

Pilot project for the environmental technology verification
In the field of simplified chemical monitoring technology

Protocol for the verification tests on simplified chemical monitoring technology

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Main section

I. Introduction

1. Target technologies

Simplified chemical monitoring technologies covered by this verification test are chemical monitoring technologies that are characterized by simple operation and maintenance and sensitive quantification, and can be expected to be applied to screening or to simplified monitoring of outliers.

A target technology, which should have potential application to general environmental monitoring, shall satisfy the following requirements and be a simple technology based on the enzyme-linked immunosorbent assay (ELISA*), an antigen-antibody reaction-based test method.

Dioxin, which has been studied separately by the Ministry of the Environment, shall be excluded from the chemicals to be monitored by target technologies.

- Monitors chemical substances designated under the Law Concerning Reporting, etc. of Releases to the Environment of Specific Chemical Substances and Promoting Improvements in Their Management (PRTR Law**), suspected endocrine-disturbing chemicals, etc.
- Can be used for general environmental monitoring (other than monitoring around sources)
- Monitors trace amounts of chemicals in the general environment that have social significance, present a pressing problem, or need monitoring by some means or other
- Easier to operate and maintain than instrumental analyses
- Commercially available
- Can determine the concentrations of chemicals in the order of ppb ($\mu\text{g/L}$)

* ELISA: Enzyme-Linked Immuno-Sorbent Assay

** PRTR: Pollutant Release and Transfer Register

2. Types and outline for verification tests

(1) Types of verification tests

In this verification test, the following points shall be checked for a target verification product presented by an environmental technology developer:

- Reliability of product performance
- Practicability in general environmental monitoring
- Ease of operation

A verification test will provide information that helps to determine whether or not the target verification product can be used for general environmental monitoring.

(2) Verification testing process

The verification test will primarily be conducted according to the steps specified below:

i. Planning

The plan for the verification test (hereinafter referred to as the “Test Plan”) will be prepared by a Verification Organization in cooperation with an environmental technology developer.

In the Test Plan, the following shall be specified:

- Verification test system

- Purpose of the verification test
- Verification test items
- Specific work items in the verification test
- Schedule of the verification test

ii Verification test

A verification test will be conducted according to the Test Plan described above. The verification test verifies the conformity of a target verification product with its objectives specified in the planning stage. The Verification Organization may, if necessary, subcontract part of the verification test to external test organizations.

iii Data assessment and reporting

Based on the results of the verification test, all data collected will be analyzed for verification, and a report on the verification test (hereinafter referred to as the “Verification Report”) will be compiled. A Verification Organization is responsible for analysis of the data and reporting.

To accelerate the above process, the Verification Organization may subcontract an external organization to prepare a draft of the Verification Report.

The Verification Report will be submitted by the Verification Organization to the Ministry of the Environment. The suitability of the verification tests will be discussed by the working group on the simplified chemical monitoring technology (hereinafter referred to as the “working group”) of the committee on the pilot project for the environmental technology verification. After being approved by the Ministry of the Environment, the report will be returned to the Verification Organization. The approved Verification Report will then be issued by the Verification Organization to the environmental technology developer and simultaneously disclosed to the public.

3. Definitions of terms and phrases

The definitions of the major terms and phrases are in accordance with those of the Japanese Industrial Standards (hereinafter referred to as “JIS”). The standard in JIS particularly relevant to this protocol for the verification (hereinafter referred to as “Protocol”) is as follows:

JIS K3600:2000 “Biotechnology Dictionary”

In addition, the terms and phrases used in this Protocol are defined as set forth in Table 1.

Table 1 Definitions of terms and phrases used in this Protocol

Term/Phrase	Definition
Target verification technology	Theoretical concept or performance of a technology under verification test
Target verification product	An apparatus to be used in the verification test among the apparatuses/equipments representing the embodiments of the target verification technology
Verification items	Items to be analyzed for determination of the performance of a target verification product
Verification applicant	A person wishing to have his/her own technology verified
Environmental technology developer	Developer or distributor of a target verification technology

II. Verification test system

1. Ministry of the Environment

- Comprehensively administer the entire pilot project for the environmental technology verification
- Comprehensively discuss the verification test system
- Select target verification technology fields for the verification test
- Establish and administer the committee on the pilot project for the environmental technology verification and its working groups
- Create a protocol for the verification
- Select Verification Organizations
- Approve reports on verification tests
- Create an Environmental Technologies Verification database (hereinafter referred to as “ETV database”) for their dissemination

2. The committee on the pilot project for the environmental technology verification

- Offer advice on the management of the entire pilot project for the environmental technology verification
- Offer advice on the comprehensive evaluation of verification test results

3. Working group on the simplified chemical monitoring technology of the pilot project for the environmental technology verification

- Offer advice on management of the entire pilot project for the environmental technology verification in the field of simplified chemical monitoring technology
- Offer advice on creating a protocol for the verification
- Offer advice on the selection of Verification Organizations
- Offer advice on approval of the Verification Report

4. Verification Organizations

- Administer all processes of the pilot project for the environmental technology verification in the target verification technology field under the auspices of the Ministry of the Environment
- Construct the quality management system shown in Appendix 0
- Invite the public to register the technologies and products that are suitable as the target of the verification tests
- Establish and administer respective Technology Panels
- Establish a Test Plan in cooperation with environmental technology developers
- Conduct and manage the target verification tests based on the Test Plan
- Operate target verification products according to their specifications presented by the respective environmental technology developers
- Restrict entry to the location of verification tests during the test period
- Ensure the health and safety of all persons involved in verification tests
- Set and adjust the test schedule by assuring the means of communication among all participants in the verification test, and providing assistance as necessary
- When the verification test is subcontracted to an external organization, ensure that the

quality management system which is required in the Protocol is indeed functioning properly at the subcontractor

- Audit the procedures for the verification test
- Manage the data/information obtained in the verification tests
- Prepare the Verification Report based on analysis/evaluation of the data on the verification test
- Register in the ETV database the contents of the approved Verification Report

5. Technology Panels

- Offer advice on the Test Plan
- Offer advice on the problems occurred during the verification tests
- Offer advice on the preparation of the Verification Report
- Offer advice on dissemination of the technologies verified in the verification test

6. Environmental Technology Developers

- Provide an application for a verification test and a target verification product required in a verification test
- Provide information on the target verification product
- Cooperate with Verification Organizations in establishment of the Test Plan, such as by providing information required for the verification test
- Provide technical information required in a verification test, such as methods for operating the target verification product for measurement.
- Cooperate with the Verification Organization in preparing the Verification Report

III. Selection of target verification technologies

1. Application

A verification applicant may apply to a Verification Organization for verification of the applicant's proprietary technology/product.

Items to be specified in the application form are described below. The verification applicant should fill in the necessary information in the "Application form for verification" set forth in Appendix 1, and submit the application form together with the designated documents to the Verification Organization.

- a. Technical specifications of the target verification product
- b. Instruction manual
- c. Results of performance tests
- d. Company profile, etc.
- e. Other reference materials

2. Selection of target verification technologies

Based on the description of the application and the advice from the Technology Panel and others from a comprehensive viewpoint, a Verification Organization selects target verification technologies and obtains approval from the Ministry of the Environment. The selection criteria are as follows:

- a. Technological requirements:
 - The applied technology falls under the target verification technology field.
 - The application form is properly filled in.
 - The technology is at a commercialization stage.
- b. Possibility of verification
 - It is possible to complete the verification from cost and organizational standpoints.
 - It is possible to establish a suitable Test Plan.
 - The technology provides basic performance and has a practical use.
- c. Environmental protective effect, etc.
 - It is possible to scientifically explain the principle and mechanism of the technology.
 - There is no possibility that the technology causes environmental side effects.
 - It provides a high environmental protective effect.
 - It is an innovative technology.

IV. Preparation for the verification tests

1. Points to check in a verification test

The following points shall be checked in a verification test: reliability, practicability, and ease of operation.

Table 2 Points to check in a verification test

Point to check	Description
Reliability	Determine whether or not the target verification product provides reliable measurement of chemicals in the general environment.
Practicability	Examine the product specifications or measurement performance to determine whether or not the target verification product can be used for general environmental monitoring.
Ease of operation	Examine whether or not the product specifications or operational procedures are simpler and easier than conventional measurement technologies.

2. Establishment of the Test Plan

The Verification Organizations establish the Test Plan based on information provided by the environmental technology developers and the advice of the Technology Panel. The items to be included in the Test Plan are listed in Appendix 2.

3. Distribution of test expenses

The cost of measuring the environmental effects of the target technology and other costs shall be borne by the Ministry of the Environment. As a rule, the verification applicant shall provide as many sets of the target verification product as required to the Verification Organization.

4. Exclusion

Participants involved in this pilot project disclaim legal responsibility as follows:

- The verification applicant shall be held liable for any losses or damage incurred by a third party resulting from defects in the product, except in cases of intentional wrongdoing or gross negligence by the third party. The Ministry of the Environment, the Verification Organization, the organization operating the database, and other parties participating in the pilot project shall not be held liable.
- The Ministry of the Environment, the Verification Organization, the organization operating the database, and other parties participating in the pilot project shall not be held liable for any disputes arising between the verification applicant and a third party as a result of the public release of the Verification Report.
- If changes are made to the specifications of the product based on the target technology, data contained in the Verification Report does no longer apply to the product after such changes.

V. Verification test method

1. Chemicals to be monitored

This verification test assumes that target verification technologies monitor chemicals that have social significance, present a pressing problem, or need monitoring by some means or other, and will be used for general environmental monitoring. Based on this assumption, chemicals to be monitored in the verification test shall be selected from the following:

- Chemical substances designated under the Law Concerning Reporting, etc. of Releases into the Environment of Specific Chemical Substances and Promoting Improvements in Their Management (PRTR Law)
- Suspected endocrine-disturbing chemicals, etc.

Where possible, select chemicals that can be monitored by instrumental analyses too. In addition, two or more companies should provide similar products to monitor the chemicals.

2. Details of verification items

In a verification test, verification items regarding the basic performance and practical performance of a target verification product based on the ELISA shall be evaluated.

Basic performance is important in determining whether or not the specifications of the target verification product presented by the environmental technology developer are reliable. Practical performance is also important in determining whether or not the target verification product can be used for general environmental monitoring in light of foreign substances contained in environmental samples.

The term “plate” used in the following sections refers to a microplate. If a product of a different type, such as a tube, is used, replace the term with an appropriate one when reading the following sections.

Table 3 Points to check and methods for verification items

Item	Parameter	Point to check			Method	
		Reliability	Practicability	Ease of operation	Documents	Test
1. Basic performance						
(1) Measurement range	Correlation, etc.					(S1)
(2) Lower detection and determination limits	Deviation, etc.					(S1)
(3) Repeatability	Variation, etc.					(S1)
(4) Day-to-day reproducibility	Variation, etc.					(S1)
(5) Period-to-period reproducibility	Variation, etc.					(S1)
(6) Plate-to-plate reproducibility	Variation, etc.					(S1)
(7) Cross-reactivity	Cross-reaction, etc.					(S1)
2. Practical performance						
(1) Recovery characteristics	Variation, etc.					(S2)
(2) Measurement accuracy, etc.	Correlation, etc.					(S3) *

Note: A double circle (⊙) in the Method column indicates that significance is increased at the stage of discussion, and an asterisk (*) indicates that instrumental analyses are conducted at the same time.

S1 and S2 indicate that samples prepared from commercial standard products that represent chemicals to be monitored or chemicals similar to them are used. S3 indicates that actual samples in the general environment, such as river water, are used.

Table 4 Description of verification items

Item	Description
1. Basic performance	
(1) Measurement range	Based on presented documents, variations in measurements obtained by the ELISA using test samples (known concentrations) prepared from commercial standard products, and other parameters, verify numerical settings.
(2) Lower detection and determination limits	Based on presented documents, the standard deviations of measurements obtained by the ELISA repeated under the same conditions using test samples (known concentrations) prepared from commercial standard products, and other parameters, verify numerical settings.
(3) Repeatability	Based on presented documents, variations in measurements obtained by the ELISA repeated under the same conditions using test samples (known concentrations) prepared from commercial standard products, and other parameters, verify reproducibility.
(4) Day-to-day reproducibility	Based on presented documents, variations in measurements obtained by the ELISA repeated under different conditions (on different dates) using test samples (known concentrations) prepared from commercial standard products, and other parameters, verify reproducibility.
(5) Period-to-period reproducibility	Based on presented documents, variations in measurements obtained by the ELISA using test samples (known concentrations) prepared from commercial standard products with a product produced a certain period ago, and other parameters, verify reproducibility.
(6) Plate-to-plate reproducibility	Based on presented documents, variations in measurements obtained by the ELISA using test samples (known concentrations) prepared from commercial standard products and different plates with several sets of the target verification product produced in different lots, and other parameters, verify reproducibility.
(7) Cross-reactivity	Based on presented documents, differences between similar but different chemicals in measurements obtained by the ELISA using test samples (known concentrations) prepared from commercial standard products, and other parameters, examine cross-reactivity.
2. Practical performance	
(1) Recovery characteristics	Based on presented documents, comparison between measurements obtained by the ELISA using test samples (known concentrations) prepared by mixing commercial standard products to imitate environmental samples, and other parameters, examine recovery characteristics.
(2) Measurement accuracy	Based on variations in measurements obtained by the ELISA using environmental samples (unknown concentrations), the features of operational procedures, and other parameters, determine the applicability of the target verification product to the monitoring of environmental samples from the measurement accuracy, the adequacy of preprocessing, ease of operation, and other factors.

3. Details of a verification test

In a verification test, the basic performance and practical performance of a target verification product shall be examined.

A Verification Organization shall prepare a detailed Test Plan based on the target verification product and the application form and other documents presented with the product, and conduct a verification test with the approval of the Technology Panel.

Details of items to be verified in a verification test are described below:

(1) Basic performance

In order to examine the basic performance of a target verification product, the reliability of the product specifications and other factors shall be tested using standard samples.

Detailed methods for testing verification items are described below. A Verification Organization may test additional verification items for a more effective verification test with the approval of the Technology Panel.

i. Measurement range

Prepare test sample solutions with adjusted concentrations from commercial standard products (standard samples) that represent the chemicals to be monitored, and use the target verification product to assay the test sample solutions in the same plate at the same time. Use a standard curve prepared according to the method in the instruction manual to calculate the concentrations of the test sample solutions (observed concentrations).

By comparing the adjusted concentrations of the test sample solutions with the observed concentrations by the target verification product, and determining the variation coefficient of the observed concentrations, examine variations in measurements and other parameters.

This test requires a single kit of the target verification product.

Follow the steps below to conduct this test with standard samples:

a. Prepare standard solutions from which a standard curve is prepared

Prepare standard solutions from which a standard curve is prepared using the accessories included with the product according to the instruction manual. For example, an undiluted solution of a standard product may come with the product. In that case, dilute the undiluted solution with a solution specified in the instruction manual, such as a buffer solution included with the product, in stages to prepare a dilution series of standard solutions from which a standard curve is prepared.

Prepare as many standard solutions with different dilution ratios as specified in the instruction manual.

b. Prepare test sample solutions

Prepare test sample solutions from commercial standard products (standard samples) obtained in advance. Dilute the standard samples in stages in the measurement range of the product.

Use a solution specified in the instruction manual, such as a buffer solution included with the product, to dilute them.

Prepare as many types of test sample solutions as the number of standard solutions with different dilution ratios required to prepare a standard curve, which is specified in the instruction manual.

c. Operate the product

Use the target verification product to assay the standard solutions from which a standard curve is prepared for the number of times specified in the instruction manual. At the same time, assay the test sample solutions with adjusted (known) concentrations in the same plate (triple or denser measurement).

Operate the product according to the instruction manual.

d. Measure absorbance

After chromogenic reaction, use a microplate reader to measure the absorbance of the test sample solutions. Record the mean value of the measurements for the test sample solutions as their absorbance.

e. Prepare a standard curve

Prepare a standard curve based on the concentrations of the standard solutions from which a standard curve is prepared and the corresponding absorbance, according to the instruction manual.

If a software product is used to prepare a standard curve, give the name of the software.

f. Calculate observed concentrations

Calculate the concentrations (observed concentrations) of the test sample solutions from the absorbance and the standard curve according to the instruction manual.

g. Summarize the measurement range

Summarize the standard deviation, the coefficient of variation ($CV = \text{standard deviation}/\text{mean} \times 100$), and other parameters from the observed concentrations of the test sample solutions.

h. Discussion

Verify the measurement range of the target verification product from the coefficient of variation and other parameters obtained in these steps.

ii. Lower detection and determination limits

Prepare test sample solutions with adjusted concentrations around the lower limit of the measurement range of the product from commercial standard products (standard samples) that represent the chemicals to be monitored, and use the target verification product to assay the test sample solutions repeatedly in the same plate at the same time. Use a standard curve prepared according to the method in the instruction manual to calculate the concentrations of the test sample solutions (observed concentrations).

Determine the lower detection and determination limits of the target verification product from the standard deviation of the observed concentrations.

This test requires a single kit of the target verification product.

Follow the steps below to conduct this test with standard samples:

a. Prepare standard solutions from which a standard curve is prepared

Prepare standard solutions from which a standard curve is prepared using the accessories included with the product according to the instruction manual. For example, an undiluted solution of a standard product may come with the product. In that case, dilute the undiluted solution with a solution specified in the instruction manual, such as a buffer solution included with the product, in stages to prepare a dilution series of standard solutions from which a standard curve is prepared.

Prepare as many standard solutions with different dilution ratios as specified in the instruction manual.

b. Prepare test sample solutions

Prepare test sample solutions from commercial standard products (standard samples) obtained in advance. Dilute the standard samples to around the lower limit of the measurement range of the product.

Use a solution specified in the instruction manual, such as a buffer solution included with the product, to dilute them.

c. Operate the product

Use the target verification product to assay the standard solutions from which a standard curve is prepared for the number of times specified in the instruction manual. At the same time, assay the test sample solutions with adjusted (known) concentrations in the same plate (8 or more times).

Operate the product according to the instruction manual.

d. Measure absorbance

After chromogenic reaction, use a microplate reader to measure the absorbance of the test sample solutions. Record the mean value of the measurements for the test sample solutions as their absorbance.

e. Prepare a standard curve

Prepare a standard curve based on the concentrations of the standard solutions from which a standard curve is prepared and the corresponding absorbance, according to the instruction manual.

If a software product is used to prepare a standard curve, give the name of the software.

f. Calculate observed concentrations

Calculate the concentrations (observed concentrations) of the test sample solutions from the absorbance and the standard curve according to the instruction manual.

g. Summarize the lower detection and determination limits

Summarize the standard deviation from the observed concentrations of the test sample solutions with the same adjusted concentration.

h. Discussion

Verify the lower detection and determination limits of the target verification product from the standard deviation obtained in these steps.

iii. Repeatability

Prepare test sample solutions with adjusted concentrations from commercial standard products (standard samples) that represent the chemicals to be monitored, and use the target verification product to assay the test sample solutions repeatedly under the same conditions at the same time. Use a standard curve prepared according to the method in the instruction manual to calculate the concentrations of the test sample solutions (observed concentrations).

By comparing the adjusted concentrations of the test sample solutions with the observed concentrations by the target verification product, and determining the variation coefficient of the observed concentrations, examine variations in measurements and other parameters.

This test requires a single kit of the target verification product.

Follow the steps below to conduct this test with standard samples:

a. Prepare standard solutions from which a standard curve is prepared

Prepare standard solutions from which a standard curve is prepared using the accessories included with the product according to the instruction manual. For example, an undiluted solution of a standard product may come with the product. In that case, dilute the undiluted solution with a solution specified in the instruction manual, such as a buffer solution included with the product, in stages to prepare a dilution series of standard solutions from which a standard curve is prepared.

Prepare as many standard solutions with different dilution ratios as specified in the instruction manual.

b. Prepare test sample solutions

Prepare test sample solutions from commercial standard products (standard samples) obtained in advance. Dilute the standard samples in a linear area of the measurement range of the product.

Use a solution specified in the instruction manual, such as a buffer solution included with the product, to dilute them.

c. Operate the product

Use the target verification product to assay the standard solutions from which a standard curve is prepared for the number of times specified in the instruction manual. At the same time, assay the test sample solutions with adjusted (known) concentrations in the same plate (triple or denser measurement, 8 or more times).

Operate the product according to the instruction manual.

d. Measure absorbance

After chromogenic reaction, use a microplate reader to measure the absorbance of the test sample solutions. Record the mean value of the measurements for the test sample solutions as their absorbance.

e. Prepare a standard curve

Prepare a standard curve based on the concentrations of the standard solutions from which a standard curve is prepared and the corresponding absorbance, according to the instruction manual.

If a software product is used to prepare a standard curve, give the name of the software.

f. Calculate observed concentrations

Calculate the concentrations (observed concentrations) of the test sample solutions from the absorbance and the standard curve according to the instruction manual.

g. Summarize repeatability

Summarize the standard deviation, the coefficient of variation ($CV = \text{standard deviation}/\text{mean} \times 100$), and other parameters from the observed concentrations of the test sample solutions with the same adjusted concentration.

h. Discussion

Verify the repeatability of the target verification product from the coefficient of variation and other parameters obtained in these steps.

iv. Day-to-day reproducibility

Prepare test sample solutions with adjusted concentrations from commercial standard products (standard samples) that represent the chemicals to be monitored, and use 3 or more sets of the target verification product produced in the same lot to assay the test sample solutions on different dates and times. Use a standard curve prepared according to the method in the instruction manual to calculate the concentrations of the test sample solutions (observed concentrations).

By comparing the adjusted concentrations of the test sample solutions with the observed concentrations by the target verification product, and determining the variation coefficient of the observed concentrations, examine variations in measurements and other parameters.

This test requires 3 or more kits of the target verification product.

Follow the steps below to conduct this test with standard samples:

a. Prepare standard solutions from which a standard curve is prepared

Prepare standard solutions from which a standard curve is prepared using the accessories included with the product according to the instruction manual. For example, an undiluted solution of a standard product may come with the product. In that case, dilute the undiluted solution with a solution specified in the instruction manual, such as a buffer solution included with the product, in stages to prepare a dilution series of standard solutions from which a standard curve is prepared.

Prepare as many standard solutions with different dilution ratios as specified in the

instruction manual.

b. Prepare test sample solutions

Prepare test sample solutions from commercial standard products (standard samples) obtained in advance. Dilute the standard samples in stages in the measurement range of the product.

Use a solution specified in the instruction manual, such as a buffer solution included with the product, to dilute them.

Prepare as many types of test sample solutions as the number of standard solutions with different dilution ratios required to prepare a standard curve, which is specified in the instruction manual.

c. Operate the product

Use the target verification product to assay the standard solutions from which a standard curve is prepared for the number of times specified in the instruction manual. At the same time, assay the test sample solutions with adjusted (known) concentrations in different plates on different dates and times (triple or denser measurement).

Operate the product according to the instruction manual.

d. Measure absorbance

After chromogenic reaction, use a microplate reader to measure the absorbance of the test sample solutions. Record the mean value of the measurements for the test sample solutions as their absorbance.

e. Prepare a standard curve

Prepare a standard curve based on the concentrations of the standard solutions from which a standard curve is prepared and the corresponding absorbance, according to the instruction manual.

If a software product is used to prepare a standard curve, give the name of the software.

f. Calculate observed concentrations

Calculate the concentrations (observed concentrations) of the test sample solutions from the absorbance and the standard curve according to the instruction manual.

g. Summarize day-to-day reproducibility

Summarize the standard deviation, the coefficient of variation ($CV = \text{standard deviation}/\text{mean} \times 100$), and other parameters from the observed concentrations of the test sample solutions with the same adjusted concentration.

h. Discussion

Verify the day-to-day reproducibility of the target verification product from the coefficient of variation and other parameters obtained in these steps.

v. Period-to-period reproducibility

Prepare test sample solutions with adjusted concentrations from commercial standard products (standard samples) that represent the chemicals to be monitored, and use the target verification product produced several months ago to assay the test sample solutions at the same time. Use a standard curve prepared according to the method in the instruction manual to calculate the concentrations of the test sample solutions (observed concentrations).

By comparing the adjusted concentrations of the test sample solutions with the observed concentrations by the target verification product, and determining the variation coefficient of the observed concentrations, examine variations in measurements and other parameters.

This test requires a single kit of the target verification product.

Follow the steps below to conduct this test with standard samples:

a. Prepare standard solutions from which a standard curve is prepared

Prepare standard solutions from which a standard curve is prepared using the accessories included with the product according to the instruction manual. For example, an undiluted solution of a standard product may come with the product. In that case, dilute the undiluted solution with a solution specified in the instruction manual, such as a buffer solution included with the product, in stages to prepare a dilution series of standard solutions from which a standard curve is prepared.

Prepare as many standard solutions with different dilution ratios as specified in the instruction manual.

b. Prepare test sample solutions

Prepare test sample solutions from commercial standard products (standard samples) obtained in advance. Dilute the standard samples in stages in the measurement range of the product.

Use a solution specified in the instruction manual, such as a buffer solution included with the product, to dilute them.

Prepare as many types of test sample solutions as the number of standard solutions with different dilution ratios required to prepare a standard curve, which is specified in the instruction manual.

c. Operate the product

Use the target verification product to assay the standard solutions from which a standard curve is prepared for the number of times specified in the instruction manual. At the same time, assay the test sample solutions with adjusted (known) concentrations (triple or denser measurement).

Operate the product according to the instruction manual.

d. Measure absorbance

After chromogenic reaction, use a microplate reader to measure the absorbance of the test sample solutions. Record the mean value of the measurements for the test sample solutions as their absorbance.

e. Prepare a standard curve

Prepare a standard curve based on the concentrations of the standard solutions from which a standard curve is prepared and the corresponding absorbance, according to the instruction manual.

If a software product is used to prepare a standard curve, give the name of the software.

f. Calculate observed concentrations

Calculate the concentrations (observed concentrations) of the test sample solutions from the absorbance and the standard curve according to the instruction manual.

g. Summarize period-to-period reproducibility

Summarize the standard deviation, the coefficient of variation ($CV = \text{standard deviation}/\text{mean} \times 100$), and other parameters from the observed concentrations of the test sample solutions with the same adjusted concentration.

h. Discussion

Verify the period-to-period reproducibility of the target verification product from the coefficient of variation and other parameters obtained in these steps.

vi. Plate-to-plate reproducibility

Prepare test sample solutions with adjusted concentrations from commercial standard products (standard samples) that represent the chemicals to be monitored, and use 2 or more sets of the target verification product produced in the same lot and another 1 or more sets of the product produced in a different lot (a total of 3 or more sets) to assay the test sample solutions repeatedly in different plates at the same time. Use a standard curve prepared

according to the method in the instruction manual to calculate the concentrations of the test sample solutions (observed concentrations).

By comparing the adjusted concentrations of the test sample solutions with the observed concentrations by the target verification product, and determining the variation coefficient of the observed concentrations, examine variations in measurements and other parameters.

This test requires 2 or more kits of the target verification product produced in the same lot and another 1 or more kits of the product produced in a different lot.

Follow the steps below to conduct this test with standard samples:

a. Prepare standard solutions from which a standard curve is prepared

Prepare standard solutions from which a standard curve is prepared using the accessories included with the product according to the instruction manual. For example, an undiluted solution of a standard product may come with the product. In that case, dilute the undiluted solution with a solution specified in the instruction manual, such as a buffer solution included with the product, in stages to prepare a dilution series of standard solutions from which a standard curve is prepared.

Prepare as many standard solutions with different dilution ratios as specified in the instruction manual.

b. Prepare test sample solutions

Prepare test sample solutions from commercial standard products (standard samples) obtained in advance. Dilute the standard samples in stages in the measurement range of the product.

Use a solution specified in the instruction manual, such as a buffer solution included with the product, to dilute them.

Prepare as many types of test sample solutions as the number of standard solutions with different dilution ratios required to prepare a standard curve, which is specified in the instruction manual.

c. Operate the product

Use the target verification product to assay the standard solutions from which a standard curve is prepared for the number of times specified in the instruction manual. At the same time, assay the test sample solutions with adjusted (known) concentrations in different plates (triple or denser measurement).

Operate the product according to the instruction manual.

d. Measure absorbance

After chromogenic reaction, use a microplate reader to measure the absorbance of the test sample solutions. Record the mean value of the measurements for the test sample solutions as their absorbance.

e. Prepare a standard curve

Prepare a standard curve based on the concentrations of the standard solutions from which a standard curve is prepared and the corresponding absorbance, according to the instruction manual.

If a software product is used to prepare a standard curve, give the name of the software.

f. Calculate observed concentrations

Calculate the concentrations (observed concentrations) of the test sample solutions from the absorbance and the standard curve according to the instruction manual.

g. Summarize plate-to-plate reproducibility

Summarize the standard deviation, the coefficient of variation ($CV = \text{standard deviation}/\text{mean} \times 100$), and other parameters from the observed concentrations of the test sample solutions with the same adjusted concentration.

h. Discussion

Verify the plate-to-plate reproducibility of the target verification product from the coefficient of variation and other parameters obtained in these steps.

vii. Cross-reactivity

Prepare test sample solutions with adjusted concentrations from commercial standard products (standard samples) that represent the chemicals to be monitored and chemicals similar to them, use the target verification product to assay the test sample solutions, and then prepare concentration-response curves, etc.

Examine the cross rates for the similar chemicals relative to the chemicals to be monitored based on IC50 and other parameters of the test sample solutions with different concentrations determined by the target verification product.

This test requires a single kit of the target verification product.

Follow the steps below to conduct this test with standard samples:

a. Prepare standard solutions from which a standard curve is prepared

Prepare standard solutions from which a standard curve is prepared using the accessories included with the product according to the instruction manual. For example, an undiluted solution of a standard product may come with the product. In that case, dilute the undiluted solution with a solution specified in the instruction manual, such as a buffer solution included with the product, in stages to prepare a dilution series of standard solutions from which a standard curve is prepared.

Prepare as many standard solutions with different dilution ratios as specified in the instruction manual.

b. Prepare test sample solutions

Prepare test sample solutions from commercial standard products (standard samples) that represent the chemicals to be monitored and chemicals similar to them (1 or more types), which are to be obtained in advance. Dilute the standard samples in stages in the measurement range of the product. However, if high cross-reactivity is shown, change the dilution ratios. Select chemicals similar to those to be monitored in light of their characteristics. Existing reports of environmental research may help you select appropriate chemicals.

Use a solution specified in the instruction manual, such as a buffer solution included with the product, to dilute them.

Prepare as many types of test sample solutions as the number of standard solutions with different dilution ratios required to prepare a standard curve, which is specified in the instruction manual.

c. Operate the product

Use the target verification product to assay the standard solutions from which a standard curve is prepared for the number of times specified in the instruction manual. At the same time, assay the test sample solutions with adjusted (known) concentrations (triple or denser measurement).

Operate the product according to the instruction manual.

d. Measure absorbance

After chromogenic reaction, use a microplate reader to measure the absorbance of the test sample solutions. Record the mean value of the measurements for the test sample solutions as their absorbance.

e. Prepare a standard curve

Prepare a standard curve based on the concentrations of the standard solutions from which a standard curve is prepared and the corresponding absorbance, according to the instruction

manual.

If a software product is used to prepare a standard curve, give the name of the software.

f. Calculate observed concentrations

Calculate the concentrations (observed concentrations) of the test sample solutions from the absorbance and the standard curve according to the instruction manual.

g. Summarize cross-reactivity

Summarize the cross rates for the similar chemicals relative to the chemicals to be monitored from IC50 and other parameters obtained for the different types of test sample solutions.

h. Discussion

Verify the cross-reactivity of the target verification product from the cross rates and other parameters obtained in these steps.

(2) Practical performance

In order to examine the practical performance of a target verification product, the applicability of the product to monitoring of environmental samples and other factors shall be tested with use of environmental samples.

Detailed methods for testing verification items are described below. A Verification Organization may test additional verification items for a more effective verification test with the approval of the Technology Panel.

I. Recovery characteristics

Prepare test sample solutions from environmental samples (simulated) prepared by mixing commercial standard products that represent chemicals to monitor and chemicals similar to them, and use the target verification product to assay the test sample solutions. Use a standard curve prepared according to the method in the instruction manual to calculate the concentrations of the test sample solutions (observed concentrations).

By comparing the adjusted concentrations of the test sample solutions with the observed concentrations by the target verification product, examine variations in measurements and other parameters.

This test requires a single kit of the target verification product.

Follow the steps below to conduct this test with standard samples:

a. Prepare standard solutions from which a standard curve is prepared

Prepare standard solutions from which a standard curve is prepared with use of accessories included with the product according to the instruction manual. For example, an undiluted solution of a standard product may come with the product. In that case, dilute the undiluted solution with a solution specified in the instruction manual, such as a buffer solution included with the product, in stages to prepare a dilution series of standard solutions from which a standard curve is prepared.

Prepare as many standard solutions with different dilution ratios as specified in the instruction manual.

b. Prepare test sample solutions

Prepare test sample solutions from environmental samples (simulated) prepared in advance by mixing commercial standard products that represent chemicals to monitor and chemicals similar to them. Dilute the environmental samples to around the middle of the measurement range of the product. Select chemicals similar to those to monitor in light of their characteristics. Existing reports of environmental research may help you select appropriate chemicals.

Use a solution specified in the instruction manual, such as a buffer solution included with the

product, to dilute them.

c. Operate the product

Use the target verification product to assay the standard solutions from which a standard curve is prepared for the number of times specified in the instruction manual. At the same time, assay the test sample solutions with adjusted (known) concentrations (triple or denser measurement).

Operate the product according to the instruction manual.

d. Measure absorbance

After chromogenic reaction, use a microplate reader to measure the absorbance of the test sample solutions. Record the mean value of the measurements for the test sample solutions as their absorbance.

e. Prepare a standard curve

Prepare a standard curve based on the concentrations of the standard solutions from which a standard curve is prepared and the corresponding absorbance, according to the instruction manual.

If a software product is used to prepare a standard curve, give the name of the software.

f. Calculate observed concentrations

Calculate the concentrations (observed concentrations) of the test sample solutions from the absorbance and the standard curve according to the instruction manual.

g. Summarize recovery characteristics

Compare the observed concentrations of the test sample solutions and the adjusted concentrations of the chemicals to monitor and summarize the results.

h. Discussion

Verify the recovery characteristics of the target verification product from the comparison of the concentrations obtained in these steps.

ii. Measurement accuracy, etc.

Use the target verification product to assay chemicals to monitor in samples obtained from environmental media, such as river water, and use a standard curve prepared according to the method in the instruction manual to calculate the concentrations of the test sample solutions (observed concentrations).

Use samples cleaned up by two different methods: a preprocessing method specified in the product specifications presented by the environmental technology developer and a preprocessing method generally employed in instrumental analyses.

This test requires a single kit of the target verification product.

Follow the steps below to conduct this test with environmental samples:

a. Prepare standard solutions from which a standard curve is prepared

Prepare standard solutions from which a standard curve is prepared with use of accessories included with the product according to the instruction manual. For example, an undiluted solution of a standard product may come with the product. In that case, dilute the undiluted solution with a solution specified in the instruction manual, such as a buffer solution included with the product, in stages to prepare a dilution series of standard solutions from which a standard curve is prepared.

Prepare as many standard solutions with different dilution ratios as specified in the instruction manual.

b. Prepare test sample solutions

- (a) Prepare environmental samples

Use a sampling method specified by the Ministry of the Environment to obtain samples from environmental media. Select a sampling area where substances contained are different in quality and quantity from one location to another and that reflects various contamination characteristics, such as a river.

For example, river water can be collected from several locations believed to contain chemicals to monitor in different concentrations, or from those that reflect the contamination characteristics of domestic wastewater (organic systems), industrial drainage (inorganic systems), agricultural drainage (agricultural chemicals), and other drainage sources. Existing reports of environmental research may help you select appropriate locations.

(b) Preprocess environmental samples

Preprocess the environmental samples by the following two methods:

- Method 1: Preprocessing presented (recommended) by the environmental technology developer
- Method 2: Preprocessing generally employed in instrumental analyses

(c) Prepare test sample solutions

Use the samples preprocessed by the above two methods as test sample solutions.

Use a solution specified in the instruction manual, such as a buffer solution included with the product, to dilute them.

In addition, consider preparing samples mixed with commercial standard products that represent chemicals to monitor.

c. Operate the product

Use the target verification product to assay the standard solutions from which a standard curve is prepared for the number of times specified in the instruction manual. At the same time, assay the test sample solutions preprocessed by method 1 (triple or denser measurement).

Operate the product according to the instruction manual.

Make an instrumental analysis of the test sample solutions preprocessed by method 2 for reference.

d. Measure absorbance

After chromogenic reaction, use a microplate reader to measure the absorbance of the test sample solutions. Record the mean value of the measurements for the test sample solutions as their absorbance.

e. Prepare a standard curve

Prepare a standard curve based on the concentrations of the standard solutions from which a standard curve is prepared and the corresponding absorbance, according to the instruction manual.

If a software product is used to prepare a standard curve, give the name of the software.

f. Calculate observed concentrations

Calculate the concentrations (observed concentrations) of the test sample solutions from the absorbance and the standard curve according to the instruction manual. Also calculate the observed concentrations of the test sample solutions by the instrumental analysis.

g. Summarize measurement accuracy, etc.

Compare the observed concentrations of the test sample solutions by the target verification product and by the instrumental analysis and summarize the results.

h. Discussion

Compare the concentrations obtained in the test and examine the general operability. Discuss the following to determine the reliability, practicability, and ease of operation of the target verification product:

- Measurement accuracy (of concentrations) indicated by differences with the values obtained by the instrumental analysis
- Adequacy of preprocessing for the different contamination characteristics of samples (effects of foreign substances)
- Ease of operation throughout all the processes (required time and number of operations)
- Applicability of the product to monitoring of environmental samples shown by the measurement results

(3) Summary

Test procedures for items to verify in a verification test are summarized in the following tables:

Table 5 Overview of procedures for testing verification items (1/3)

Verification item/test procedure		1. Basic performance				
		(i) Measurement range	(ii) Lower detection and determination limits	(iii) Repeatability	(iv) Day-to-day reproducibility	
Test with standard samples	Standard solutions from which a standard curve is prepared	Reagent	Standard product included with the product			
		Number of solutions with different dilution ratios*	Number specified in the instruction manual			
	Test sample solutions	Reagent	Standard sample (single commercial standard product)			
		Material ^A	Chemical to be monitored (1)			
		Diluent solvent	Solution specified in the instruction manual			
		Concentrations	Dilute in stages in the measurement range	Dilute to around the lower limit of the measurement range	Dilute in a linear area of the measurement range	Dilute in stages in the measurement range
		Number of solutions ^B	More than 1 (same as *)	1	1	More than 1 (same as *)
	Operate the product	Timing	Simultaneously	Simultaneously	Simultaneously	More than 1 (same as *)
		Plate/lot	Single plate	Single plate	Single plate	Same lot, different plates
		Number of kits required ^C	1	1	1	3
	Measure absorbance		Mean value (triple or denser measurement)	Individual measurements	Mean value (triple or denser measurement)	
	Prepare a standard curve		Prepare from the concentrations and absorbance of standard solutions from which a standard curve is prepared			
	Instrumental analysis					
	Calculate and summarize observed concentrations by the ELISA	Number of calculations	Same as *	Same as the number of measurements	Same as the number of measurements	Same as *
		Parameter	Standard deviation Coefficient of variation	Standard deviation	Standard deviation Coefficient of variation	Standard deviation Coefficient of variation
Discussion		Validity of the measurement range	Validity of the lower detection and determination limits	Validity of repeatability	Validity of day-to-day reproducibility	

Note: Number of observed concentrations = A × B × C

Some verification items can be tested simultaneously in the same plate ((i), (iv), (vi)).

Table 6 Overview of procedures for testing verification items (2/3)

Verification item/test procedure		1. Basic performance			
		(v) Period-to-period reproducibility	(vi) Plate-to-plate reproducibility	(vii) Cross-reactivity	
Test with standard samples	Standard solutions from which a standard curve is prepared	Reagent	Standard product included with the product		
		Number of solutions with different dilution ratios*	Number specified in the instruction manual		
	Test sample solutions	Reagent	Standard sample (single commercial standard product)		Standard sample (more than one commercial standard product)
		Material ^A	Chemical to be monitored (1)		Chemical to be monitored (1) Similar chemical (1 or more)
		Diluent solvent	Solution specified in the instruction manual		
		Concentrations	Dilute in stages in the measurement	Dilute in stages in the measurement range	Dilute to around the middle of the
		Number of solutions ^B	More than 1 (same as *)	More than 1 (same as *)	More than 1 (same as the number)
	Operate the product	Timing	Simultaneously	Simultaneously	Simultaneously
		Plate/lot	Single plate	Same lot, different plates Different lots,	Single plate
		Number of kits required ^C	1	3	1
	Measure absorbance		Mean value (triple or denser measurement)		
	Prepare a standard curve		Prepare from the concentrations and absorbance of standard solutions from which a standard curve is prepared		
	Instrumental analysis		-	-	-
	Calculate and summarize observed concentrations by the ELISA	Number of calculations	Same as *	Same as *	Same as the number of chemicals
		Parameter	Standard deviation Coefficient of variation	Standard deviation Coefficient of variation	Cross rate
	Discussion		Validity of period-to-period reproducibility	Validity of plate-to-plate reproducibility	Validity of cross-reactivity

Note: Number of observed concentrations = A × B × C

Some verification items can be tested simultaneously in the same plate ((i), (iv), (vi)).

Table 7 Overview of procedures for testing verification items (3/3)

Verification item/test procedure		2. Practical performance			
		(i) Recovery characteristics	(ii) Measurement accuracy, etc.		
Test with environmental samples	Standard solutions from which a standard curve is prepared	Reagent	Standard product included with the product		
		Number of solutions with different dilution	Number specified in the instruction manual		
	Test sample solutions	Reagent	Environmental sample (simulated) (more than one commercial standard product)	Environmental sample	
		Material ^A	Mixture of a chemical to be monitored (1) and a similar chemical(s) (1 or more)	Chemicals that reflect different contamination characteristics	
		Diluent solvent	Solution specified in the instruction manual		
		Concentrations	Mix commercial standard products that simulate an actual environment	Actual environmental sample	
		Number of solutions ^B	1 or more	More than 1 (same as the number of	
	Operate the product	Timing	Simultaneously	Simultaneously	
		Plate/lot	Single plate	Single plate	
		Number of kits required ^C	1	1	
	Measure absorbance		Mean value (triple or denser measurement)		
	Prepare a standard curve		Prepare from the concentrations and absorbance of standard solutions from which a standard curve is prepared		
	Instrumental analysis		-	Conduct in parallel	
	Calculate and summarize observed concentrations by the ELISA	Number of calculations	1 or more	Same as the number of samples	
		Parameter	Comparison with adjusted concentrations	Comparison with measurements by the instrumental analysis General operability, etc.	
	Discussion		Validity of recovery characteristics	Measurement accuracy, validity of preprocessing, ease of operation, etc.	

Note: Number of observed concentrations = A × B × C

VI. Preparation of the Verification Report

The results obtained in the verification test shall be reported in the Verification Report. All data, including the results of the verification test and problems with verification items arising during the test period, shall be described in the Verification Report.

The Verification Report shall contain the following:

- Executive summary
- Basic principle
- Identification and description of the target verification technology and product (including capacity)
- Manufacturer of product (name, address, TEL) and serial number
- Verification test system (Verification Organization and Test Site)
- Methods for testing verification items
- Test results of verification items (Data shall be shown in tables and graphs.)
- Discussion of verification test results
- Other reference materials (Test Plan)

The Verification Organization prepares a draft of the Verification Report and, after obtaining the consent of the environmental technology developer concerning the description and discussions by the Technology Panel, finalizes the Verification Report.

The Verification Report submitted to the Ministry of the Environment shall be discussed by the working group and approved by the Ministry of the Environment. In addition, the Verification Organization shall prepare a brief summary of the verification test results with reference to Appendix 3.

VII. Remarks in conducting the verification test

In conducting a verification test, the following shall be kept in mind:

- Apparatuses periodically calibrated to provide adequate measurement accuracy shall be used for a verification test.
- Verification Organizations shall prepare Test Plans based on the latest information on verification items.
- Based on the idea that data on verification items must be absolutely correct and reliable, Verification Organizations shall control the accuracy of all test processes from sampling to analytical processes and summarization of test results to avoid errors or variations in data on verification items resulting from various factors such as measurers, storage conditions of samples, reagents, and analysis environments.

Appendix 0: Quality management system to be constructed at the Verification Organizations

Introduction

The Verification Organizations participating in the pilot project for the environmental technology verification should desirably construct the quality management system in accordance with JIS Q 17025:2000 (ISO/IEC 17025:1999) "General requirements for the competence of testing and calibration laboratories." In this Appendix, some elements of the quality management system that are required to be constructed at Verification Organizations that do not have such a quality management system in accordance with the above standard will be described.

1. Scope

The quality management system specified in this Appendix is applicable to all departments or procedures relevant to the verification test in the Verification Organization. In addition, if part of the verification test is subcontracted to an external organization, that organization is also included in the scope of application.

The Verification Organization in which all departments relevant to the verification test have already received the following certification, JIS Q 17025:2000 (General requirements for the competence of testing and calibration laboratories) or JIS Q 9001:2000 (Quality management systems - Requirements), will be regarded as satisfying the requirements specified in this Appendix.

2. References

JIS Q 17025:2000 (ISO/IEC 17025:1999) General requirements for the competence of testing and calibration laboratories

JIS Q 9001:2000 (ISO 9001: 2000) Quality management systems – Requirements

3. Quality management system

(1) Organization and responsibility

The organization concerned shall be an entity that can be held legally responsible.

The responsibilities of key personnel in the organization relevant to the verification tests shall be clearly defined.

Appoint a member of the staff as a quality manager (however named) who, irrespective of his or her other duties and responsibilities, shall have defined responsibility and authority for ensuring that the quality system is implemented and followed at all times.

(2) Quality system

The organization concerned shall establish, implement, and maintain a quality management system appropriate to the scope of its activities regarding the verification test.

In the quality management system, the quality policy regarding the verification test and the procedures for the quality management system shall be documented. These documents shall be communicated to and understood by the appropriate personnel.

The policy shall include the following:

- a) The organization's commitment to ensuring the quality of verification tests
- b) The organization's statement on the quality standard of the verification tests
- c) The objectives of the quality system
- d) A description of the construction and implementation of the quality management system

In addition, the system for promoting verification tests, as well as the role, responsibility, and authority of the personnel concerned, shall be documented.

(3) Control of documents and records

The organization concerned shall control documents such as the standards regarding the verification tests (protocol for the verification and relevant standards) and the Test Plan, as well as drawings, software, specifications, written directives, and manuals.

With respect to document control, the following shall be ensured:

- a) All documents shall be reviewed and approved for use by authorized personnel prior to their issuance.
- b) All documents shall contain a description of the relevant documents to ensure that appropriate documents can be found easily and are available at any time at all Test Sites.
- c) Invalid and/or obsolete documents shall be promptly removed or be assuredly prevented from unintended use.
- d) The management method for documents as data shall be specified and maintained.
- e) The form for records and the location of documents, as well as the inspection method, shall be specified and maintained.

In addition, records regarding the verification tests shall be identified, properly collected, indexed, specified for usage, filed for applications, maintained, and adequately discharged, and the storage period for them shall be decided. In particular, records in the original copy of the test data, data and information that enable trace audits, records of calibrations, records of the persons involved, each individual report published, and copies of calibration certificates shall be stored for a predetermined period.

(4) Subcontracting of the tests

If the organization concerned subcontracts to perform the verification test, the organization shall select a competent external organization, and demand the same quality management as that of the Verification Organization.

(5) Purchase of goods and services

The organization concerned shall examine, by appropriate measures such as inspection, whether the goods and services purchased from external sources that may affect the quality of verification tests satisfy the requirements specified in the protocol for the verification, and shall not use them for the verification tests until this examination is completed.

In addition, the organization shall evaluate the suppliers of goods and services, and make a list of the approved suppliers.

(6) Control of complaints and nonconforming tests

The organization concerned shall have a system and method that shall be implemented when any of its verification tests or the results of these tests do not conform to the protocol for the verification or other specifications for any reason. The organization shall have a system and method for handling contingencies such as complaints from environmental technology developers, the inhibition of impartiality, information leaks, and others. These systems shall include the person in charge and personnel required for the handling of such cases.

(7) Corrective and preventive actions

When any of its verification tests or the results of these tests do not or may not conform to the protocol for the verification or other specifications, the organization concerned shall investigate the reasons and take corrective or preventive actions.

(8) Audit

The organization concerned shall conduct audits to judge whether the verification test has been properly conducted. When the verification test is subcontracted to an external organization, the operations of the subcontracted organization shall be audited.

The audit shall be conducted at least once during the test period. If the verification test lasts for 2 years or more, the audit shall be conducted periodically, and the frequency of the audit shall desirably be more than once per year.

In addition, the audit shall be conducted by personnel who are independent of the verification test to as great an extent as possible. The results of the audit shall be reported to the superintendent of the organization concerned.

4. Technical requirements

(1) Personnel

The organization concerned shall ensure the competence of all who operate specific equipment for the verification test, perform tests, evaluate results, and sign test reports. The personnel performing specific tasks shall be qualified on the basis of appropriate education, training, and/or demonstrated skills, as required.

(2) Accommodation and environmental conditions

The facilities for the verification test, including but not limited to energy sources, lighting, and environmental conditions, shall be such as to facilitate correct performance of the tests. The organization concerned shall ensure that the environmental conditions do not invalidate the results or adversely affect the required quality of any measurement. Particular care shall be taken when the verification test is undertaken at sites other than a permanent laboratory facility.

The organization concerned shall monitor, control, and record environmental conditions of the test in accordance with the protocol for the verification, the Test Plan, and other standards. Tests shall be stopped when the environmental conditions jeopardize the results of the tests.

(3) Test methods and method validation

The organization concerned shall use appropriate methods and procedures for all tests within its scope and determine the test methods in accordance with the protocol for the verification.

When the method to be used is not specified in the protocol for the verification, the organization concerned shall select either an appropriate method disclosed in international standards, regional or national standards, scientific texts, or the like, or a method specified by the manufacturer of the equipment. When it is necessary to use methods not covered by standard methods, these shall be subject to agreement with the verification applicant, and their validity shall be appropriately examined prior to use. Validation is the confirmation by examination that the requirements for a specific intended use are fulfilled. The validation shall be conducted based on discussion and subsequent approval by the Technology Panel.

When computers or automated equipment are used for data management, the organization concerned shall provide suitable environmental and operational conditions for the purpose of managing the computers and automated equipment properly, to ensure that there is no loss or improper conversion of data as a result of accidental erasure.

(4) Equipment

The organization concerned shall be furnished with (or leased) all items of the equipment required for the execution of verification tests. If a piece of equipment can only be operated by authorized personnel, the organization concerned shall specify the equipment. Equipment that has been subjected to overloading or mishandling, gives suspect results, or has been shown to be defective or outside specified limits, shall be taken out of service until it has been repaired and confirmed to perform correctly.

(5) Measurement traceability

All equipment used for tests that has a significant effect on the accuracy or validity of the result of the verification test shall be calibrated before being put into service.

(6) Sampling

The organization concerned shall take samples of reagents, materials, or products in accordance with the protocol for the verification.

(7) Handling of test and calibration items

If necessary, the organization concerned shall transport, receive, handle, protect, store, retain, and/or dispose of test items in accordance with the protocol for the verification.

(8) Verification of data and assurance of test result quality

The data resulting from the verification test shall be recorded in such a way that trends are detectable and, where practicable, statistical techniques shall be applied to the review of the results. This verification shall be conducted by a person other than the one who conducts the verification test.

(9) Reporting the results

The organization concerned shall report the results of the test conducted accurately, clearly, unambiguously, and objectively in accordance with the protocol of the verification tests.

Appendix 1: Application form for verification

A verification applicant shall submit the application form specified below.

[1] Applicant

Company name	
Address	
Division and name of person in charge	
Contact address	TEL: _____ FAX: _____
	e-mail: _____
Name of technology/product	

[2] Product data

Item	Description
Product name	
Serial number	
Distributor/manufacturer	
Weight (g)	g
Price (yen)	yen
Chemical(s) to be monitored	
Environmental media to be monitored	Water quality, bottom sediment, organisms, etc. ()
Use	
Standard reagent/type	Accessories (prepared/need to prepare)
Operational environment (room temperature)	degrees C to degrees C
Storage condition	degrees C or lower
Period of guarantee	months after production
Maximum number of samples that can be tested at a time	Samples
Measurement time	hours

[3] Performance test results

Item	Description
1. Basic performance	
(i) Measurement range	to $\mu\text{g/L}$ (attached sheet number)
(ii) Lower detection and determination limits	Lower detection limit: $\mu\text{g/L}$ Lower determination limit:
(iii) Repeatability	Standard deviation: Coefficient of variation:
(iv) Day-to-day reproducibility	Standard deviation: Coefficient of variation:
(v) Period-to-period reproducibility	Standard deviation: Coefficient of variation:
(vi) Plate-to-plate reproducibility	Standard deviation: Coefficient of variation:
(vii) Cross-reactivity	Cross rate: % (Chemical name)
(viii) Others	
2. Practical performance	
(i) Recovery characteristics	
(ii) Measurement accuracy, etc.	Correlation with instrumental analysis:
(iii) Others	
Test director	
Test date	

Note: Provide performance test results or other reference materials on these items that provide verification of the following:

- Validity of performance test methods
- Validity of interpretations of performance test results
- Practical validity of the target verification product

[4] Novelty of the technology

Describe the novelty of the technology, patent or utility model applications, patents or utility models obtained for the technology, papers presented previously, or prize history, if any.

[5] Other relevant or unique features (if any)

[Documents to be attached to this application form]

- Instruction manual of the target verification product (manual included with the product and delivered to users)
- Technical specifications (containing technical specifications particular to the product)
- Results of performance tests (related to the verification items), etc.

Appendix 2: Test Plan

The Test Plan provides a general description of the objectives and design of a verification test and test procedures. Verification Organizations and the Technology Panel are basically responsible for preparing the Test Plan based on information provided by environmental technology developers.

Some parts of the Test Plan may depend on the characteristics of the product presented by the environmental technology developer. However, the following information must be contained:

1. Cover sheet/approval of the verification test participants/table of contents
2. Overview and objectives of the verification test
3. Participating organizations and personnel responsibilities
4. Overview of the simplified chemical monitoring technology to be tested
5. Design of the verification test (method, schedule, etc.)
6. Quality control of data
7. Management, analysis and presentation of data
8. Audit
9. Appendix
 - Product information presented by the environmental technology developer
 - Instruction manual
 - Results of in-house performance tests
 - Other reference documents or data, etc.

[3] Verification test results (measurement range)

1. Test conditions

Item	Description
Verification item	Basic performance/measurement range (test with standard samples)
Chemical(s) to be monitored	
Product name	
Product number	Lot No.
Production date	
Measurement range (product specifications)	to $\mu\text{g/L}$
Test date	From : to :
Test Site	
Room temperature during the test	Degrees C
Commercial standard product used in the test	Name Reagent company name Product No. Lot No.
Software used to prepare a standard curve	
Test laboratory/person in charge	Laboratory

2. Test results

Item	Unit	Test sample solutions				
		Solution B1	Solution B2	...	Solution BN	Whole
Adjusted concentration	$\mu\text{g/L}$					-
Standard deviation	-					
Coefficient of variation	%					

Note: Perform triple or denser measurements.

3. Special notes

[Reference] Sample recording of test results (measurement range)

1. Recording of preparation of a standard curve

Item	Unit	Standard solutions from which a standard curve is prepared				
		Solution A1	Solution A2	Solution A3	...	Solution AN
Prescribed concentration	ug/L					
Number of actual measurements						
Measurement by ELISA		ug/L				
	2	ug/L				
	...	ug/L				
	n	ug/L				

Note: Perform actual measurements as many times as specified in the instruction manual (ex.: double measurement).

2. Regression equation factors used $[Y = D + (A-D) / (1 + (X / C)^B)]$

Regression equation factor	A	B	C	D	R ²
Value					

Note: Use regression equations specified in the instruction manual. Attach graphs too.

3. Recording of test results

Item	Unit	Test sample solutions				
		Solution B1	Solution B2	Solution B3	...	Solution BN
Adjusted concentration	μg/L					
Number of actual measurements						
Measurement by ELISA	1	ug/L				
	2	ug/L				
	...	ug/L				
	n	ug/L				
	Mean	ug/L				
Standard deviation	-					
Coefficient of variation	%					
Comparison between adjusted and observed concentrations	ug/L					

Perform triple or denser measurements.

Reference

.Brief overview of the pilot project for the environmental technology verification

1. Objectives

Many innovative environmental technologies that are already at a practically applicable stage and seem to be useful have not come into wide use because end users, including local municipal entities, companies, consumers, and the like, cannot use the technologies with confidence due to the lack of objective evaluation of the environmental protective effect and the like.

Accordingly, in this pilot project for the environmental technology verification, with respect to the innovative environmental technologies that have not been widely accepted as described above, the environment protective effect and others will be objectively verified by an independent organization on an experimental basis.

It is hoped that the pilot project for the environmental technology verification will accelerate the dissemination of the environmental technologies developed by venture companies and the like, and contribute to the activation of economic activity through environmental protection and the advancement of regional environmental industries.

2. What the “verification” means

In the pilot project for the environmental technology verification, the environmental protective effect and the like of particular environmental technologies will be verified through the collection of objective data based on various tests and others. There is a similar term, “certification,” in which the suitability to the standard of an environmental technology is judged in terms of the performance that a technology should provide. The present project does not conduct such “certification.”

3. System for promoting the project

The pilot project for the environmental technology verification will be conducted by the Ministry of the Environment in cooperation with the “Verification Organizations” (local municipal entities, etc.), which are independent organizations that conduct technology verification under the entrustment and contract of the Ministry of the Environment.

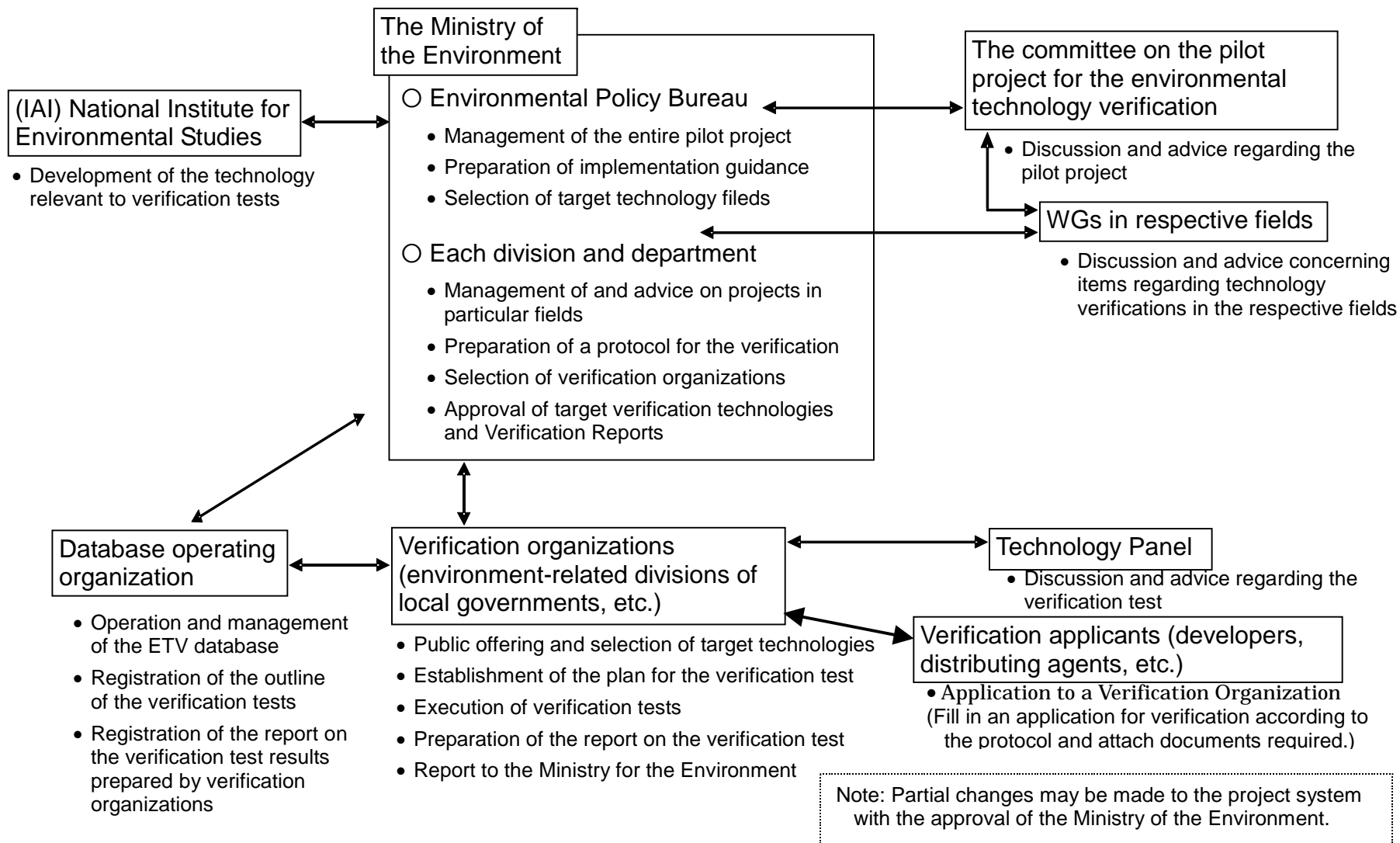
4. Procedures of the project

The pilot project for the environmental technology verification will generally be conducted in accordance with the following procedures:

- (1) The Ministry of the Environment will identify the needs of the technology developers / distributing agents, users, and others, through the use of questionnaires or the like.
- (2) The Ministry of the Environment will select target verification technology fields based on discussions in the committee on the pilot project for the environmental technology verification.
- (3) The Ministry of the Environment will prepare a “protocol for the verification,” which establishes the specific methods of technology verification regarding the selected target verification technology fields.
- (4) The Ministry of the Environment will select the “Verification Organizations,” which are the independent organizations that conduct the verification tests.
- (5) The Verification Organizations will invite applications from companies and the like for the technology to be verified.
- (6) The Verification Organization will select the technologies to be verified in the project from among the applications, following discussion in a panel consisting of the specialists.
- (7) The Verification Organizations will conduct verification tests on the selected technologies in accordance with the protocol for the verification.

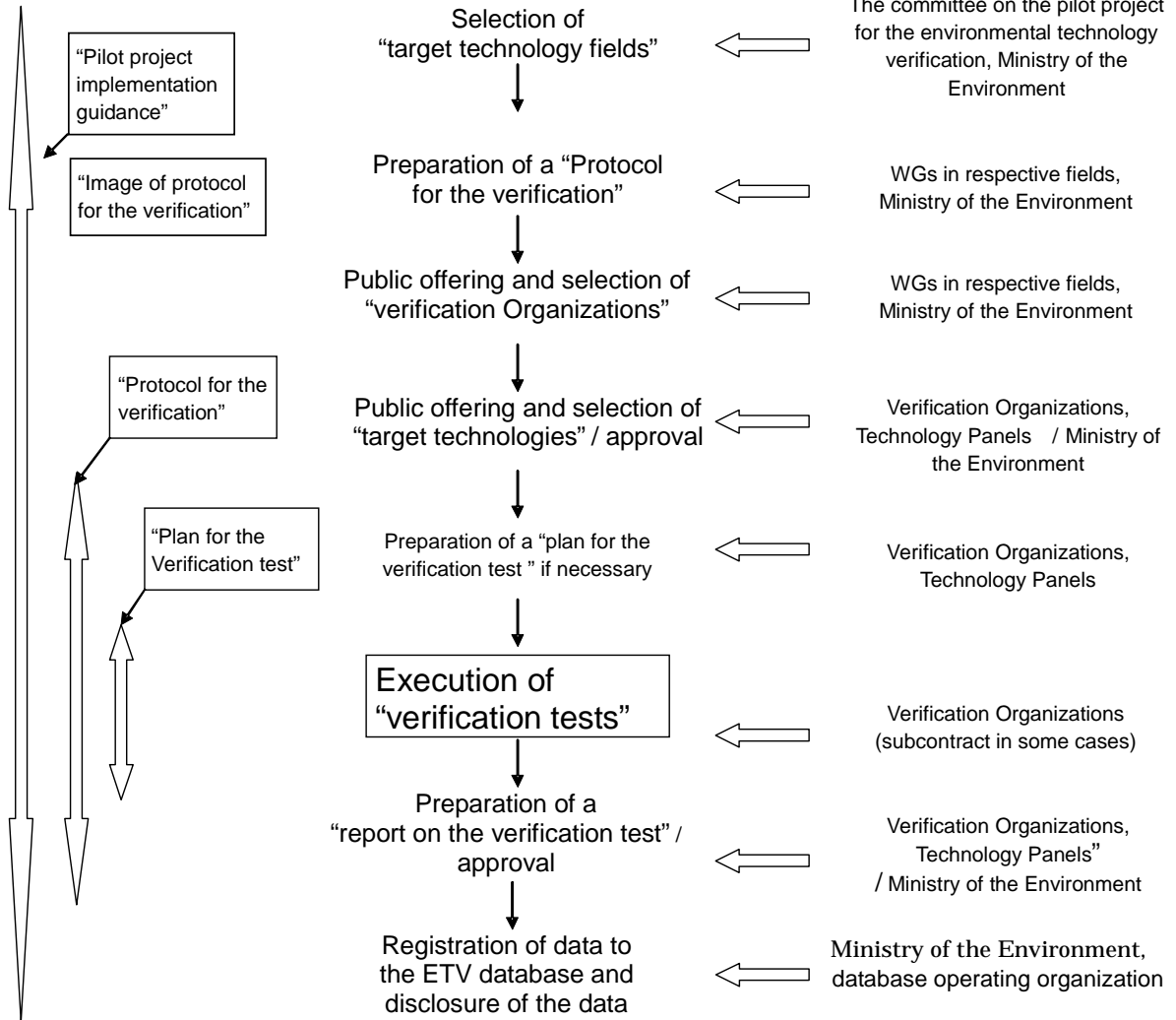
(8) The Verification Organizations will summarize the results of the test in report form, notify the technology developers/distributing agents of them, and report to the Ministry of the Environment. In addition, the report will be registered in a database on the Internet and made available to the public.

II. System for promotion of the “pilot project for the environmental technology verification”



III. Flow of the pilot project for the environmental technology verification

Basic reference for promotion of the project and its period of application



IV. Prospectus for organizing the working group on the simplified chemical monitoring technology

(Committee on the pilot project for the environmental technology verification for 2004)

1. Objective of the working group

The working group on the simplified chemical monitoring technology is established for the purpose of discussing based on specialized knowledge “simplified chemical monitoring technology,” a technology field in which technology verification is scheduled to be conducted in 2004, in the pilot project for the environmental technology verification and thus promoting the project smoothly and efficiently.

2. Items to be investigated and discussed

(1) Field of simplified chemical monitoring technology

- (i) Preparation of a protocol for the verification
- (ii) Selection of Verification Organizations
- (iii) Confirmation of Verification Reports
- (iv) Other items relevant to execution of the project

(2) How future verification tests shall be conducted, and the selection of candidate technology fields

3. Organization and others

- (1) The working group consists of experts on simplified chemical monitoring technology.
- (2) The working group has a chairperson.
- (3) The chairperson will supervise the working group.
- (4) The members will be appointed by Towa Kagaku Co., Ltd. with the approval of the Environmental Health and Safety Division of the Environmental Health Department of the Ministry of the Environment.
- (5) The members will be under commission for the period from the date of appointment by Towa Kagaku Co., Ltd. to the end of the same fiscal year.

4. Disclosure of the discussion and others, etc.

Meetings of the working group will, in principle, be held in public. However, the chairperson may hold a closed meeting of the working group if a public meeting may cause significant obstacles to fair and neutral discussion, and provide particular individuals with unfair benefit or detriment.

5. Secretariat

The general affairs of the working group will be processed by Towa Kagaku Co., Ltd.

6. Additional information

Other affairs of the working group that are not specified in this prospectus shall be settled by the chairperson in consultation with the working group.

List of the members of the working group on the simplified
chemical monitoring technology

(Committee on the pilot project for the environmental
technology verification for 2004)

Koji Arizono,	Professor, Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto
Kiwao Kadokami,	Section Chief, Aqua Research Center, Kitakyushu City Institute of Environmental Sciences
Norihisa Tatarazako	Chief Researcher, Ecochemistry Laboratory, National Institute for Environmental Studies
Hiroyuki Nakazawa	Processor, School of Pharmacy and Pharmaceutical Sciences, Hoshi University
Tetsuharu Nishimura	Chief, Section 3, Division of Environmental Chemistry, National Institute of Health Sciences
Minoru Fukushima	Deputy Senior Researcher, Osaka City Institute of Public Health and Environmental Sciences

* Names are listed in the order of the Japanese syllabary. Titles are omitted.

< Secretariat (Ministry of the Environment) >

Kazuko Kamiya	Manager, Environmental Health and Safety Division, Environmental Health Department, Environmental Policy Bureau
Yoshitoku Yoshida	Deputy Director for Health and Safety, Environmental Health and Safety Division, Environmental Health Department, Environmental Policy Bureau
Taro Kawamura	Assistant Research Manager, Environmental Health and Safety Division, Environmental Health Department, Environmental Policy Bureau
Kenji Ueda	Deputy Director, Office of Environmental Research and Technology, General Affairs Division, Environmental Policy Bureau

< Secretariat >

Takanori Kanetsuna	New Technology Development Division, Towa Kagaku Co., Ltd.
Zennosuke Hamada	New Technology Development Division, Towa Kagaku Co., Ltd.
Misako Kobayashi	New Technology Development Division, Towa Kagaku Co., Ltd.