MINISTRY OF THE ENVIRONMENT
PROTOCOL FOR ACCEPTANCE OF ALTERNATE METHODS (PAAM)

VERSION 1.4

JANUARY 2005

PREFACE

LIST OF ACROYNMS

ANOVA	Analysis of Variance
CAEAL	The Canadian Association for Environmental Analytical Laboratories
CITAC	Co-Operation on International Traceability in Analytical Chemistry
CRM	Certified Reference Material
ISO/IEC	The International Organization for Standardization
IUPAC	The International Union of Pure and Applied Chemistry
MDL	Method Detection Limit
MOE	The Ministry of the Environment
ODWQS	Ontario Drinking-Water Quality Standard
PAAM	Protocol for Acceptance of Alternate Methods
PT	Performance Testing
QC	Quality Control
RDL	Reporting Detection Limit
SDWA	*Safe Drinking Water Act, 2002*

1. Introduction

As required by the *Safe Drinking Water Act, 2002* (SDWA), the Ministry of the Environment (MOE) has documented test procedures (analytical methods) for analytical testing in the Drinking-Water Testing Services Regulation compendium document" Protocol of Accepted Drinking-Water Testing Methods" as amended from time to time.

These test methods have well documented performance characteristics and are compiled from many recognized sources. The MOE recognizes that the list of referenced methods does not include all existing methods which would be suitable for the quality requirements of drinking-water analysis. The MOE also is well aware that improvements in environmental analytical equipment need to be considered for acceptance in analysis of drinking-water samples where there are sound reasons for such considerations e.g. cost of analysis, improved accuracy or precision, improved selectivity etc.

The requirement for ISO/IEC17025 method accreditation as a condition of a drinking-water testing licence ensures that the laboratory is using a well documented and validated method and that the laboratory is competent to carry out the method as written.

This assessment however, does not always consider whether the analytical result is to be compared to some jurisdictional or regulatory standard. It is the responsibility of MOE to ensure that analytical methods in use for testing of Ontario drinking water adequately provide information which can be used to ensure proper operation and maintenance of drinking-water supply and distribution systems.

The following protocol has been developed to provide flexibility in the use of analytical methods for chemical parameters while maintaining the quality standards needed for drinking-water analysis. For microbiological methods, MOE will require the method developer to establish method equivalence following the protocol described in the international standard ISO/FDIS 17994 “Water quality-Criteria for establishing equivalence between microbiological methods”[1].

2. Modified Methods

Under the MOE "Protocol for Acceptance of Alternate Methods" (PAAM), an organization or individual may use a modified version of the MOE designated reference methods without prior MOE approval, provided that the laboratory demonstrates and documents that the modified method produces analytical data equal to or superior to these reference methods and the data validation instructions in this document are followed. The title of the method must clearly describe the front-end and determinative technique employed in the method.

A modified method must be accredited and included in the laboratory licence prior to use for drinking-water sample analysis. The method should be identified as a modification of a reference method in the method description.

3. Types of Method Modifications

a) Adding New Target Analytes

Method developers can add new analytes to an accredited method if the conditions below are met:

- It can be demonstrated that the analyte does not interfere with the determination of the other analytes of concern in that method

- QC acceptance criteria are developed by the applicant and employed for determination of the target analyte(s) and meet any data quality objectives determined for the analyte(s) by MOE

- The reason for adding the analyte is not to avoid the sample preservation or sample (or extract) holding time conditions that are already required for that analyte in another accredited method. (This criterion precludes "method shopping," whereby a user might add analytes to an approved method with less rigid sample collection or holding time criteria.)

b) Modifications to Front-end Techniques

A front-end technique is any technique in the analytical process conducted at the laboratory that precedes the determinative technique. Front-end techniques include all procedures, equipment, solvents, etc., that are used in the preparation and cleanup of a sample prior to instrument analysis. Laboratories may modify any and all front-end techniques provided the modification can be demonstrated to produce results equal or superior to results produced by MOE referenced methods for each combination of analyte and determinative technique.

Changing the chemistry of the method might affect the extract holding times specified in the accredited method. If so, a new extract holding time study must be performed. Training records are also required to be maintained for proficiency in the new front-end technique.

c) New Determinative Technique

Instrument and equipment performance is vital to the overall data quality produced from an analytical method. Changes in determinative technique are allowed without MOE approval provided that it can be demonstrated and documented to produce results equal or superior to results produced by the referenced methods and the following three conditions are met:

- The alternate determinative technique measures the analyte using a physical or chemical property similar to the prescribed technique.

- The alternative technique is demonstrated to be equally or more specific and/or equally or more sensitive for the analyte of concern than the determinative technique in the referenced methods.

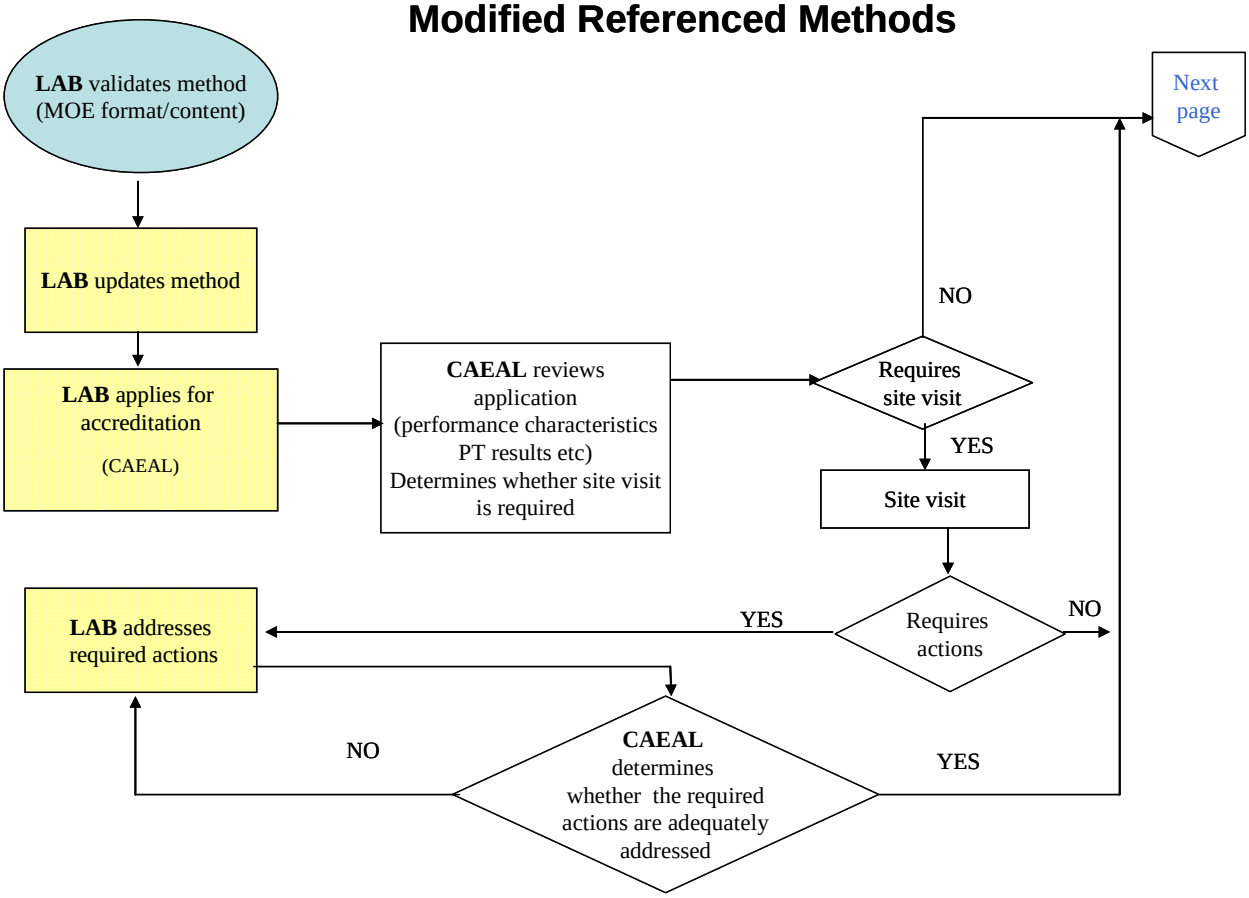
- The use of the alternate determinative technique has not been identified in scientific publications as inappropriate for use with the analyte of interest or the front-end technique.

PAAM for modified referenced methods is presented in Flow-charts 1 & 2.

Flow-chart 1

PROPOSED PROTOCOL FOR ACCEPTANCE OF ALTERNATE METHODS (PAAM)

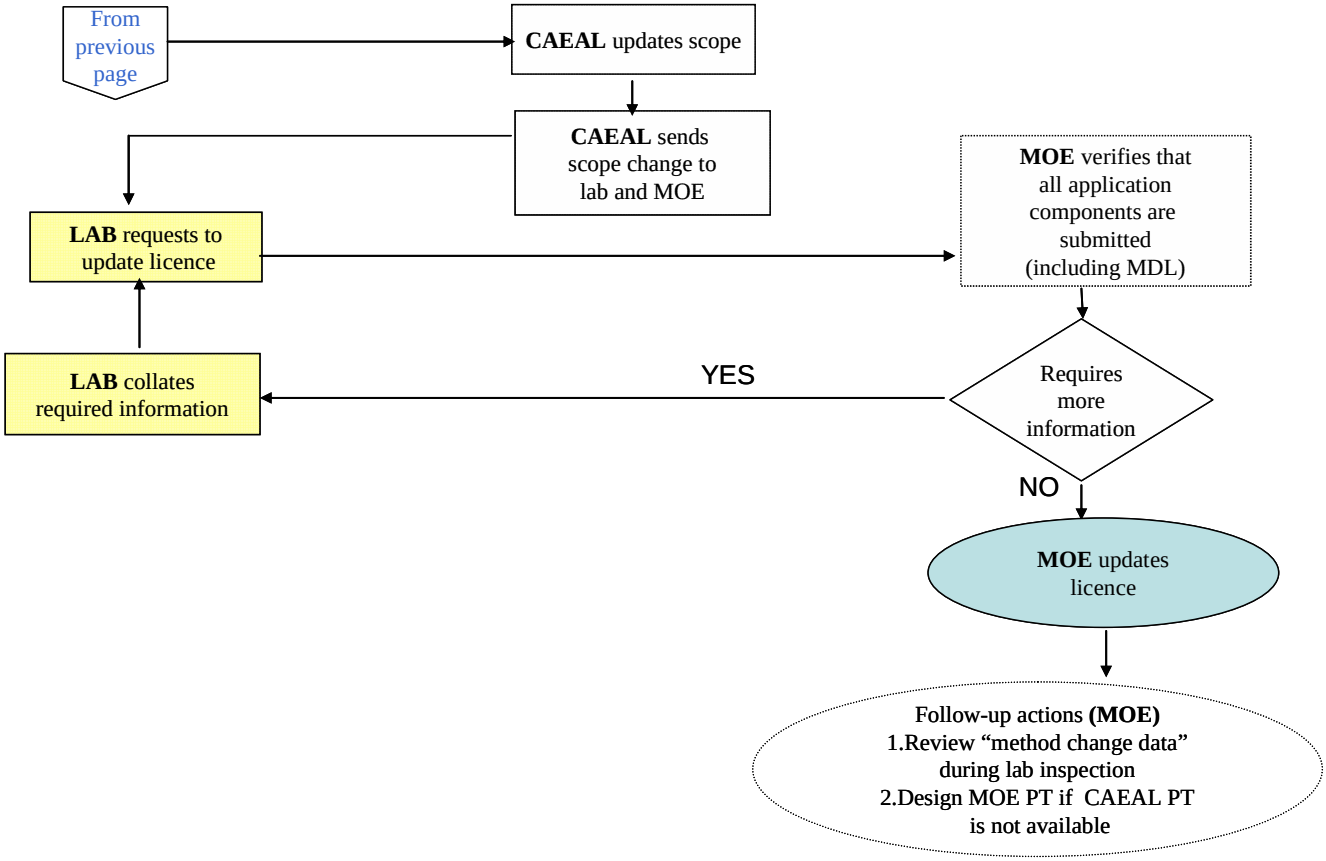
Modified Referenced Methods



Flow-chart 2

PROPOSED PROTOCOL FOR ACCEPTANCE OF ALTERNATE METHODS (PAAM)

Modified Referenced Methods (continued)



4. New Methods

Licensed laboratories will require MOE approval for new accredited methods, prior to use for drinking-water analysis. A new method uses an alternate determinative technique that measures the analyte of concern using a physical or chemical property different from the prescribed technique. A new method will be considered for approval if it:

- Is documented in accordance with the format outlined in this document (page 15)
- Contains the appropriate standard QC elements contained in the data validation instructions
- Meets or exceeds the QC acceptance criteria outlined in the referenced methods
- Employs a determinative technique that is more sensitive and/or selective than the determinative techniques listed in the referenced methods

Approval for a new method will consider the benefit of the new procedure to reduce analytical costs, overcome matrix interference problems, improve laboratory productivity, or reduce the amount of hazardous materials used and/or produced in the laboratory.

An MOE expert will review all submitted requests for new method approval and will determine if the application is to proceed or is rejected. Because there is a possibility that the method would be rejected, the laboratory is advised to submit the new method to MOE prior to application for accreditation, unless the method is also to be used for samples other than drinking water. When a new method is approved, it will be included in the list of reference methods

If a peer review is warranted, MOE will select the peer review panel, assemble all of the information needed for peer review, provide instructions and timelines for peer review and assemble the findings of the peer review process. MOE retains the right to make the final decision on method acceptance after peer review.

Generally, the more novel or complex the science or technology, the greater the cost implications of the impending decision and the more controversial the issue, then the stronger the need for peer review.

A new method must be accredited and included in the laboratory licence prior to its use for drinking-water sample analysis.

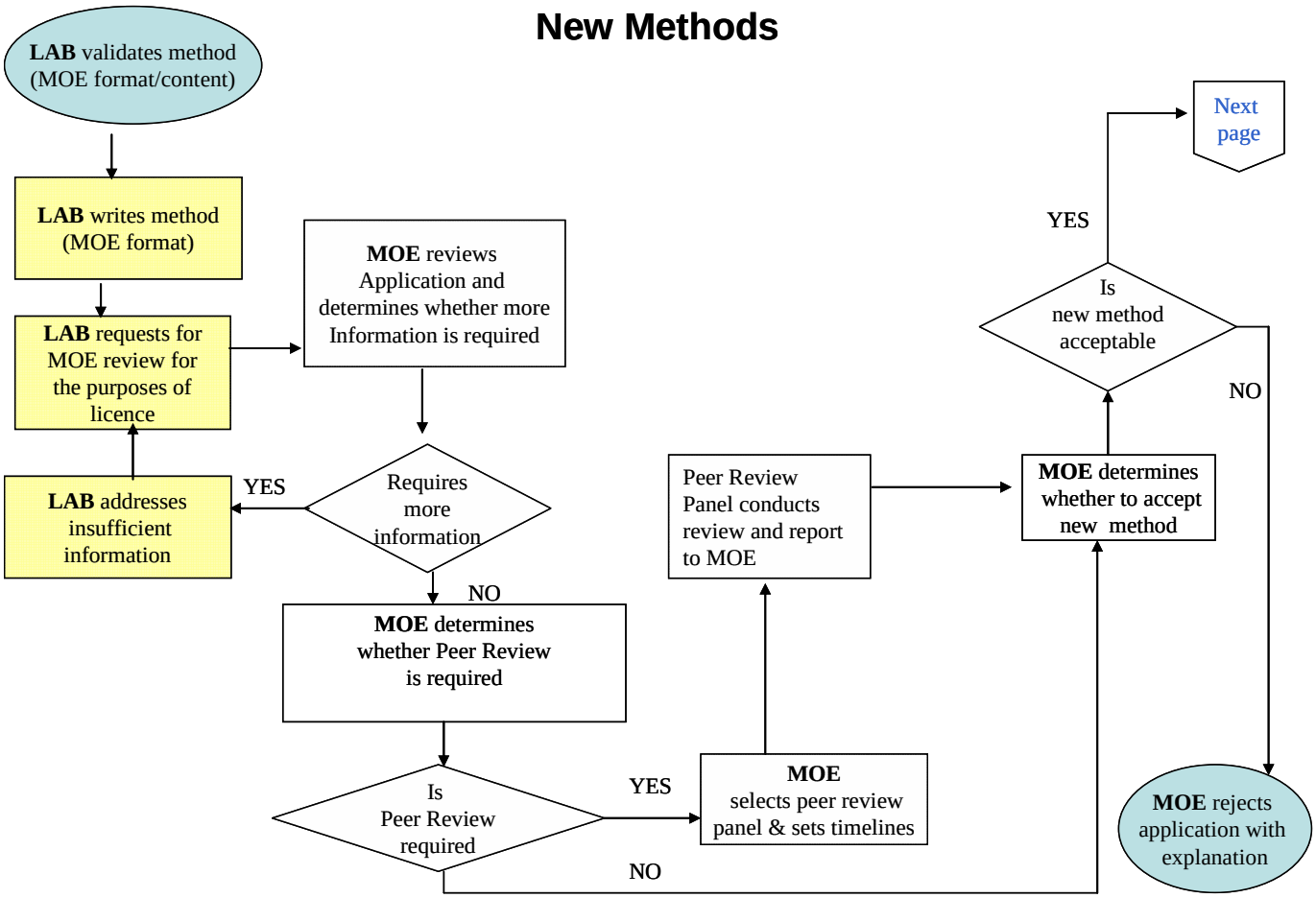
NOTE: The method developer always has the option of asking MOE for a technical opinion on the acceptability of the developer's validation data that supports any method modification or the development of a new method. All method validation data is subject to inspection by MOE.

PAAM for new methods is presented in Flow-charts 3 & 4.

Flow-chart 3

PROPOSED PROTOCOL FOR ACCEPTANCE OF ALTERNATE METHODS (PAAM)

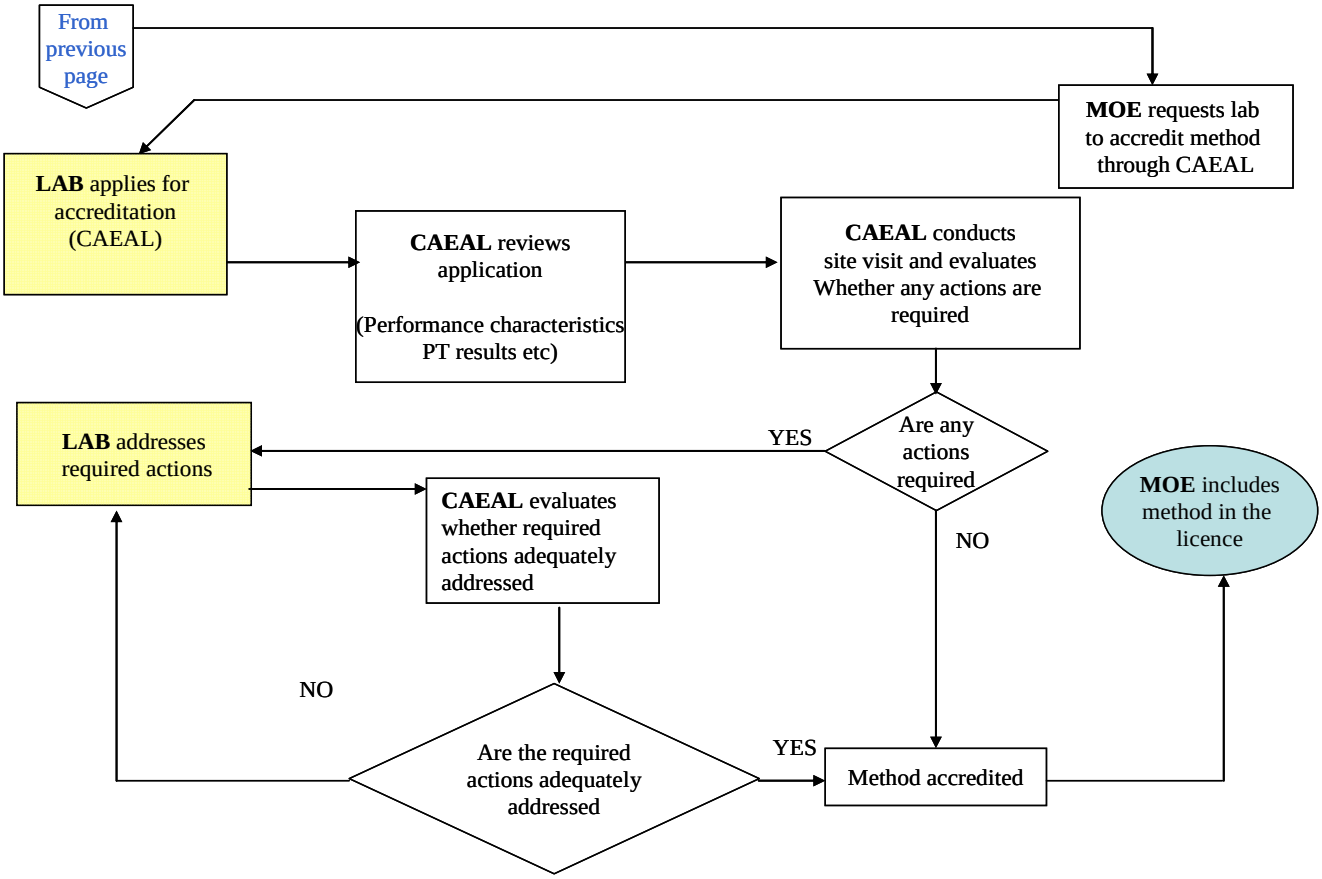
New Methods



Flow-chart 4

PROPOSED PROTOCOL FOR ACCEPTANCE OF ALTERNATE METHODS (PAAM)

New Methods (Continued)



5. Method Validation

Method validation is the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires. To do this, it is necessary to evaluate the method's performance capabilities. The assessment of fitness for the application goes beyond strictly evaluating performance parameters. At the end of the process, a decision is made as to the method's "fitness-for-use", based on a comparison of method performance versus requirements.

Use of this Guideline will ensure that the performance capabilities of the method under development will be determined in a manner that will allow their assessment against the data quality requirements.

It is important that analytical performance be established and made apparent so that measurement results are shown to be useful to the intended application, and interpreted appropriately.

5.1 Analytical Method Performance Parameters

It is implicit that studies to determine method performance be carried out using equipment that is within specification, working correctly and adequately calibrated. The software associated with the application must be loaded and running without error messages or failures.

a) Confirmation of Identity

It is required to demonstrate that the response produced by the method is due to the analyte of concern. Analyte identity is often confirmed by analyzing an authentic compound and or Certified Reference Material (CRM). Techniques that are used to confirm identity include:

- emission spectral patterns;
- retention and or relative retention time;
- dual columns;
- high resolution mass spectra and comparison to spectral library;
- Fourier Transform Infrared Spectrometry;
- etc.

Validation documentation must contain identity of the source of all authentic compounds and CRMs and all relevant chromatograms and mass spectra.

b) Selectivity/specificity

These two terms are often used interchangeably but they are not the same. Selectivity of a method is its ability to measure analyte(s) of concern in the presence of other chemicals. The method is said to be specific if it is able to measure unequivocally a particular analyte at the exclusion of all other compounds. This is seldom achieved. International Union of Pure and Applied Chemistry (IUPAC) has stated that "*specificity is the ultimate of selectivity*".

The selectivity is often achieved by employing a variety of sample preparation and sample clean-up techniques and separatory techniques such as Gas Chromatography which allows only the analyte of

interest to be presented to the measurement device.

The effects of interferences include co-eluting peaks and analyte degradation due to interaction with either injector port, transfer line or column (chromatographic methods), overlapping of spectral lines causing either positive or negative signal enhancement (spectroscopic methods), etc.

Demonstration of selectivity is accomplished by analyzing samples containing various suspected interfering materials in the presence of the analytes of interest and by using appropriate statistical techniques to establish that there are no significant differences in the measurement results between those samples containing interfering chemical(s) and those that are free of interfering chemical(s). The choice of such potentially interfering materials should be based on scientific judgement with consideration of the interference that could occur. Paired t-test and analysis of variance (ANOVA) / Factorial analysis are statistical tools that may be effectively employed to demonstrate selectivity [2,3]. The number of tests, N should be based on the power required.

Validation documentation must contain the identity of all potentially interfering materials and all the relevant statistical calculations.

c) Low Level Detection

Drinking-water methods must meet the requirements of Reporting Detection Limits (RDL) as specified under the SDWA. Ontario Drinking-Water Quality Standard (ODWQS) and relevant RDL are listed in the Schedules of O.Reg. 169/03[4] and ministry document "Protocol of Accepted Drinking-Water Testing Methods" [5] respectively. Typically RDLs are one tenth ODWQS. The Method Detection Limit (MDL) of the method must be equal or lower than RDL. The statistical confidence interval for the estimated MDL must be established. The upper limit of this confidence interval [2] must be lower than RDL.

Procedures to estimate MDL are described in MOE document entitled *'Estimation of Analytical Method Detection Limits (MDL)'* [6].

All raw data and computations must be included in the validation documentation.

d) Working and Linear Ranges

A working range over which the method may be applied for each analyte must be established. The working range must include RDL and twice the concentration of ODWQS for each analyte, where applicable.

It is recognized that there are cases where this general rule will not apply.
For example:

i Concentration - response relationship may not be linear beyond ODWQS
ii For very sensitive methods, ODWQS levels may cause detector saturation resulting in carry over and loss of production time

In such cases, it is sufficient to state the reason for the exception and outline steps that will be taken, such as dilution if samples of concentration greater than the maximum of the dynamic range are encountered. Also for certain analytes, historical data may warrant the working range to be extended as much as 20 times the ODWQS.

The working range should be evaluated by visual examination of the plot of response versus analyte concentration. If there is a linear relationship, appropriate statistical methods must be applied to check for linearity and calculation of the regression line by the method of least squares. The lowest and highest result that can be reported must be established by analyzing appropriate standards. For establishment of linearity, a minimum of 5 concentrations between upper and lower limit of the working range must be prepared in triplicate and analyzed.

Sometimes it is difficult to establish deviations from linearity by visual inspection. In such cases, plot the deviations from the regression line versus the concentrations. For linear ranges, negative and positive values must be approximately equally distributed.

Working and linear range must be clearly stated in the validation documentation. Also the correlation coefficient, y-intercept, slope and residual sum of squares along with the graph(s) must be included in the validation documentation.

e) Accuracy

Accuracy is a measure of the closeness of the result to the true value. It has two components namely:

Trueness expressed as bias
Precision expressed as repeatability (within and between run)

Bias is established by analyzing certified reference materials (CRMs). The concentration of the analyte(s) should be between RDL and twice the concentration of ODWQS. If suitable CRMs are not available, samples spiked with known amount of analytes may be substituted. Results obtained must be compared with the true or designed value. A sample size of 10 or more and performance of the t-test are recommended to demonstrate ‘no bias’.

Precision is measured as the difference between duplicate results of analyses of the same sample or duplicate spikes. This difference should not exceed twice RDL for samples of concentrations between RDL and twice the concentration of ODWQS.

Associated with bias and precision is recovery, which may be estimated by analysing CRM or fortified sample. The expected recovery will very much depend on sample concentration. Typically ± 20% and ± 10% is acceptable for organics and inorganics respectively. For samples of concentrations below 5 times MDL, the acceptable range is wider as shown in the table below.

Concentration	Acceptable Recovery
1 x MDL	± 100%
2 x MDL	± 50%
3 x MDL	± 33.3%
4 x MDL	± 25%

Validation documentation must contain all raw data, calculations and conclusions.

f) Sensitivity

Sensitivity is the ability to detect small changes in concentrations. For drinking-water methods, the data reporting increment should be equal to or less than one third RDL. Therefore, it must be demonstrated that the method on average is able to discern concentrations differing by one third RDL. This requirement is usually met if the MDL is below the RDL.

g) Ruggedness

Ruggedness is the ability of the method to not be affected by slight changes in operational parameters including laboratory environmental conditions. Examples of operational parameters include a number of chromatographic parameters, i.e. flow rate, column temperature, injection volume, detection wavelength or mobile phase composition etc. Other operational parameters include digestion temperature, pH of buffers, normality of acids etc. Ideally, ruggedness is demonstrated by varying operational parameters and laboratory environmental conditions within previously specified tolerance and establishing that these changes do not significantly affect the measurement result, employing factorial analysis and/or Youden Ruggedness Tests[3,7]. An interim proof of ruggedness may by provided by demonstrating that the between analysts (within the same laboratory) precision does not exceed 1.5 times the single analyst precision.

h) Uncertainty

The International Vocabulary of Basic and General Terms in Metrology[8] defines Uncertainty as a parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand. Uncertainty of measurement must be estimated and documented. There are several guidelines for the estimation of measurement uncertainty including those published by MOE[9] and EURACHEM/CITAC[10]. Every possible source of uncertainty must be evaluated, but only those exceeding 1/3rd the largest source need to be included in estimating combined uncertainty. If method performance data are used to estimate uncertainty, studies should be conducted such that the number and range of effects,

concentrations and matrices are varied to ensure that the conditions encountered under normal use of the method are represented.

Uncertainty of measurement must be estimated for all analytes and expressed as expanded uncertainty (k=2).
The documentation must list all the uncertainty components and include all raw data and computations.

6. Bibliography

1. *Water quality-Criteria for establishing equivalence between microbiological methods,* ISO/FDIS 17994 (final draft).

2. John K. Taylor, *Statistical Techniques for Data Anaysis*, ISBN 0-87371-250-1, Lewis Publishers, Inc.

3. J.C.Miller and J.N.Miller, *Statistics for Analytical Chemistry*, (3rd Edition), ISBN 0-13-030990-7, Ellis Horwood PTR Prentice Hall

4. Ontario Regulation 169/03, printed in *The Ontario Gazette*: May 17, 2003

5. *Protocol for Accepted Drinking Water Testing Methods*, Laboratory Services Branch, Ministry of the Environment, June 2003.

6. Crawford G., *Estimation of Analytical Method Detection Limits (MDL)*, MOEE Publication, Queen's Printer for Ontario, 1991 (PIBS299).

7. Youden W.J., *The Collaborative Test*, presented at the Seventy sixth Annual Meeting of the Association of Official Agricultural Chemists, October 16, 1962.

8. *International Vocabulary of Basic and General Terms in Metrology, ISO*, Geneva, (1993). (ISBN 92-67-10175-10)

9. *Laboratory Services Branch Guidelines for the Determination and Documentation of Uncertainty of Chemical Measurements*, Procedures Manual, Laboratory Services Branch, Ministry of the Environment

10. *Quantifying Uncertainty in Analytical Measurement*, EURACHEM/CITAC Guide (second edition, 2000).

APPENDIX

Minimum Contents for Method Documentation

1. Title (Includes scope, description of front-end and determinative techniques)

2. Principle of the method
   -relationship to other methods
   -shortcomings
      interferences
      biases
      limitations

3. Analytical Performance summary
   MDL
   selectivity/specificity
   working and linear range
      accuracy
      precision
      bias
      recovery
   interlaboratory performance
   uncertainty

4. Sample processing
   Clean-up
   digestion
   extraction etc

5. Instrumentation
   standard preparation
   calibration

6. Calculations

7. Reporting Format

8. Bibliography