codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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**Agenda Item 9** **CX/RVDF 04/15/7**
**July 2004**

**JOINT FAO/WHO FOOD STANDARDS PROGRAMME**

**CODEX COMMITTEE ON RESIDUES OF VETERINARY DRUGS IN FOODS**

**Fifteenth Session**

**Washington, DC (metro area), United States of America, 26-29 October 2004**

# PROPOSED DRAFT REVISED PART II "GENERAL CONSIDERATION ON ANALYTICAL METHODS FOR RESIDUE CONTROL" OF THE CODEX GUIDELINES FOR THE ESTABLISHMENT OF A REGULATORY PROGRAM FOR THE CONTROL OF VETERINARY DRUG RESIDUES IN FOODS

Governments and international organizations wishing to submit comments at Step 3 on the attached proposed draft revised Part II "General Considerations on Analytical Methods for Residues Control" of the Guidelines for the Establishment of a Regulatory Programme for Control of veterinary Drugs Residues in Foods (CAC/GL 16-1993) are invited to do so **no later than 15 September 2004** as follows: U.S. Codex Office, Food safety and Inspection Service, US Department of Agriculture, Room 4861, South Building, 14th Independence Avenue, S.W., Washington DC 20250, USA (Telefax: +1 202 720 3157 ; or ***preferably*** E-mail: uscodex@usda.gov, with a copy to the Secretary, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Viale delle Terme di Caracalla, 00100 Rome, Italy (Telefax: +39.06.5705.4593; E-mail: Codex@fao.org).

## BACKGROUND

1. The 14th Session of the Codex Committee on Residues of Veterinary Drugs in Foods agreed that a drafting group would review Part II "General Considerations on Analytical Methods for Residues Control" of the Guidelines for the Establishment of a Regulatory Programme for Control of Veterinary Drugs Residues in Foods (CAC/GL 16-1993) for circulation comments at Step 3 and further consideration at its 15th meeting.[1]

2. Governments and interested international organizations are invited to comment **at Step 3** on the proposed draft revised Part II "General Considerations on Analytical Methods for Residues Control" of the Guidelines for the Establishment of a Regulatory Programme for Control of Veterinary Drugs Residues in Foods (CAC/GL 16-1993)" annexed to this document, as directed above.

1 ALINORM 03/31A, para. 105.

**Annex**

## PROPOSED DRAFT REVISED PART II "GENERAL CONSIDERATIONS ON ANALYTICAL METHODS FOR RESIDUES CONTROL" OF THE GUIDELINES FOR THE ESTABLISHMENT OF A REGULATORY PROGRAMME FOR CONTROL OF VETERINARY DRUGS RESIDUES IN FOODS (CAC/GL 16-1993)"PART II [2]

1. Analytical methods used to determine compliance with MRLVDs should be effective and practical for the detection, quantification, and confirmation *of all residues of veterinary drugs and* substances which may also be used as veterinary drugs, such as certain pesticides, *that may be present in commodities within the terms of reference of this Codex Committee. These methods* should be suitable for routine use *by regulatory control authorities of member governments for their residue testing programmes.* Applications for such methods in a regulatory programme include analysis of randomly selected survey samples in a national programme to determine compliance with established MRLVDs and analysis of targeted samples where there is reason to suspect non-compliance with MRLVDs. Other uses may include analysis of samples to meet a commercial requirement or analyses used to estimate consumer exposure to residues though food. In addition, methods may be required to detect residues of substances prohibited for use in food animals or for which MRVLDs have not yet been established.

2. There may be some differences in requirements for method performance, depending on the intended use of the method. While the focus of assessing method performance is usually within a relatively narrow analytical range bracketing a target concentration for those substances for which an MRLVD has been established, application to estimates of dietary intake can require methods with a broader analytical range and capability to detect smaller quantities of a target substance than is required to monitor compliance with an MRLVD. For those substances for which an MRVLD has not been established, the so-called "zero residue" becomes a lower target concentration as technology and analytical detection capabilities evolve. While the primary focus of this document is on methods intended for determination of compliance with MRLVDs, some consideration and guidance is included for the other applications of residue methods for veterinary drug residues and related substances in foods.

3. *Methods with the capabilities mentioned above are not* routinely *available for* all possible *compounds of interest* in all potential sample materials *because of the extensive number of potential veterinary drug residues which may find their way into foods within the terms of reference of the CCRVDF. To optimize the effectiveness of regulatory programmes to test for veterinary drug residues, residue control programmes must* identify and select for use suitable *residue methodology to assure compliance with Codex MRLVDs and, as necessary, take appropriate regulatory action against adulterated products, consistent with the reliability of the analytical data. To assist regulatory authorities in determining their analytical needs for residue control programmes, this document will describe the types of methods available and* identify attributes of methods to establish that the methods are fit for purpose *in residue control programmes* intended to carry out the missions described above.

4. *The principal attributes of analytical methods* used in *residue control programmes are* dependent on the use of the method and the information that it is intended to provide. The requirements differ, depending on whether a method is intended to simply detect, to quantify or to confirm the presence of a target residue. Methods which provide quantitative results must perform in good statistical control within the analytical range that brackets the MRLVD. In such cases, performance of the method within that range and the inclusion of appropriate calibration points (including the lowest calibrated level, or LCL) may be more important than a characterization of a limit of detection (LD) or limit of quantification (LOQ). For methods applied in studies to assess daily intake of a selected residue, the capability of the method to accurately measure concentrations orders of magnitude below the MRLVD may be important, so that the LOQ and linearity of response over an extended analytical range become primary considerations.

2 Text retained from the original Part II is in italics

5. *There are some methods for which additional analysis is required to support regulatory action. This category may include methods that do not provide adequate information of structure or residue concentration. However, these methods may be useful* to screen for substances at an established minimum concentration to identify commodities which may contain residues which are not in compliance with MRLVDs. *Results* obtained using *such methods should be considered only as estimates of analyte concentration or identification without additional supporting analytical information. Results from these methods can be useful for gathering residue information, such as determining whether or not a veterinary drug residue problem exists in a sampling population, and determining whether there is a need to* apply *a more definitive method* to particular samples. *These methods should not be used alone for residue control purposes on official samples without additional information (e.g., such as the presence of an injection site in the sample)* and without the availability of suitably validated determinative and/or confirmatory methods to apply to any samples identified as potentially not in compliance with an MRLVD.

6. Some methods may be applied in regulatory control programmes for the detection of residues of substances for which MRLVDs have not been established by the Codex Alimentarius Commission because the toxicology of an analyte does not allow an ADI or MRLVD to be established. Methods for analytes such as chloramphenicol would be in this category. For such substances, the determination of the lowest concentration at which the residue can be detected and the identity confirmed in a food is a primary concern in the method validation. Performance characteristics related to quantitative analyses may be less critical for such substances, where detection and confirmation of the presence of the substance as a residue is the major issue. Confirmation of identity of a residue is based on the comparison of a set of characteristics of a detected substance with those of a known standard of the suspected residue.

7. *The performance* attributes, or characteristics, which must be determined during method validation for each type of method – screening, determinative, confirmatory – are *presented in a subsequent section of this paper.*

## CONSIDERATIONS FOR SELECTION OF ANALYTICAL METHODS

8. *Various* types of methods are available to food safety agencies and programmes to conduct analyses that *may be* consistent *with their requirements*. Decisions on the use of a specific analytical method *should be based* on the intended objectives of the regulatory programme and the *resulting* analytical performance characteristics *required* of *the selected* methods. Methods that are suitable for determining compliance with MRLVDs are those that *have been successfully validated for the analysis of specific veterinary drug residue, tissue and species combinations*. These methods provide analytical results for either quantification or confirmation that are appropriate to *support* regulatory action without the need for additional analyses. In some cases, these methods may be considered reference methods, but reference methods frequently *are not those selected for routine use*.

9. Relatively few of the analytical *methods currently being used* in *residue control programmes have successfully completed a multi-laboratory study. Multi-laboratory method performance studies generally satisfy the analytical requirements for use in a regulatory programme,* as valuable information on method performance in the hands of different analysts in different laboratories is obtained through these studies. Multi-laboratory validated methods are subjected to a properly designed inter-laboratory study with analysts in independent laboratories, so that different sources of reagents, chromatographic media and equipment are used by the participants. *Collaborative study methods conducted* prior to 1995 *have successfully completed method evaluation in* a minimum of *six laboratories in an acceptable, statistically designed study.* Quantitative methods studied collaboratively according to the revised harmonized protocol adopted in 1995 have been evaluated in a minimum of 8 laboratories, unless highly complex equipment or other unusual requirements were identified (in such cases, a minimum of 5 participating laboratories are required). Collaborative studies of qualitative methods currently require a minimum of 10 participating laboratories.

10. Multi-laboratory and collaborative studies of methods usually do not encompass all possible combinations of residue, tissue and species to which the method may subsequently be applied. These *methods may be extended to* related analytes, *additional tissues, species products, or combinations of these, not included in the original multi-laboratory study by completing additional properly designed* within-laboratory *laboratory studies. On a case by case basis, analytical results from method extension studies may require additional analysis and/or review before* use in a regulatory programme. Whenever possible, analytical results obtained using *methods that have not been validated by traditional inter-laboratory study* should *be correlated and compared with* results *obtained* using *a* method which has been validated through a *collaborative* or multi-laboratory *study. The* comparison should be based on *a statistically acceptable study design using portions of the same (homogeneous) samples. The data from* such *studies should be* independently *reviewed by a* qualified third party (such as a QA unit, a *peer group of regulatory scientists,* auditors of national accreditation body) *to determine the comparability of method performance*.

11. *Some residue control methods that have demonstrated their usefulness for determining compliance with MRLVDs have an historical origin. These methods* with a history of use *were considered to be the best available at the time of initial regulatory use and have continued in use over an extended period of time* either *in the absence of* alternative *validated methods*, or because they remain a preferred choice for reasons which may include such considerations as readily available technology, cost, reliability and suitability for use within the constraints of a national programme. Although evidence of a formal collaborative or multi-laboratory method trial is lacking, the method performance has been demonstrated through successful use in various laboratories over time.

12. Most regulatory laboratories must rely on the use of *veterinary drug residue methods* which have *not have been subjected to an inter-laboratory study*. Factors which have contributed to this situation include a *require*ment for *specialized expertise or equipment*, cost of such studies, lack of suitable collaborating laboratories, analyte and/or sample instability and rapidly changing technologies. While for many years the focus on equivalency of analytical results was based on the use of standardized methods which had performance characteristics defined based on collaborative study, accredited laboratories now operate in an environment where it is the responsibility of the individual laboratory to demonstrate that the methods used and the analytical results produced meet performance criteria established in consultation with a client.

13. The Codex Alimentarius Commission has provided guidance for laboratories involved in the import/export testing of foods[3]. This includes the recommendations that such laboratories should:

- use internal quality control procedures which comply with the *Harmonised Guidelines for Internal Quality Control in Analytical Chemistry*[4];
- participate in proficiency testing schemes designed and conducted in accordance with the *International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories*[5];
- become accredited according to ISO/IEC Guide 25 :*General requirements for the competence of calibration and testing laboratories*[6,7]; and
- whenever available, use methods which have been validated according to the principles laid down by the Codex Alimentarius Commission.

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3 CAC/GL 27-1997. Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Food.

4 *Thompson, M. and Wood, R. 1995. Harmonized Guidelines for Internal Quality Control in Analytical Chemistry Laboratories. Pure & Appl. Chem. 67: 649-666.*

5 Thompson, M. and Wood, R. 1993. International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories. Pure & Appl. Chem. 65: 2132-2144.

6 ISO/IEC. 1990. ISO Guide 25: General requirements for the competence of calibration and testing laboratories. International Organization for Standardization, Geneva.

7 ISO/IEC Guide 25 has been replaced by ISO/IEC-17025: General requirements for the competence of calibration and testing laboratories. International Organization for Standardization, Geneva (1999).

14. For regulatory laboratories involved in the analysis of veterinary drug residues in foods, typical requirements would include that the methods are capable of detecting the compounds included in the residue control programme in the target foodstuffs with analytical recovery and precision which meets the criteria stated elsewhere in this document, and that the methods are used within an established laboratory quality assurance system which is consistent with the principles in the document on internal quality control referenced above. When methods which have not been subjected to a multi-laboratory performance trial are used in a regulatory programme for control of veterinary drug residues in foods, the *quality control and quality assurance procedures applied with these methods* require careful definition, implementation and monitoring. In the case of methods which have been through multi-laboratory trials, performance characteristics, such as recovery and precision, are defined through the results obtained during the study. For a method validated within a single laboratory, data must be generated to define the performance characteristics expected of the method when used by analysts within that laboratory, then the on-going performance must be monitored through the quality system in place in the laboratory.

15. A guidance document on single laboratory validation of methods, "Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis", has been published as a technical report by the International Union of Pure and Applied Chemistry[8]. Requirements for the use of single-laboratory validation of methods for Codex purposes have also been considered by the Codex Committee on Methods of Analysis and Sampling[9]. The following conditions have been proposed for addition to the Procedural Manual to establish "criteria for cases where single laboratory validated methods can be used:

a) No inter-laboratory validated method is appropriate.

b) The single-laboratory validated methods must fulfill the following criteria

   i) the method is validated according to an internationally recognized protocol (for example, the IUPAC protocol referenced above);

   ii) the use of the method is embedded in a quality assurance system under accreditation;

   iii) when available, external reference is given by systematic participation in proficiency schemes, by calibration using reference materials and by comparison of results with those obtained using other methods."

**METHOD DEVELOPMENT CONSIDERATIONS**

16. *Developing an analytical method requires analysts* experienced in the analytical techniques to be used, *laboratory space, equipment, and financial support. To optimize the benefit of these resources, it is important to provide introductory and background information to establish a perspective for planning an analytical method development project, and for evaluating the performance of the analytical method. Residue control programmes should use methodology suitable to the analytes of interest to assure a safe and wholesome food supply. Necessary and appropriate regulatory action should be taken against adulterated products, consistent with the reliability of the analytical data. Before initiating method development activities, the intended use and need for a method in a residue control programme should be established,* including the required performance parameters*. Other considerations include the* required scope of the method *(compound or class of compounds of interest and types of sample materials) potential interfering substances, potential measurement systems and their properties, the pertinent physical and chemical properties that may influence method performance, the specificity of the desired testing system and how it* will be *determined, analyte and reagent stability data and purity of reagents, the acceptable operating conditions for meeting method performance factors, sample preparation guidelines, environmental factors that may influence method performance, safety items, and any other specific information pertinent to programme needs.* In particular, analyte stability of standards and during processing of samples should be assessed. Analyte stability during typical conditions of sample storage prior to analysis should also be determined, including any period for which a sample may be held pending a potential re-analysis for confirmatory purposes.

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8 Thompson, M., Ellison, S.L.R. & Wood, R. (2002) Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis. *Pure & Appl. Chem*. **74**: 835-852.

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**ANALYTICAL PERFORMANCE CHARACTERISTICS**

17. The ability of an analytical method to detect and discriminate the signal response from a compound in the presence of other compounds which may be present in the sample material is of particular importance in defining the performance characteristics of methods used in regulatory control programmes for veterinary drug residues in foods. There are two aspects which must be considered – the ability of the method to provide a signal response which is free from interferences from other compounds which may be present in a sample or sample extract and the ability of the method to unequivocally identify a signal response as being exclusively related to a specific compound. These two related attributes of an analytical method are usually referred to as the method selectivity and specificity, respectively.

18. Specificity is defined in the Procedural Manual as "the property of a method to respond exclusively to the characteristic or analyte defined in the Codex standard. For an analytical method used to support MRLVDs in a regulatory programme, *specificity is* considered as *the ability of a method to distinguish between the analyte of interest and other substances which may be present in the test sample. A* confirmatory *residue control method must be able to provide unambiguous identification of the compound being measured. The ability to quantitatively differentiate the analyte from homologues, analogues, or metabolic products under the experimental conditions employed is an important consideration of specificity.* CCMAS have proposed that the term selectivity, defined as "the extent to which a method can determine particular analyte(s) in mixtures or matrices without interferences from other components", is "the recommended term in analytical chemistry to express the extent to which a particular method can determine analyte(s) in the presence of interferences from other components"[10]. This property should be determined by the analysis of known blank sample materials. No interfering substances should be detected when the method is applied to typical sample materials representative of those which would be submitted for analysis. The method should be able to discriminate the analyte in the presence of potential interfering substances (selectivity), such as other drugs which might be expected to be present as residues in typical field samples.

19. Information on the specificity and selectivity associated with the analysis of a particular veterinary drug residue in a sample may be developed from various sources, which include[11]:

i) data from the chemistry used in the extraction and clean-up procedure;

ii) data from the subsequent chromatography;

iii) data from the detecting spectroscopy or electrochemistry;

iv) data from the "blank" reagents;

v) data from the "blank" samples;

vi) data from library searches for potential interferences or matches;

vii) critical evaluation of available data and subsequent interpretation as to why potential interferences in practice should not interfere;

viii) available information on availability and potential uses of a particular compound; and

ix) other data of interest or importance, such as sampling and transport/storage history.

20. The above information can be captured in a structured logging document of all the information that leads to the conclusion a method has detected a particular compound in a sample, at a measured concentration as reported. While no single measurement or analysis may provide the unequivocal proof of compound identity and/or quantity present that is desired, the combined information that has been compiled provides evidence that the analyst has made a conscientious effort to arrive at a logical result consistent with the data and other information available.

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11 Stephany, R.W. (2003). SPECLOG – The Specificity Log. CRD-9, Codex Committee on Residues of Veterinary Drugs in Foods, 14th Session, Arlington, VA., U.S.A., March 4-7.

21. Precision is defined in the Procedural Manual as the "closeness of agreement between independent test results obtained under stipulated conditions". It may be expressed in terms of repeatability (intra-laboratory) and reproducibility (inter-laboratory). For a single laboratory method validation, precision as repeatability should be determined from experiments conducted on different days, using different reagent batches and preferably by different analysts. Precision expressed as reproducibility requires a multi-laboratory trial of the method. *Precision of a method is usually expressed as standard deviation. Another useful term is relative standard deviation, or coefficient of variation (the standard deviation, divided by the absolute value of the arithmetic mean). It may be reported as a percentage by multiplying by one hundred.*

22. *Method variability achieved in the developing laboratory after considerable experience with a method, is usually less than the variability achieved by other laboratories that may later also use the method.. If a method cannot achieve a suitable level of performance in the developing laboratory, it cannot be expected to do any better in other laboratories*.

23. Accuracy, sometimes referred to as "trueness" or "bias", is defined in the Procedural Manual as the "closeness of agreement between the reported result and the accepted reference value". The Procedural Manual also defines "trueness" as the "closeness of agreement between the average value obtained from a series of test results and an accepted reference value" and "bias" as the "difference between the expectation of the test results and an accepted reference value". Accuracy is therefore the ability of a method to provide a result consistent with the true concentration of the analyte present in the test material. The accuracy of a method may be determined by analysis of a certified reference material, by comparison of results with those obtained using another method for which the performance parameters have previously been rigorously established (that is, a recognized reference method) or, in the absence of reference materials or methods, by determination of the recovery of analyte fortified into known blank sample material.

24. The *accuracy* of a measurement *is closely related to systematic error (analytical method bias) and analyte recovery (measured as percent recovery). The accuracy requirements of methods will vary depending upon the planned regulatory use of the results. Generally, the accuracy at and below the MRLVD or level of interest must be equal to or greater than the accuracy above the level of interest.*

25. *The percent recovery of analyte added to a blank test sample is a related measurement that compares the amount found by analysis with the amount added to the sample.* Recovery has been defined as the proportion of the amount of analyte present or added to the test material which is extracted and presented for measurement [8]. It is typically expressed as the percentage of analyte experimentally determined after fortification of sample material at a known concentration and should be assessed at concentrations which cover the analytical range of the method. *In interpreting recoveries, it is necessary to recognize that analyte added to a sample may not behave in the same manner as the same biologically incurred analyte (veterinary drug residue).* In many situations, the amount of an incurred residue that is extracted (the yield or recovered fraction) is less than the total incurred residues present, due to losses during extraction, intra-cellular binding of residues, presence of conjugates or other factors that are not fully represented by recovery experiments conducted with analyte-fortified blank tissues. This has been addressed by some regulatory authorities in the establishment of requirements for the performance of regulatory methods of analysis[12]. *At relatively high concentrations, analytical recoveries are expected to approach one hundred percent. At lower concentrations and, particularly with methods involving a number of steps including extraction, isolation, purification, and concentration, recoveries may be lower. Regardless of what average recoveries are observed, recovery with low variability is desirable.*

[12] Commission Decision 2002/657/EC, implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, *Official Journal of the European Communities, L221/8*, August 17, 2002.

26. *The sensitivity of a method is a measure of its ability to detect the presence of an analyte and to discriminate between small differences in analyte concentration.* For a determinative method, the sensitivity is defined in the Procedural Manual as "the change in the response divided by the corresponding change in the concentration of a standard (calibration curve); i.e. the slope, $s_i$, of the analytical calibration curve". It has also been defined as "the gradient of the calibration function"[6]. *Sensitivity also requires the ability to differentiate between analyte, related compounds and background interferences. For analytical instruments used in residue analysis, sensitivity is determined by two factors: instrumental response to the analyte and background interference, or instrument noise, the response produced by an instrument when no analyte is present in the test sample.* For measurements at or near the MRVLD, a method with inadequate sensitivity may not permit the analyst to distinguish with confidence whether residue concentrations are above or below the MRVLD.

27. For a screening test, the term sensitivity usually refers to the lowest concentration at which the target analyte may be reliably detected. In the AOAC Performance Tested Program ™ for test kits, this is determined experimentally by testing a minimum of 30 residue-free sample materials fortified with the analyte at the target concentration. The sample materials should be from at least six different sources (that is, at least 5 replicates from each of at least 6 sources), all of which should yield a positive result when fortified at the target concentration. Three or more negative results constitute a failure of the sensitivity test. If one or two of the results are negative, the experiment should be repeated and two negative results would then constitute failure. The experiment should be repeated with known incurred material at the target concentration, if such material is available.

28. The *calibration curve* should be determined to assess the detector response to standards. The concentrations (a minimum of five, plus blank) should cover the full range of analytical interest and the resultant curve should be statistically expressed. The *analytical function* relates the response for the analyte recovered from sample material at various concentrations throughout the range of analytical interest. For analytes for which an MRLVD has been established in a particular sample material (matrix), response is typically determined for known blank sample material and for blank sample material fortified at each of 0.5x, 1.0x and 2.0x the MRLVD (use of 6 different sources of blank materials is recommended). The analytical function experiment can be combined with the recovery experiment described above and is of particular importance when the presence of matrix co-extractives modifies the response of the analyte as compared to analytical standards. It is increasingly common in methods for veterinary drug residues in foods to base the quantitative determination on a standard curve prepared by addition of standard to known blank representative matrix material at a range of appropriate concentrations which bracket the target value. Use of such a "tissue standard curve" for calibration incorporates a recovery correction into the analytical results obtained. Typically, a linear response is desirable for the calibration curve and the analytical function, statistically expressed in terms of linear correlation.

29. Linearity has been defined in as "the ability of a method of analysis, within a certain range, to provide an instrumental response or results proportional to the quality of analyte to be determined in the laboratory sample".[13] This proportionality is expressed by an *a priori* defined mathematical expression. The linearity limits are the experimental limits of concentrations between which a linear calibration model can be applied with a known confidence level (generally taken to be 1%). For a method in which fortified blank matrix material is used for quantification, the linearity is determined from the analytical function experiments as described and is the statistical expression of the curve obtained for the analysis of sample materials fortified at the target concentrations bracketing the Maximum Residue Limit. It is typically determined from a linear regression analysis of the data, assuming there is a linear response.

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30. The limit of detection or detection limit is conventionally defined as field blank + 3s, where s is the standard deviation of the field blank signal [9]. It is also defined as "the smallest amount or concentration of analyte in the test sample that can be reliably be distinguished from zero"[6]. Typically, the calculation of the mean signal for the field blanks and the standard deviation is made from 20 or more determinations . This approach can yield an optimistic estimate of the limit of detection. An alternative approach involves the calculation of the limit of detection from the standard deviation $s_{y/x}$ from the linear regression analysis of the standard curve generated in the analytical function experiment described above[14]. The limit of detection is then calculated using the y-intercept of the curve plus three times $s_{y/x}$. This approach provides a more conservative estimate of the limit of detection.

31. The limit of quantification (also referred to as limit of quantitation or quantification limit)[15], which has been defined in practical terms as the lowest concentration of analyte in a defined matrix which can be determined with the required precision and accuracy[16]. It may be expressed conservatively as 10 times the standard deviation of the mean value for 20 or more determinations of the response from known blank matrix material. For methods used to support MRLVDs established by the Codex Alimentarius Commission, the limit of quantification should meet the criteria for precision and accuracy (recovery) in Table 1 and should be equal to or less than one-half the MRVLD. However, given that the limit of quantification of a method may be considerably lower than the actual concentrations monitored for compliance with a MRLVD, the validation and subsequent application of the method may be based on a *lowest calibrated level*, which is typically 0.5x the MRVLD. For use in a regulatory program, the limits of detection and quantification are important parameters when the method will be applied to estimate exposures to residues, where there may be an interest in monitoring residues at concentrations below the MRVLD. For monitoring compliance with an MRVLD, it is important that a lowest calibrated level (LCL) be included in the analysis which adequately demonstrates that the MRL concentration may be reliably determined. The lowest calibrated level of a method used to support an MRVLD should not be less than the limit of quantification.

32. The Procedural Manual recommends the term "determination limit" under "Terms to be Used in the Criteria Approach". This is defined as 6 or 10 times the standard deviation of the mean value signal of a field blank, consistent with the definitions of limit of quantification.

33. *There are a number of collateral attributes suitable for analytical methods for regulatory control programmes beyond these principle method attributes. Methods should be rugged or robust, cost effective, relatively uncomplicated, portable, and capable of simultaneously handling a set of samples in a time effective manner.*

34. The Procedural Manual defines ruggedness as "the ability of a chemical measurement process to resist changes in results when subjected to minor changes in environmental and procedural variables, laboratories, personnel, etc." Ruggedness testing should be conducted using the standard factorial design approach to determine any critical control points[17]. Typical factors to include in a design include variations in reagent volumes or concentrations, pH, incubation or reaction time and temperature, reagent quality, and different batch or source of a reagent or chromatographic material.

35. *Cost-effectiveness is the use of relatively common reagents, instruments, or equipment customarily available and used in a laboratory devoted to veterinary drug residue analyses.* Methods should use reagents and supplies which are readily available in the required purity from local suppliers and equipment for which parts and service are also readily available.

36. Portability is the analytical method characteristic that enables it to be transferred from one location to another without loss of established analytical performance characteristics.

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14 Miller, J.C., & Miller, J.N. (1993) *Statistics for Analytical Chemistry, 3rd Edition,* Ellis Horwood Ltd., Chichester.

15 Inczedy, J.; Lengyel, T. and Ure, A.M. (1998) Compendium of Analytical Nomenclature (definitive rules 1997), 3rd edition, Blackwell Science, 1998.

16 Holland, P.T. (1996) Glossary of Terms Relating to Pesticides. *Pure & Appl. Chem.*, **68**: 1167-1193.

17 Youden, W.J., & Steiner, E.H. (1975) *Statistical Manual of the Association of Official Analytical Chemists,* AOAC International, Gaithersburg, VA.

37. *The capability of a residue control method to simultaneously analyze a set of samples aids in method efficiency by allowing sets or batches of samples to be analyzed at the same time. This attribute reduces the analytical time requirements of sample analysis* and usually also results in a lower cost per sample, as there are certain fixed costs associated with the analysis of samples, whether done singly or in larger sets. The ability of a method to accommodate multiple samples in a batch *is important when large numbers of samples must be analyzed in short or fixed time frames.*

38. *Establishing method performance attributes is very important. These attributes provide the necessary information for food safety agencies to develop and manage their public health programmes. Performance attributes for analytical methods also provide a basis for good management decisions in future planning, evaluation, and product disposition. For the animal health care industry, it provides a guideline for knowing exactly what performance must be achieved in developing analytical procedures. All will benefit by having well defined analytical method performance factors.* Method performance requirements will vary, depending on whether the method is used for the detection, determination or confirmation of a residue for which Maximum Residue Limits have been established, or for residues of a drug which has been formally banned from use in food-producing animals. In the latter case, the competent authority may establish a minimum performance standard which must be met by methods used by regulatory authorities. However, since no safe concentrations of these compounds in foods can be established, the competent authority will feel obliged to adjust such limits to lower concentrations, as required to reflect improvements in technology and analytical capability. When such limits have not been formally established by the competent authority, they are usually established *de facto* by the detection capabilities of the methods used in the regulatory laboratories.

## INTEGRATING ANALYTICAL METHODS FOR RESIDUE CONTROL

39. *Residue control and standard setting organizations have different terminologies to describe application of analytical methods. Methods of analysis for veterinary drug residues in foods must ultimately be able to reliably detect the presence of an analyte of interest, determine its concentration, and correctly identify the analyte.* For residues resulting from the use of approved substances, the presence of residues *at and above an established maximum residue limit (MRLVD)* should be confirmed *for regulatory enforcement actions to be taken*. For substances which have been banned from use in food-producing animals, the confirmed presence of residues at any concentration in a food will usually result in regulatory action. Methods which confirm the identity of an analyte are *classified as confirmatory methods. These confirmatory methods may or may not have a quantitative or semi-quantitative component.*

40. *Other types of methods that may be used in residue control programmes, and which can strengthen such a programme, may be classified into two additional categories. These categories are quantitative,* or determinative, *methods and screening methods. Quantitative methods provide precise information concerning the amount of an analyte that may be present, but may only provide indirect information about the structural identity of the analyte. Screening methods may quickly determine the presence of one or more compounds, based upon one or more common characteristic of a class of veterinary drugs in a qualitative or semi-quantitative manner at a specified concentration limit. They may also determine that an analyte is below the limit of detection of the screening method.*

41. *These three categories of methods, confirmatory, quantitative, and screening, often share a common set of performance characteristics described above. In addition, they may have other specific considerations. Understanding the relationship between these three categories of methods is important in the development and operation of a balanced residue control programme. Screening methods are useful because they provide greater analytical efficiency (i.e., a greater number of analyses may be performed in a given time frame) than quantitative and/or confirmatory methods. In many circumstances screening methods can be performed in non-laboratory environments. Screening methods suitable for use in non-laboratory environments may be less expensive for regulatory control programmes than conducting all testing within a laboratory setting. Screening methods can be* used *to separate test samples with no detectable residue from those that indicate the presence of a veterinary drug residue at or below an MRLVD or an appropriate level of interest. This would allow a laboratory to focus more of its efforts on quantitation of the presumptive positive test samples of regulatory interest.*

42. *Screening tests may also be used efficiently in a laboratory setting because they analyze a larger numbers of samples in a given time frame than their corresponding quantitative methods. The cost savings may not be as great as when screening methods are used in non-laboratory environments because the costs associated with the handling and shipping of samples must still be incurred. Presumptive positive results obtained from laboratory screening methods should not be used independently in taking regulatory action. Data obtained from such methods may be used to determine the need for additional testing and/or the development of a method suitable for routine enforcement of MRLVDs.*

**METHOD DEVELOPMENT AND VALIDATION CONSIDERATIONS FOR RESIDUE CONTROL METHODS**

43. Laboratories must demonstrate that the methods in use for analysis of regulatory samples have been suitably validated. Traditionally, the *multi-laboratory method validation study has been the preferred approach to provide analytical data to define method performance characteristics*. However, other models have been developed which include multi-laboratory trials with smaller numbers of laboratories than are required to conduct a full collaborative study and single laboratory validation[6] based on rigourous in-house evaluation of method performance, supported by a quality system, independent audits and analysis of proficiency or reference materials, when available. *In developing a residue control method, whenever possible, data should be collected from three types of samples. Control test material from non-treated animals provides information about analytical background and matrix interferences. Fortified test material, containing known amounts of the analyte added to the control material, yields information about the method's ability to recover the analyte of interest under controlled conditions. Dosed or biologically incurred tissue, from food producing animals and birds that have been treated with the drug, provide additional analytical performance information about biological or other interactions that may occur when analyzing residue control samples.* Tissues should be obtained from multiple sources to cover the variations resulting from factors such as different diets, husbandry practices, sex and breed of animals.

44. *Residue methods should be designed with as much simplicity as possible. Analytical simplicity helps minimize the variety, size, and type of glassware and equipment needed, minimizes the potential for analytical errors, and reduces laboratory and method costs. Reagents and standards must be available commercially or from some other reliable source. Instrumentation should be selected based on its performance characteristics rather than a particular manufacturer*. Laboratories should provide their clients with information on the measurement uncertainty associated with the quantitative results produced by each determinative method. This requires a review of the method to determine the potential error that may be introduced at each step of the method, from preparation of standards, selection and weighing of test portions, through each step in the analysis to final measurement. the more complex and involved the method, the more difficult this becomes to accomplish.

45. *Residue methods are sometimes designed using internal standards for analytical control. A properly used internal standard will compensate for some of the analytical variability of an analysis, improving precision. However, an improperly used internal standard may obscure variables that are an important part of the analytical measurement. If an internal standard is used, it should be added to a sample as early as possible in the procedure, preferably to the test material before analysis begins.* The internal standard must reflect the recovery of the target analyte in a uniform and predictable fashion. An internal standard that does not mirror the behavior of the target analyte in the method will lead to significant errors in calculation of the final result. *Caution must be taken in the choice of internal standards to ensure that they do not alter the percent recovery of the analyte of interest or interfere with the measurement process. It is important to know the extent and predictability of the effects of the internal standard on an analytical method. Internal standards can greatly enhance method performance when used properly*.

46. *Residue control methods that may be subjected to widely variable physical test environments will place some additional requirements on methods. Addressing these may help improve method ruggedness. Warmer environments may require reagents to be more thermally stable, while solvents used in the analysis will have to be less volatile, and test sample requirements to be more lenient. Cooler environments may require reagents and solvents to have different physical properties, such as lower freezing point and greater solvating characteristics, to ensure effective extraction of an analyte. Environmental temperatures may influence the time required to perform an analysis, as well as influencing reaction rates, gravitational separations and colour development. These considerations may strain efforts to standardize methods for use in broadly differing environments because of the need to adapt methods to compensate for these factors.* It is important when considering the physical environment in which a method will be used to remember that volumetric glassware and many analytical instruments are calibrated to be used at specific temperatures, or within a controlled range of temperature. Operation outside these temperatures may compromise test results.

47. *An analytical method developed and used in only one laboratory may have limited use in a residue control programme* unless care is taken to meet the rigourous expectations for single laboratory method validation associated with accreditation under ISO/IEC-17025 or equivalent accreditation procedures for testing laboratories. *The reliability of reported values may be a concern even though strong quality control procedures may have been employed, unless supported by data from an on-going proficiency programme, comparison with a suitable reference method or other forms of inter-laboratory comparison of results. As a minimum,* it was previously recommended that *three laboratories expected to use these methods should develop performance characteristics for residue control, including analytical variability, and obtain statistically acceptable agreement on the same samples divided among the testing laboratories*. Such an approach is still preferred, whenever possible. However it is also recognized that the rapid changes in technology and the ever-increasing range of compounds which may be included in a residue control programme requires from a practical approach that laboratories focus first on internal validation of methods to meet the time constraints. Methods which have been carefully validated in a single laboratory with inclusion of properly designed ruggedness tests *should be able to successfully undergo a collaborative study involving at least eight different laboratories*.

48. *The principles for conducting either a* single laboratory *validation*, a multi-laboratory method trial *or* a *collaborative study of a residue control method are the same. Samples for evaluating method performance should be unknown to the analyst*, in randomized duplicates, *contain*ing *the residue near the MRLVD* or other target concentration, *as well as samples with the analyte above and below the level of interest, and test material blanks. All study samples should be analyzed over a limited number of days, preferably with replicate analysis, to improve statistical evaluation of method performance. It should be noted that these are only minimal requirements.* The establishment of statistically-based performance standards for methods is enhanced by increasing the number of independent analysts and laboratories testing the method, as well as by the number of samples tested. In a single-laboratory validation, it is recommended that the method should be tested by multiple analysts to provide appropriate measures of within-laboratory performance. Expanding the validation to include other laboratories, preferably to the number required for a collaborative study, is recommended. *Duplicate analyses in only* eight *laboratories with one or two animal species and tissues yield limited quality estimates for* overall *repeatability and reproducibility*. The validation of a collaboratively studied method can be extended to include additional tissues and species in a subsequent study conducted by a single expert laboratory, as required.

49. *Quality control and quality assurance principles are essential components of residue analysis. They provide the basis for ensuring optimum method performance for all methods, regardless of method attributes, whenever they are used. Quality control monitors those factors associated with the analysis of a sample by a tester, while quality assurance provides the oversight by independent reviewers to ensure that the analytical programme is performing in an acceptable manner. Quality control and quality assurance programmes are invaluable to support decision-making for residue control agencies, improving the reliability of analytical results, and providing quality data for residue control programmes to demonstrate food safety to consumers, producers, and law making bodies regarding residues of veterinary drugs in food.* The establishment of quality measures consistent with the principles published by the International Union of Pure and Applied Chemistry is recommended for regulatory control laboratories[1].