codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 8 **CX/RVDF 06/16/9**
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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON RESIDUES OF VETERINARY DRUGS IN FOODS

Sixteenth Session

Cancun, Quintana Roo*, Mexico, 8 -12 May 2006

PROPOSED DRAFT REVISED PART I, II, III OF THE CODEX GUIDELINES FOR THE ESTABLISHMENT OF A REGULATORY PROGRAM FOR THE CONTROL OF VETERINARY DRUG RESIDUES IN FOODS

(at Step 3 of the Elaboration Procedure)

Document prepared by Canada and the Netherlands with the assistance of Australia, Brazil, Republic of Korea, Poland, Sweden, Thailand, the United Kingdom and the United States.

Governments and international organizations wishing to submit comments at Step 3 on the following subject matter are invited to do so **no later than 28 February 2006** 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 15th Session of the CCRVDF agreed to return the Proposed Draft Revised Guidelines to Step 2, and agreed that a Working Group led by Canada would redraft all sections on methods of analysis and sampling in the Guidelines (Part I, II and III), for comments and consideration by the next session (ALINORM 05/28/31, para. 132).

PROCEEDINGS OF THE WORKING GROUP:

2. The Working Group worked primarily by e-mail exchange of documents and comments, with several meetings held by the members from Canada and the United Kingdom to consolidate comments from other members and edit the draft documents. A first draft of revisions of Parts II and III was prepared by Canada incorporating previously received comments and circulated to other members of the Working Group in early February, 2005, with a deadline for comments of April 30, 2005. Members of the Working Group from Canada and the United Kingdom met in early May to consolidate comments received from other members of the drafting group and prepare a revised document. This was circulated to all members in late May, with a request for comments by June 30, 2005. Members of the Working Group from Canada and the United Kingdom met again in early July and again consolidated the comments of other Working Group members into a revised draft.

* To be confirmed

3. Part I, dealing with sampling, was circulated to all members of the Working Group in late April, 2005, with a request for comments by May 31, 2005. Comments on this document were also consolidated by the Working Group members from Canada and the United Kingdom during their meeting in July and a revised draft document was prepared incorporating comments from Working Group members. The revised drafts of Parts I, II and III were again circulated to all members with a request for final comments by September 15. Final editing and revisions were completed in late September. During the drafting and comment process, comments from Working Group members and the response, either a revision or no change with explanatory comments, were included with each version so that all members were aware of all comments made and all revisions. As requested by the Committee in assigning the work to this Working Group, all drafts were sent to the chair of the Working Group led by New Zealand assigned to revision of the main body of the guidelines concerning regulatory programmes to ensure coordination of effort between the Working Groups (ALINORM 05/28/31, para. 133).

4. The revisions to Part I dealing with sampling are primarily editorial, with consolidation of the specific instructions on sampling. Revisions to Parts II and III reflect changes in analytical science and laboratory management practices since the adoption of the original text of Parts I-III by the Committee, including laboratory accreditation, proficiency testing and alternative approaches to method validation, such as the single laboratory validation model. These revisions reflect the general directions given in the *Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Foods* (CAC/GL 27-1997), the recommendations of several expert consultations referenced in the text and on-going work in the Codex Committee on Methods of Analysis and Sampling and the Codex Committee on Pesticide Residues to incorporate the "analytical system" model into Codex guidance documents.

RECOMMENDATION TO THE CCRVDF:

5. The Working Group recommends that the Committee may wish to consider this document at its 16th Meeting as a revision to the current text of the relevant sections of the Codex Alimentarius, Volume 3 (CAC/GL 16-1993, Parts I-III).

PROPOSED DRAFT REVISED PART I, II, III OF THE CODEX GUIDELINES FOR THE ESTABLISHMENT OF A REGULATORY PROGRAM FOR THE CONTROL OF VETERINARY DRUG RESIDUES IN FOODS

(at Step 3 of the Elaboration Procedure)

PART I - SAMPLING FOR THE CONTROL OF RESIDUES OF VETERINARY DRUGS IN FOODS

I.1. INTRODUCTION

I.1.1 BASIS FOR THE SAMPLING PRINCIPLE

5. The Codex Alimentarius Commission has decided that recommended sampling procedures for food additives, pesticide residues and residues of veterinary drugs in food are exempted from the general sampling procedures of food commodities developed by the Codex Committee on Methods of Analysis and Sampling - Normal Practice. That committee's work is concerned mainly with sampling procedures for the visible and measurable qualities and attributes of various commodities and foods; sampling to determine whether standards of identity and composition have been met and to measure traditional attributes of quality, such as dust and moisture content in grain. The Codex Committees that are responsible for establishing permitted levels of regulated added substances - food additives, pesticides, veterinary drugs in food, have been given authority to prepare their own recommendations for methods of analysis and sampling. In this regard, the Codex Committee on Residues of Veterinary Drugs in Foods established an *Ad Hoc* Working Group on Methods of Analysis and Sampling at its first meeting.

I.1.2 GENERAL PRINCIPLES

6. Sampling for analytical testing is only one element of a country's residue control programme and, by itself, cannot accomplish the entire objective of protecting public health. Sampling is a tool used as part of the system for developing information to determine if a supply of foodstuffs meets public health requirements, in this case, that the concentration of veterinary drug residues are within specified limits.

7. Sampling has varying purposes and statistical parameters. This guideline discusses the various objectives which sampling may address and provides technical guidance to be applied for sampling products within the terms of reference of this Codex Committee. By using Codex standards, including agreed upon sampling methods, member countries can comply with Article III of the General Agreement on Tariffs and Trade.

8. In sampling for residues of an added, regulated substance such as a veterinary drug, it is important to sample as near as possible to where animals raised for food are cared for and slaughtered in herds or flocks. The most meaningful sampling for tissue residues will occur in conjunction with slaughter. For other food products within the scope of this Committee, such as honey, the most meaningful sampling for residues will occur at the time of collection, prior to commingling of samples from different producers.

9. Samples can be taken from animals (including fish) or animal products (e.g. milk or honey) prior to further processing or slaughter in order to provide information to the inspector on the residue status. The samples collected may be in the form of body fluids from live animals or tissues from a small number of animals representative of the herd or flock. Residue testing of body fluids is generally only appropriate as a screening mechanism to test for the presence of a veterinary drug residue. When MRLVDs are established testing to confirm compliance should be undertaken on tissues sample for which an MRLVD is set.

10. Samplers should be aware that processing schedules in production plants (including abattoirs) are such that lots or batches of animals or animals products may be mixed during processing. Such mixing may dilute the residue from an individual animal or product batch. If residues are found in samples, post-production tracing of the source of the residue could be compromised. For example, processed products such as sausage or minced fish may be made with tissues from different days' or even different establishments' production. It is therefore recommended that sampling of animals or animal products is conducted while the single production source (herd, flock or batch) can be readily identified.

I.2. OBJECTIVES OF SAMPLING

I.2.1 PRIMARY POINT OF ORIGIN SAMPLING

I.2.1.1 Non-biased sampling

11. Non-biased sampling is designed to provide profile information on the occurrence of residues in specified food producing populations on an annual, national basis. For residue testing, the focus is on gathering information on the prevalence of residue non-compliances; therefore, only compounds with established safe limits such as MRLVDs are usually considered for residue control programmes. Compounds selected for statistically designed non-biased sampling are usually based on risk profiles (considering toxicity of residues and use) and the availability of analytical methods suitable for regulatory control purposes. Information is obtained through a statistically based selection of random samples from animals presented for inspection. Limited or geographical area sampling may be conducted where a localized potential drug residue problem appears. The information obtained from this type of sampling should be reviewed periodically to assess residue control programmes and to allocate resources according to specific needs.

12. In addition to profile information, residue data provides a basis for further regulatory action. In particular, the results can be used to identify producers marketing animals, or other food commoditieswithin the terms of reference of this Committee, which contain concentrations of residues in excess of MRLVDs or residues of banned substances. When these producers subsequently bring animals, fish or honey for inspection, they will be subjected to more directed and specific sampling and testing until compliance with MRLVDs is demonstrated. Other auxiliary uses of the data are to indicate prevalence and concentrations of residue non-compliances, to evaluate residue trends, and to identify residue problem areas within the industry where educational or other corrective efforts may be needed. Thus, non-biased sampling gathers information and assists in deterring practices that lead to residue non-compliances.

13. As a general practice, samples collected by inspectors are sent for residue analysis to a laboratory designated by national authorities. Now, however, advances in analytical technology provide inspection authorities an opportunity for performing residue screening tests on commodities at an abattoir or similar facility. In these situations, inspectors may send tissue samples to a laboratory designated by national authorities for more definitive analyses when results obtained from the screening test suggest a positive residue finding.

14. In some cases and situations where samples are sent directly to a designated laboratory for residue testing, the laboratory results may not be available until after the product has moved into consumer markets and become untraceable. Because of this pragmatic limitation, some animals, fish or honey containing residues which exceed MRLVDs may inevitably pass into consumer markets, regardless of the regulatory control efforts to limit this occurrence as much as possible. The consequences to human health, however, are minimal as long as the frequency of such occurrences is low. This is because MRLVDs represent the maximum residue concentration determined to be safe for daily consumption within the limits of the acceptable daily intake (ADI) over a lifetime. As a result of employing safety factors for determining an ADI, and subsequently the MRLVD, the occasional consumption of products with slightly higher residue concentrations than the MRLVD is unlikely to result in adverse health effects.

15. Non-biased sampling should have a statistically specified reliability. This may be expressed in reference to a confidence level and a prevalence rate. For example, sampling may be designed to detect, with 95% certainty, a prevalence occurring in 1% of healthy animals submitted for inspection. When a confidence level and prevalence rate is established, the number of samples necessary to achieve the desired objective can be determined from Table 1.

Table 1: Number of samples required to detect at least one non-compliance with predefined probabilities (i.e., 90, 95, and 99 percent) in a population having a known non-compliance prevalence.

Non-compliance prevalence (% in a population)	Minimum number of samples required to detect a non-compliance with a confidence level of:		
	90%	95%	99%
35	6	7	11
30	7	9	13
25	9	11	17
20	11	14	21
15	15	19	29
10	22	29	44
5	45	59	90
1	230	299	459
0.5	460	598	919
0.1	2302	2995	4603

I.2.1.2 Directed sampling

16. Directed sampling is designed to investigate and control the movement of potentially adulterated products. The sampling is often purposely biased and is directed at particular carcasses, products or producers in response to information from statistically based sampling (or other regulatory control agency data), or from inspector observations during ante-mortem or post-mortem inspection indicating that residues which are non-compliant may be present. In-plant or on site residue testing procedures may be performed by the inspector, or samples may be submitted for analysis to a laboratory designated by national authorities. Depending upon the weight of evidence for testing in support of directed sampling, product may be retained until test results indicate the appropriate regulatory disposition. Laboratory analysis of directed residue test samples should be completed as rapidly as possible and take precedence over routine, statistically based samples. In directed sampling situations, herds of animals, flocks of birds, lots of fish or honey, may be considered unacceptable until it can be demonstrated that they are in compliance with Codex MRLVDs or national regulations in the country of origin for the specific commodity.

17. The probability of failing to detect a residue non-compliance with an MRLVD and accepting the lot depends upon the directed sampling programmes' sample size and prevalence of the residue non-compliance frequency. Table 2 shows the probability of failing to detect a residue non-compliance using different sample sizes from an "infinite" population with a specified proportion of non-compliances. For example, selecting 5 samples from a large lot in which 10 percent of the units contain residues not in compliance would, on the average, fail to detect a residue non-compliance in 59.0 percent of such lots (i.e., 59.0 percent of the lots would be accepted). Assuming the same conditions as the previous example, but using a sample size of 50, would result in only 0.5 percent of such lots being accepted.

Table 2: Probability of failing to detect a residue non-compliance with a MRLVD

Prevalence (%)	Number of animals in sample tested									
	5	10	25	50	75	100	200	250	500	1000
1	0.951	0.904	0.779	0.605	0.471	0.366	0.134	0.081	0.007	0.000
2	0.904	0.817	0.603	0.364	0.220	0.133	0.018	0.006	0.000	
3	0.859	0.737	0.467	0.218	0.102	0.048	0.002	0.000		
4	0.815	0.665	0.360	0.130	0.047	0.017	0.000			
5	0.774	0.599	0.277	0.077	0.021	0.006				
6	0.734	0.539	0.213	0.045	0.010	0.002				
7	0.696	0.484	0.163	0.027	0.004	0.001				
8	0.659	0.434	0.124	0.015	0.002	0.000				
9	0.624	0.389	0.095	0.009	0.001					
10	0.590	0.349	0.072	0.005	0.000					
12	0.528	0.279	0.041	0.002						
14	0.470	0.221	0.023	0.001						
16	0.418	0.175	0.013	0.000						
18	0.371	0.137	0.007							
20	0.328	0.107	0.004							
24	0.254	0.064	0.001							
28	0.193	0.037	0.000							
32	0.145	0.021								
36	0.107	0.012								
40	0.078	0.006								
50	0.031	0.001								
60	0.010	0.000								

18. Risk and cost factors should be considered in determining the sample sizes used in a directed sampling programme. Also, because of possible gains in the probability of detecting unacceptable herds of animals, flocks of birds, lots of fish or honey due to residue non-compliance with MRLVDs, the feasibility of selecting separate samples from separate lots instead of from a single lot should be considered.

I.2.2 SECONDARY POINT OF SAMPLING

I.2.2.1 Port of entry sampling

19. Port of entry testing of products derived from food producing animals, poultry, or fish, and honey, imported by member countries of Codex Alimentarius is a means of verifying the effectiveness of the exporting country's residue control programme. Such testing should be statistically based and should reflect both the frequency and the volume of the trade in the product. The purpose of port of entry sampling and testing is not to replace an exporting country's residue control programmes.

20. Results of residue testing that indicate imported product is in compliance with Codex MRLVDs should permit the product to move into commerce. When test results indicate that imported product contains non-compliant residues, subsequent shipments of the same product group from that establishment or company should be retained at the port of entry until laboratory results indicating compliance with MRLVDs are known by regulatory control authorities. Consideration should be given to placing all subsequent shipments of similar products from the country of origin on an increased testing schedule until a record of compliance with Codex MRLVDs is re-established.

21. Compounds selected for residue testing at port of entry should take into account the compounds approved for use in the exporting country, as well as those included in the domestic residue control programme of the importing and exporting country. Guidance for collecting samples for port of entry testing is summarized in Appendix A, Table A, Appendix B, Table B and Appendix C.

Appendix A

SAMPLING FOR THE CONTROL OF VETERINARY DRUG RESIDUES IN ANIMALS, ANIMAL PRODUCTS AND ANIMAL-DERIVED FOODS (EXCEPT HONEY)

1. OBJECTIVE

22. To provide instructions for sampling a lot of animals (including fish), animal products or animal-derived foods to determine compliance with Codex Maximum Residue Limits for Veterinary Drugs (MRLVDs).

2. DEFINITIONS

2.1 Lot

23. An identifiable group of animals or quantity of animal product intended for food use and determined to have common characteristics, such as origin, variety, type of packing, packer or consignor, or markings, by the sampling official. Several Lots may make up a consignment.

2.2 Consignment

24. An identifiable group of animals or quantity of animal product intended for food use as described on a particular contractor's shipping document. Lots in a Consignment may have different origins or may be delivered at different times.

2.3 Primary Sample

25. A quantity of representative biological material taken from a single animal (or group of animals) or from one place in the Lot. When the quantity is inadequate for residue analysis, samples from more than one animal (or group of animals) or more than one location in the Lot can be combined for the Primary Sample (such as poultry organs).

2.4 Bulk Sample

26. The combined total of all the Primary Samples taken from the same Lot.

2.5 Final (Laboratory) Sample

27. The Primary or Bulk sample, or a representative portion of the Primary or Bulk Sample, intended for laboratory analysis.

2.6 Laboratory Test Portion

28. The representative portion of the Final (Laboratory) Sample on which an analysis is conducted. The entire Laboratory Sample may be used for analysis in some cases but typically will be sub-divided into representative test portions for analysis.

3. COMMODITIES TO WHICH THE GUIDELINE APPLIES

3.1 Selected Class B: Primary Food Commodities of Animal Origin

Type 06 Mammalian Products

No. 030 Mammalian Meat
No. 031 Mammalian Fats
No. 032 Mammalian Edible Offal
No. 033 Milks

Type 07 Poultry Products

No. 036 Poultry Meats
No. 037 Poultry Fats
No. 038 Poultry Edible Offal

No. 039 Eggs

Type 08 Aquatic Animal Products

No. 040 Freshwater Fish
No. 041 Diadromous Fish
No. 043 Fish Roe and Edible Offal of Fish
No. 045 Crustaceans

Type 09 Amphibians and Reptiles

No. 048 Frogs, Lizards, Snakes and Turtles

Type 10 Invertebrate Animals

No. 049 Molluscs and Other Invertebrate Animals

3.2 Selected Class E: Processed Products of Animal Origin made from only Primary Food Nos. 030, 032, 036, and 038

Type 16 - Secondary Products

Type 17 - Derived Edible Products of Aquatic Animal Origin

Type 18 - Manufactured (single ingredient) Products of a Minimum of One Kilogram Container or Unit Size

Type 19 - Manufactured (multiple ingredient) Products of a Minimum of One Kilogram Container or Unit Size

4. PRINCIPLE ADOPTED

29. For purposes of control, the MRLVD is applied to the residue concentration found in each Laboratory Sample taken from a Lot. Lot compliance with a Codex MRLVD is achieved when the mean result for analysis of the Laboratory Test Portions does not indicate the presence of a residue which exceeds the MRLVD.

5. EMPLOYMENT OF AUTHORIZED SAMPLING OFFICIALS

30. Samples must be collected by officials authorized for this purpose.

6. SAMPLING PROCEDURES

6.1 Product to Sample

31. Each Lot to be examined must be sampled separately.

6.2 Precautions to Take

32. During collection and processing, contamination or other changes in the samples which would alter the residue, affect the analytical determination, or make the Laboratory Test Portion not representative of the Bulk or Laboratory Sample, must be prevented.

6.3 Collection of a Primary Sample

33. Detailed instructions for collection of a Primary Sample of various products are provided in Tables A and B. Quantities to collect are dependent on the analytical method requirements. Minimum quantity requirements are included in Table A: Meat and Poultry Products; Table B: Milk, Eggs, Dairy Products and Aquatic Animal Products. The following are general instructions.

a. Each Primary Sample should be taken from a single animal (or group of animals) or unit in a Lot, and when possible, be selected randomly.

b. When multiple animals are required for adequate sample size of the Primary Sample (i.e., poultry organs), the samples should be collected consecutively after random selection of the starting point.

c. Frozen product should not be thawed before sampling.

d. Canned or packaged product should not be opened for sampling unless the unit size is at least twice the amount required for the Final (Laboratory) Sample. The Final (Laboratory Sample) should contain a representative portion of juices surrounding the product.

- Unopened cans or packages which constitute a Final (Laboratory) Sample should be sent unopened and intact to the laboratory for analysis.

e. e. The contents of cans or packages opened by the inspector should then be frozen as described in paragraph 6.8.d before dispatch to the laboratory for analysis.

f. Large, bone-containing units of product (i.e., prime cuts) should be sampled by collecting edible product only as the Primary Sample.

g. Remaining portions of Final (Laboratory) Samples, after removal of Laboratory Test Portions for analysis, should be frozen and stored in conditions which will maintain the sample integrity.

6.4 The Number of Primary Samples to Collect from a Lot

34. The number of Primary Samples collected will vary depending on the status of the Lot. A Lot may be considered suspect if there is a history of non-compliance with the MRLVD, evidence of contamination during transport, signs of toxicosis observed during ante- or post-mortem inspection, or other relevant information available to the inspection official. If there is no reason to suspect adulteration, the Lot is designated as non-suspect.

6.4.1 Sampling Suspect Lots

35. A minimum of six to a maximum of thirty Primary Samples should be collected from a Suspect Lot. When the suspected adulteration is expected to occur throughout the Lot or is readily identifiable within the Lot, the smaller number of samples is sufficient.

6.4.2 Sampling Non-Suspect Lots

36. A statistically-based, non-biased sampling programme is recommended for Non-Suspect Lots. Any of the following types of sampling can be used.

a. Stratified Random Sampling

37. In a complex system where commodities must be sampled at many locations over extended time periods, it is very difficult to apply simple random criteria in the design of a sampling programme. A useful alternative sampling design is Stratified Random Sampling which separates population elements into non-overlapping groups, called strata. Primary Samples are selected within each stratum by a simple random design. Homogeneity within each stratum is better than in the whole population. Countries or geographic regions are considered natural strata based on uniformity in agricultural practices. Time strata (e.g., month, quarter) are commonly used for convenience, efficiency, and detection of seasonal variability. Random number tables or other objective techniques should be used to ensure that all elements of a population have an equal and independent chance of being included in the sample.

b. Systematic Sampling

38. Systematic Sampling is a method of selecting a sample from every 'K' quantity of product to be sampled, and then sampling every 'K' unit thereafter. Systematic Sampling is quicker, easier, and less costly than non-biased sampling, when there is reliable information on product volumes to determine the sampling interval that will provide the desired number of samples over time. If the sampling system is so predictable that it may be abused, it is advisable to build some randomness around the sampling point within the sampling interval.

c. Biased or Estimated Worst Case Sampling

39. In Biased or Estimated Worst Case Sampling, the investigators should use their judgement and experience regarding the population, Lot, or sampling frame to decide which Primary Samples to select. As these are non-random samples, no inferences should be made about the population sampled from the data collected. The population group anticipated to be at greatest risk may be identified. Exporting countries should conduct a comprehensive residue control programme and provide results to importing countries. Based on an importing country's data, testing may be conducted as applied to non-suspect products. Countries that do not provide residue testing results showing compliance with MRLVDs should be sampled as suspect lots.

6.5 Preparation of the Bulk Sample

40. The Bulk Sample is prepared by combining and thoroughly mixing the Primary Samples.

6.6 Preparation of the Final (Laboratory) Sample

41. The Primary or Bulk Sample, or a representative portion of the Primary or Bulk Sample, which constitutes the Laboratory Sample, should be submitted to the laboratory for analysis.

42. Some national legislation may require that the Final (Laboratory) Sample is sub-divided into two or more portions for separate analyses. Each portion should be representative of the Final (Laboratory) Sample. Precautions in paragraph 6.2 should be observed.

6.7 Preparation of the Laboratory Test Portion

43. The Laboratory Test Portion should be prepared from the Final (Laboratory) Sample by an appropriate method of reduction.

6.8 Packaging and Transmission of Final (Laboratory) Samples

44. a. Each sample should be placed in a clean, thermally insulating, chemically inert container to protect the sample from contamination, defrosting and damage in shipping.

b. The container should be sealed so that unauthorized opening is detectable.

c. The container should be sent to the laboratory as soon as possible, after taking precautions against leakage and spoilage.

d. For shipping, all perishable samples should be frozen to minus 20^{o}C, immediately after collection, and packed in a suitable container that retards thawing. Freezer packs or other suitable refrigerants should be used to maintain freezer temperatures during shipment. Samples and freezer packs should be fully frozen to minus 20^{o}C prior to dispatch.

e. Replicate portions of the Final (Laboratory) Sample which may be retained as required by national legislation or as an administrative policy should be placed in a clean, chemically inert container to protect the sample from contamination, sealed so that unauthorized opening is detectable and stored under suitable conditions to prevent a change in the product or any residues it may contain in case future analysis is required for comparison with analytical results obtained on the sample material submitted to the laboratory.

7. RECORDS

45. Each Primary or Bulk Sample and each Final (Laboratory) Sample should be uniquely linked to a record with the type of sample, analyses required, its origin (e.g., country, state, or town), its location of collection, date of sampling, and additional information required for follow-up action if necessary.

8. DEPARTURE FROM RECOMMENDED SAMPLING PROCEDURES

46. If there is a departure from recommended sampling procedures, records accompanying the sample should fully describe procedures actually followed.

TABLE A: MEAT AND POULTRY PRODUCTS		
Commodity	**Instructions for collection**	**Minimum quantity required for laboratory sample**
I. Group 030 (Mammalian Meats)		
A. Whole carcass or side, unit weight normally 10 kg or more	Collect diaphragm muscle, supplement with cervical muscle, if necessary, from one animal.	500 g
B. Small carcass (e.g., rabbit)		500 g after removal of skin and bone
C. Fresh/chilled parts		
1. Unit minimum weight of 0.5 kg, excluding bone (e.g., quarters, soulders, roasts)	Collect muscle from one unit.	500 g
2. Unit weighing less than 0.5 kg (e.g., chops, fillets)	Collect the number of units from selected container to meet laboratory sample size requirements.	500 g after removal of bone
D. Bulk frozen parts	Collect a frozen cross-section from selected container, or take muscle from one large part.	500 g
E. Retail packaged frozen/chilled parts, or individually wrapped units for wholesale	For large cuts, collect muscle from one unit or take sample from number of units to meet laboratory sample size requirements.	500 g after removal of bone
Ia. Group 030 (Mammalian Meats where MRL is expressed in carcass fat)		
A. Animals sampled at slaughter	See instructions under II. Group 031.	
B. Other meat parts	Collect 500 g of visible fat, or sufficient product to yield 50-100 g of fat for analysis. (Normally 1.5-2.0 kg of product is required for cuts without trimmable fat).	Sufficient to yield 50-100 g of fat
II. Group 031 (Mammalian Fats)		
A. Large animals sampled at slaughter, usually weighing at least 10 kg	Collect kidney, abdominal, or subcutaneous fat from one animal.	500 g
B. Small animals sampled at slaughter[1]	Collect abdominal and subcutaneous fat from one or more animals.	500 g
C. Bulk fat tissue	Collect equal size portions from 3 locations in container.	500 g
III. Group 032 (Mammalian Edible Offal)		
A. Liver	Collect whole liver(s) or portion sufficient to meet laboratory sample size requirements.	400 - 500 g

TABLE A: MEAT AND POULTRY PRODUCTS		
Commodity	**Instructions for collection**	**Minimum quantity required for laboratory sample**
B. Kidney	Collect one or both kidneys, or kidneys from more than one animal, sufficient to meet laboratory sample size requirement. Do not-collect from more than one animal if size meets the low range for sample size.	250 - 500 g
C. Heart	Collect whole heart or ventricle portion sufficient to meet laboratory sample size requirement.	400 - 500 g
D. Other fresh/chilled orfrozen, edible offal product	Collect portion derived from one animal unless product from more than one animal is required to meet laboratory sample size requirement. A cross-section can be taken from bulk frozen product.	500 g
IV. Group 036 (Poultry Meats)		
A. Whole carcass of large bird, typically weighing 2-3 kg or more (e.g., turkey, mature chicken, goose, duck)	Collect thigh, leg, and other dark meat from one bird.	500 g after removal of skin and bone
B. Whole carcass of bird typically weighing between 0.5-2.0 kg (e.g., young chicken, duckling, guinea fowl)	Collect thigh, legs, and other dark meat from 3-6 birds, depending on size.	500 g after removal of skin and bone
C. Whole carcasses of very small birds typically weighing less than 500 g (e.g., quail, pigeon)	Collect at least 6 whole carcasses	. 250 - 500 g of muscle tissue
D. Fresh/chilled or frozen parts		
1. Wholesale packaged a. Large parts b. Small parts	 Collect an interior unit from a selected container. Collect sufficient parts from a selected layer in the container.	500 g after removal of skin and bone
2. Retail packaged	Collect a number of units from selected container to meet laboratory sample size requirement.	500 g after removal of skin and bone
IVa. Group 036 (Poultry Meats where MRLVD is expressed in carcass fat)		
A. Birds sampled at slaughter	See instructions under V. Group 037	
B. Other poultry meat	Collect 500 g of fat or sufficient product to yield 50-100 g of fat. (Normally, 1.5-2.0 kg is required.)	500 g of fat or enough tissue to yield 50-100 g of fat

TABLE A: MEAT AND POULTRY PRODUCTS		
Commodity	**Instructions for collection**	**Minimum quantity required for laboratory sample**
V. Group 037 (Poultry Fats)		
A. Birds sampled at slaughter	Collect abdominal fat from 3-6 birds, depending on size.	Sufficient to yield 50-100 g of fat
B. Bulk fat tissue	Collect equal size portions from 3 locations in container.	500 g
VI. Group 038 (Poultry Edible Offal)		
A. Liver	Collect 6 whole livers or a sufficient number to meet laboratory sample requirement.	250 - 500 g
B. Other fresh/chilled or frozen edible offal product	Collect appropriate parts from 6 birds. If bulk frozen, take a cross-section from container.	250 - 500 g
VII. Class E - Type 16 (Secondary Meat and Poultry Products)		
A. Fresh/chilled or frozen comminuted product of single species origin	Collect a representative fresh or frozen cross-section from selected container or packaged unit.	500 g
B. Group 080(Dried Meat Products)	Collect a number of packaged units in a selected container sufficient to meet laboratory sample size requirements.	500 g, unless fat content is less than 5% and MRLVD is expressed on a fat basis. Then 1.5-2.0 kg is required.
VIII. Class E-Type 18 (Manufactured, single ingredient product of animal origin)		
A. Canned product (e.g., ham, beef, chicken), unit size of 1 kg or more	Collect one can from a lot. When unit size is large (greater than 2 kg), a representativesample including juices may be taken.	500 g, unless fat content is less than 5% and MRLVD is expressed on a fat basis. Then 1.5-2.0 kg is required.
B. Cured, smoked, or cooked product (e.g., bacon slab, ham, turkey, cooked beef), unit size of at least 1 kg	Collect portion from a large unit (greater than 2 kg), or take whole unit, depending on size.	500 g, unless fat content is less than 5% and MRLVD is expressed on a fat basis. Then 1.5-2.0 kg is required.
IX. Class E - Type 19 (Manufactured, multiple ingredient, product of animal origin)		
A. Sausage and luncheon meat rolls with a unit size of at least 1 kg	Collect cross-section portion from a large unit (greater than 2 kg), or whole unit, depending on size.	500 g

[1] When adhering fat is insufficient to provide a suitable sample, the sole commodity without bone, is analyzed and the MRL will apply to the sole commodity.

TABLE B: MILK, EGGS, DAIRY PRODUCTS AND AQUATIC ANIMAL PRODUCTS		
Commodity	**Instructions for collection**	**Minimum quantity required for laboratory sample**
I. Group 033 (Milks)		
Whole liquid milk raw, pasteurized, UHT & sterilized	In bulk. Mix thoroughly and immediately take a sample by means of a dipper. In retail containers. Take sufficient units to meet laboratory sample size requirements.	500 mL
II. Group 082 (Secondary Milk Products)		
A. Skimmed milk skimmed and Semiskimmed	As for whole liquid milk.	500 mL
B. Evaporated milk evaporated full cream & skimmed milk	Bulk containers (barrels, drums). Mix the contents carefully and scrape adheri material from the sides and bottom of the co tainer. Remove 2 to 3 litres, repeat the stirri and take a 500 mL sample. Small retail containers. Take sufficient units to meet laboratory sample size requirements.	500 mL
C. Milk powders 1. Whole	Bulk containers. Pass a dry borer tube steadily through the powder at an even rate of penetration. Remove sufficient bores to make up a sample of 500 g. Small retail containers. Take sufficient units to meet laboratory sample size requirements.	500 g
2. Low fat	As for whole milk powders.	500 g
III. Group 087 **(**Derived Milk Products)		
A. Cream fresh, frozen & UHT; single, whipping, whipped, double & clotted	Bulk containers. Plunge to ensure thorough mixing moving the plunger from place to place avoiding foaming, whipping and churning. Take a 200 ml sample by means of a dipper. Small containers. Take sufficient units to meet laboratory sample size requirements.	200 mL

TABLE B: MILK, EGGS, DAIRY PRODUCTS AND AQUATIC ANIMAL PRODUCTS		
Commodity	**Instructions for collection**	**Minimum quantity required for laboratory sample**
B. Butter including whey butter and low fat spreads containing butterfat	In bulk. Take two cores or more of butter so that the minimum total sample weight is not less than 200 g In pats or rolls. For units weighing over 250 g divide into four and take opposite quarters. For units weighing less than 250 g take one unit as sample.	200 g
C. Butteroil including anhydrous butte roil and an-hydrous milkfat	Mix thoroughly and take a 200 g sample.	200 g
IV. Group 090 (Manufactured Milk Products - single ingredient)		
A. Yoghurt natural, low fat through to full cream	Select number of units sufficient to meet laboratory requirements.	500 g
B. Cheeses all varieties	Make two cuts radiating from the centre of the cheese if the cheese has a circular base, or parallel to the sides if the base is rectangular. The piece removed should meet the laboratory sample size requirements. For small cheeses and wrapped portions of cheese take sufficient units to meet laboratory sample requirements.	200 g
V. Group 092 (Manufactured MilkProducts - multi-ingredient)		
A. Dairy ice cream only ice cream containing 5% or greater of milk fat	Select block or units sufficient to meet laboratory sample size requirements.	500 mL
B. Processed cheese preparations	Select units sufficient to meet laboratory sample size requirements.	200 g
C. Flavoured yoghurt	As for natural yoghurt.	500 g
D. Sweetened condensed Milk	As for evaporated milk.	500 mL
VI. Group 039 (Eggs and Egg Products)		
A. Liquid and frozen eggs	Use sample schedule. Subsample size will be 25 mL liquid or 500 mL packed shavings from aseptic drillings into containers.	500 g
B. Dried egg products	Use sample schedule. For containers of 500 g or less or 25 mL or less, collect a minimum of 2 units per subsample. For containers of 500 g to 10 kg select 1 unit per subsample. For containers of 10 kg or more collect 1 kg from each unit sampled. Collect with aseptic technique.	500 g

TABLE B: MILK, EGGS, DAIRY PRODUCTS AND AQUATIC ANIMAL PRODUCTS		
Commodity	**Instructions for collection**	**Minimum quantity required for laboratory sample**
C. Shell eggs 1. Retail packages	Use sample schedule. Subsample size is 12 eggs.	500 g or 10 whole eggs
2. Commercial cases	For 15 cases or less collect 12 eggs from each case, minimum of 24 eggs. For 16 or more cases collect 12 eggs from 15 random cases.	500 g or 10 whole eggs
VII. Class B - Type 08 (Aquatic Animal Products)		
A. Packaged fish fresh, frozen, smoked,cured, or shellfish (except oysters)	Collect 12 subsamples randomly. Minimum subsample size is 1 kg.	1000 g
B. Bulk fish 0.5 - 1.5 kg	Collect 12 subsamples randomly. Each subsample should total 500 g of edible fish.	1000 g
C. Bulk shellfish	Collect 12 subsamples randomly.	1000 g
D. Other fish and shellfish Products (including oysters)	Collect 12 subsamples	1000 g
VIII. Class E - Type 17 (Derived Edible Products of Aquatic Animal Origin)		
A. Canned fish and shellfish products (except oysters)	Collect 12 subsamples of 5 cans per subsample.	1000 g
B. Other fish and shellfish products - fish flour and meal	Use sample schedule. Collect 1 kg per subsample.	1000 g

Appendix B

SAMPLING FOR THE CONTROL OF VETERINARY DRUG RESIDUES IN HONEY

1. OBJECTIVE

47. To provide instructions for sampling a lot of honey to determine compliance with Codex Maximum Residue Limits for Residues of Veterinary Drugs (MRLVDs).

2. DEFINITIONS

2.1 Lot

48. An identifiable quantity of food (honey) delivered for distribution at one time, and determined to have common characteristics, such as origin, variety, type of packing, packer or consignor, or markings, by the sampling official. Several Lots may make up a consignment.

2.2 Consignment

49. A quantity of food (honey) as described on a particular contractor's shipping document. Lots in a Consignment may have different origins or may be delivered at different times.

2.3 Primary Sample

50. A quantity of honey taken from one place in the Lot, unless this quantity is inadequate for the residue analysis. When the quantity is inadequate, samples from more than one location can be combined for the Primary Sample.

2.4 Bulk Sample

51. The combined total of all the Primary Samples taken from the same lot.

2.5 Final (Laboratory) Sample

52. The Primary or Bulk sample, or a representative portion of the Primary or Bulk sample, intended for laboratory analysis.

2.6 Laboratory Test Portion

53. The representative portion of the Final (Laboratory) Sample on which an analysis is conducted. The entire Laboratory Sample may be used for analysis in some cases but typically will be sub-divided into representative test portions for analysis.

3. COMMODITIES TO WHICH THE GUIDELINE APPLIES

3.1 Selected According to Origin

54. Blossom or nectar honey that comes mainly from nectaries of flowers.

55. Honeydew honey that comes mainly from secretions of or on living parts of plants.

3.2 Selected According to Mode of Processing

56. Comb honey that is stored by bees in the cells of freshly built broodless combs, and sold in sealed whole combs or sections of such combs.

57. Extracted honey that is obtained by centrifuging decapped broodless combs.

58. Pressed honey that is obtained by pressing broodless combs with or without the application of moderate heat.

4. PRINCIPLE ADOPTED

59. For purposes of control, the maximum residue limit (MRLVD) is applied to the residue concentration found in each Final (Laboratory) Sample taken from a Lot. Lot compliance with a Codex MRLVD is achieved when none of the Final (Laboratory) Samples contain a residue greater than the MRLVD.

5. EMPLOYMENT OF AUTHORIZED SAMPLING OFFICIALS

60. Samples must be collected by officials authorized for this purpose.

6. SAMPLING PROCEDURES

6.1 Product to Sample

61. Each Lot to be examined must be sampled separately.

6.2 Precautions to Take

62. During collection and processing, contamination or other changes in the samples must be prevented which would alter the residue, affect the analytical determination, or make the Final (Laboratory) Sample not representative of the Bulk Sample.

6.3 Collection of a Primary Sample

63. Quantities to collect are dependent on the analytical method requirements. Minimum quantity requirements and detailed instructions for collection of a primary sample of honey are provided in Appendix B, paragraph 9. The following are general instructions.

a. Each Primary Sample should be taken from a single unit in a Lot, and when possible, be selected randomly.

b. Packaged product should not be opened for sampling unless the unit size is at least twice the amount required for the Final (Laboratory) Sample. The Primary Sample should contain a representative portion of the product. Each sample should be prepared for analysis as referenced in paragraph 6.5.

6.4 The Number of Primary Samples to Collect from a Lot

64. The number of Primary Samples collected will vary depending on the status of the Lot. If adulteration is suspected by origin from a source with a past history of residue non-compliances with the MRLVD, by evidence of contamination during transport or by the availability of other relevant information to the inspection official, the Lot is designated a suspect Lot. If there is no reason to suspect adulteration, the Lot is designated a non-suspect Lot.

6.5 Preparation of the Primary Sample

65. The Primary Sample is prepared as described in paragraph 9.

6.6 Preparation of the Final (Laboratory) Sample

66. The Primary Sample (or the Primary Samples pooled as a Bulk Sample)should, if possible, constitute the Final (Laboratory) Sample. The Final (Laboratory) Sample should be submitted to the laboratory for analysis. If the Primary Sample (or Bulk Sample from pooled primary Samples) is too large to be submitted to the laboratory, a representative subsample should be prepared. Some national legislation may require that the final sample be sub-divided into two or more portions for separate analysis. Each portion should be representative of the Final (Laboratory) Sample. Precautions in paragraph 6.2 should be observed.

6.7 Preparation of the Laboratory Test Portion

67. The Laboratory Test Portion should be prepared from the Final (laboratory) Sample by an appropriate method of reduction.

6.8 Packaging and Transmission of Final (Laboratory) Samples

68. Each Final (Laboratory) Sample should be placed in a clean, chemically inert container to protect the sample from contamination and from being damaged in shipping.

69. The container should be sealed so that unauthorized opening is detectable.

70. The container should be sent to the laboratory as soon as possible, after taking precautions against leakage and spoilage.

71. Replicate portions of the Final (Laboratory) Sample which may be retained as required by national legislation or as an administrative policy should be placed in a clean, chemically inert container to protect the sample from contamination, sealed so that unauthorized opening is detectable and stored under suitable conditions to prevent a change in the product or any residues it may contain in case future analysis is required for comparison with analytical results obtained on the sample material submitted to the laboratory.

7. RECORDS

72. Each Primary or Bulk Sample and each Final (Laboratory) should be correctly identified by a record with the type of sample, its origin (e.g., country, state, or town), its location of collection, date of sampling, and additional information useful to the analyst or to regulatory officials for follow-up action if necessary.

8. DEPARTURE FROM RECOMMENDED SAMPLING PROCEDURES

73. If there is a departure from recommended sampling procedures, records accompanying the sample should fully describe procedures actually followed.

9. SAMPLING INSTRUCTIONS

9.1 Liquid or Strained Honey

74. If sample is free from granulation, mix thoroughly by stirring or shaking; if granulated, place closed container in water-bath without submerging, and heat 30 min at 60°C; then if necessary heat at 65°C until liquefied. Occasional shaking is essential. Mix thoroughly and cool rapidly as soon as sample liquefies. If foreign matter, such as wax, sticks, bees, particles of comb, etc., is present, heat sample to 40°C in water-bath and strain through cheesecloth in hot-water-funnel before sampling.

75. Collect 250 ml of liquid or strained honey.

9.2 Comb Honey

76. Cut across top of comb, if sealed, and separate completely from comb by straining through a sieve the meshes of which are made by so weaving wire as to form square opening of 0.500 mm by 0.500 mm (ISO 565-1983)[2]. When portions of comb or wax pass through sieve, heat samples as in paragraph 9.1 and strain through cheesecloth. If honey is granulated in comb, heat until wax is liquefied; stir, cool and remove wax.

77. Collect 250 ml of liquid honey.

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[2] Such sieve could be replaced by US sieve with No. 40 standard screen (size of opening 0.420 mm).

PART II - GENERAL CONSIDERATIONS ON ANALYTICAL METHODS FOR RESIDUE CONTROL

II.1. INTRODUCTION

78. Analytical methods used to determine compliance with MRLVDs should be suitable for routine use by compentent authorities of member governments for their testing programmes for all residues of veterinary drugs and substances which may be used as veterinary drugs. This includes certain pesticides which have veterinary uses and that may be present as residues in commodities within the terms of reference of this Codex Committee. These methods may be used for the analysis of randomly selected survey samples in a national regulatory control programme to determine compliance with established MRLVDs, for the analysis of targeted samples when there is reason to suspect non-compliance with MRLVDs or for the collection of data for use in estimation of intake.

79. Methods may also be required in regulatory control programmes for the detection of residues of substances for which ADIs and MRLVDs have not been established by the Codex Alimentarius Commission. For some substances, the toxicological evaluation leads to the conclusion that an ADI or MRLVD should not be established. 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 generally based on the comparison of a set of characteristics of a detected substance with those of a known standard of the suspected residue.

80. Suitably validated methods are not always available for all possible combinations of veterinary drug residues and foods within the terms of reference of the CCRVDF. Competent authorities responsible for designing national residue control programmes should ensure that appropriate residue methods of analysis are used to assure compliance with Codex MRLVDs. This may sometimes require the development and validation of a new analytical method or the extension of the validation of an existing analytical method to include a new combination of analyte and matrix. Appropriate regulatory action may then be taken against adulterated products, consistent with the reliability of the analytical data.

II.2 INTEGRATING ANALYTICAL METHODS FOR RESIDUE CONTROL

81. Analytical methods for veterinary drug residues in foods must reliably detect the presence of an analyte of interest, determine its concentration and correctly identify the analyte. When residues resulting from the use of approved veterinary drugs are detected at concentrations above an established maximum residue limit (MRLVD), the results should be confirmed before regulatory enforcement actions are taken. In the case of substances which have been banned from use in food-producing animals by a competent authority, or for which an ADI and MRLVDs have not been established, the confirmed presence of residues at any concentration in a food may result in regulatory action.

82. The principal performance attributes of analytical methods used in residue control programmes are dependent on whether a method is intended to simply detect, to quantify, or to confirm the presence of a target residue. The CCRVDF has designated three categories of methods for use in regulatory programmes for the control of veterinary drug residues in foods. Completion of a full collaborative study[1] is not a requirement for recognition of a method to be placed in one of these three categories.

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[1] Horwitz, W. 1995. Protocol for the design, conduct and interpretation of method performance studies. Pure and Applied Chemistry, 67:331-343.

83. Level III methods are qualitative or semi-quantitative in nature and are used as screening methods to identify the presence (or absence) of samples from a herd or lot which may contain residues which exceed an MRLVD or other regulatory action limit established by a competent authority. These methods may not provide adequate information to accurately define the concentration present or, to confirm the structure of a residue but may be used to quickly determine which products require further testing and which can be released. They may be applied to a sample at the point of entry into the food chain, site of inspection or on receipt of a sample at the laboratory to determine if the sample contains residues which may exceed a regulatory limit. Such methods usually provide greater analytical efficiency, can sometimes be performed in non-laboratory environments and may be less expensive for use in regulatory control programmes than tests conducted within a laboratory. Use of Level III methods allows the laboratory resources to be focused on analysis of the presumptive positive (suspect) samples identified using this test. These methods, which should have a defined and low false negative rate, should not be used alone for residue control purposes on official samples without the availability of suitably validated quantitative and/or confirmatory methods to apply to any samples identified as potentially not in compliance with an MRLVD.

84. Level II methods provide quantitative information which may be used to determine if residues in a particular sample exceed an MRLVD or other regulatory action limit, but do not provide unequivocal confirmation of the identity of the residue. Such methods which provide quantitative results must perform in good statistical control within the analytical range that brackets the MRLVD or regulatory action limit.

85. Level I methods provide unequivocal confirmation of the identity of the residue and may also confirm the quantity present. Level I methods are the most definitive and frequently are based on combined chromatographic and mass spectrometric techniques, such as liquid chromatography – mass spectrometry (LC/MS). Such methods when used for confirmation of residue identity should provide reliable structural information within established statistical limits. When the Level I method does not provide quantitative information, the quantification result of the original Level II method should be verified by analysis of replicate test portions using the original quantitative method or a suitably validated alternative quantitative method.

86. These three categories of methods – screening, quantitative and confirmatory - often share some performance characteristics. In addition, each category has 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. These three categories of methods may be applied sequentially in a residue control programme.

87. Samples which test “positive” with the Level III method are considered as suspect and are usually designated for further laboratory testing using more definitive methods. This could include repeat testing of replicate test portions with a Level III method, but typically Level II and/or Level I methods are used in the laboratory to establish that the sample does contain residues in excess of the regulatory limit. Such tests should be conducted on new test portions of the sample material used in the initial screening test to confirm that the analyte detected in the initial test is definitely the suspected compound and that the MRLVD (or other regulatory action limit established by the authority) has indeed been exceeded. The performance attributes, or characteristics, which must be determined during method validation for each type of method – screening, quantitative, confirmatory – are presented in PART III: ATTRIBUTES OF ANALYTICAL METHODS FOR RESIDUES OF VETERINARY DRUGS IN FOODS.

II.3 CONSIDERATIONS FOR SELECTION AND VALIDATION OF ANALYTICAL METHODS

II.3.1 IDENTIFICATION OF METHODS REQUIREMENTS

II.3.1.1 Method scope

88. The intended purpose of the method is usually defined in a statement of *scope* which defines the analytes (residues), the matrices (tissues, milk, honey, *etc.*) and the concentration range to which the method applies. It also states whether the method is intended for screening, quantitative, or confirmatory use. The competent authority must establish an appropriate *marker residue* for each drug for which an MRLVD has been established and should also designate a preferred *target tissue* to be sampled for testing.

II.3.1.2 Marker residue

89. The MRLVD is expressed in terms of the marker residue, which may be the parent drug, a major metabolite, a sum of parent drug and/or metabolites or a reaction product formed from the drug residues during analysis. In some cases, the parent drug or the metabolite may be present in the form of a bound residue which requires chemical or enzymatic treatment or incubation to be released for analysis. It is important that the marker residue should, whenever possible, provide unequivocal evidence of exposure to the drug. In rare situations, it is necessary to use compounds as marker residues which may also result from sources other than exposure to the drug. In such cases, additional information is required to ascertain the probable source of the residue is exposure to the drug. An example of such a situation is the use of semi-carbazide, which may occur from other sources, as a marker residue for the drug nitrofurazone.

II.3.1.3 Target Tissue

90. The usual target tissue selected by competent authorities to be tested for veterinary drug residues in a residue control programme is the edible tissue in which residues of the marker residue occur at the highest concentrations and are most persistent. For lipophilic substances, the usual target tissue is fat. For most other substances, the target tissue is liver or kidney, depending on the primary route of elimination. One of these tissues is usually the target tissue designated for use in testing of domestically produced foods of animal origin. The organ tissues may not be available for testing imported products, so muscle tissue may be the target tissue for testing of these commodities. In some cases, such as drugs which are normally administered as injectable formulations, testing of muscle tissue from suspected injection sites may be required. The regulatory programme manager and the laboratory managers need to clearly identify the testing objectives and the analytical requirements required in terms of target tissues, marker residues and concentration ranges to ensure suitable methods are used in the regulatory control programme. In certain situations, competent authorities may also use biological fluids such as urine or serum to indicate the presence or absence of residues of interest.

II.3.2 IMPLEMENTING CODEX ALIMENTARIUS COMMISSION GUIDELINES

91. The Codex Alimentarius Commission has issued a guideline for laboratories involved in the import/export testing of foods[2] which recommends that such laboratories should:

a. use internal quality control procedures which comply with the Harmonised Guidelines for Internal Quality Control in Analytical Chemistry[3];

b. participate in appropriate proficiency testing schemes designed and conducted in accordance with the International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories[4];

c. become accredited according to ISO/IEC-17025:1999 General requirements for the competence of calibration and testing laboratories[5];and

d. whenever available, use methods which have been validated according to the principles laid down by the Codex Alimentarius Commission.

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

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

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

5 The original guideline CAC/GL 27 referred to ISO/IEC Guide 25: General requirements for the competence of calibration and testing laboratories. International Organization for Standardization, Geneva (1990), which has been superceded by ISO/IEC-17025: General requirements for the competence of calibration and testing laboratories. International Organization for Standardization, Geneva (1999).

92. Methods used for analyses of veterinary drug residues in foods should be capable of detecting the compounds included in the residue control programme. The analytical recovery and precision for the target foodstuffs should meet the criteria stated elsewhere in this document. The methods should be 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. The ongoing performance must be monitored through the quality system in place in the laboratory.

II.3.3 METHOD VALIDATION AND FITNESS FOR PURPOSE

93. The process of method validation is intended to demonstrate that a method is *fit-for-purpose*. This means that in the hands of a properly trained analyst using the specified equipment and materials, and following the procedures described in the method, reliable and consistent results can be obtained within specified statistical limits for the analysis of a sample. The validation should address the issues of marker residue, target tissue and concentration range identified by the laboratory in consultation with the residue programme manager. When the method protocol is followed, using suitable analytical standards, results within the established performance limits should be obtained on the same or equivalent sample material by a trained analyst in any experienced residue control laboratory.

94. Multi-laboratory method performance studies generally satisfy the analytical requirements for use in a regulatory programme. These methods are subjected to a properly designed inter-laboratory study with analysts in independent laboratories, so that different sources of reagents, materials, and equipment are used by the participants.

95. Quantitative methods studied collaboratively according to the revised harmonized protocol adopted in 1995 by AOAC International, the International Union of Pure and Applied Chemistry (IUPAC), and the International Standards Organization (ISO) 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 is required)[1]. Collaborative studies of qualitative methods currently require a minimum of 10 participating laboratories. Collaborative studies conducted prior to 1995 completed method evaluation in a minimum of six laboratories in an acceptable, statistically designed study. These multi-laboratory method performance studies generally satisfy the analytical requirements for use in a regulatory programme, as information on method performance in the hands of different analysts in different laboratories is obtained through these studies. However, relatively few of the analytical methods currently used in residue control programmes for veterinary drug residues in foods have been validated by such a multi-laboratory study. Collaborative study designs are based on the analyses of coded duplicate test materials which represent the combinations of analytes, matrices, and concentrations included in the scope of the method and include an independent peer-review of both the study design and the results. In some situations, multi-laboratory studies may be conducted which do not have the minimum number of laboratories required to qualify as a collaborative study. Such studies, when conducted using the same scientific principles of design, evaluation, and review as are applied in collaborative studies, can provide useful information on method performance in the hands of multiple analysts in different laboratories, but do not provide the same level of statistical confidence obtained from the results of a collaborative study.

96. 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. Methods may be extended to include related analytes, additional tissues, species or products (or combinations of these not included in the original multi-laboratory study) by completing additional within-laboratory studies. Analytical results from method extension studies may require additional 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 compared with results obtained using a method which has been validated through a collaborative or multi-laboratory study or tested using sample materials from a recognized proficiency programme. 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.

97. Some residue control methods that have been demonstrated to be suitable to determine compliance with MRLVDs have a history of use in one or more expert laboratories, but have not been subjected to a formal multi-laboratory study. These methods were demonstrated to be suitable 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 use of 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 and from quality control data in one or more laboratories over time.

98. Most regulatory laboratories rely on the use of veterinary drug residue methods which have not have been subjected to a multi-laboratory study. Factors which have contributed to this situation include a requirement 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. In the absence of methods validated through inter-laboratory method trials, regulatory laboratories must frequently use analytical methods which have been subjected to validation studies conducted within their own laboratory to characterize the method performance.

II.3.4 SINGLE LABORATORY VALIDATION – THE CRITERIA APPROACH

99. 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 IUPAC[6]. 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[7]. The Procedural Manual[8] recognizes that inter-laboratory validated methods are not always available or applicable, particularly for multi-analyte/ multi-substrate methods and new analytes. In such cases, methods may be validated in a single laboratory to meet the General Criteria for the Selection of Methods of Analysis, as well as the additional criteria:

a. the method is validated according to an internationally recognized protocol (for example, the IUPAC Guidelines for Single Laboratory Validation of Methods of Analysis, referenced above);

b. use of the method is embedded in a quality assurance system in compliance with the ISO/IEC 17025 (1999) Standard or with the Principles of Good Laboratory Practice.

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6 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.

7 CX/MAS 02/11

8 FAO/WHO. 2004. Codex Alimentarius Commission Procedural Manual, 14th Ed., Food and Agriculture Organization of the United Nations, Rome.

c. the method should be complemented with information on accuracy demonstrated for instance by:

   i) regular participation in proficiency schemes, where available;

   ii) calibration using certified reference materials, where applicable;

   iii) recovery studies performed at the expected concentration of the analytes;

   iv) verification of result with other validated method where available.

100. The criteria approach, which combines a single laboratory validation model with a requirement that methods meet specific performance specifications, has been adopted by some regulatory authorities, such as the European Commission[9].

[9] 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.

PART III - ATTRIBUTES OF ANALYTICAL METHODS FOR RESIDUES OF VETERINARY DRUGS IN FOODS

III.1 INTRODUCTION

101. The performance characteristics of analytical methods used to determine compliance with MRLVDs must be defined and proposed methods evaluated accordingly. This will assure reliable analytical results and provide a secure basis for determining residues of veterinary drugs in foods for commodities in international trade. Part II, *General Considerations of Analytical Methods for Residue Control*, presents a discussion of general types or categories of regulatory methods, and provides a scheme for using these analytical methods based upon their intended purpose in a regulatory framework. In the discussion below, attributes common to the three categories of methods (referred to as Level I, Level II and Level III methods) defined by CCRVDF for determining compliance with Codex MRLVDs are presented. The additional attributes that are applicable to only one or two categories of methods are also discussed. **(Not**e: This Part contains numerous definitions. The CCRVDF has attempted to harmonize these definitions with those provided in the "Analytical Terminology for Codex Use" in the Procedural Manual and those used by the Joint FAO/WHO Expert Committee on Food Additives in assessment of veterinary drug residues and analytical methods.)

III.2 METHOD DEVELOPMENT CONSIDERATIONS

102. The development of an analytical method requires analysts experienced in the analytical techniques to be used, as well as appropriate laboratory space, equipment, and financial support. 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[9]. 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 considerations, and any other specific information pertinent to programme needs. In particular, stability of standards, both under normal conditions of storage and use and during processing of samples, should be assessed. Analyte stability in samples 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.

103. Establishing method performance attributes is essential, as these 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 screening, quantification, or confirmation of a residue for which Maximum Residue Limits have been established, or for residues of a drug for which an ADI and MRLVDs have not been recommended. In the latter case, the competent authority may establish a minimum performance standard which must be met by analytical methods used for regulatory control purposes. However, when no safe concentrations of these compounds in foods have been established, the competent authority may review such limits periodically to ensure they 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.

III.3 ANALYTICAL PERFORMANCE CHARACTERISTICS

III.3.1 PERFORMANCE CHARACTERISTICS OF SCREENING (LEVEL III) METHODS

104. Screening methods are usually either qualitative or semi-quantitative in nature, with the objective being to discriminate samples which contain no detectable residues above a threshold value ("negatives") from those which may contain residues above that value ("positives"). The validation strategy therefore focuses on establishing a threshold concentration above which results are "positive", determining a statistically based rate for both "false positive" and "false negative" results, testing for interferences and establishing appropriate conditions of use.

105. For a screening test, particularly those involving test kit technologies, the term "*sensitivity*" refers to the lowest concentration at which the target analyte may be reliably detected within defined statistical limits. 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.

106. The "*selectivity"* of a screening method refers to the ability of the test to determine that samples which give a negative response are truly negative. The test must also be able to distinguish the presence of the target compound, or group of compounds, from other substances which may be present in the sample material. It normally is not as great as that of a quantitative method, because screening methods often take advantage of a structural feature common to a group or class of compounds. These methods, which generally fit into the Level III methods category, are often based on microbiological growth inhibition, immunoassays, or chromogenic responses which may not unambiguously identify a compound. The selectivity of a screening method may be increased when it is used as a detection system after chromatographic or other separation technique. To demonstrate a selectivity rate of at least 90% with 95% confidence is recommended for screening tests, 30 replicate analyses are conducted on representative blank sample matrix materials from a minimum of six different sources. All results should be negative. Additional tests for potential interferences and cross-reactivity may then be conducted by testing blank matrix material fortified with potential interfering substances, such as other drugs which might be used in animal treatment, potential environmental contaminants, drug metabolites, or chemically related compounds. Again, responses should be negative when these compounds are present at concentrations which might reasonably be expected to be present in a sample.

107. The "cut-off" or threshold for the test for a particular compound is established by conducting concentration-response experiments, typically using 30 replicates (from at least six sources) fortified at each of a series of increasing concentrations. Once the concentrations have been established where all 30 replicates give a negative response and all 30 replicates give a positive response, the experiment is repeated using the blank matrix materials fortified at four evenly spaced concentrations between the "all negative" and "all positive" concentrations. An additional set is tested at a concentration 20% above the "all positive" concentration. Statistical analysis of the results enables the user to establish a reliable detection concentration at the required confidence level (usually 95%)[10].

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[10] Finney, D.J. (1978) *Statistical Method in Biological Assay, 3rd. edition*. MacMillan Publishing Co., New York.

III.3.2 PERFORMANCE CHARACTERISTICS FOR QUANTITATIVE (LEVEL II) METHODS

108. *Selectivity*, 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. For a Level II method, the requirement is that the signal used for quantification should relate only to the target analyte and not contain contributions for co-extracted materials. Chromatographic analyses based on peaks which are not fully resolved provide less reliable quantitative results. Use of element-specific detectors or detection wavelengths or mass-selective detectors which are more specific to a particular compound or structure, combined with chromatographic separation, improves the selectivity of quantitative methods for veterinary drug residues in foods.

109. In addition to the selectivity of a method, the ability of the method to provide a quantitative result which is reliable must be demonstrated. This consists of two factors:

a. the closeness of the result to the true or accepted value for the concentration of analyte present in the sample material, expressed in terms of *accuracy*, *trueness,* or *bias*; and

b. the ability of the method to provide consistent results on replicate determinations, expressed in terms of *precision* (*repeatability,* and *reproducibility).*

110. CCRVDF has recommended that methods used to support MRLVDs established by the Codex Alimentarius Commission should meet the performance standards for trueness and precision listed in Table 1, where CV_A refers to the coefficient of variation determined by test portions of blank matrix fortified prior to extraction and CV_L is the overall laboratory variability which includes a 10% estimate for variability of sample processing [11].

Table 1. Performance criteria which should be met by methods suitable for use as quantitative (Level II) analytical methods to support MRLVDs for residues of veterinary drugs in foods [12]

Concentration µg/kg	Coefficient of Variability (CV)				Trueness
	Repeatability (Within-Laboratory, CV_A) %	Repeatability (Within-Laboratory, CV_L) %	Reproducibility (Between-Laboratory, CV_A) %	Reproducibility (Between-Laboratory, CV_L) %	Range of Mean % Recovery
≤ 1	35	36	53	54	50 -120
1 to 10	30	32	45	46	60 -120
10 to 100	20	22	32	34	70 -120
100 to 1000	15	18	23	25	70 -110
≥ 1000	10	14	16	19	70 – 110

[11] Alder, L, Holland, PT, Lantos, J, Lee, M, MacNeil, JD (chairman), O'Rangers, J, van Zoonen, P, Ambrus, A (scientific secretary). 2000. Report of the AOAC/FAO/IAEA/IUPAC Expert Consultation on Single-Laboratory Validation of Analytical Methods for Trace-Level Concentrations of Organic Chemicals, Miskolc, Hungary, November 8-11, 1999. Report published on the website of the International Atomic Energy Agency (IAEA). http://www.iaea.org/trc/pest-qa_val2.htm (accessed 2005/05/20).

111. 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 (typically, a collaboratively studied method) or, in the absence of reference materials or methods validated by inter-laboratory trial, by determination of the *recovery* of analyte fortified into known blank sample material. The determination of accuracy as recovery is frequently used in validation of methods for veterinary drug residues in foods, as both certified reference materials and methods validated by inter-laboratory trial are often not available. 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. The accuracy should be carefully characterized at concentrations near the MRLVD or target concentration for regulatory action (typically at concentrations from 0.5 to 2.0 times that target concentration) to ensure that regulatory action is only taken on samples containing residues which can be demonstrated to exceed the regulatory action limit with a defined statistical confidence.

112. *Recovery* is usually expressed as the percentage of analyte experimentally determined after fortification of sample material at a known concentration and should be assessed over 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. This may be due to losses during extraction, intra-cellular binding of residues, the 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[10]. At relatively high concentrations, analytical recoveries are expected to approach one hundred percent. At lower concentrations, particularly with methods involving extensive extraction, isolation, and concentration steps, recoveries may be lower. Regardless of what average recoveries are observed, recovery with low variability is desirable so that a reliable correction for recovery can be made to the final result, when required. Recovery corrections should be made consistent with the guidance provided by the Codex Alimentarius Commission[12].

113. *Precision*, which quantifies the variation between replicated measurements on test portions from the same sample material, is also an important consideration in determining when a residue in a sample should be considered to exceed an MRLVD or other regulatory action limit. Precision of a method is usually expressed in terms of the within-laboratory variation (*repeatability*) and the between-laboratory variability (*reproducibility*) when the method has been subjected to a multi-laboratory trial. For a single laboratory method validation, precision as repeatability should be determined from experiments conducted on different days, using a minimum of six different tissue pools, different reagent batches(and different equipment?, etc.) and preferably by different analysts. Precision of a method is usually expressed as the 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.

114. 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 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.

115. The *sensitivity* of a quantitative method is a measure of its ability to discriminate between small differences in analyte concentration. Although the term has been applied in other ways, such as in defining the detection capabilities (see below) of test kit technologies, the use of the term sensitivity with such meaning when discussing quantitative methods is discouraged. For analytical instruments used in residue analysis, sensitivity is determined by two factors: instrumental response to the analyte and instrument noise. For measurements at or near the MRLVD, a method with inadequate sensitivity may not permit the analyst to distinguish with confidence whether residue concentrations are above or below the MRLVD.

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[12] *CAC/GL 37-2001* Harmonized IUPAC Guidelines for the use of Recovery Information in Analytical Measurement; see also Thompson, M., Ellison, S., Fajgelj, A., Willetts, P., & Wood, R. (1999) Harmonised Guidelines for the Use of Recovery Information in Analytical Measurement, *Pure Appl. Chem.*, **71**:. 337 – 348.

116. Quantitative methods are usually based on a comparison of the response from an analyte in a sample with the response from standards of the analyte in solution at known concentrations. In method development and validation, the calibration curve should first be determined to assess the detector response to standards over a range of concentrations. These concentrations (a minimum of five, plus blank) should cover the full range of analytical interest and the resultant curve should be statistically expressed. However, although it is recommended practice to include a suitable blank with the calibration samples, this does not imply that it is acceptable to extrapolate into the region of the curve below the low standard to obtain a quantitative result. 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 or regulatory action limit 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).

117. The analytical function experiment data can also be used to calculate the analytical recovery at each concentration and is of particular importance when the presence of matrix co-extractives modifies the response of the analyte as compared to analytical standards. The *linearity* is determined from the analytical function experiments and is the statistical expression of the curve obtained for the analysis of sample materials fortified at the target concentrations. It is typically determined from a linear regression analysis of the data, assuming there is a linear response. 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 (the analytical function). Use of such a "tissue standard curve" for calibration incorporates a recovery correction into the analytical results obtained.

118. It is also necessary to establish the lower limits at which reliable detection, quantification, or confirmation of the presence of an analyte may be performed using a particular analytical method. The *detection limit* may be described in practical terms as the lowest concentration where the analyte can be identified in a sample. It can be estimated using the standard deviation ($s_{y/x}$) from the linear regression analysis of the standard curve generated in the analytical function experiment described above[13]. Using this approach, the limit of detection is calculated using the y-intercept (assuming a positive value) of the curve plus three times $s_{y/x}$. This approach provides a conservative estimate of the detection limit.

119. The *limit of quantification* (LOQ), also referred to as limit of quantification or quantification limit) may be established from the same experiments using the y-intercept of the curve plus ten times $s_{y/x}$. 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 MRLVD. However, when the limit of quantification of a method is lower than the actual concentrations monitored for compliance with a MRLVD, the validation and subsequent application of the method should be based on a *lowest calibrated level*, which is typically 0.5x the MRLVD. For use in a regulatory programme, 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 MRLVD, or when conducting residue analyses for substances which do not have ADIs or MRLVDs. For monitoring compliance with an MRLVD, 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 LCL of a method used to support an MRLVD should not be less than the LOQ. The Procedural Manual recommends the term *determination limit* under "Terms to be Used in the Criteria Approach"[9]. CCMAS has recently recommended replacing the term "*determination limit" with quantification limit* 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 LOQ.

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

III.3.3 PERFORMANCE CHARACTERISTICS FOR CONFORMATORY (LEVEL I) METHODS

120. *Selectivity*, the ability of the method to unequivocally identify a signal response as being exclusively related to a specific compound, is the primary consideration for confirmatory methods**.** Certain instrumental techniques such as Fourier transform infrared spectroscopy or mass spectrometry may be sufficiently selective to provide unambiguous identification. These are often the techniques on which Level I methods are based.

121. Typically, a minimum of four identification points is required to meet accepted performance criteria for regulatory methods. Methods based on high resolution mass spectrometry are considered to give a higher reliability through more precise measurement of mass than can be obtained using low resolution mass spectrometry techniques. Method performance requirements for confirmatory methods based on low resolution GC/MS and LC/MS, as recently published by an international expert body[14] and several regulatory authorities [10,15], are given in Table 2.

Table 2: Performance requirements for relative ion intensities (sample compared to standard) using various mass spectrometric analytical techniques[7].

Relative ion intensity (% of base peak)	GC-MS (EI) (relative)	GC-MS (CI), GC-MS/MS LC-MS, LC-MS/MS (relative)
>50 %	∀10 %	∀ 20 %
20% to 50%	∀ 15 %	∀ 25 %
10% to 20%	∀ 20 %	∀ 30 %
< 10%	∀ 50 %	∀ 50 %

122. It is considered that one identification point should be assigned to each structurally significant ion fragment detected using a low resolution mass spectrometric method. When a tandem low resolution instrument, such as a "triple quadrupole" mass spectrometer is used, secondary fragments are detected from a primary fragment that is isolated in the first stage of the spectrometer. The fact that these structurally significant fragments are produced from the fragmentation of a major fragment (parent or precursor ion) associated with the molecule provides greater confidence and each such daughter or product ion is assigned a value of 1.5 identification points. A combination of a precursor ion and two product ions provides the 4 required identification points when low resolution MS/MS instruments are used in a confirmatory method.

123. Additional confidence is provided when high resolution mass spectrometers are used in a confirmatory method, as the high resolution provides more precise identification of the mass and may be used to predict the elemental composition of each fragment. For a single high resolution mass spectrometer, each structurally significant fragment detected is assigned a value of two identification points, while product ions generated in high resolution MS/MS experiments are assigned an identification point value of 2.5 each. In addition, at least one ion ratio must also be measured to eliminate the potential for fragments of the same mass arising from isobaric compounds of similar structure.

124. Other techniques, when they are used in combination, may be capable of achieving a comparable degree of selectivity as confirmatory techniques. For example, identification may be verified by combinations of methods such as:

- thin layer chromatography,
- element-specific gas-liquid chromatography and accompanying detection systems,
- formation of characteristic derivatives followed by additional chromatography,or
- determining compound specific relative retention times using several chromatographic systems of differing polarity.

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14 Bethem, R., Boison, J.O., Gale, J., Heller, D., Lehotay, S., Loo, J., Musser, S., Price, P., and Stein, S. (2003) Establishing the Fitness for Purpose of Mass Spectrometric methods. Journal of the American Society for Mass Spectrometry 14, 528-541

15 Guidance for Industry: Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues. U.S. Food & Drug Administration. http://www.fda.gov/cvm/guidance/guide118.doc (Accessed January 20, 2005)

125. Such procedures must be applicable at the designated MRLVD of the analyte. When a confirmatory method such as mass spectrometry is not available, information on the selectivity associated with the analysis of a particular veterinary drug residue in a sample may be developed from various sources[16]. This information may 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. Examples of analytical techniques which may be suitable to meet criteria for confirmatory analytical methods are summarized in Table 3.

Table 3. Examples of detection methods suitable for the confirmatory analysis of substances, as recommended by the Miskolc Consultation[12]

Detection method	*Criterion*
LC or GC and Mass Spectrometry	if sufficient number of fragment ions are monitored
LC-DAD	if the UV spectrum is characteristic
LC – fluorescence	in combination with other techniques
2-D TLC – (spectrophotometry)	in combination with other techniques
GC-ECD, NPD, FPD	only if combined with two or more separation techniques[a]
Derivatisation	if it was not the first choice method
LC-immunogram	in combination with other techniques
LC-UV/VIS (single wavelength)	in combination with other techniques

[a] Other chromatographic systems (applying stationary and/or mobile phases of different selectivity) or other techniques.

126. Although Level I methods are generally instrumental procedures, observation of a pathologic or other morphologic change that specifically identifies exposure to a class of veterinary drugs, could potentially be a Level I method, if it has sufficient sensitivity and precision.

III.3.4 GENERAL PERFORMANCE CHARACTERISTICS FOR METHODS FOR USE IN A REGULATORY CONTROL PROGRAMME

127. There are some additional considerations for selection of suitable methods for use in a regulatory control programme for veterinary drug residues in foods. Methods should be rugged (robust), cost effective, relatively uncomplicated, portable, and capable of simultaneously handling a set of samples in a time effective manner. The stability of analytes must also be established.

128. *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. Ruggedness testing of a confirmatory method may be required if the method differs significantly from the quantitative method previously validated (if the method uses different extraction or derivatization procedures than are used in the quantitative method).

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16 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.

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

129. *Cost-effectiveness* is the use of 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. The *method efficiency* is increased when multiple samples can be analyzed at the same time. This reduces the analytical time requirements per sample and usually reduces the 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. *Portability* is the analytical method characteristic that enables it to be transferred from one location to another without loss of established analytical performance characteristics.

130. *Analyte stability* during analysis must be established for both standards and analyte in the presence of sample material, during processing through the complete analysis for all methods used in a regulatory control programme and for typical conditions of storage while a sample is awaiting analysis. The period chosen for stability during storage should cover the expected time when sample material may be stored for all required analyses, including the use of the screening, quantitative, and confirmatory methods. It is prudent to conduct the storage study for a period which extends to at least 90 days beyond the expected time for all screening, quantitative, and confirmatory analyses to be completed and the results reported in case there is a challenge and a request for re-analysis.

III.4 METHOD DEVELOPMENT AND VALIDATION CONSIDERATIONS FOR RESIDUE CONTROL METHODS

III.4.1 SELECTION OF APPROPRIATE TEST MATERIAL FOR VALIDATION

131. 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[7] 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.

132. In developing and validating a residue control method, data should be derived from three types of sample material. 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. 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. A minimum of six different sources of material is recommended by CCRVDF.

133. Finally, analysis of biologically incurred tissue from food producing animals that have been treated with the drug provides information about biological or other interactions that may occur when analyzing residue control samples.

III.4.2 MEASUREMENT UNCERTAINTY

134. Laboratories should provide their clients on request with information on the measurement uncertainty associated with the quantitative results produced by each quantitative method[4]. 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. An alternative approach uses method validation and/or on-going QC data generated in the laboratory to estimate the measurement uncertainty. Guidance on estimation of measurement uncertainty is being developed by IUPAC and has been published by other independent scientific bodies.[18]

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[18] EURACHEM/CITAC Guide to Quantifying Measurement Uncertainty in Analytical Measurement, http://www.measurementuncertainty.org/mu/guide/index.html, accessed May 20, 2005.

III.4.3 USE OF INTERNAL STANDARDS

135. 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 behaviour 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.

III.4.4 ENVIROMENTAL CONSIDERATIONS

136. When residue control methods that may be subjected to widely variable physical test environments, this should be taken into account in the development and validation of these methods. Addressing these issues 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 tolerant. Cooler environments may require reagents and solvents to have different physical properties, such as lower freezing point and greater solvating characteristics, to provide 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.

III.4.5 CHOICE OF VALIDATION MODEL

137. 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 method validated in an inter-laboratory trial or other forms of inter-laboratory comparison of results. As a minimum, CCRVDF 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 recommended, 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 require 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.

138. The principles for conducting a single laboratory method 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 replicates, containing the residue near the MRLVD or other target concentration, as well as samples with the analyte above and below the concentration of interest, and test material blanks. All study samples should be analysed over a minimum number of days, preferably with replicate analysis, to improve statistical evaluation of method performance and provide an estimate of inter-day variability. 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. Analyses of blind duplicates, as required in the collaborative study protocol[6], in only eight laboratories, with one or two animal species and tissues, yields 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.

III.4.6 QUALITY CONTROL AND QUALITY ASSURANCE

139. 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 IUPAC is recommended for regulatory control laboratories[2].