and quality-control samples (see Reagents and Standards).

7. In individual wells of a mixing plate (ordinary polystyrene 96-well microtiter plate), mix 200 µL of each 1-naphthol standard, quality control sample, and buffered well water sample with 50 µL of the antibody solution. For the blanks, add 250 µL of 1×

PBST to separate wells with no antibody solution. For the zero-analyte standards, mix 200 µL of 1× PBST with 50 µL of the antibody solution.

Each well of the mixing plate contains enough sample to perform two replicates in the ELISA plate (see Fig. 2C.8.1 for a typical distribution of the samples and standards on the ELISA plate).

8. Cover the microtiter plate with an adhesive plate sealer and incubate overnight at 4°C.

Perform the ELISA (day 2)

9. Wash the coated ELISA plate (from step 3) five times, each time by adding ~300 µL 1× PBST to every well, then aspirating the liquid. Tap the plates dry on absorbent paper and use them immediately for the assay.

   With this procedure, unbound reagents are removed from the wells. Dry, coated ELISA plates can also be stored at –20°C for several days with no significant change in the assay characteristics. However, longer coating times result in an increase in well-to-well variability.

10. Begin the competition step by transferring 100 µL from each well of the mixing plate (from step 8) to the corresponding well of the ELISA plate (Fig. 2C.8.1).

11. Cover the plate with an adhesive plate sealer and incubate 1 hr at room temperature.

12. Prepare a working solution of the second antibody reagent, GAR-HRP, by diluting the stock solution 1:6000 in 1× PBST.

    For one microtiter plate assay, mix 2 µL of the second antibody stock solution and 12 mL of 1× PBST to obtain the above concentration.

13. Wash the plate as described in step 9.

14. Add 100 µL of the GAR-HRP working solution prepared in step 12 to each well of the coated plate.

15. Cover the plate with an adhesive plate sealer and incubate 1 hr at room temperature.

Develop the color

16. Prepare the substrate solution (see Reagents and Standards).

17. Wash the plate as described in step 9.

18. Add 100 µL substrate solution to each well of the ELISA plate and cover with an adhesive plate sealer. Incubate 30 min at room temperature.

19. Add 50 µL of 2 M sulfuric acid to each well.

20. Measure the absorbances of the wells at 450 nm using a microtiter plate reader.

Calculate the results

21. Prepare the calibration curve for 1-naphthol by fitting the data for the standards using a four-parameter logistic equation. Calculate the concentration of 1-naphthol present in the well water samples and in the quality-control samples.

    If the deviation of the calculated concentrations of the quality control samples from the expected value is >15%, the method is out of control. See Quality Control and Commentary for strategies to bring the method back into control.

22. Determine that the parameters of the four-parameter logistic equation (slope, IC_{50}, maximum and minimum absorbance, and coefficient of correlation) are within expected values, and that the curve is well defined by standards used.

The four-parameter logistic equation has the following formula:

$$y = \frac{A - D}{1 + \left(\frac{x}{C}\right)^B} + D$$

where y is the absorbance, x is the concentration of the analyte, A and D are the upper and lower asymptotes of the sigmoid curve, respectively, B is the slope, and C is the IC_{50} or the analyte concentration defining the midpoint of the linear portion of the curve.

23. To determine if the 1-naphthol concentrations for well water samples and quality control samples are within the working range of the assay, normalize the absorbances by dividing the absorbance value (B) for each well water sample and quality control sample by the mean of the absorbance at zero analyte concentration. (B_0).
24. Calculate the absorbance value corresponding to 90%, 80%, and 20% of the B_0. From these absorbances, use the calibration curve parameters determined in step 21 to calculate the corresponding concentrations of 1-naphthol.

    The 1-naphthol concentration at 90% of B_0 (D-90) is the limit of detection. The 1-naphthol concentrations at 80% and 20% of B_0 (D-80 and D-20) are the working range of the assay.

25. If well water samples give absorbances <20% of B_0, dilute and reanalyze to give a more accurate 1-naphthol concentration value.
26. If well water samples give absorbances >80% of B_0, concentrate samples by solid-phase extraction so that the resulting absorbances fall into the the working range (i.e., 20% to 80% of B_0).
27. Calculate the concentration of 1-naphthol in the well water samples using absorbance values that fall within the working range.

REAGENTS AND STANDARDS

NOTE: Prepare all buffers with Milli-Q-purified water. Although 0.02% (w/v) sodium azide or 0.005% (w/v) sodium ethylmercurithiosalicylate can be added as preservatives, their use can be eliminated when buffers are used within 1 to 2 weeks and stored at 4°C. Because sodium azide inhibits horseradish peroxidase activity, better assay performance may be obtained by eliminating the preservative from the final wash step. Preservative should never be added to substrate buffer.

Anti-1-naphthol polyclonal antibody R3907

The primary antibody used in the Basic Protocol was developed in New Zealand white rabbits immunized with 7-KLH (Fig. 2C.9.1), a conjugate of 5-hydroxy-2-naphthoic acid and keyhole limpet hemocyanin (KLH). It should be stored in 50-µL aliquots at –20°C. This reagent may be provided by Prof. Bruce D. Hammock at the Department of Entomology, University of California, Davis, upon request. The description and derivation of this reagent are reported in Krämer et al. (1994).

Coating antigen 2b-BSA

The coating antigen used in the Basic Protocol is a conjugate of (5-hydroxy-2-naphthylenyl) oxy acetic acid covalently coupled to bovine serum albumin (BSA). The coating antigen should be stored freeze-dried or frozen in aliquots. Stock solutions at concentrations of 1 mg/mL in 1× PBS (see below) can be stored at 4°C for ~4 months without loss of performance. This reagent may be provided by Prof. Bruce D. Hammock at the Department of Entomology, University of California, Davis, upon request. The description and derivation of this reagent are reported in Krämer et al. (1994).

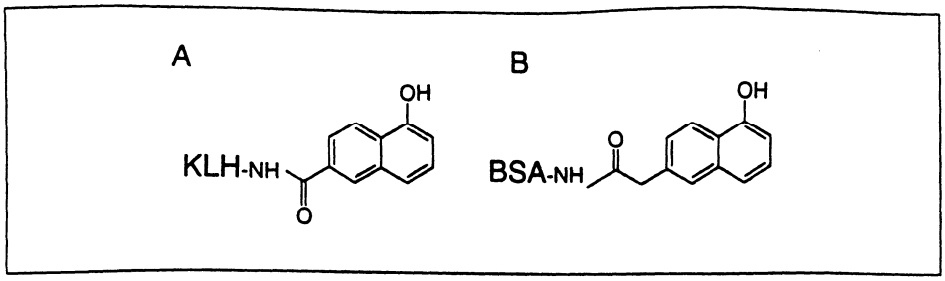

Figure 2C.9.1 Chemical structures of (**A**) 7-KLH, which used as the immunogen for producing antibodies against 1-naphthol, obtained by covalent coupling of the hapten 5-hydroxy-2-naphthoic acid to keyhole limpet hemocyanin (KLH), and (**B**) 2b-BSA, which is used as coating antigen in the indirect ELISA for 1-naphthol.

Coating buffer

To a beaker containing ~350 mL of water, add 0.795 g Na_2CO_3 and 1.465 g $NaHCO_3$. Adjust to pH 9.5 and bring to a final volume of 500 mL with water.

Goat anti-rabbit IgG–horseradish peroxidase conjugate (GAR-HRP)

To make a stock solution, reconstitute horseradish peroxidase–conjugated goat anti-rabbit IgG (Sigma, cat. no. R-2004) with 1 mL of 0.135 M NaCl. Divide into 50-mL aliquots and store up to 6 months at –20°C.

Thawed aliquots may be stored 1 to 2 weeks at 4°C.

1-Naphthol standard stock and working solutions

Prepare the primary 2.9 mg/mL stock solution by weighing 2.9 mg of analytical-grade 1-naphthol and dissolving it in 10 mL dimethyl sulfoxide (DMSO). Store up to 2 weeks at room temperature. At the time the standards are to be prepared (see below), dilute 100 µL of the primary stock solution in 900 µL DMSO to make a 0.29 mg/mL working solution.

Phosphate-buffered saline (PBS), 10× and 1×

For 10× solution, add the following to ~700 mL H_2O:

80 g NaCl
2 g KCl
2 g KH_2PO_4
11.5 g Na_2HPO_4 (add slowly to prevent clumping)
Adjust pH to 7.5
Adjust volume to 1 L with H_2O
Store up to 4 weeks at room temperature

For 1× solution: Dilute 50 mL of 10× PBS to a final volume of 500 mL with H_2O. Adjust pH to 7.5 if necessary. Store up to 2 weeks at 4°C.

PBS-Tween (PBST), 10× and 1×

For 1× solution: Mix 50 mL of 10× PBS (see above) and 5 mL of 5% (v/v) Tween 20. Adjust to 500 mL with H_2O. Store up to 2 weeks at room temperature.

For 10× solution: Mix 450 mL of 10× PBS (see above) and 50 mL of 5% (v/v) Tween 20. Store up to 2 weeks at room temperature.

Quality control (QC) samples

The accuracy of the assay and absence of matrix effect may be monitored by preparing a negative control (zero-analyte; QC-0) and positive controls (analyte concentrations giving 20%, 50%, and 80% of the maximal absorbance; QC-20,

continued

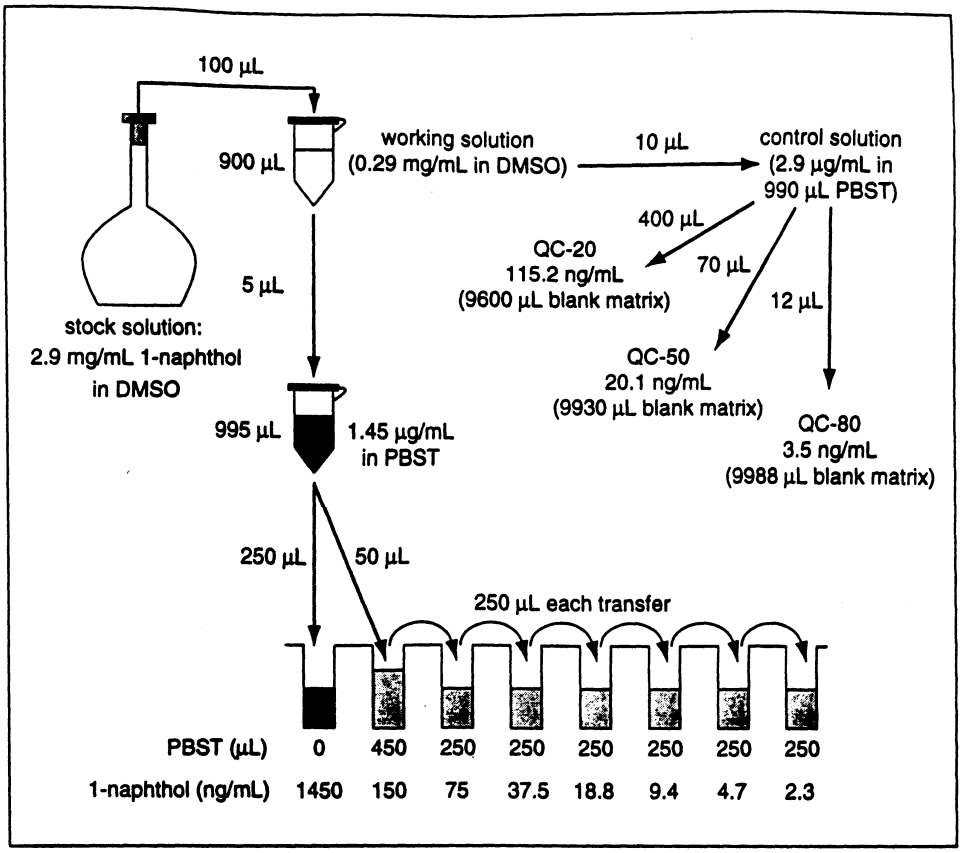

Figure 2C.9.2 Scheme showing the steps involved in the preparation of the standard curve and the quality-control samples for the ELISA for 1-naphthol.

QC-50, and QC-80) in well water of the source to be analyzed containing no 1-naphthol (blank matrix). Otherwise similar controls can be prepared with 1× PBST (see above). For this purpose prepare a 2.9 µg/mL control solution of 1-naphthol in PBST by adding 10 µL of 0.29 mg/mL 1-naphthol working solution (see above) to 990 µL of 1× PBST (see above), then dilute as follows.

QC-0; zero analyte concentration; matrix only

QC-20: 115.2 ng/mL 1-naphthol; 400 µL of 2.9 µg/mL control solution/ 9600 µL matrix

QC-50: 20.1 ng/mL 1-naphthol; 70 µL of 2.9 µg/mL control solution/9930 µL matrix

QC-80: 3.5 ng/mL 1-naphthol; 12 µL of 2.9 µg/mL control solution/9988 µL matrix

See Fig. 2C.9.2 for an illustration of this dilution series.

Standards

Dilute 5 µL of the 0.29 mg/mL 1-naphthol working solution (see above) in 995 µL PBST (see above) to make a 1.45 µg/mL solution (which will be the highest concentration to be tested in the ELISA). Dilute 50 µL of the 1.45 µg/mL solution with 450 µL of PBST to make a 0.15 µg/mL (150 ng/mL) solution (the second standard concentration on the calibration curve). From the 150 ng/mL standard solution, prepare two-fold serial dilutions with 1× PBST in the wells of a mixing plate (ordinary polystyrene 96-well microtiter plate) to obtain the following 1-

continued

naphthol standard concentrations: 75, 37.5, 18.8, 9.4, 4.7, and 2.3 ng/mL (see Fig. 2C.9.2). In addition to the eight 1-naphthol concentrations, a zero-analyte standard and a blank (containing no anti-1-naphthol antibody) are included in the calibration curve.

This procedure for the preparation of the standard curve utilizes an amount of analytical standard that can be weighed with a reasonable amount of accuracy. DMSO is a very polar, water-miscible solvent of low volatility. The final concentration of organic solvent in the well containing the 1.45 µg/mL standard is only 5%. The use of cluster tubes in a 96-microtiter plate format to prepare the standard curve further simplifies performance of the steps of the Basic Protocol.

Substrate buffer

To a beaker containing ~350 mL of Milli-Q water, add 6.81 g of sodium citrate. Adjust pH to 5.5 with acetic acid and bring to final volume of 500 mL with Milli-Q water. Store up to 2 weeks at 4°C.

Preservative should never be added to the substrate buffer.

Substrate solution

Purchase 3,3′,5,5′-tetramethylbenzidine (TMB) of >95% purity (store dry at 2° to 8°C). Prepare a 6 mg/mL stock solution in DMSO (store up to 3 weeks at room temperature, protected from light). Just prior to use, mix 44 µL of 1% H_2O_2 (store up to ~1 month in a plastic container at 4°C), 176 µL of 6 mg/mL TMB stock solution, and 11 mL substrate buffer (see above).

The TMB solution should be colorless. Do not use the TMB stock if the solution is yellow or brown colored. Use of low-purity TMB will result in a colored solution even when freshly prepared in DMSO.

COMMENTARY

Background Information

1-Naphthol is the major metabolite of the insecticide carbaryl (Sevin, 1-naphthyl-*N*-methylcarbamate). Carbaryl has been reported to have low to moderate mammalian toxicity (Ripley and Chau, 1982). However, some adverse effects have been reported, such as alteration of liver microsomal enzymes (Lechner and Abdel-Raman, 1985), subchronic toxicity after long-term exposure to carbaryl when used as household insecticide (Branch and Jacqz, 1986), and changes in the immunological functions of in vitro cultures of granular lymphocytes (Bavari et al., 1991). The carbamate function of this insecticide is highly susceptible to chemical hydrolysis and biodegradation, producing 1-naphthol as the major metabolite. Moreover, Dikshith et al. (1990) analyzed 1-naphthol in soil samples taken around Bhopal, India, where carbaryl was produced for more than a decade, and found alarming levels of 1-naphthol in water ranging from 153 to 656 µg/L. Similarly, the finding of 1-naphthol in well water samples may be indicative of a previous contamination by carbaryl (Marco et al., 1995; see Method Performance). This hydrolytic process may also have adverse effects on the ecosystem, since, for example, a synergetic toxicological effect of carbaryl in combination with 1-naphthol on green algae and cyanobacteria has been described (Megharaj et al., 1990). In mammals, biotransformation occurs via oxidative and hydrolytic pathways leading to 1-naphthol, which is rapidly excreted in urine, feces and respiratory gases. It has been reported that the urine of formulators of technical carbaryl at a manufacturing plant contained extremely high levels of 1-naphthol, ranging from 6.2 to 78.8 mg/L. Similarly the urine of agricultural workers who used carbaryl for pest control had concentration levels between 0.07 and 1.7 mg/L. Because of the widespread use of carbaryl and the environmental instability of this compound resulting in the formation of 1-naphthol, the analytical method for carbaryl (*UNIT 2C.8*) needs to take into account the determination of 1-naphthol as well. Therefore, this unit represents the procedure for analysis of 1-naphthol by ELISA as a complementary method to the immunoassay procedure for carbaryl described in *UNIT 2C.8*.

Definitions

See *UNIT 2C.8* for definitions.

Safety

NOTE: *Review all relevant Material Safety Data Sheets (MSDSs) prior to the analysis and take appropriate safety precautions. MSDSs are available from reagent manufacturers and on the World Wide Web.*

If using sodium azide to prepare the buffers, avoid breathing vapors or skin contact. 1-Naphthol has very low mammalian toxicity; however, samples and particularly the concentrated stock solutions should be handled with care. DMSO is known to increase skin absorption.

Critical Parameters

It is extremely important that measurements take place in the linear range of the sigmoid curve. Therefore, on each plate, the working range (80% to 20% of the maximal absorbance) should be determined. It will also speed sample analysis to prepare several dilutions of the sample to ensure that one of them is within the working range.

Allow all the reagents and buffers to reach room temperature before use. Careful control of the incubation times is also advisable to ensure reproducibility. Because temperature and time cannot be stringently controlled when multiple plates are run simultaneously or when conducting analysis in the field, each ELISA plate should contain a calibration curve. Since this technique is based on the use of biological reagents, it is imperative that the storage instructions given by the supplier be closely followed.

Whenever a solid-phase extraction technique is used for the clean-up or concentration of the sample, be sure that the amount of organic solvent employed does not interfere with the assay, or prepare the standard curve in the presence of the same amount of solvent as contained in the sample to be analyzed (see discussion on the evaluation of the matrix effects in *UNIT 2C.8*). The interference of three solvents commonly used in liquid-chromatography procedures—acetone, acetonitrile, and methanol—has been examined (Krämer et al., 1994). Each of these solvents can be used at concentrations up to 10% without significant variation of the assay parameters. When the concentration of solvent is increased to 50%, it is still possible to obtain a sigmoid curve, but the sensitivity of the assay decreases.

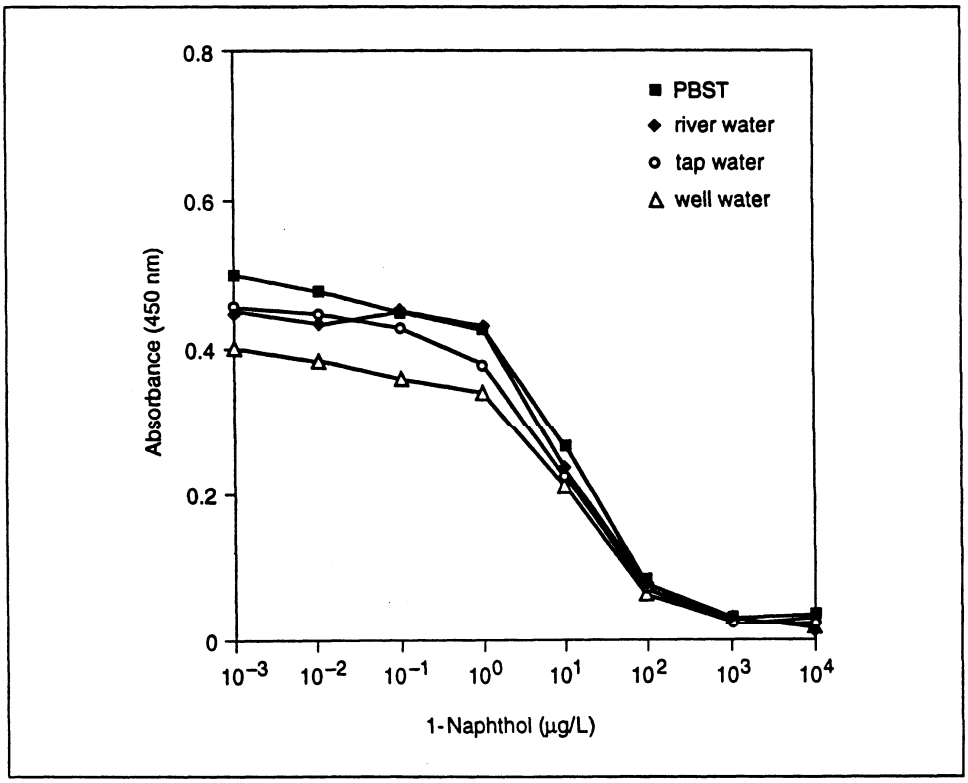

Figure 2C.9.3 Curves showing the parallelism between the standard curve prepared in the assay buffer (PBST) and those prepared in different water matrices. Water samples were used without any dilution or cleanup step.

Troubleshooting

The reader is directed to other publications for general guides to the troubleshooting of immunoassays (Schneider et al., 1995; Gee et al., 1996).

Method Performance

The routine use of quality-control samples will serve to evaluate the method accuracy within the linear range at three different levels. In this context, Figure 2C.9.3 shows that the effect of several water matrices on the immunoassay performance was negligible. In a program that monitored the input of agrochemicals into the groundwater of Almería in Andalucia, Spain (Marco et al., 1995), carbamates were only detected sporadically due to their instability and the particular hydrolytic conditions. For this reason the authors also analyzed these water samples for the presence of 1-naphthol. The accuracy and precision of the ELISA method described in this protocol are demonstrated in Table 2C.9.1. The recoveries and standard deviations obtained when well water samples were spiked at different levels and analyzed by ELISA and on-line solid-phase extraction/liquid chromatography/fluorescence detection (on-line SPE-LC-PCR-FD) are compared. The recoveries were always close to 100% and the ELISA showed a clear tendency to overestimate the concentration value. The inter- and intra-plate variation observed and the standard deviation of the calibration parameters of the standard curve observed during this study are shown in Table 2C.9.2. With these samples, the correlation between the LC-FD

Table 2C.9.1 Recoveries Obtained by On-Line SPE-LC-PCR-FD and ELISA for Well Water Samples Spiked with 1-Napththol at Different Levels[a,b]

Spike level (μg/mL)	SPE-LC-PCR-FD (% recovery)	ELISA (% recovery)	ELISA (% RSD)
1	93	110	36
4	112	98	15
14	100	130	22
22	90	115	8
29	98	85	6
50	102	103	4
58	80	85	20
86	90	89	11
130	115	120	18
216	78	80	35

[a]Data shown correspond to the average and standard deviation of the recoveries found using two plates measured on different days; each plate had three well replicates. Data from Marco et al., 1995.

[b]Abbreviations: SPE-LC-PCR-FD, solid-phase extraction/liquid chromatography/post-column derivitization/fluorescence detection; RSD, relative standard deviation.

Table 2C.9.2 Working Range and Coefficient of Variation of the ELISA[a]

Parameter		Average	Standard deviation	*n*
	IC_{50} (μg/L)[b]	19.41	4.45	16
	Slope[b]	1.1	0.15	16
Working range[c]	Upper limit (μg/L)	118	29.3	16
	Lower limit (μg/L)	3.2	1.16	16
Coefficient of variation[d]	Intra-plate (%)	2.5	0.7	8
	Day-to-day (%)	23	9	5

[a]Data from Marco et al., 1995.

[b]The IC_{50} and slope values are the average of the parameters extracted from the four-parameter logistic equations used to fit the standard curves.

[c]The working range defines the linear portion of the curve and the upper and lower limits are concentrations giving 20% and 80% of the B_0, respectively.

[d]Coefficients of variation correspond to the IC_{50} values observed. Data from Marco et al., 1995.

method and the ELISA protocol was determined. The parameters of the linear regression analysis are given in Table 2C.9.3.

Nearly 30 compounds have been evaluated for their potential interference in the ELISA for 1-naphthol. Other than the haptens used to raise antibodies or to prepare coating antigens, 1,6-dihydroxynaphthalene showed the highest cross-reactivity value, interfering with the assay at 66%. See Table 2C.9.4 for information on the cross-reactivity of other compounds.

Table 2C.9.5 summarizes the results obtained during a 4-month monitoring period of five wells of the Aquifer Campo de Nijar in Almería (Andalucia, Spain). The carbaryl concentration was highest in May to June, corresponding to the time when application of these pesticides began. During the same period, 1-naphthol was also detected in some of the well water samples analyzed. The levels of carbaryl were below the detection limit of the ELISA for carbaryl in July and August. However, the levels of 1-naphthol were still high, especially in samples from wells 3 and 4 during the month of July. In August, 1-naphthol was present in all the well water samples analyzed, indicating that significant hydrolysis of carbaryl had occurred during this period. Results obtained by ELISA were validated by on-line SPE-LC-PCR-FD and on-line SPE-LC-TSP-MS (Marco et al., 1995).

Time Considerations

Aside from the coating step, processing the ELISA plates takes ~2.5 hr. Although dry, coated ELISA plates can be stored frozen for several days, the authors prefer to coat fresh plates for each experiment. The authors have

Table 2C.9.3 Linear Regression Analysis of the Correlation Between On-Line SPE-LC-PCR-FD and ELISA Using Spiked Well Water Samples[a]

Procedure	Slope	Intercept	Correlation coefficient
SPE-LC-PCR-FD vs. ELISA for 1-naphthol	1.02	0.76	0.997

[a]Data from Marco et al., 1995. Abbreviation: SPE-LC-PCR-FD, solid-phase extraction/liquid chromatography/post-column derivatization/fluorescence detection.

Table 2C.9.4 Cross-reactivities of Compunds Structurally Related to 1-Naphthol and to the Hapten Used to Raise Antibodies[a]

Compound	Cross-reactivity (%)
1-Naphthol	100
2-Naphthol	1.1
Carbaryl	0.5
3-Hydroxycarbaryl	2.6
4-Hydroxycarbaryl	0.4
5-Hydroxycarbaryl	1.6
Carbofuran	3
Naphthalene	<0.001
1-Naphthaleneacetamide	<0.001
1-Naphthoic acid	0.3
1-Naphthoxyacetic acid	0.3
2-Naphthoic acid	0.3
2-Naphthoxyacetic acid	0.2
1,5-Dihydroxynaphthalene	3.2
1,6-Dihydroxynaphthalene	66
5-Hydroxy-2-naphthoic acid	25
Phenol	0.5
4-Nitrophenol	0.7
Pentachlorophenol	<0.001

[a]Data extracted from Krämer et al., 1994.

Table 2C.9.5 Results of the Analysis of Carbaryl and 1-Naphthol by ELISA in Well Water Samples from Almería (Spain)[a,b]

Well no.	May		June		July		August	
	Carbaryl	1-Naphthol	Carbaryl	1-Naphthol	Carbaryl	1-Naphthol	Carbaryl	1-Naphthol
1	0.11 ± 0.2	ND	1.37 ± 0.2	ND	ND.	ND.	ND	0.22 ± 0.12[c]
2	0.09 ± 0.00	0.31 ± 0.17[c]	0.64 ± 0.22	ND	ND	ND	ND	0.36 ± 0.19[c]
3	0.12 ± 0.1	0.89 ± 0.2[c]	0.41 ± 0.23	ND	ND	2.39 ± 0.51	ND	0.42 ± 0.21[c]
4	0.08 ± 0.01	ND	0.56 ± 0.19	0.2 ± 0.06[c]	ND	1.64 ± 0.42	ND	0.20 ± 0.09[c]
5	0.17 ± 0.01	ND	0.34 ± 0.18	0.15 ± 0.02[c]	NT	NT	NT	NT

[a]Concentration values are expressed in µg/L. Samples were collected monthly and split to analyze carbaryl and 1-naphthol by ELISA and on-line SPE-LC-PCR-FD (data not shown). Data from Marco et al., 1995.

[b]Abbreviations: ND, not detected; NT., samples not tested.

[c]Values measured outside of the working range of the ELISA.

also found that the sensitivity of the assay is improved by preincubating the samples (and standards) with the antibody overnight at 4°C. Thus, assay setup should begin the day before the analysis. An advantage of using ELISA to screen environmental samples is that multiple samples can be run on the same ELISA plate. Additionally, several plates can be processed simultaneously, increasing the number of samples that can be processed on each assay day.

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The protocol development and validation studies were supported in part by the National Institute of Environmental Health Sciences Superfund Research Program, 5 P42 ES04699 and the Environmental Protection Agency Center for Ecological Health Research, CR819658. UC Davis is an NIEHS Health Science Center (1 P30 ES05707). Although the work reported here was sponsored in part by the U.S. Environmental Protection Agency, no endorsement by the agency is implied.