

**CODEX GUIDELINES FOR THE ESTABLISHMENT OF A REGULATORY PROGRAMME
FOR CONTROL OF VETERINARY DRUG RESIDUES IN FOODS
CAC/GL 16-1993**

Governments need regulatory control programmes to ensure their citizens of a safe and wholesome food supply. Specifications of a residue control programme are determined by the importance of the various health risks that could be incurred by consumers of products derived from animal food products.

One type of risk may occur if meat is handled and consumed from animals excessively contaminated with microorganisms or toxins that could affect the health of consumers. This type of health risk can be minimized by establishing meat inspection programmes that emphasize appropriate and provide specific procedures on how to recognize the signs of disease in food producing animals.

Another kind of risk can occur if food animals have been raised using veterinary drugs or pesticides in an inappropriate manner. The improper use of such chemicals can result in unsafe residues of these substances in food derived from the treated animals. The safety of the human food requires a full scientific evaluation of the relative hazard as well as quantity of a drug residue remaining in the tissues of treated livestock and poultry when used according to good veterinary practices, and a systematic set of procedures that will ensure effective control of such residues in human food.

In addition to the health protection benefits in having an effective residue control programme, a country with such a programme has the capability to participate in the community of food trading nations with greater confidence. This is because an effective residue control programme can also serve as the foundation for certifying the safety of the country's exported food products, as well as provide assurance of safety of such products imported into the country.

When establishing a programme for control of residues in foods, it is important to distinguish between the notion of "unbiased statistical sampling", where the samples are obtained from animals that are presented for inspection, and the notion of "biased or directed sampling", where samples are obtained from suspect food products. The purpose of unbiased statistical sampling is to determine the frequency of occurrence of contaminated products among those presented for inspection.

Samples are taken at random from food considered safe, and it is not necessary to retain these food products while waiting for the results of analytical testing. The sampling plan is determined beforehand, using statistical rules to ensure that the results are representative of the overall quality of the product(s) under consideration. The results may be used to certify the exported food products are in compliance with Codex MRLVDs. Conversely, directed sampling focuses on food products suspected of having residue concentrations that exceed the maximum residue limits. The food products are detained while waiting for results of laboratory testing, and are not released for human consumption should test results be unfavourable. The number of samples to be taken during the year for directed sampling may not, by definition, be predetermined. The results of directed sampling do not have statistical representativeness.

In establishing an effective residue control programme, a country should first establish a comprehensive system for determining the safety of veterinary drugs. This may be accomplished, for example, through an organization with suitable technical expertise and administrative authority. Veterinary drugs may be approved taking into consideration several relevant criteria, among which will be the safety evaluation of the veterinary drug for animals and for human food consumption. The scientific evaluation of the safety of veterinary drugs is a long and rigorous task, that, perhaps, may not be necessary to perform in each country, especially in developing countries. Evaluation could be performed by the

interested country, using the technical expertise of international organizations such as the Joint FAO/WHO Expert Committee on Food Additives (for veterinary drugs), or the technical evaluation results in other countries having an acceptable, technically qualified safety assessment organizations.

To establish an effective programme for the control of residues of veterinary drugs in food, a country should include but not necessarily be limited to the following items:

1. Establishing the regulatory authority responsibility for implementing inspection programmes and laboratory analyses.
2. Elaborating an integrated inspection programme, including a residue control programme for the inspection of foods. The organization in charge of implementing this inspection programme should be granted the authority to take all the steps necessary to control products when residues exceed the maximum residue limits established for a food commodity.
3. Compiling a register of veterinary drugs and/or pure chemical; substances used in the country, including the products manufactured in the country and those products that are imported into the country.
4. Elaborating regulations concerning the distribution of veterinary drugs as a whole, providing for procedures for the authorized sale, manufacture, distribution and use of such products.
5. Elaborating procedures for determining the safety and efficacy of veterinary drugs in animals and residues in food from use of such veterinary drugs. This should include describing procedures for determining maximum residue limits for veterinary drugs in food and procedures for analysis of test samples intended to verify compliance with those limits.
6. Establishing procedures for sampling food products of animal origin, indicating the specific drug residues of greatest health concern, the number of samples to be taken for unbiased statistical sampling, and the nature of the tissue and quantity of sample to be taken. Procedures for sampling for residue control in a country may be required for certain substances for purposes other than the enforcement of MRLVDs. These analyses, for example, come within the scope of exploratory surveys for determining residues in foods where unapproved substances may be used in food producing animals or poultry. This type of data is essential to provide a residue control programme the flexibility necessary to be adapted to national needs.
7. Selecting the methods of analysis to be used. As an initial step, a residue control programme should include screening methods. The use of these methods should not require investment in complex laboratory instrumentation nor in costly reagents or personnel training, and should provide analysis of samples in a cost effective manner. Screening methods are generally defined as qualitative or semi-quantitative methods of analysis that detect the presence of a substance at a concentration that is equal to or lower than the maximum residue limits. A positive result indicates the possibility that the maximum residue limit has been exceeded. Additional testing measures should be required, as determined by the objectives set forth in a country's residue control programme, to verify or confirm the results of screening methods.
8. Implementing a quality assurance programme to assure the highest quality results for methods of analysis. Such a programme will assure regulatory control authorities that the methods used will give reliable results that are compatible with the MRLVD or within the limits established by national regulations.

9. Developing an educational programme(s) for producers and veterinarians providing instruction in the proper use of veterinary drugs, and encouraging the use of preventive measure to reduce the occurrence of residues in food animals and poultry.

For determining maximum residue limits, the Joint FAO/WHO Expert Committee on Food Additives (for veterinary drugs) may constitute a useful resource for obtaining these data.

10. Specific details concerning the establishment of a regulatory programme for control of veterinary drug residues in foods, as based on the above general principles, are attached to these guidelines as follows:

- PART 1: Sampling for the Control of Residues of Veterinary Drugs in Foods
 - Appendix A: Sampling for the Control of Veterinary Drug Residues in Meat and Poultry Products
 - Appendix B: Sampling for the Control of Veterinary Drug Residues in Fish, Milk, and Egg Products
 - Appendix C: Sampling for the Control of Veterinary Drug Residues in Honey
- PART 2: General Considerations on Analytical Methods for Residue Control
- PART 3: Attributes of Analytical Methods for Residues of Veterinary Drugs in Foods

PART I

SAMPLING FOR THE CONTROL OF RESIDUES OF VETERINARY DRUGS IN FOODS

1. INTRODUCTION

1.1 Basis for the Sampling Principle

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.

1.2 General Principles

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.

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.

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.

Sampling at an abattoir in conjunction with slaughter of a herd or flock or with preliminary slaughter of a small number of test animals or birds, may involve testing samples drawn from live animals or birds. In these situations, analyses performed on tissues drawn from test animals or body fluids from live animals may provide test results for an inspector before a herd or flock is presented for slaughter or shipment. Analyses associated with pre-slaughter must be designed to prevent subsequent administration of drugs. In a like manner, for processed foods such as might be obtained from fish or honey, any sampling and testing must be designed to prevent subsequent administration of drugs. When body fluids are used for residue testing, care must be taken to have established tissue-fluid relationships between the analytic results in these fluids and results in tissues where the MRLVDs are established.

Shortly after slaughter or after appropriately harvesting the principle food products, these products may be commingled to an extent that it destroys the possibility of drawing a representative sample.

Samples for fresh meat or poultry or fresh chilled meat or poultry may be drawn from different days' production, for example. Processed products such as sausage or minced fish may be made with meat tissues from different days' or even different establishments' production. Although under some circumstances lots for sampling have been defined as products from the same consignor or packer, sample homogeneity can best be guaranteed when it is taken in conjunction with slaughter or primary collection point.

2. OBJECTIVES OF SAMPLING

2.1 Primary Point of Origin Sampling

2.1.1 Non-biased sampling

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 violations; therefore, only compounds with established safe limits such as MRLVDs are usually considered for residue testing 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 laboratory 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.

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 commodity within the terms of reference of this Committee, with violative concentrations of residues. 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 violations, 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 violations.

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.

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 violative residues 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 violative residues 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.

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 violation with predefined probabilities (i.e., 90, 95, and 99 percent) in a population having a known violation prevalence.

Violation prevalence (% in a population)	Minimum number of samples required to detect a violation 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

2.1.2 Directed sampling

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 violative residues 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, should 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.

The probability of failing to detect a residue violation and accepting the lot depends upon the directed sampling programmes' sample size and prevalence of the residue violation frequency. Table 2 shows the probability of failing to detect a residue violation using different sample sizes from an "infinite" population with a specified proportion of violations. For example, selecting 5 samples from a large lot in which 10 percent of the units contain violative residues would, on the average, fail to detect a residue violation 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 violation

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								

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 violations, the feasibility of selecting separate samples from separate lots instead of from a single lot should be considered.

2.2 Secondary Point of Sampling

2.2.1 Port of entry sampling

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. The purpose of port of entry sampling and testing is not to replace an exporting country's residue control programmes.

Results of residue testing that indicate imported product is in compliance with Codex MRLVDs should be permitted to move into commerce. When test results indicate that imported product contains violative 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.

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 MEAT AND POULTRY PRODUCTS

1. OBJECTIVE

To provide instructions for sampling a lot of meat or poultry products to determine compliance with Codex Maximum Residue Limits for Veterinary Drugs (MRLVDs).

2. DEFINITIONS

2.1 Lot

An identifiable quantity of food delivered for slaughter or 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

A quantity of food 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

A quantity of tissue taken from a single animal or 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 animal or location can be combined for the primary sample (such as poultry organs).

2.4 Bulk Sample

The combined total of all the primary samples taken from the same lot.

2.5 Final Sample

The primary sample or a representative portion of the primary sample to be used for control purposes.

2.6 Laboratory Sample

The sample intended for laboratory analysis. A whole primary sample may be used for analysis or the sample may be subdivided into representative portions, if required by national legislation.

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

Type 07 Poultry Products

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

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 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

For purposes of control, the maximum residue limit (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 none of the laboratory samples contains a residue greater than the MRLVD.

5. EMPLOYMENT OF AUTHORIZED SAMPLING OFFICIALS

Samples must be collected by officials authorized for this purpose.

6. SAMPLING PROCEDURES

6.1 Product to Sample

Each lot to be examined must be sampled separately.

6.2 Precautions to Take

During collection and processing, contamination or other changes in the samples which would alter the residue or affect the analytical determination must be prevented.

6.3 Collection of a Primary Sample

Detailed instructions for collection of a primary sample of various products are provided in Table A. Quantities to collect are dependent on the analytical method requirements. Minimum quantity requirements are included in Table A. The following are general instructions.

- a. Each primary sample should be taken from a single animal 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. Canned or packaged product should not be opened for sampling unless the unit size is at least twice the amount required for the primary laboratory sample. The primary sample should contain a representative portion of juices surrounding the product. Each sample should then be frozen as described in paragraph 6.8.d.
- d. Frozen product should not be thawed before sampling.
- e. Large, bone-containing units of product (i.e., prime cuts) should be sampled by collecting edible product only as the primary sample.

6.4 The Number of Primary Samples to Collect from a Lot

The number of primary samples collected will vary depending on the status of the lot. If a residue violation is suspected because of its origin from a source with a past history of residue violations of the MRLVD, by evidence of contamination during transport, by signs of toxicosis observed during ante- or post-mortem inspection, or by other relevant information available 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.

6.4.1 Sampling suspect lots

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

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

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. Then 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 natural strata because of 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

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 too predictable, 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

In biased or estimated worst case sampling, the investigator should use their judgement and experience regarding the population, lot, or sampling frame to decide which samples to select. As a non-random technique, no inferences should be made about the population sampled based on data collected. The population group anticipated to be at greatest risk may be identified.

Exporting countries should conduct a comprehensive residue testing 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

The bulk sample is prepared by combining and thoroughly mixing the primary samples.

6.6 Preparation of the Final Sample

The primary sample should, if possible, constitute the final sample. If the primary sample is too large, the final sample may be prepared from it by a suitable method of reduction.

6.7 Preparation of the Laboratory Sample

The final sample should be submitted to the laboratory for analysis. If the final sample is too large to be submitted to the laboratory, a representative subsample should be prepared. Some national legislation may require the final sample be subdivided into two or more portions for separate analysis. Each portion should be representative of the final sample. Precautions in paragraph 6.2 should be observed.

6.8 Packaging and Transmission of Samples

- a. Each sample should be placed in a clean, chemically inert container to protect the sample from contamination and from being damaged 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°C, immediately after collection, and packed in a suitable container that retards thawing. If possible, the shipping container should be placed in a freezer for 24 hours prior to packing and shipping the frozen sample.

7. RECORDS

Each primary sample 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

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)	Collect hind quarter or whole carcass from one or more animals.	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, shoulders, 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 found 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

Commodity	Instructions for collection	Minimum quantity required for laboratory sample
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
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 or frozen, 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

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.

Commodity	Instructions for collection	Minimum quantity required for laboratory sample
D. Fresh/chilled or frozen parts		
1. Wholesale packaged		500 g after removal of skin and bone
a. Large parts	Collect an interior unit from a selected container.	
b. Small parts	Collect sufficient parts from a selected layer in the container.	
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
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

Commodity	Instructions for collection	Minimum quantity required for laboratory sample
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 representative sample 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

Appendix B**SAMPLING FOR THE CONTROL OF VETERINARY DRUG RESIDUES
IN FISH, MILK AND EGG PRODUCTS****1. OBJECTIVE**

To provide instructions for sampling a lot of eggs, milk, or aquatic animal products, to determine compliance with Codex Maximum Residue Limits for Veterinary Drugs (MRLVDs).

2. DEFINITIONS**2.1 Lot**

An identifiable quantity of food delivered for slaughter or 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

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

2.3 Primary Sample

A quantity of food taken from a single animal or 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 in the lot can be combined for the primary sample.

2.4 Bulk Sample

The combined total of all the primary samples taken from the same lot.

2.5 Final Sample

The bulk sample or a representative portion of the bulk sample to be used for control purposes.

2.6 Laboratory Sample

The sample intended for laboratory analysis. A whole primary sample may be used for analysis or the sample may be subdivided into representative portions, if required by national legislation.

3. COMMODITIES TO WHICH THE GUIDELINE APPLIES**3.1 Selected Class B: Primary Food Commodities of Animal Origin**

Type 06 Mammalian Products

No. 033 Milks

Type 07 Poultry Products

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. 033, 039, 040, 041, 043, 045, 048, and 049

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

For purposes of control, the maximum residue limit (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 none of the laboratory samples contains a residue greater than the MRLVD.

5. EMPLOYMENT OF AUTHORIZED SAMPLING OFFICIALS

Samples must be collected by officials authorized for this purpose.

6. SAMPLING PROCEDURES

6.1 Product to Sample

Each lot to be examined must be sampled separately.

6.2 Precautions to Take

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 laboratory sample not representative of the bulk or final sample.

6.3 Collection of a Primary Sample

Detailed instructions for collection of a primary sample of various products are provided in Table B. Quantities to collect are dependent on the analytical method requirements. Minimum quantity requirements are included in Table B. 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. Canned or packaged product should not be opened for sampling unless the unit size is at least twice the amount required for the primary laboratory sample. Each primary sample should contain a representative portion of juices surrounding the product. Each sample should then be frozen as described in paragraph 6.8.d.
- c. Frozen product should not be thawed before sampling.

6.4 The Number of Primary Samples to Collect from a Lot

The number of primary samples collected will vary depending on the status of the lot. If a residue violation is suspected because of its origin from a source with a past history of residue violations of the MRLVD, by evidence of contamination during transport or by 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.

6.4.1 Sampling suspect lots

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

A statistically-based, random sampling programme is recommended for non-suspect lots. Any of the following types of sampling can be used.

a. Stratified random sampling

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. Then 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 natural strata because of 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

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 random sampling, when there is reliable information on product volumes to be used to determine the sampling interval that will provide the desired number of samples over time. If the sampling system is too predictable, 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

In biased or estimated worst case sampling, the investigator should use their own judgement and experience regarding the population, lot, or sampling frame to decide which samples to select. As a non-random technique, no inferences should be made about the population sampled based on data collected. The population group anticipated to be at greatest risk may be identified.

Exporting countries should conduct a comprehensive residue testing 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 which do not provide residue testing results showing compliance with MRLVDs should be sampled as suspect lots.

6.5 Preparation of the Bulk Sample

The bulk sample is prepared by combining and thoroughly mixing the primary samples.

6.6 Preparation of the Final Sample

The primary sample should, if possible, constitute the final sample. If the primary sample is too large, the final sample may be prepared from the primary sample by a suitable method of reduction.

6.7 Preparation of the Laboratory Sample

The final sample should be submitted to the laboratory for analysis. If the final sample is too large to be submitted to the laboratory, a representative subsample should be prepared. Some national legislation may require the final sample be subdivided into two or more portions for separate analysis. Each portion should be representative of the final sample. Precautions in paragraph 6.2 should be observed.

6.8 Packaging and Transmission of Samples

- a. Each sample or subsample should be placed in a clean, chemically inert container to protect the sample from contamination and from being damaged 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°C, immediately after collection, and packed in a suitable container that retards thawing. If possible, the shipping container should be placed in a freezer for 24 hours prior to packing and shipping the frozen sample.

7. RECORDS

Each sample must be correctly identified by a record with the type of sample, origin of the sample (e.g., country, state, or town), location of collection of the sample, 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

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

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 semi-skimmed	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 adhering material from the sides and bottom of the container. Remove 2 to 3 litres, repeat the stirring 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

Commodity	Instructions for collection	Minimum quantity required for laboratory sample
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.	200 ml
	Small containers. Take sufficient units to meet laboratory sample size requirements.	
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	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.	
C. Butteroil including anhydrous butteroil 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 Milk Products - multi- ingredient)		

Commodity	Instructions for collection	Minimum quantity required for laboratory sample
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 0.25 litre liquid or 0.5 litre packed shavings from aseptic drillings into containers.	500 g
B. Dried egg products	Use sample schedule. For containers of 0.5 kg or less or 0.25 litre or less, collect a minimum of 2 units per subsample. For containers of 0.5 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
C. Shell eggs		
1. Retail packages	Use sample schedule. Subsample size is 1 dozen.	500 g or 10 whole eggs
2. Commercial cases	For 15 cases or less collect 1 dozen from each case, minimum of 2 dozen eggs. For 16 or more cases collect 1 dozen 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 0.5 kg of edible fish.	1000 g
C. Bulk shellfish (except oysters)	Collect 12 subsamples randomly.	1000 g

Commodity	Instructions for collection	Minimum quantity required for laboratory sample
D. Other fish and shellfish products (including oysters)	Collect 12 - 0.25 litre 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 C

SAMPLING FOR THE CONTROL OF VETERINARY DRUG RESIDUES IN HONEY

1. OBJECTIVE

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

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

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

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

The combined total of all the primary samples taken from the same lot.

2.5 Final Sample

The bulk sample or a representative portion of the bulk sample to be used for control purposes.

2.6 Laboratory Sample

The sample intended for laboratory analysis. A whole primary sample may be used for analysis or the sample may be subdivided into representative portions, if required by national legislation.

3. COMMODITIES TO WHICH THE GUIDELINE APPLIES

3.1 Selected According to Origin

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

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

3.2 Selected According to Mode of Processing

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.

Extracted honey that is obtained by centrifuging decapped broodless combs.

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

4. PRINCIPLE ADOPTED

For purposes of control, the maximum residue limit (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 none of the laboratory samples contain a residue greater than the MRLVD.

5. EMPLOYMENT OF AUTHORIZED SAMPLING OFFICIALS

Samples must be collected by officials authorized for this purpose.

6. SAMPLING PROCEDURES

6.1 Product to Sample

Each lot to be examined must be sampled separately.

6.2 Precautions to Take

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 laboratory sample not representative of the bulk or final sample.

6.3 Collection of a Primary Sample

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 C, 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 primary 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

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 violations of 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

The primary sample is prepared as described in paragraph 9.

6.6 Preparation of the Laboratory Sample

The primary sample should, if possible, constitute the final sample. If the primary sample is too large, the final sample may be prepared from it by a suitable method of reduction.

6.7 Preparation of the Laboratory Sample

The final sample should be submitted to the laboratory for analysis. If the final sample 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 subdivided into two or more portions for separate analysis. Each portion should be representative of the final sample. Precautions in paragraph 6.2 should be observed.

6.8 Packaging and Transmission of Primary Samples

- a. Each primary sample should be placed in a clean, chemically inert container to protect the sample from contamination and from being damaged 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.

7. RECORDS

Each primary sample 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

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

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.

Collect 250 ml of liquid or strained honey.

9.2 Comb Honey

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.

Collect 250 ml of liquid honey.

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

It would be ideal to have analytical methods available for determining compliance with MRLVDs that are effective and practical to detect, quantify, and identify all residues of veterinary drugs and pesticides (used as veterinary drugs) that may be present in commodities within the terms of reference of this Codex Committee. These methods could be routinely used by regulatory control authorities of member governments for their residue testing programmes to assure compliance with food safety requirements.

Methods with the capabilities mentioned above are not available for many compounds of interest because of the extensive number of potential veterinary drug residues which may find their way into food within the terms of reference of the CCRVDF. To optimize the effectiveness of regulatory programmes to test for veterinary drug residues, residue control programmes must use available residue methodology to assure compliance with Codex MRLVDs and, as necessary, take appropriate regulatory action against adulterated products, consistent with the reliability of the analytical data.

To assist regulatory authorities in determining their analytical needs for residue control programmes, this document will describe the types of methods available and a set of attributes which residue control programmes may utilize in carrying out their missions.

The principal attributes of analytical methods for residue control programmes are specificity, precision, accuracy (measured as systematic error and recovery), and sensitivity. Determining these principal attributes in a method requires well designed multi-laboratory studies. The attributes noted above will be presented in a subsequent section of this paper in more detail.

TYPES OF ANALYTICAL METHODS

Several types of methods are available to food safety agencies and programmes to conduct analyses that are consistent with the needs of residue testing programmes. Decisions on the use of a specific analytical method depends on the intended objectives of the regulatory programme and the analytical performance characteristics of methods.

Methods that are suitable for determining compliance with MRLVDs are those that have successfully completed an extensive multi-laboratory study for specific tissue and species combinations. These methods provide analytical results for either quantitation or identification that are appropriate to take regulatory action without the need for additional analyses. In some cases, these methods may be considered reference methods, but reference methods frequently are not routine.

Many methods currently being used by residue control programmes have successfully completed a multi-laboratory study. Multi-laboratory method performance studies generally satisfy these analytical requirements. Validated methods are those subjected to a properly designed inter-laboratory study with three or more analysts, and preferably, in three different laboratories. Collaborative study methods have successfully completed method evaluation in six or more laboratories in an acceptable, statistically designed study. Some residue control methods that have demonstrated their usefulness for determining compliance with MRLVDs have an historical origin. These history based methods were considered to be the best available at the time of initial regulatory use and have continued in use over an extended period of time in the absence of more effective validated methods.

Collaborative study and validated methods may be extended to additional tissues, species, products, or combinations of these, not included in the original multi-laboratory study by completing additional properly designed laboratory studies. On a case by case basis, analytical results from method extension studies may require additional analysis and/or review before reporting results or taking regulatory action.

Methods that have not been validated by traditional inter-laboratory study, but provide results that may be correlated and compared with data obtained from a collaborative study or validated method, may serve a regulatory purpose. The validated and non-validated methods must be compared in a statistically acceptable study design using portions of the same (homogeneous) samples prepared for this comparison. The data from these studies should be reviewed by a peer group of regulatory scientists to determine the comparability of method performance.

There are some non-routine veterinary drug residue methods suitable for enforcement of MRLVDs. These methods may not have been subjected to an inter-laboratory study because they require specialized expertise or equipment. Good quality control and quality assurance procedures must be applied with these methods. Analytical data obtained from these methods should be reviewed by a peer group of regulatory analysts before recommending any regulatory action. These analytical methods may require analysis by another method to corroborate the initial experimental findings.

Occasionally, a method may be suitable for Codex purposes because the toxicology of an analyte does not allow an MRLVD to be established. Methods for analytes such as chloramphenicol would be in this category. Some methods in this category will include those presented above which are not sufficiently sensitive to quantitate and/or identify analyte(s) at or below the MRLVD. Such methods also may not meet other performance factors stated above.

There are some methods for which additional analysis is required to support regulatory action. This category may include methods that do not provide adequate information of structure or residue concentration. Analytical methods that may have been subjected to ruggedness testing, but not successfully to a multi-laboratory study to evaluate method performance, may have limited usefulness in a residue control programme. However, these methods may be useful in non-recurring or infrequent residue analyses, but they commonly require use of a rigorous protocol for sample analysis. Results from such methods should be considered only as estimates of analyte concentration or identification without additional supporting analytical information. Results from these methods can be useful for gathering residue information and determining whether there is a need to develop a more definitive method. These methods should not be used alone for residue control purposes on official samples without additional information (e.g., such as the presence of an injection site in the sample).

Certain methods may only be suitable for determining whether or not a veterinary drug residue problem exists in a sampling population. Methods in this category are used for information gathering, or exploratory residue control studies. Exploratory studies may also be undertaken using methods which have not been subjected to inter-laboratory study. These non-routine methods may be complex, or require highly specialized instrumentation, and may have been developed and used only in a single laboratory. Analytical results from these methods should not be used independently for taking regulatory action, but may be used to determine the need for additional testing and/or development of a method suitable for routine enforcement of MRLVDs.

Methods designed to analyze large numbers of samples quickly may be useful for determining the presence or absence of one or more compounds in a quantitative or semi-quantitative manner, at or above a specified concentration. Results at or above the MRLVD commonly require additional analysis using a method with acceptable performance characteristics before taking regulatory action. Results from methods

of this type that are below the MRLVD but above a level of reliable measurement of a more definitive method, may have limited use in determining exposure patterns.

METHOD DEVELOPMENT CONSIDERATIONS

Developing an analytical method requires analysts, laboratory space, equipment, and financial support. To optimize the benefit of these resources, it is important to provide introductory and background information to establish a perspective for planning an analytical method development project, and for evaluating the performance of the analytical method.

Residue control programmes should use methodology suitable to the analytes of interest to assure a safe and wholesome food supply. Necessary and appropriate regulatory action should be taken against adulterated products, consistent with the reliability of the analytical data. Before initiating method development activities, the intended use and need for a method in a residue control programme should be established. Other considerations include the compound or class of compounds of interest (and 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 was determined, analyte and reagent stability data and purity of reagents, the acceptable operating conditions for meeting method performance factors, sample preparation guidelines, environmental factors that may influence method performance, safety items, and any other specific information pertinent to programme needs.

ANALYTICAL PERFORMANCE CHARACTERISTICS

Specificity is the ability of a method to distinguish between the analyte of interest and other substances which may be present in the test sample. A residue control method must be able to provide unambiguous identification of the compound being measured. The ability to quantitatively differentiate the analyte from homologues, analogues, or metabolic products under the experimental conditions employed is an important consideration of specificity.

Precision of a method is the closeness of agreement between independent test results obtained from homogeneous test material under the stipulated conditions of use. Analytical variability between different laboratories is defined as reproducibility, and variability from repeated analyses within a laboratory is repeatability. Precision of a method is usually expressed as standard deviation. Another useful term is relative standard deviation, or coefficient of variation (the standard deviation, divided by the absolute value of the arithmetic mean). It may be reported as a percentage by multiplying by one hundred. Method variability achieved in the developing laboratory after considerable experience with a method, is usually less than the variability achieved by other laboratories that may later also use the method. For this reason, analytical data from a method should be statistically analyzed by procedures described by Youden and Steiner (Ref: Statistical Manual of the AOAC, AOAC INTERNATIONAL, Gaithersburg, MD, 1975) before preparing a final method write up. 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.

Accuracy refers to the closeness of agreement between the true value of the analyte concentration and the mean result that is obtained by applying the experimental procedure a large number of times to a set of homogeneous samples. Accuracy is closely related to systematic error (analytical method bias) and analyte recovery (measured as percent recovery). The accuracy requirements of methods will vary depending upon the planned regulatory use of the results. Generally, the accuracy at and below the MRLVD or level of interest must be equal to or greater than the accuracy above the level of interest.

The percent recovery of analyte added to a blank test sample is a related measurement that compares the amount found by analysis with the amount added to the sample. 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). At relatively high concentrations, analytical recoveries are expected to approach one hundred percent. At lower concentrations and, particularly with methods involving a number of steps including extraction, isolation, purification, and concentration, recoveries may be lower. Regardless of what average recoveries are observed, recovery with low variability is desirable.

The sensitivity of a method is a measure of its ability to detect the presence of an analyte and to discriminate between small differences in analyte concentration. Sensitivity also requires the ability to differentiate between analyte, related compounds and background interferences. For analytical instruments used in residue analysis, sensitivity is determined by two factors: instrumental response to the analyte and background interference, or instrument noise. Response is measured by the slope of the calibration curve with analyte standards at concentrations of interest. An ideal situation would be afforded by a linear curve. Instrument noise is the response produced by an instrument when no analyte is present in the test sample.

There are a number of collateral attributes suitable for analytical methods for regulatory control programmes beyond these principle method attributes. Methods should be rugged or robust, cost effective, relatively uncomplicated, portable, and capable of simultaneously handling a set of samples in a time effective manner. Ruggedness of a method refers to results being relatively unaffected by small deviations from the optimal amounts of reagents used in the analytical method, time factors for extractions or reactions, or temperature. This does not provide latitude for carelessness or haphazard techniques. Cost-effectiveness is the use of relatively common reagents, instruments, or equipment customarily available and used in a laboratory devoted to veterinary drug residue analyses. An uncomplicated method uses simple, straightforward mechanical or operational procedures throughout the method.

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

The capability of a residue control method to simultaneously analyze a set of samples aids in method efficiency by allowing sets or batches of samples to be analyzed at the same time. This attribute reduces the analytical time requirements of sample analysis. It provides, for example, the capability of completing four or more analyses in a normal working day. This is important when large numbers of samples must be analyzed in short or fixed time frames.

Establishing method performance attributes is very important. These attributes provide the necessary information for food safety agencies to develop and manage their public health programmes. Performance attributes for analytical methods also provide a basis for good management decisions in future planning, evaluation, and product disposition. For the animal health care industry, it provides a guideline for knowing exactly what performance must be achieved in developing analytical procedures. All will benefit by having well defined analytical method performance factors.

INTEGRATING ANALYTICAL METHODS FOR RESIDUE CONTROL

Residue control and standard setting organizations have different terminologies to describe application of analytical methods. Methods of analysis for veterinary drug residues in foods must ultimately be able to reliably detect the presence of an analyte of interest, determine its concentration, and correctly identify the analyte at and above an established maximum residue limit (MRLVD) for regulatory enforcement actions to be taken. The latter methods would be classified as confirmatory methods. These confirmatory methods may or may not have a quantitative or semi-quantitative component.

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

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

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

METHOD DEVELOPMENT AND VALIDATION CONSIDERATIONS FOR RESIDUE CONTROL METHODS

The multi-laboratory method validation study is the most important factor in providing analytical data to define method performance characteristics.

In developing a residue control method, whenever possible, data should be collected from three types of samples. Control test material from non-treated animals provides information about analytical background and matrix interferences. Fortified test material, containing known amounts of the analyte added to the control material, yields information about the method's ability to recover the analyte of interest under controlled conditions. Dosed or biologically incurred tissue, from food producing animals

and birds that have been treated with the drug, provide additional analytical performance information about biological or other interactions that may occur when analyzing residue control samples.

Residue methods should be designed with as much simplicity as possible. Analytical simplicity helps minimize the variety, size, and type of glassware and equipment needed, minimizes the potential for analytical errors, and reduces laboratory and method costs. Reagents and standards must be available commercially or from some other reliable source. Instrumentation should be selected based on its performance characteristics rather than a particular manufacturer.

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. 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.

Residue control methods that may be subjected to widely variable physical test environments will place some additional requirements on methods. Addressing these may help improve method ruggedness. Warmer environments may require reagents to be more thermally stable, while solvents used in the analysis will have to be less volatile, and test sample requirements to be more lenient. Cooler environments may require reagents and solvents to have different physical properties, such as lower freezing point and greater solvating characteristics, to ensure effective extraction of an analyte. Environmental temperatures may influence the time required to perform an analysis, as well as influencing reaction rates, gravitational separations and colour development. These considerations may strain efforts to standardize methods for use in broadly differing environments because of the need to adapt methods to compensate for these factors.

An analytical method developed and used in only one laboratory may have limited use in a residue control programme. The reliability of reported values may be a concern even though strong quality control procedures may have been employed. As a minimum, three laboratories expected to use these methods should be used to develop performance characteristics for residue control, including analytical variability, and obtain statistically acceptable agreement on the same samples divided among the testing laboratories. Methods with higher reliability for residue testing should be able to successfully undergo a collaborative study involving at least six different laboratories (Ref: *Use of Statistics to Develop and Evaluate Analytical Methods* (by G.T. Wernimont and W. Spendley, AOAC INTERNATIONAL, Gaithersburg, MD), and *Compound Evaluation and Analytical Capability National Residue Programme Plan 1990*, (section 5, USDA, Food Safety and Inspection Service, Washington, D.C.)).

The principles for conducting either a validation or collaborative study of a residue control method are the same. Samples for evaluating method performance should be unknown to the analyst, contain the residue near the MRLVD as well as samples with the analyte above and below the level of interest, and test material blanks. All study samples should be analyzed over a limited number of days, preferably with replicate analysis, to improve statistical evaluation of method performance. It should be noted that these are only minimal requirements. Duplicate analyses in only six laboratories with one or two animal species and tissues would yield limited quality estimates for repeatability and reproducibility.

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.

PART III

ATTRIBUTES OF ANALYTICAL METHODS FOR RESIDUES OF VETERINARY DRUGS IN FOODS

The performance characteristics of analytical methods for determining compliance with MRLVDs must be defined and proposed methods evaluated accordingly. This will ensure 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 Regulatory 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 three categories of methods for determining compliance with Codex MRLVDs referred to as Level I, Level II and Level III methods will be presented followed by additional attributes that are applicable to only one or two categories of methods.

(**Note:** This Part contains numerous definitions. The CCRVDF has attempted to harmonize these definitions with those provided in the "Definitions for the Purpose of the Codex Alimentarius" in Volume 1.)

GENERAL CRITERIA FOR ATTRIBUTES

All methods may be characterized by a set of attributes or properties that determine their usefulness: *specificity* - what is being measured; *precision* - the variability of the measurement; and *systematic error* or *bias* - measured as analytical recovery. Another attribute, *accuracy*, usually refers to the closeness of agreement, or trueness of an analytical result, between the true value and the mean value obtained by analyzing a large number of samples of the test material. For semi-quantitative methods and screening methods, accuracy may also be defined as a measure of false negative and false positive responses. The *limit of detection*, *method sensitivity*, *practicality of use*, *tissue/species applicability*, *limit of detection* and *limit of quantitation* are additional attributes that have varying relevance to some methods, depending upon the intended use of the analytical results.

Methods may be described according to performance attributes as an alternative to classifying them by intent of use or purpose. This alternative approach defines methods by the analytical information and detail provided concerning the amount and nature of the analyte(s) of interest. Level I methods are the most definitive, while Level III methods usually provide general information about the presence of an analyte and semi-quantitative information about the amount of material present.

Level I methods quantify the amount of a specific analyte or class of analytes and positively identify the analyte, providing the greatest amount of reliability for quantitation and structure identification of the analyte at the level of interest. These methods may be a single procedure that determines both the concentration and identity of the analyte, or a combination of methods to quantify and confirm the structure of a veterinary drug residue. A good example of the latter is a chromatographic technique combined with a mass spectrometry procedure. 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.

Level I methods may be limited to analytes with appropriate physical and chemical properties amenable to chromatographic and other instrumental methods of analysis. For example, at the present

time, there are very few antibiotic drugs for veterinary use that have mass spectrometric procedures useful to determine compliance with MRLVDs because of the relatively low volatility and stability of antibiotic drugs to chemical techniques commonly employed for mass spectrometry analysis. However, new technology and instrumentation is now making development of these confirmatory methods possible. Level I methods are sometimes referred to as reference methods.

Level II methods commonly determine the concentration of an analyte at the level of interest, but do not provide unequivocal structure identification. These methods may use structure, functional group, or immunological properties as the basis for the analytical scheme. A common practice is to use one Level II method as the determinative assay and a second Level II method as the positive identification procedure. These methods may also be used to verify the presence of a compound or class of compounds. Two Level II methods may provide information suitable for a Level I method, when they use different chemical procedures. The majority of analytical methods commonly used to support MRLVDs are quantitative Level II laboratory methods.

Level III methods are those that generate less definitive but useful information. These testing procedures generally determine the presence or the absence of a compound or class of compounds at some designated level of interest. They are often based on non-instrumental techniques. For these reasons, Level III methods are commonly referred to as screening or semi-quantitative methods. Results on a given sample are not as reliable as Level I or II methods and usually need corroborating information for regulatory action. For example, Level III methods may provide good semi-quantitative information, but poor identification. Alternatively, they may provide strong or unequivocal identification with very little quantitative information. Level III methods are not poorly described or sloppy methods. They must have a well-defined operating protocol, operating characteristics and performance data.

Many of the microbiological agar plate assay procedures, enzyme inhibition assays and immunology based systems are in this category. They are useful for residue control programmes because of their high sample capacity, portability, convenience and potential suitability to non-laboratory environments. The limitation of Level III type methods is that action based on individual positive results usually requires verification using Level I or II methods. Individual results may be verified by epidemiological information.

Level III methods may offer substantial advantages to a residue control programme. Their advantages include analytical speed, sample efficiency through batch analysis, portability to non-laboratory environments, good sensitivity, or the ability to detect classes of compounds. Even though a Level III method may not detect a specific compound at a regulatory limit (i.e., an MRLVD) with every sample, it may be better than relying on Level I and II methods because of their ability to test more samples.

The decision to use Level III methods should be determined in part by performance characteristics, as well as the need to test large numbers of samples within a given time frame. Two key characteristics to consider for Level III methods are the percent false positives and percent false negatives, determined by comparison with a validated quantitative assay in a statistically designed protocol. The percent false negatives must be quite low at the levels of interest, while slightly more flexibility may be acceptable for false positives. Residue detection limits can be described based on these two parameters.

METHOD ATTRIBUTES

Specificity is the ability of a method to distinguish between the analyte being measured and other substances which may be present in the test material. A proposed method also must provide the required specificity for the compound being measured and discriminate between other structurally similar substances. This characteristic is predominately a function of the measuring principle or detection system used. Certain instrumental techniques such as Fourier transform infrared spectroscopy or mass spectrometry may be sufficiently specific by themselves to provide unambiguous identification. These are often referred to as confirmatory methods. Positive identification from a confirmatory method is usually considered necessary before regulatory action is taken in those instances when an analytical result is not sufficiently specific for regulatory purposes. Confirmatory methods may be considered Level I methods when they provide a determinative result to quantify and tentatively identify a given analyte, and a procedure which verifies the identity of the analyte of interest.

Other techniques, when they are used in combination, may be capable of achieving a comparable degree of specificity as confirmatory techniques. For example, specificity 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. Such procedures must be applicable at the designated maximum residue limit (MRLVD) of the analyte.

The specificity of a screening method normally is not as great as that of a determinative method, because screening methods often take advantage of a structural feature common to a group or class of compounds. These methods generally fit into the Level III methods category. Techniques based on biological assays, immunoassays, or chromogenic responses are not expected to be as specific as those techniques which unequivocally identify a compound. Specificity of a screening method may be increased by the use of chromatographic or other separation technique.

If a non-specific response or some ambiguity in a test result is obtained (i.e., cross-reactivity with components of the matrix other than that for which the analysis was designed), studies that approximate the concentration of the non-specific response of the analytical method may be required to identify the compounds that respond to the detection system. If the method is not sufficiently specific, then a confirmatory or identification procedure will be needed to characterize the analyte of interest.

Precision is an important performance characteristic of residue control methods. This attribute is common to all methods, and as noted below, acceptable precision may not be a function of the type of method, but of the concentration of the analyte in the original sample. There are several types of precision. Inter-laboratory precision, or reproducibility, is the closeness of agreement between test results obtained with the same method on identical test material in different laboratories. The variation in replicate analyses of a test material within a laboratory when performed by one analyst is repeatability. The intra-laboratory variability among analysts performing the same analysis is within-laboratory bias, and is primarily due to random error. Precision is usually expressed as a standard deviation (an absolute value determined experimentally). More useful is the relative standard deviation, or coefficient of variation. This parameter expresses variability as a function of concentration, and is relatively constant over a given concentration interval.

Precision limits for analytical methods, as a function of concentration, are presented below. The recommended values take into consideration the wide variety of methods, analytes, matrices, and species within the terms of reference of the Committee and that are usually applied in a broad-based residue control programme.

Concentration	Coefficient of Variability (CV) (Repeatability)
$\leq 1 \mu\text{g/kg}$	35%
$\geq 1 \mu\text{g/kg} \leq 10 \mu\text{g/kg}$	30%
$\geq 10 \mu\text{g/kg} \leq 100 \mu\text{g/kg}$	20%
$\geq 100 \mu\text{g/kg}$	15%

The variability achieved in the laboratory where a method was developed, and where there is considerable experience, is usually smaller than that attained by laboratories that may later use the method and have less experience with it. The final version of the method should be optimized by using procedures such as ruggedness testing to identify its critical control points and ensure that its performance will not be adversely affected by small changes in using the analytical procedure. If a method cannot achieve acceptable performance in the sponsor's laboratory, its performance usually will not be any better in other laboratories.

When developing analytical data to be used to define expected method variability and other performance characteristics, methods should be performed by an analyst who has not been directly involved in developing the method. This procedure will verify the adequacy of the method's written description and help identify critical parameters which affect method performance.

The within laboratory coefficient of variation should be ≤ 15 percent when the designated concentration of the analyte is greater than or equal to $100 \mu\text{g/kg}$. When the designated concentration of the analyte is $10 - 100 \mu\text{g/kg}$, the within laboratory coefficient of variation should be ≤ 20 percent. When the concentration of interest is below $10 \mu\text{g/kg}$, a coefficient of variation of ≤ 30 percent is acceptable.

A Level III method should be capable of identifying samples that contain a residue concentration at the level of interest. When a sample contains a residue that exceeds the MRLVD using a semi-quantitative (screening) method, regulatory action requires additional analysis. In this situation, the sample will require analysis using a determinative method and a confirmatory method with defined performance characteristics. A useful attribute for Level III methods is its precision at and just below the MRLVD. Precision may be somewhat less important above the MRLVD.

Systematic error, or method bias, is the difference between the experimentally determined (measured) value and the mean result that would be obtained by applying the experimental procedure a very large number of times to the test material. Systematic errors are always of the same sign and magnitude. Random error, however, is variable in magnitude and sign and the mean of random errors may approach zero if sufficient samples are tested. Accuracy is generally expressed as the percent recovery of the analyte of interest. Recovery is obtained experimentally by adding known quantities of the analyte directly to separate portions of the test material and comparing the amount recovered with the amount added. The percent recovery of an analyte added directly to the sample matrix is generally a higher value than is obtained experimentally when isolating the same biologically incurred analyte from a given sample matrix. At relatively high analyte concentrations, recoveries are expected to approach 100 percent. At lower concentrations or with multi-step methods that require extractions, solvent transfers,

concentration steps, and absorption chromatography, recoveries will be lower. Variability of analyte recovery is usually as important as the percent recovery itself and should be small.

Average recoveries of 80 to 110 percent should be obtained when the MRLVD for the analyte is 100 µg/kg or greater and when the analytical method can be performed with acceptable precision.

Recommended acceptable recoveries at lower MRLVDs are 70 to 110 percent when the MRLVD is 10 µg/kg to 100 µg/kg, and 60 to 120 percent when the MRLVD is less than 10 µg/kg. These recovery limits are reasonable when viewed within the context of the wide variety of residues, methods, matrices, and species normally included in a broad-based residue testing programme. Variability in recovery should be small regardless of the percent recovery.

Correction factors for more or less than 100 percent recovery may be appropriate when analytical methods use isotope dilution procedures or other appropriate internal reference standards for quantitation purposes.

The accuracy requirements of different types of methods will vary with the intended use for the results. In general, methods should have their greatest accuracy at the MRLVD. The accuracy requirements of confirmatory methods may not be as great as is required for quantitative methods, because in most residue control programmes these methods are only performed after a residue concentration greater than the MRLVD has been determined by a quantitative method. Most confirmatory methods have a quantitative aspect built into them which serves as an additional check on the previously performed quantitative method. Suggested accuracy requirements for methods are given below, and are based upon the previously stated considerations of a broad-based residue testing programme.

Concentration	Acceptable range
≤ 1 µg/kg	-50 to +20%
≥ 1 µg/kg ≤ 10 µg/kg	-40 to +20%
≥ 10 µg/kg ≤ 100 µg/kg	-30 to +10%
≥ 100 µg/kg	-20 to +10%

Level III methods may be useful for residue control programmes in several scenarios. For example, they may be used in situations where no MRLVD can be established or where one does not otherwise exist, and regulatory action may be taken if any amount of the drug residue is found. Non-quantitative methods may also be used when the MRLVD or the level of interest is less than the limit of detection of the screening method. In both cases, it is necessary to evaluate proposed methods for the specified residue test to experimentally determine the lowest concentration at which an analyte can be detected and to determine method accuracy and limits by using data on false negatives (i.e., a negative analytical result is obtained when the analyte is present), and false positives, (i.e., a positive result is obtained when the analyte is not present) at or above the MRLVD.

If Level III methods involve a manufactured test kit, at a minimum, the accuracy, precision, specificity and lowest detection limit data should be provided by the manufacturer. The users should verify the validity of this data through their own studies and evaluate performance by quality control checks. The lowest detectable concentration of an analyte should represent the smallest amount of an individual analyte that can be reliably observed or found in the test sample. The method accuracy, expressed in terms of false negatives and false positives, should be determined by a statistically valid, scientifically correct study with appropriate controls.

In general, non-quantitative methods should produce less than 5 percent false negatives and less than 10 percent false positives when analysis is performed on the test sample. These values may vary depending on the type of action that will be taken as a result of the analytical test. Conservative values should be chosen appropriate to residue testing needs.

The limit of detection is the smallest measured concentration of an analyte from which it is possible to deduce the presence of the analyte in the test sample with acceptable certainty. This determination should consider matrix related interferences with an instrumental signal to noise (S/N) ratio greater than 5:1 or the concentration determined by a factor of 3 standard deviations of the signal response for blank tissue, whichever is less.

Sensitivity is a measure of the ability of a method to detect the presence of an analyte and to discriminate between small differences in analyte content. This may be determined by the slope of the standard curve at concentrations of interest.

COLLATERAL PARAMETERS FOR METHODS SUITABLE FOR ROUTINE USE FOR ENFORCEMENT OF MAXIMUM RESIDUE LIMITS

Residue control methods should be capable of analyzing several samples simultaneously, normally in groups of four or more during a normal work period. These methods should ideally require no more than about 2 hours of analytical time per sample. This does not require that results for a set of analytical samples must be completed within 2 hours. Several hours may be necessary to prepare a set of extracts or complete a microbiological incubation, for example, before analysis of test sample results can be completed. Regulatory methods should be able to be completed within reasonable time periods consistent with regulatory objectives.

The applicability of a method refers to the tissue matrices and animal species that a particular method has demonstrated acceptable method performance for compliance with an MRLVD.

The limit of quantitation corresponds to the smallest measured concentration of residue from endogenously incurred test material above which a determination of the analyte can be made with a specified degree of certainty to its accuracy and precision.

For determining compliance with an MRLVD, an analytical method should require only instrumentation generally available in a laboratory devoted to trace analyses in the appropriate test material. The methods should be capable of analyzing analytes at or below the MRLVD. In addition, the methods should have written protocols that include extensive quality assurance and quality control components. These quality assurance plans should also include analyst training needs.

Whenever applicable, methods should be evaluated in an inter-laboratory study using some test samples with biologically incurred analyte. Experience suggests that using biologically incurred residues for method evaluation provides a better description of the expected performance characteristics of the method as it would be used routinely by regulatory authorities.

Residue testing methods must demonstrate that they can be performed at their described performance characteristics by experienced analysts who have received adequate method training. Acceptable methods performance can be demonstrated by successfully analyzing sets of samples containing the analyte of interest in sample matrices within the scope of the CCRVDF terms of reference.

Methods to determine compliance with MRLVDs should utilize commercially available reagents and equipment. Methods may become impractical and potentially unreliable if new or unusual reagents

are not readily available. New or unusual reagents and standards must be assured by the method sponsor upon request.

Regulatory methods for residue control should not use large quantities of solvents, reagents, and supplies which would render the method economically impractical. Methods for determining compliance with Codex MRLVDs should be designed for safe performance by trained analysts.

Several other indicators of satisfactory performance may be helpful in determining whether or not a method is acceptable for Codex purposes. These include: (a) calibration (standard) and analytical (recovery) curves; (b) information on the effectiveness of extraction for removing specific potential interferences; (c) adequate method sensitivity (slope of the standard calibration curve) with a linear dynamic range at the concentration of interest; (d) adequate resolution from matrix components; (e) sufficiently low and reproducibly consistent blanks; and (f) stability studies performed on the matrix, the analyte within the matrix, and reagents used in the procedure. The analytical response of the blank should be no more than 10% of the analyte response at the MRLVD, whenever an MRLVD is established. Critical control points within the analytical procedure, those steps where extreme care must be taken to insure optimum method performance, and stopping points within the method need to be identified and noted in the written procedure.

SPECIFIC DATA NEEDED

The developer of a method must provide pertinent information and supporting data necessary to familiarize other intended users of a method so they can achieve satisfactory methods performance. This necessary information should include the following:

For Codex methods, the developer of a method should collect and provide data from three types of samples: (a) control tissue samples from animals that are known not to have been exposed to the analyte; (b) tissue samples that are fortified or spiked at the levels of interest by the addition of known amounts of the analyte to uncontaminated control tissue; and (c) dosed or incurred tissue samples at the concentration of interest (MRLVD) obtained from animals treated with the veterinary drug according to good veterinary practices.

Methods provided by developers, drug sponsors and commercially available test kits intended for use with Codex MRLVDs should only be recommended for use after it can be demonstrated that the method(s) will meet established performance characteristics or provide an improvement to current methods, regulatory decision making and regulatory consistency.

The developer of the method must determine: (a) the analytical response obtained when the matrix is known to be free from chemical interferences; (b) the method variability, and (c) the lowest concentration at which the amount of analyte present can be detected with reasonable statistical certainty. The data should demonstrate that the proposed method can satisfactorily recover and identify known amounts of the analyte that have been added to the test sample. Finally, the developer should demonstrate that the proposed method can satisfactorily recover the analyte from the target tissue matrix in which it has been biologically bound or incurred. Recovery studies must demonstrate absence of responses from substances that may interfere or adversely affect the reliability of the analysis.

The method must demonstrate acceptable method performance in controlled laboratory environments and in field trials which represent anticipated operating conditions, if that is the intended use of the method. The results must be verified by appropriate quality assurance and quality control procedures, including analysis of known blank and positive control samples. Analysis of sufficient numbers of both positive and negative control samples must be performed to establish false positive and

false negative rates, with a statistically appropriate number of these samples analyzed by a separate method to verify the results.

A complete description of the method must be provided which includes the scientific principle(s) upon which the method is based, preparation of analytical standards, appropriate tissues the method is suitable for, shelf-life and storage conditions for the analyte in solution and in the target tissue matrix, reagent and standard shelf-life stability, instrumentation as well as their performance standards and calibration procedures, and identification of critical steps and stopping places. Test limitations as well as appropriate and inappropriate uses of the test must be described. Critical test components and reagents must be identified and specifications described. The developer must provide procedures for demonstrating evidence of satisfactory method performance as well as guarantee the long term availability of all components necessary to successfully perform the test.

For rapid test procedures, the quality control criteria needed to verify and maintain acceptable method performance and to determine that a test kit is operating properly must be provided. Information to verify proper test data interpretation associated with the quality control criteria must be specified. A standard curve prepared for the analyte of interest of known purity is needed. A typical analytical curve prepared by fortifying blank test material with the analyte of interest must be provided.

Data from uncontaminated, fortified, and dosed test material is required to show that the method meets the specificity, precision, systematic error, and accuracy attributes for its intended use. Test samples should be fortified at 0.5 (where practical), 1 and 2 times the MRLVD. Additional samples within these concentration limits may be included.

Data from inter-laboratory studies should be provided on the analytical worksheet developed for evaluating methods for Codex MRLVDs. The method should be tested in three or more laboratories for ease in evaluating multi-laboratory study reports. Each laboratory should analyze samples fortified as stated previously and should test biologically incurred samples containing the analyte at the same concentrations.

Test kits should utilize simple, unambiguous procedures. The analytical procedures designed into test kits to be used by field personnel should be successfully evaluated by at least ten trained individuals in a properly designed study before being placed into general use. The study environment must be similar to that expected for routine use of the test. The design should provide sufficient data for a statistical description of false positive and false negatives, and allow determination of the analytical limits of the test. Participants should include those individuals who have been trained by the developer of the test to determine that training procedures are sufficient to provide acceptable method performance.

STANDARD REFERENCE MATERIALS FOR VETERINARY DRUG RESIDUE ANALYSIS

At the present time it is usually not practical to develop standard reference materials for determination of residues of veterinary drugs in foods. There are specific difficulties in developing standard reference materials for international use as noted below.

Some drugs are not sufficiently stable in test materials at ordinary freezer temperatures. Veterinary drug residue concentrations commonly deplete with time, dependent upon the analyte and test material, at ordinary freezer temperatures. These test materials must be stored and shipped at ultra-cold temperatures or use lyophilized, irradiated, or treated otherwise to reduce enzymatic activity and prevent loss of analyte. The relevant studies for most compounds of interest to CCRVDF have not been published at this time, so it is not known whether treatments noted above will affect the extent to which the drugs of

interest are bound to the tissues, whether drug residues remain stable in tissues, or whether they might chemically alter the trace residues.

Recognized standard reference materials are generally very expensive and, considering their other limitations, they are generally not cost effective for residue analysis. Commercial reference standards for veterinary drugs have limited availability at the present time. Because of these and other limitations, such as analytical variability of a method versus the concentration of the analyte (i.e., low mg/kg to $\mu\text{g/kg}$), standard reference materials are generally inappropriate.