CODE OF HYGIENIC PRACTICE FOR EGGS AND EGG PRODUCTS

CAC/RCP 15-1976

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INTRODUCTION

This *Code of Hygienic Practice for Eggs and Egg Products* is intended to provide guidance for the safe production of eggs and egg products. A hazard analysis approach was used in determining the controls presented in this Code. The FAO/WHO document below was used to provide a risk-based foundation for the revised Code.

- *Risk assessments of* Salmonella *in eggs and broiler chickens*. Microbiological Risk Assessment Series 1. FAO/WHO 2002 (ISBN 92-5-104873-8). http://www.fao.org/DOCREP/005/Y4393E/Y4393E00.HTM

This *Code of Hygienic Practice for Eggs and Egg Products* takes into consideration, to the extent possible, the differing egg and egg product production systems and processing procedures used by countries. This Code focuses primarily on eggs produced from domesticated chickens. The principles may also be applied to the hygienic practices for egg production from other domesticated egg producing bird species (e.g. duck, quail and goose). Therefore, the code is, of necessity, a flexible one to allow for different systems of control and prevention of contamination of eggs and egg products.

This Code addresses the two main sources of contamination of eggs:

1. internally during egg formation, and
2. externally, at any point at or after laying.

It takes into consideration the possibility of illness in the general population due to the consumption of eggs or egg products contaminated by *Salmonella* species, other enteric pathogens or other contaminants, as well as the susceptibility to illness of sectors of the population such as the elderly, children, and immunocompromised individuals. For microbiological contamination, this approach is consistent with the approach identified by the Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods (Rome, Italy, 30 April – 4 May 2001).

1. OBJECTIVES

The objective of this Code is to ensure the safety and suitability[1] of eggs and egg products by applying the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969) to the particular case of eggs and egg products. The

[1] Safety and suitability as defined in the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

document describes the specific considerations for food hygiene and safety associated with all methods of primary production and processing of eggs and egg products, including the adequate measures for small-scale producers and processors.

2. SCOPE AND USE OF THE DOCUMENT

2.1 Scope

This Code applies to the primary production, sorting, grading, storing, transport, processing, and distribution of eggs in shell and egg products of such eggs produced by domesticated birds and intended for human consumption. Traditional delicacy eggs (e.g. Balut, 1 000-year-old eggs) are not within the scope of this Code.

2.2 Use of the document

The provisions of this document are supplemental to and should be used in conjunction with, the Recommended *International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

The Code also references other Codex Standards, Codes or Guidelines, including the labelling standards and the Codex *Code of Hygienic Practice for the Transport of Foods in Bulk and Semi-Packed Food* (CAC/RCP 47-2001), when they apply to the hygienic production of eggs and egg products.

This document consists of a series of principles, explanatory narratives and guidelines.

Principles, shown in **bold text**, are a statement of the goal or objective that is to be achieved. *Explanatory narratives*, shown in *italicized text,* serve to explain the purpose of the stated principle. Additional information to assist in the application of the stated principle is shown in normal text.

Principles that are applicable to all phases of production, handling and processing of eggs and egg products are given in Section 2.3.

This Code is flexible to allow for different productions systems, size of operation and different systems of control of hazards during production, handling and processing of eggs and egg products.

Recognition of the production and processing of eggs by small-scale/less developed egg producers/businesses

In the context of this Code, the expression "small-scale egg producer" refers to production systems based on the number of birds, or where automated collecting and sorting/grading machines are not generally used, or where water and other requirements are in poor supply thus limiting the number of birds that can be kept. The maximum number of birds permitted in small-scale establishments may be set down in national legislation, codes of practice or other guidelines.

Flexibility in the application of these requirements in this Code may apply to less developed egg producers, i.e. those producers with larger flocks that have less developed systems, and/or economic, water and/or power supply constraints, preventing investment in modern grading and packaging processes and infrastructure.

Flexibility in the application of requirements on the primary production of eggs by small-scale and/or less developed egg producers can be exercised, where necessary. However, any microbiological or other control measures used should be sufficient to obtain safe and suitable eggs and egg products.

Such flexibility is indicated throughout the Code by the use of a parenthetical statement "where practicable" placed next to the particular provision where the flexibility is needed.

Further guidance on the issues facing small and less developed businesses, particularly in relation to implementing HACCP is under development and can be found in FAO/WHO *Guidance to Governments on the Application of HACCP in Small and/or Less Developed Businesses* (FAO/WHO, October 2006)

2.3 Principles applying to the production, handling and processing of all eggs and egg products

The following principles should apply, where appropriate and practicable, to the production, handling and processing of all eggs and egg products.

- **From primary production to the point of consumption, eggs and egg products should be subject to control measures intended to achieve the appropriate level of public health protection.**
  The Code is aimed at encouraging the safe production of eggs and egg products for human consumption, and gives relevant guidance to producers and processors, large and small, on the application of control measures throughout the entire food chain. It recognizes that there is a need for continuous, effective effort or controls, which should be applied, by primary producers in addition to processors, in assuring the safety and suitability of eggs and egg products.
  Good hygienic, agricultural and manufacturing practices should be identified during primary production, shell egg processing and egg product processing. Such practices should be applied throughout the food production chain so that eggs and egg products are safe and suitable for their intended use.
  Both the relationship and impact of one part of the food production chain on another part should be identified to ensure that potential gaps in the chain are dealt with through communication and interaction between those in the production chain. Information should be obtained to cover one step forward and one step back through to final food preparation.
  No part of this Code should be used without consideration of what takes place in the production chain prior to the particular measure being applied or what will take place subsequent to a particular step. The Code should only be used within the context of an understanding that there is a continuous system of controls

that are applied from the breeding flock and sourcing of the laying flock to consumption of the end product. Good hygienic practice should also apply when handling eggs during food preparation.

- **Wherever appropriate, hygienic practices for eggs and egg products should be implemented within the context of HACCP systems as described in the Annex to the *Recommended International Code of Practice – General Principles of Food Hygiene.***
  There should be an understanding of the hazards associated with eggs, at each stage in egg production, handling, grading, packaging, transporting and processing so as to minimize contamination. It is principally the responsibility of the producer, where practicable, to conduct a hazard analysis within the context of developing a control system based on HACCP and thus to identify and control hazards associated with flock management and egg production. Similarly it is principally the responsibility of the processor to conduct a hazard analysis to identify and control hazards associated with egg processing.
  This principle is presented with the recognition that there are limitations to the full application of HACCP principles at the primary production level of eggs. In the case where HACCP is not implemented at the producer level, good hygienic, agricultural and animal husbandry practices should be followed.

- **Control measures should be effective and validated, where practicable.**
  The overall effectiveness of the control measures should be validated according to the prevalence of hazards in the egg, taking into consideration the characteristics of the individual hazards(s) of concern, established Food Safety Objectives/Performance Objectives and level of risk to the consumer.
  Small and less developed businesses that do not have resources to validate the effectiveness of their control measures should implement appropriate control measures required by their country. Where there are no legal requirements, such businesses should follow recommendations in industry-recognized guidelines or follow practices established as safe, where practicable.

2.4 Relative roles of egg producers, processors and transporters

All parties involved in the egg production chain share responsibility for food safety. This can include those involved in primary production, handling, grading, packaging, processing, supplying, distributing and commercial cooking of eggs and egg products for human consumption. In order to achieve this common goal, respective parties should pay attention to the following responsibilities:

- Good communication and interaction should exist between egg producers, processors and others in the chain so that an effective chain of controls is maintained from breeding of the laying flock to production of eggs to consumption. This can help to ensure that appropriate and complementary hygiene practices are applied at each stage of the chain and that appropriate and timely action is taken to resolve any food safety problems that may arise.

- Primary producers should apply good hygienic, agricultural and animal husbandry practices consistent with food safety, and adapt their operations as appropriate and practicable to meet any specifications for specific hygiene controls to be applied and/or any standards to be achieved as may be agreed with the processor, distributor, transporter or warehouser.
- Processors should follow good manufacturing and good hygienic practices, especially those presented in this Code and in the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969) or those required by the competent authority. The processor may have to implement controls, or adapt their manufacturing processes, based on the ability of the egg producer to minimize or prevent associated hazards.
- Producers and/or processors should communicate any recommendations for safe handling and storage of eggs and egg products during distribution and transportation, and their subsequent use by food businesses.
- Distributors and transporters, wholesalers, retailers and those involved in food preparation at any facility should ensure that eggs and egg products under their control are handled and stored properly and according to the producers and/or processors instructions.
- Information to consumers should include advice on safe handling, storage and preparation of eggs.

2.5 Definitions

Definitions of general expressions are included in the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969). For the purpose of this Code, the following terms have the definition stated:

Breaking – the process of intentionally cracking the egg shell and separating its pieces to remove the egg contents.

Breeding flock – a group of birds kept for the purpose of production of the laying flock.

Broken/leaker egg – an egg showing breaks of both the shell and the membrane, resulting in the exposure of its contents.

Candling – examining the interior condition of an egg and the integrity of the shell by rotating or causing the egg to rotate in front of or over a light source that illuminates the contents of the egg.

Cracked egg – an egg with a damaged shell, but with intact membrane

Dirty egg – an egg with foreign matter on the shell surface, including egg yolk, manure or soil.

Domesticated birds – members of the Class Aves that are kept for the production of eggs intended for human consumption.

Egg laying establishment – the facilities and the surrounding area where primary production of eggs takes place.

Egg product – all, or a portion of, the contents found inside eggs separated from the shell, with or without added ingredients, intended for human consumption.

Incubator egg – an egg that has been set in an incubator.

Microbiocidal treatment is a control measure that practically eliminates the number of micro-organisms, including pathogenic micro-organisms present in a food or reduces them to a level at which they do not constitute a health hazard.

Pasteurization – a microbiocidal control measure where eggs or egg products are subjected to a process, using heat to reduce the load of pathogenic micro-organisms to an acceptable level to ensure safety.

Shelf life – the period during which the egg or egg product maintains its safety and suitability.

Table egg – an egg destined to be sold to the end consumer in its shell and without having received any treatment significantly modifying its properties.

3. PRIMARY PRODUCTION

It is recognized that some of the provisions in this Code may be difficult to implement in areas where primary production is conducted in small holdings in both developed and developing countries and also in areas where traditional farming is practised. Therefore, the Code is, of necessity, a flexible one to allow for different systems of control and prevention of contamination of eggs during primary production.

These principles and narratives supplement those contained in Section 3 of the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969) and the general principles presented in Section 2.3 above.

Egg producers should take all reasonable measures to reduce the likelihood of hazards occurring in or on eggs during primary production.
Primary production activities can significantly impact on the safety of eggs and egg products. Bacterial contamination of eggs can occur during formation, thus the practices used at this phase of production are a key factor in reducing the potential for micro-organisms to be present in or on eggs.

It is recognized that microbiological hazards can be introduced both from the primary production environment and from the breeding and laying flocks themselves. Pathogens such as Salmonella *Enteritidis (SE) can be transmitted vertically from breeder flocks to commercial laying flocks, and horizontally from other layers, feed and/or environment and hence to eggs. Importantly, the presence of* Salmonella *in the laying and/or breeding flock increases the possibility of* Salmonella *in the egg.*

Thus the preventative role of good hygienic and agricultural practice in the primary production of eggs is critically important. Appropriate animal husbandry practices should be respected and care should be taken to assure that proper health of the breeding and laying flocks is maintained. Further, lack of good agricultural, animal feeding and veterinary practices and inadequate general hygiene by personnel and equipment during egg handling, and/or collection may lead to unacceptable levels of bacterial and other contamination (such as physical and chemical) during primary production.

The focus for primary producers is to reduce the likelihood that such hazards will occur during the primary production phase of the chain. Likewise, in certain primary production situations, the occurrence of food safety hazards may be less avoidable which may result in the application of more stringent control measures during subsequent processing in order to ensure safety and suitability of the finished product. The degree to which primary production practices control the likelihood of occurrence of a food safety hazard in or on eggs will have an impact on the nature of controls needed during the subsequent processing of eggs.

Contamination of eggs during primary production should be minimized.
Producers should obtain domesticated birds from breeding stock that have been subject to control measures to reduce and, if possible eliminate, the risk of introducing into laying flocks, poultry diseases and pathogenic organisms transmissible to humans. The breeding flock should be subject to a programme which will monitor the effect of the control measures.

Laying flock management is key to safe primary production of eggs. Laying flocks are managed under a wide range of climatic conditions using various agricultural inputs and technologies, and on farms of various sizes. However in backyard poultry farms and small scale producers, the number of birds maintained is very small and, accordingly, the systems and hygienic conditions of production may vary. Hazards may vary between one type of production system and another. In each egg laying establishment, it is necessary to consider the particular agricultural practices that promote the safe production of eggs, the type of products (e.g., unsorted eggs, eggs for the table egg market, eggs strictly for breaking) and production methods used.

The microbial load of eggs should be as low as achievable, using good egg production practices, taking into account the requirements for subsequent processing. Measures should be implemented at the primary production level to reduce as far as possible the initial load of pathogenic micro-organisms affecting safety and suitability. Such measures would permit the application of microbiological control measures of lesser stringency and still ensure product safety and suitability.

3.1 Environmental hygiene

The egg laying establishment should be appropriate for the primary production of eggs such that sources of potentially harmful substances are minimized and are not present at unacceptable levels in or on eggs.
Where practicable, producers could identify and evaluate the immediate surroundings and previous use (indoor and outdoor) of the egg laying establishment in order to identify hazards. Potential sources of contamination from the egg laying establishment including the immediate environment should be identified. This could include contamination associated with previous uses of the land, presence of contaminants, polluted surface water, potential microbial and chemical hazards from contamination by faeces, and other organic waste that could be introduced into the egg laying establishment. This is particularly relevant in the case of free range foraging by domesticated birds.

Primary production should not be carried out in areas where the presence of potentially harmful substances in the egg laying establishment would lead to an unacceptable level of such substances in or on eggs. The potential for contamination from, for example, agricultural chemicals, hazardous wastes, etc. should be considered. The potential for the introduction of disease from wild birds and animals should also be considered.

The evaluation process could include the following:

- Identification of previous and present usage of the primary production area and the adjoining sites to determine potential microbial, chemical and physical hazards and determine sources of environmental contamination, for example by faeces or other organic waste, that could be introduced into the egg laying establishment.
  - Sites/uses of concern can include crops grown, feed lot, animal production, hazardous waste site, sewage treatment site, and mining extraction site.
- Identification of points of access to the site by domesticated and wild animals, including access to water sources used in primary production, to determine potential faecal and other contamination of the soils and water and the likelihood of contamination of eggs.
  - Existing practices should be reviewed to assess the prevalence and likelihood of uncontrolled deposits of animal faeces coming into contact with eggs.
  - As much as possible, domestic and wild animals, including wild birds as well as rodents should be prevented from entering egg laying establishments.
- Identification of the potential for contamination of egg laying establishments by leaking, leaching or overflowing manure storage sites and flooding from polluted surface waters.

If previous uses cannot be identified, or the evaluation leads to the conclusion that hazards exist, where practicable, the sites should be tested for contaminants of concern. Additionally, periodic monitoring of the environment and forage, and judicious selection and use of fertilizers and agricultural chemicals should occur.

If contaminants are present at levels which may result in the egg or egg product being harmful to human health, and corrective or preventive actions have not been taken to minimize identified hazards, the sites should not be used until such actions have been applied.

Care should be taken to minimize access to contaminated water or to environmental contaminants to the extent practicable in order to avoid diseases transmissible to birds or to humans or the likelihood of contamination of eggs.

3.2 Hygienic production of eggs

Provisions in this section are equally relevant to all egg producers.

3.2.1 Flock management and animal health

Eggs should come from flocks (both breeding and laying) in good health so that flock health does not adversely affect the safety and suitability of the eggs.

Good animal husbandry practices should be used to help maintain flock health and resistance to colonization by pathogenic organisms. These practices should include timely treatment for parasites, minimizing stress through proper management of human access and environmental conditions and use of appropriate preventive measures for example, veterinary medicines and vaccines.

The Salmonella *Enteritidis Risk Assessment has shown that reducing the prevalence of* Salmonella *Enteritidis infected flocks is anticipated to result in a reduction in the risk of human illness from the consumption of* Salmonella *Enteritidis positive eggs.*[2]

Flock management is critical in reducing the risk of human illness from the consumption of eggs. Good husbandry practices should also be used to reduce the likelihood of pathogens (i.e. avian disease) and thus reduce the use of veterinary drugs. Where drug treatment occurs, its use should be appropriate and should consider possible antimicrobial resistance.[3] In particular, measures to prevent disease could include:

- Evaluating the health status of domesticated birds relative to avian diseases and where practicable, colonization by pathogenic organisms transmissible to humans and always taking action to ensure only healthy birds are used.
- Taking preventive measures, including managing human access, to reduce the risk of transferring micro-organisms that may impact on food safety to, or from, or between, flocks.
- Using, where permitted, appropriate vaccines as part of an overall flock management programme, including as measures when introducing new birds.
- Regularly checking the flock and removing dead and diseased birds, isolating sick birds, and investigating suspicious or unknown causes of illness or death to prevent further cases.
- Disposing of dead birds in a manner that prevents recycling of diseases to the laying flock by either pests or handlers.
- Treating birds only with veterinary drugs where permitted, prescribed by a veterinarian and in a manner that will not adversely impact on the safety and suitability of eggs, including adhering to the withdrawal period specified by the manufacturer or veterinarian.
  - Only those medicinal products and medicinal premixes that have been authorized by the relevant authority for inclusion in animal feed should be used.
  - Where birds/flocks have been treated with veterinary drugs that can be transferred to eggs, their eggs should be discarded until the withholding period for the particular veterinary drug has been achieved. Established

[2] *Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods*, FAO Headquarters, Rome, Italy 30 April – 4 May 2001, page 13.

[3] *Code of Practice to Minimize and Contain Antimicrobial Resistance* (CAC/RCP 61-2005).

maximum residue levels (MRLs), including those established by Codex, for residues of veterinary drugs in eggs, may be used to verify such measures.
  - The veterinarian and/or the producer/layer establishment owner/manager or the collection centre should keep a record of the products used, including the quantity, the date of administration, the identity of the flock and withdrawal period.
  - Appropriate sampling schemes and testing protocols should be used to verify the effectiveness of on-farm controls of veterinary drug use and in meeting established MRLs.
  - Veterinary drugs should be stored appropriately and according to manufacturer's instructions.
- Particularly for countries where *Salmonella* Enteritidis has been associated with poultry or eggs, monitoring for SE through faecal testing and the use of a vaccination protocol may reduce the risk of human illness.[4] If a vaccine is used, it should be approved by the competent authority. Monitoring for SE can also include environmental testing of litter, dust, ventilation fans etc.
- Disposing of eggs from infected flocks still in production that represent a risk to human or flock health, in a safe manner or specifically diverting them to a process that ensures elimination of a hazard.
- Where practicable, destruction of *Salmonella* Enteridis positive flocks or slaughter in accordance with country requirements.
- Ensuring visitors, where necessary, wear appropriate protective clothing, footwear and head covering to reduce the risk of introducing hazards or spreading hazards between flocks. Visitor movement should be controlled to minimize likelihood of transfer of pathogens from other sources.

3.2.2 Areas and establishments for egg laying systems

Egg laying areas and establishments should, to the extent practicable, be designed, constructed, maintained and used in a manner that minimizes exposure of domesticated birds or their eggs to hazards and pests.

Improperly protected and maintained areas and premises for the housing of flocks and laying of eggs, particularly for free range and barn production systems may contribute to the contamination of eggs.

Taking into account climatic conditions, production systems including those used to provide feed, water, shelter, control temperature and predators and manage interactions between birds should be designed, constructed, maintained and used in a manner to minimize the likelihood of transfer of foodborne pathogens to the egg, either directly or indirectly.[5]

[4] *Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods,* FAO Headquarters, Rome, Italy 30 April – 4 May 2001, page 17.

[5] Although evaluation of the importance of such interventions for reducing the risk of human illness based on existing data was inconclusive. *Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods*, FAO Headquarters, Rome, Italy 30 April – 4 May 2001, page 17.

The following should be considered, where practicable, in the assessment of areas and establishments used for egg laying:

- The internal design and layout of housing should not adversely affect the health of the birds and should permit compliance with good hygienic practices.
- The facilities used to house flocks should be cleaned and disinfected in a way that reduces the risk of transfer of pathogens to the next flock. An 'all-in, all-out' step for each poultry house should be followed, where feasible, taking into consideration multi-aged poultry houses. Such a process would give the opportunity to eliminate rodents and insects before the next flock is introduced.
- A plan should be in place to detect any failure in cleaning and disinfection programmes and ensure that corrective actions are taken.
- Use of litter should be managed to reduce the risk of introducing or spreading hazards.
- Water delivery systems should be protected, maintained and cleaned, as appropriate, to prevent microbial contamination of water.
- Drainage systems and systems for storing and removal of manure should be designed, constructed and maintained so as to prevent the likelihood of contaminating the water supply or eggs.

Access to egg laying establishments by other animal species (i.e. dogs, cat, wild animals and other birds) that may adversely affect the safety of the eggs should be minimized.

The egg laying establishments should, as far as practicable, be kept clean. Accumulations of broken eggs, manure, or any other objectionable materials should be minimized in order to reduce the likelihood of contact with eggs and to minimize attracting pests into the establishment.

3.2.3 General hygienic practice

3.2.3.1 Watering

Water should be managed in a way that minimizes the potential for the transmission of hazards, directly or indirectly, into or on the egg.

Water used in primary production operations should be suitable for its intended purpose and should not contribute to the introduction of microbiological or chemical hazards into or on eggs.
Contaminated water may contaminate feed, equipment or laying birds leading to the potential introduction of hazards in or on eggs.
As water can be a source of contamination, treatment of drinking water to reduce or eliminate pathogens including *Salmonella* should be considered.

- Potable water should be used, or if potable water is not available for some or all purposes, water should be of a quality that does not introduce hazards

to humans consuming the eggs.[6] Access to surface water, where it introduces hazards, should be denied.

- Potential sources of contamination of water from chemical runoff or improperly managed faeces should be identified and controlled to the extent practicable to minimize the likelihood of contaminating eggs.
- Appropriate safety and suitability criteria that meet the intended outcomes should be established for any water used in egg production.
- Where practicable, good purchasing practices for water could be used to minimize the risk associated with hazards in the water and may include using vendor assurances or contractual agreements.
- Where possible, water should be regularly tested to ensure that water supplied to the birds is of a quality that does not introduce hazards in or on the egg.

Any reuse of water should be subject to a hazard analysis including assessment of whether it is appropriate for reconditioning. Critical control point(s) should be identified, as appropriate, and critical limit(s) established and monitored to verify compliance.

- Water recirculated or recycled for reuse should be treated and maintained in such a condition that no risk to the safety and suitability of eggs results from its use.
- Reconditioning of water for reuse and use of reclaimed, recirculated and recycled water should be managed in accordance with HACCP principles.

3.2.3.2 Feeding[7]

Feed for the laying and/or breeding flock should not introduce, directly or indirectly, microbiological or chemical contaminants into eggs that present an unacceptable health risk to the consumer or adversely affect the suitability of eggs and egg products.

The improper procurement, manufacturing and handling of animal feed may result in the introduction of pathogens and spoilage organisms to the breeding and laying flock and the introduction of chemical hazards, such as pesticide residues and other contaminants, which can affect the safety and suitability of eggs and egg products.

Producers should take care where appropriate, during production, transportation, preparation, processing, procurement, storage, and delivery of feed to reduce the likelihood of introducing hazards into the production system.

- To minimize the risk associated with hazards in the feed, good purchasing practices for feed and feed ingredients should be employed. This may include using vendor assurances, contractual agreements and/or purchasing batches of feed that have had microbiological and chemical analysis and are accompanied by certificates of analysis.

[6] *Safe Use of Wastewater, Excreta and Greywater. Volume II, Wastewater Use in Agriculture.* WHO/FAO/UNEP, 2006 and the *Code of Hygienic Practice for Meat* (CAC/RCP 58-2005).

[7] *Code of Practice on Good Animal Feeding* (CAC/RCP 54 – 2004).

- Feed should be managed so that it does not become mouldy or contaminated from waste including faeces.
- As feed can be a source of contamination, heat or other treatment of feed to reduce or eliminate pathogens including *Salmonella* should be considered.
- When the egg producer processes their own feed, information should be kept about its composition, the origin of the ingredients, relevant processing parameters and where practicable, the results of any analyses of the finished feed.
- The owner should keep a record of relevant information concerning feed.

3.2.3.3 Pest control

Pests should be controlled using a properly designed pest control programme as they are recognized as vectors for pathogenic organisms.

Any pest control measures should not result in unacceptable levels of residues, such as pesticides, in or on eggs.

Pests such as insects and rodents are known vectors for the introduction of human and animal pathogens into the production environment. Improper application of chemicals used to control these pests may introduce chemical hazards into the production environment.

A properly designed pest control programme should be used, that considers the following:

- Before pesticides or rodenticides are used, all efforts should be made to minimize the presence of insects, rats and mice and reduce or remove places which could harbour pests.
  - As cages/pens/enclosures/coops (if used) attract such pests, measures such as proper design, construction and maintenance of buildings (if applicable), effective cleaning procedures and removal of faecal waste should be used to minimize pests.
  - Mice, rats and wild birds are attracted to stored feed. Any feed stores should be located, designed, constructed and maintained so as to be, where practicable, inaccessible to pests. Feed should be kept in pest proof containers.
- Bait should always be placed in "bait stations" so that they are obvious, cannot be accessed by animals or insects they are not intended for and can be identifiable and found easily for checking.
- If it is necessary to resort to chemical pest control measures, the chemicals should be approved for use in food premises and used in accordance with the manufacturer's instructions.
- Any pest control chemicals should be stored in a manner that will not contaminate the laying environment. Such chemicals should be stored in a safe manner. They should not be stored in wet areas or close to feed stores or be accessible by birds. It is preferable to use solid baits, wherever possible.

3.2.3.4 Agricultural and veterinary chemicals

Procurement, transport, storage and use of agricultural and veterinary chemicals should be undertaken in such a way that they do not pose a risk of contaminating the eggs, flock or the egg-laying establishment.

- Transport, storage and use of agricultural and veterinary chemicals should be in accordance with the manufacturer's instructions.
- Storage and use of agricultural and veterinary chemicals on the egg laying establishment should be evaluated and managed, as they may represent a direct or indirect hazard for the eggs and flock.
- Agricultural and veterinary chemical residues should not exceed limits established by the Codex Alimentarius Commission or as per national legislation.
- Workers that apply agricultural and veterinary chemicals should receive training in the proper application procedures.
- Agricultural and veterinary chemicals should be kept in their original containers. Labels should have the name of the chemical substances and the instructions for their application.
- Equipment used to apply or administer agricultural and veterinary chemicals should be stored or disposed of in a manner that does not represent a direct or indirect hazard for the eggs and flock
- Empty agricultural and veterinary containers should be disposed of according to applicable regulation and/or the manufacturer's directions and should not be used for other purposes.
- Where possible and practicable, producers should keep records of agricultural and veterinary chemical applications. Records should include information on the date of application, the chemical used, the concentration, method and frequency of application, the purpose for using the chemical applications and where it was applied.

3.3 Collection, handling, storage and transport of eggs

Eggs should be collected, handled, stored and transported in a manner that minimizes contamination and/or damage to the egg or egg shell, and with appropriate attention to time-temperature considerations, particularly temperature fluctuations.

Appropriate measures should be implemented during disposal of unsafe and unsuitable eggs to protect other eggs from contamination.
Proper collection, whether using manual or automated methods, handling, storage and transport of eggs are important elements of the system of controls necessary to produce safe and suitable eggs and egg products. Contact with unsanitary equipment and foreign materials or methods that cause damage to the shell, may contribute to egg contamination.

Whether manual or automated methods are used to collect eggs, producers should minimize the time between egg laying and further handling or processing. In particular, the time between egg laying and controlled temperature storage should be minimized.

Methods used to collect, handle, store and transport eggs should minimize damage to the shell, and avoid contamination and practices should reflect the following points:

- Cracked and/or dirty eggs should be excluded from the table egg trade.
- Cracked and/or dirty eggs should be directed to a processing or packing establishment, as appropriate, as soon as possible after collection (see Section 5.1).
- Hygienic practices, which take into account time and temperature factors, should be used to protect the egg from surface moisture in order to minimize microbial growth.
- Where appropriate, broken and/or dirty eggs should be segregated from clean and intact eggs.
- Broken eggs and incubator eggs should not be used for human consumption and be disposed of in a safe manner.

Egg processors should communicate any specific requirements at farm level (i.e. time/temperature controls) to the egg producer.

Selection

Eggs from different species of poultry and/or farm production systems (e.g. free range, barn and caged eggs) should be segregated as appropriate.

3.3.1 Egg collection equipment

Collection equipment should be made of materials that are non-toxic and be designed, constructed, installed, maintained and used in a manner to facilitate good hygiene practices.

It is important to prevent any damage to the eggshells by collecting equipment since such damage can lead to contamination and consequently adversely affects the safety and suitability of eggs and egg products. It is also important that the equipment is maintained to a standard of cleanliness adequate to prevent contamination of the eggs.

Where used, egg collecting equipment and containers should be cleaned and disinfected regularly, or if necessary replaced, and with sufficient frequency to minimize or prevent contamination of eggs.

Single use containers should not be reused.

Egg collecting equipment should be maintained in proper working condition and this should be periodically verified.

3.3.2 Packaging and storage

Egg packaging and packaging equipment should be designed, constructed, maintained and used in a manner that will minimize damage to the eggshell and avoid the introduction of contaminants in or on eggs.

Wherever eggs are stored, it should be in a manner that minimizes damage to the eggshell and avoids the introduction of contaminants, or growth of existing micro-organisms in or on eggs, giving consideration to time and temperature conditions.
Any egg packaging, storage or associated equipment should not transfer substances to eggs that will present a health risk to the consumer.
Where permanent equipment is used, it should be corrosion resistant and easy to clean and disinfect or if necessary able to be dismantled and reassembled.

Storage temperatures, times and humidity should not have a detrimental effect on the safety and suitability of eggs. The time and temperature conditions and humidity for egg storage at the farm should be established taking into account the hygienic condition of the eggs, the hazards that are reasonably likely to occur, the end use of the eggs, and the intended duration of storage.

3.3.3 Transport, delivery procedures and equipment

Whenever eggs are transported, it should be in a manner that minimizes damage to the egg or eggshell and avoids the introduction of contaminants in or on eggs.
Personnel and vehicular access should be adequate for the hygienic handling of eggs, such that contamination is not introduced onto the farm and thus in or on eggs.

Lorries, trucks or other vehicles or equipment, which carry the eggs, should be cleaned at a frequency necessary to prevent contamination flow between farms or premises and thus of eggs.

The time and temperature conditions for the transport and delivery of eggs from the producer should be established taking into account the hygienic condition of the eggs, the hazards that are reasonably likely to occur, the end use of the eggs, and the intended duration of storage.

- These conditions may be specified in legislation, in codes of practice, or by the processor receiving the eggs in collaboration with the egg producer and transporter and the relevant authority.

Delivery procedures should be adequate for the hygienic handling of eggs.

3.4 Cleaning, maintenance and personnel hygiene at primary production

3.4.1 Cleaning and maintenance of egg laying establishments

Egg laying establishments should be cleaned and maintained in a manner that ensures the health of flocks and safety and suitability of eggs.
Cleaning and disinfection programmes should be in place, and their efficacy should be periodically verified and an environmental monitoring programme implemented where possible and practicable.

These programmes should include procedures for routine cleaning while birds are in the poultry house. Full cleaning and disinfection programmes should be applied when poultry houses are empty.

De-populated poultry house cleaning procedures should cover cleaning and/or sanitizing nest boxes/cages, poultry houses, disposing of contaminated litter, nesting materials and faeces from diseased birds and, where necessary, safe disposal of eggs from infected flocks and dead or diseased birds.

The egg-laying establishment should be safe for the re-entry of new stock.

3.4.2 Personnel hygiene, health, and sanitary facilities

3.4.2.1 Personnel hygiene

Hygiene and health requirements should be followed to ensure that personnel who come directly into contact with eggs are not likely to contaminate them.

Hygiene and health requirements should be followed to ensure that personnel who come directly into contact with birds are not likely to transmit illness between birds.
Personnel should understand and follow preventative measures specifically relating to the handling of birds and/or eggs, so as to prevent introducing hazards from one to the other, from other facilities or from cross contamination of birds from personnel.

Personnel should be adequately instructed and/or trained to handle eggs and domesticated birds to ensure the use of good hygienic practices that will minimize the risk of egg or flock contamination.

3.4.2.2 Health status

Personnel should be in good health and not introduce diseases or illness likely to affect flock health or the safety and suitability of eggs.
People known, or suspected, to be suffering from, or to be a carrier of a disease or illness likely to be transmitted to birds or through eggs should not be allowed to enter any bird facility or egg collection or handling area, if there is a likelihood of their contaminating the birds or the eggs. Any person so affected should immediately report illness or symptoms of illness to the management.

3.4.2.3 Personal cleanliness

Personnel who have direct contact with eggs should maintain a high degree of personal cleanliness and, where appropriate, wear suitable protective clothing, footwear and head covering that is not likely to introduce contamination into egg laying areas.
Personnel should wash their hands before starting work that involves the handling of eggs, each time they return to handling areas after a break, immediately after using the toilet, and after handling anything which may contaminate eggs.

3.4.2.4 Sanitary facilities

Facilities should be available to ensure that an appropriate degree of personal hygiene can be maintained.
Facilities should:

- Be located in close proximity to wherever eggs or domesticated birds are handled;
- Be constructed to facilitate hygienic removal of wastes and avoid contamination of facilities, equipment, raw materials and the immediate environment;
- Have adequate means for hygienically washing and drying hands and disinfecting footwear; and
- Be maintained under sanitary conditions and in good repair at all times.

3.5 Documentation and record keeping

Records should be kept, as necessary and where practicable, to enhance the ability to verify the effectiveness of the control systems. Documentation of procedures can enhance the credibility and effectiveness of the food safety control system.
With respect to food safety, records should be kept on:

- Prevention and control of avian diseases with an impact on public health;
- Identification and movement of birds and eggs;
- Use of agricultural and pest control chemicals;
- Nature and source of feed, feed ingredients and water;
- Use of veterinary drugs/medicines;
- Results of testing where testing is performed;
- Health status of personnel;
- Cleaning and disinfection; and
- Traceability/product tracing[8] and recall.

4. ESTABLISHMENT: DESIGN AND FACILITIES

Section 4 of the *Recommended International Code of Practice – General Principles of Food Hygiene* applies to both the processing of eggs for the table egg market and the processing of egg products.

The following guidelines are supplemental to Section 4 of the *Recommended International Code of Practice – General Principles of Food Hygiene* for establishments that produce egg products.

Where practicable, separate areas should be allocated for:

- Storage of egg and untreated egg product;
- Breaking and microbiocidal treatment of eggs;

[8] Refer to *Principles for Traceability/Product Tracing as a Tool within a Food Inspection and Certification System* (CAC/GL 60-2006)

- Packing of microbiocidally treated egg product;
- Storage of microbiocidally treated liquid and frozen egg products and other liquid or frozen ingredients as appropriate;
- Storage of microbiocidally treated dried egg product and other dry ingredients as appropriate; and
- Storage of cleaning and sanitizing materials.

Work areas for raw and treated product should be separated via physical barriers.

5. CONTROL OF OPERATION

These guidelines are supplemental to those set forth in Section 5 of the *Recommended International Code of Practice – General Principles of Food Hygiene*.

This section refers to control measures that should be taken to prevent, eliminate or reduce hazards when processing eggs for the shell egg market (i.e. table eggs) and when producing egg products. These measures should be used in conjunction with good hygienic and animal husbandry practices for the primary production of eggs as per Section 3 in order to provide an effective system of control of microbiological and other hazards that can occur in or on eggs and egg products.

These principles are also intended to enhance and supplement those aspects of the *Recommended International Code of Practice – General Principles of Food Hygiene HACCP* Annex (CAC/RCP 1-1969), which are essential to the successful design of a system of food safety controls for shell eggs and egg products. The users of this document are encouraged to implement the guidelines contained in the HACCP Annex when designing a HACCP system.

5.1 Control of food hazards

Eggs and egg products should be safe and suitable.

Table egg

Unsafe or unsuitable eggs[9] include:

- Incubator eggs;
- Broken/leaker eggs;
- Eggs with bacterial or fungal rots;
- Eggs contaminated with faeces;
- Eggs stored for hatching for sufficient time to adversely affect the safety and suitability.

[9] Refer to definition of food safety and food suitability in the *Recommended Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969), Section 2.3 Definitions.

Table eggs should be clean and intact.
All efforts should be made to avoid production of dirty eggs. However, dirty eggs may be used for table eggs if permitted by the relevant authorities, in accordance with country requirements, and if cleaned appropriately.

Egg products

- *Cracked or dirty eggs that are not suitable for human consumption as table eggs should be directed to processing (e.g. washing and breaking followed by a microbiocidal treatment) or be disposed of in a safe manner.*
- *Broken/leaker eggs should not be used to produce egg products and should be disposed of in a safe manner.*
- *Cracked eggs may be used in egg products, but should be processed with minimum delay.*
- *Dirty eggs should be visibly clean prior to breaking and processing.*
- *Other unsafe or unsuitable eggs should not be used for egg products and should be disposed of in a safe manner.*

Control measures based on risk should be in place to ensure that process and product specifications are met and the hazards in or on eggs and egg products are effectively identified and controlled.
Control measures used should achieve an appropriate level of public health protection. Where possible, measures should be based on HACCP principles.

These measures should allow the identification and removal of eggs and egg products that are not suitable for human consumption. They should also address the need to control pathogen growth throughout handling, cleaning, sorting and grading, packaging, processing, storage and distribution and have a sound basis in good hygiene practice. It is important that control measures are applied during primary production and processing to minimize or prevent the microbiological, chemical or physical contamination of eggs.

Processors should only use eggs that have been produced in accordance with the Code.

5.2 Key aspects of hygiene control systems

5.2.1 Temperature and time issues

From receipt of eggs, through handling, sorting and grading, washing, drying, treatment, packing, storage and distribution to point of consumption, consideration should be given to time and temperature and humidity conditions for eggs such that the growth of pathogenic micro-organisms will be minimized and the safety and suitability of the eggs will not be adversely affected.

Temperature fluctuations should be minimized as much as possible.
Storage and handling conditions, including those during cleaning, grading and packaging should be such that moisture on the shell surface is minimized.

As eggs are perishable products, particular attention should be paid to temperature conditions throughout storage and distribution, noting that lower storage and distribution temperatures lend themselves to longer shelf life and minimize microbial growth, for example of *Salmonella* Enteritidis.

From receipt of raw/untreated egg product, through processing, treatment, packaging, storage and distribution to point of consumption, consideration should be given to time and temperature conditions for egg products such that the growth of pathogenic micro-organisms will be minimized and the safety and suitability of the egg products will not be adversely affected.
Storage conditions should be such that the potential for microbial contamination, the growth of microbial pathogens and the risk to human health is minimized.

5.2.2 Specific process steps

5.2.2.1 Handling of table eggs

Eggs should be handled during all stages of cleaning, sorting, grading, packing, storing and distribution in a manner that avoids damage, minimizes moisture on the shell surface and prevents contamination.
Handling of shell eggs can result in damage to eggs. Eggs should be handled in a manner that avoids damage and contamination, including minimizing moisture on the egg shell surface.
Activities involved in shell eggs handling may be done by the primary producer, the processor or others involved in the egg production chain. Wherever in the production chain these activities are done, they should be done in accordance with this Code.

Eggs intended for the table egg market should be visibly clean prior to grading and packing.
Sorting, grading, and where appropriate, washing processes should result in clean eggs.

(i) Sorting, grading and packing
Sorting, grading and packing of the egg refers to the stage between primary production and retail or further processing, where the whole egg may undergo one or more activities to prepare it for either the table egg market or for processing into egg products.

Cracked, dirty, and unsafe/unsuitable eggs should be segregated from clean and intact eggs.
Cracked eggs should be segregated (for example, by candling) and sent for processing (see Section 5.2.2) or disposed of in a safe manner.

Dirty eggs may be cleaned and if appropriately cleaned, used for the table egg market or the egg product industry in accordance with country requirements. Dirty eggs sent for processing should be clearly labelled that they are not suitable as table eggs.

The cleaning process used should not damage or contaminate the eggs. Incorrect cleaning of eggs can result in a higher level of contamination of eggs than existed prior to cleaning.

Broken/leaker and other unsuitable eggs should be segregated from eggs suitable for human consumption.
Broken/leaker and other unsuitable eggs should be identified in such a way that they cannot be used for human consumption, for example, by appropriate labelling or the use of a de-characterizing agent (an additive that makes it clearly visible that the eggs should not be processed into human food, e.g. a denaturing agent).

Cleaning

- Where permitted by the relevant authority, a cleaning process may be used to remove foreign matter from the shell surface, but this should be carried out under carefully controlled conditions so as to minimize damage to the shell surface.
- Cleaning can be used to reduce the bacterial load on the outside of the shell.
- If dry cleaning is undertaken, the methods used should minimize damage to the protective cuticle and, where appropriate, be followed by oiling of the shell using a suitable food grade oil.

Washing, disinfection and drying
Where washing is permitted by the relevant authority, it should be carried out under carefully controlled conditions so as to minimize damage to the shell and prevent contamination of the egg contents.

- Eggs should not be soaked prior to or during washing.
- Water used for washing should be suitable and not adversely affect the safety and suitability of the egg, giving consideration to appropriate water temperature, pH, and quality, and egg temperature.
- If cleaning compounds such as detergents and sanitizers are used, they should be suitable for use on eggs and not adversely affect the safety of the egg.
- If eggs are washed, they should be dried to minimize moisture on the surface of the shell that can lead to contamination or growth of mould.
- Washing should be followed by effective sanitizing of the shell and, where appropriate, with subsequent oiling of the shell using a suitable food grade oil.

(ii) In shell treatment

Where table eggs are treated to eliminate pathogens (e.g. in-shell pasteurization) the treatment should not adversely affect the safety or suitability of the egg.

(iii) Storage and distribution

Eggs should be stored and transported under conditions that will not adversely affect the safety and suitability of the egg.
Eggs are perishable products.

- Storage conditions should minimize moisture on the shell surface.
- Lower temperatures minimize microbial growth and extend shelf life of the eggs.
- Temperature fluctuations during storage and distribution should be minimized.

(iv) Shelf life for table eggs[10]

The growth of pathogenic and/or spoilage micro-organisms to unacceptable levels may affect the shelf life of eggs.

The shelf life of eggs is influenced by a number of factors, such as:

- Storage conditions including temperature, temperature fluctuation and humidity;
- Methods and treatments;
- Type of packaging.

Shelf life of table eggs should be established by the grader/packer, consistent with requirements of relevant authorities, based on:

- information from the producer on the time since lay, time and temperature in storage and transport;
- type of packaging;
- likelihood of microbial growth, due to reasonably anticipated temperature abuse during storage, distribution, retail, sale and handling by the consumer under reasonably foreseeable conditions of distribution, storage and use.

Where processors clearly advise on egg packaging that eggs are to be refrigerated, others in the food chain, including retailers should follow the processors' advice, unless it is expressly made as a recommendation to the consumer (e.g. that the conditions of refrigeration should be fulfilled after purchasing).

5.2.2.2 Egg product processing

Processors should be satisfied that the egg products they produce are safe and suitable for human consumption.

Eggs for processing should be visibly clean prior to breaking and separating.

Cracked eggs may be processed. Broken eggs should not be processed and should be disposed of in a safe manner.
Dirty eggs should be disposed of in a safe manner or may be cleaned in accordance with 5.2.2.1.

[10] *Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods*, FAO Headquarters, Rome, Italy 30 April – 4 May 2001, page 14.

Separating the egg contents from the shell should be done in a manner that will, as far as possible, avoid cross-contamination between the shell and egg contents, avoid contamination by personnel or from equipment, and that permits examination of egg contents.

(i) Treatments

Egg products should be subjected to a microbiocidal treatment to ensure the products are safe and suitable.

All operations subsequent to the treatment should ensure that the treated product does not become contaminated.
Hygienic manufacturing and personnel practices should be in place to manage the risk of contamination from the food contact surfaces, equipment, and personnel, packaging material and between raw egg and processed egg products.
Microbiocidal treatments, including heat treatment, should be validated to show they achieve the desired reduction in the number of pathogenic micro-organisms and result in a safe and suitable product.

Where heat treatment is used, consideration should be given to time and temperature combinations.
Pasteurized liquid egg products should be cooled rapidly immediately after pasteurization and maintained under refrigeration.

(ii) Untreated egg products

Egg products that have not had a microbiocidal treatment should only be directed to further processing to ensure their safety and suitability.
Where untreated egg products leave a grading/processing premises, they should be labelled that the product has not been treated.

(iii) Storage and distribution

Egg products should be stored and transported under conditions that will not adversely affect the safety and suitability of the product.
Egg products, including those that can be stored at ambient temperatures, should be protected against external agents and contamination, e.g. direct sun light, excessive heating, moisture, external contaminants, and from rapid temperature changes which could adversely affect the integrity of the product packaging or the safety and suitability of the product.

(iv) Shelf life for egg products

The shelf life of egg products is influenced by a number of factors, such as:

- Storage conditions including temperature, temperature fluctuation and humidity;
- Processing methods and treatments;
- Type of packaging.

Shelf life of egg products should be established by the processor, consistent with requirements of relevant authorities, based on:

- Applied microbiological control measures, including storage temperatures, e.g. storage under refrigeration, freezing or ambient;
- Methods and treatments applied to product;
- Type of packaging;
- Likelihood of post process contamination and type of potential contamination under reasonably foreseeable conditions.

The safety and suitability of the egg product should be assured and, where necessary, demonstrated that it would be retained throughout the maximum period specified.

Shelf life determination may be done at the plant level by testing products subjected to the storage conditions specified or by predicting microbial growth in the product under the specified storage conditions. Reasonably anticipated temperature abuse should be integrated into the study or be taken into account by applying an appropriate safety factor (e.g., by shortening the maximum durability specified in the labelling or by requiring lower storage temperatures).

5.2.3 Microbiological and other specifications

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (*Principles for the Establishment and Application of Microbiological Criteria for Foods* (CAC/GL 21-1997)).

Information that may be useful for establishing specifications could include:

- Flock health status (including pathogen status);
- Pathogen load in/on eggs;
- Agricultural and veterinary chemical status;
- Age of eggs;
- Handling methods; and
- Microbiocidal treatments.

Particular attention should be given to specific indicating control of pathogens such as *Salmonella* Enteritidis.

5.3 Incoming material requirements

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

Depending upon the end use of the egg, certain specific microbiological criteria for incoming ingredients may be appropriate to verify that the control systems have been implemented correctly.

5.4 Packaging

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

5.5 Water

Refer to the Recommended International Code of Practice – General Principles of Food Hygiene (CAC/RCP 1-1969).

5.6 Management and supervision

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

5.7 Documentation and records

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

5.8 Recall procedures

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

6. ESTABLISHMENT: MAINTENANCE AND SANITATION

These guidelines are supplemental to those set forth in Section 6 of the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

6.1 Maintenance and cleaning

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

6.2 Cleaning programmes

Handling, packaging and processing of eggs uses a variety of equipment with sensitive electronic controls. Where wet cleaning may damage or result in the contamination of the equipment, alternative cleaning programmes should be considered.

6.3 Pest control systems

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

6.4 Waste management

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

6.5 Monitoring effectiveness

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

7. ESTABLISHMENT: PERSONAL HYGIENE

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

8. TRANSPORTATION

These principles and guidelines are supplemental to those set forth in Section 8 of the *Recommended International Code of Practice – General Principles of Food Hygiene* and, as appropriate, those set forth in *Code of Hygienic Practice for the Transport of Food in Bulk and Semi-Packed Food* (CAC/RCP 47 – 2001).

Eggs and egg products should be transported in a manner that will minimize breakage, damage and contamination.

Mobile containers and tankers should be cleaned and disinfected prior to being refilled.
Egg haulers (driver or individual in charge of transport to and from packing facility) should use vehicles suitable for transporting eggs, which permit easy and thorough cleaning.

Piping, connectors and valves used for filling and discharge of liquid egg should be of a suitable design and be cleaned, disinfected and stored as appropriate.

Eggs should be transferred between establishments promptly. Eggs should be maintained at an appropriate temperature, including avoiding fluctuations in temperatures that will result in condensation of water on the shell surface.

9. PRODUCT INFORMATION AND CONSUMER AWARENESS

These principles and guidelines are supplemental to those contained in Section 9 of the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

9.1 Lot identification

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

Documentation can enhance the credibility and effectiveness of the food safety control system, especially when it includes measures that permit a client to refer to their supplier on the history of a product. Labelling and record keeping also aid in the implementation of other emergency and corrective actions.

Where appropriate and practicable, a system should be in place that allows the identification of the egg layer establishment, transporter, grading/packing premises and processor where eggs and egg products were produced.

The system should be easy to audit. Records should be kept for a period of time sufficient to permit efficient traceback investigations of the eggs and/or egg products. It is important to ensure that all parties involved in this system are adequately informed and trained in its implementation.

9.2 Product information

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

9.3 Labelling

Egg and egg products should be labelled in accordance with the Codex *General Standard for the Labelling of Prepackaged Foods* (CODEX STAN 1-1985).

Processors and food manufacturers awareness

Processors and food manufacturers that use egg products should follow labelling instructions.

9.4 Consumer education

Where appropriate, advice should be made available to consumers on the safe handling, use, preparation and consumption of eggs.

10. TRAINING

Refer to the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

GUIDELINES FOR THE DESIGN AND IMPLEMENTATION OF NATIONAL REGULATORY FOOD SAFETY ASSURANCE PROGRAMMES ASSOCIATED WITH THE USE OF VETERINARY DRUGS IN FOOD-PRODUCING ANIMALS

CAC/GL 71-2009

GUIDELINES FOR THE DESIGN AND IMPLEMENTATION OF NATIONAL REGULATORY FOOD SAFETY ASSURANCE PROGRAMMES ASSOCIATED WITH THE USE OF VETERINARY DRUGS IN FOOD-PRODUCING ANIMALS

CAC/GL 71-2009

INTRODUCTION

1. Modern food production systems should be designed and managed to ensure that the exposure of food-producing animals to veterinary drugs does not pose a risk to human health.

2. The commercial entities involved in the production and marketing of food have the primary responsibility for ensuring food safety. The role of competent authorities is to control the use of veterinary drugs and to verify that appropriate practices are being applied and effective measures are in place within the veterinary drug distribution and food production systems to provide effective protection for consumer health and ensure fair practice in the food trade, consistent with the goals of the Codex Alimentarius. All parties also have a responsibility to provide consumers with information and education to facilitate sound choice of food products of animal origin.

3. The application of a programme based on risk to all food types should provide the controls and verification consistent with the risk that the food type may pose to consumers. The application of an approach based on risk across all food groups and hazard classes should allow a more focused application of resources to those areas that are most likely to generate real human health protection gains.

4. Risk profiles for different hazards may vary by country, region, species and/or production system. The application of a control and verification assurance programme based on risk should provide the necessary basis for exporting countries to certify the safety of exported food, and for importing countries to have the confidence to accept such consignments.

5. It is recognized that developing countries in particular may need a transition period and/or technical assistance regarding the full implementation of these Guidelines.

SCOPE

6. This guide is intended to provide the overarching principles and guidance for governments on the design and implementation of national and trade-related food safety assurance programmes for residues of veterinary drugs. The current and future appendixes to this

guide may provide a further refinement of guidance on issues that may be relevant to the control and verification programmes for products from certain species. These appendixes should be read in conjunction with the principles outlined in this guide.

GENERAL PRINCIPLES

7. Programmes for the control of residues of veterinary drugs in foods should:

   i. be based on risk using realistic risk profiles assessed as reasonably likely to be associated with food derived from the relevant productions system(s);
   ii. be prevention-focused, based on the realistic risk profiles associated with the probable or known use of approved, non-approved and prohibited veterinary drugs in the production system;
   iii. include regulatory measures proportionate to the relative human health risk associated with these hazards compared with other food-associated hazards;
   iv. ensure all parties involved in the production, marketing and processing system of the animals and/or the food products derived from them are held accountable to ensure that unsafe animal products will not be sold as a result of their action or inaction;
   v. recognize that pre-harvest controls and practices are the primary means for ensuring safe food;
   vi. recognize that the primary role of audits and sampling programmes is to verify the implementation and effectiveness of the pre-harvest controls and practices;
   vii. focus on system- and population-based assurances; and
   viii. be cost-effective and have the support of stakeholders.

8. It should be recognized that veterinary drugs are regulated in many countries for a variety of reasons, such as animal health, animal welfare and protection of the environment. Where these uses and the related standards do not fall under the mandate of the Codex Alimentarius Commission, they should be clearly identified and justified where, for reason of efficiency, they form part of the competent authority's residue control programme.

9. The Codex Alimentarius Commission's recommended sampling procedures for 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. Accordingly, these Guidelines include sampling procedures relevant for the entire control programme.

10. The safety of foods is achieved by the implementation of appropriate rules applied from primary production or import to retail or export and requires the participation of all parties involved. Competent authorities should verify correct implementation of programmes and, where necessary, if action has been taken.

11. The reliability of laboratory results is important for the decision-making of competent authorities. Thus, official laboratories should use methods validated as fit for purpose and work under internationally accepted (e.g. ISO 17025) quality management principles.

12. A control programme designed and implemented according to these Guidelines provide reassurance for importing countries to accept consignments certified as safe by the exporting country.

APPROACH BASED ON RISK

13. An approach based on risk applied across the entire production chain and on all food groups and potential hazards will allow competent authorities to focus application of resources to areas of highest risk that are most likely to have an impact on consumer health protection.

14. Continuous application of good practices and regular control contribute more significantly to food safety than end-product testing.

15. Residues may exert an adverse effect on consumers in a number of ways, such as:
    (a) chronic toxicological adverse effects;
    (b) acute pharmacological effects on consumers and on the microflora of the gastrointestinal tract of consumers;
    (c) allergic reactions.

16. Different types of controls and monitoring programme may be justified where the risk assessment identifies one or more of these other end-points as being significant for human health. Detections of non-compliant residues (e.g. those exceeding applicable maximum residue levels [MRLs]) justify regulatory follow-up.

17. Animals and/or production systems can be exposed to a variety of veterinary drugs and other chemicals that may as a result be present in the products derived from them. Their importance for consumer health protection, however, varies with type and source.

18. An understanding of the circumstances required for each veterinary drug input actually to pose a risk to consumers of animal products, along with an estimate of the relative likelihood of this occurring, is essential to determine the appropriate controls and verification programmes that should be included in the design of national residue control and verification programmes.

19. The application of a control and verification programme based on risk should provide the necessary basis for exporting countries to certify, where required, the safety of exported food, and for importing countries, subject to any additional assessment they deem necessary, to accept such consignments.

20. The same principles should apply to export assurance programmes as are applied to the design and implementation of national assurance programmes.

DEFINITIONS (FOR THE PURPOSES OF THESE GUIDELINES)

Competent authority (authorities) means the official government organization or agency (agencies) having jurisdiction.[1]

Approved means officially authorized or recognized by a competent authority.

Based on risk means focused on and proportionate to an estimate of the probability and severity of an adverse effect occurring in consumers.

Risk profiles are defined in the *Procedural Manual*. For veterinary drugs, they relate a production system to a potential consumer health risk. They are the basis for approvals and use restrictions.

System verification means obtaining overall information on the extent of application of the practices and controls.

Risk-targeted verification programmes means inspection/audit and/or sampling/laboratory analysis of specific suppliers or products aimed at the detection of non-compliance.

Non-biased sampling refers to the random sampling of specified populations to provide information about the occurrence of residue non-compliances, typically on an annual, national basis. Compounds selected for non-biased sampling are usually based on risk profiles and the availability of laboratory methods suitable for regulatory purposes. The results of non-biased sampling are a measure of the effectiveness and appropriateness of the controls and practices within a wider segment of the production system.

Survey refers to the collection of additional data aimed at the investigation of residues linked to a specific veterinary drug use or production type.

Withdrawal time/withholding time (food harvest restriction) are defined in the *Glossary of terms and definitions (residues of veterinary drugs in foods)* (CAC/MISC 5-1993). A period of time may also be represented by a combination of events or other factors.

Production system means the methods or activities used to produce food for human consumption.

Quality control (in residue laboratories) means monitoring those factors associated with the analysis of a sample by a tester.

Quality assurance (in residue laboratories) means independent review to ensure that the analytical programme is performing in an acceptable manner.

Quality management system ensures that a laboratory is managed and operated in a manner that meets the requirements of an internationally recognized quality standard to produce quality data and results (e.g. ISO/IEC 17025:2005).

[1] Definition used in the *Guidelines for the production, processing, labelling and marketing of organically produced foods* (CAC/GL 32-1999).

REGULATORY FRAMEWORK

Roles

21. Business operators/commercial entities involved in the production, processing and marketing of food have the primary responsibility for ensuring food safety.

22. Competent authorities regulate the use of veterinary drugs, verify that appropriate practices are applied and that effective measures are in place within the veterinary drug distribution and food production system to provide effective protection of consumers and facilitate trade, consistent with the goals of the Codex Alimentarius.

23. The competent authority responsible for providing consumer assurances for foods must ensure that it has sufficient knowledge of and control over veterinary drugs that are being sold and used within the production systems and that it has sufficient knowledge of food safety.

Approval by competent authority

Criteria

24. Appropriate official approval criteria should be established. These criteria may include the acceptance of the assessments of other recognized competent authorities where use patterns are likely to be similar.

25. Approval systems should:
(a) require an evaluation of the human safety of residues of the veterinary drug relying on a risk analysis and establishing, where appropriate, MRLs;
(b) take into account the needs of the producers in order to reduce the temptation to use unapproved veterinary drugs or prohibited substances.

26. Approval systems should take into account that risk profiles and management options may vary substantially among production systems and regions.

Approval restrictions

27. The conditions for the approval of veterinary drugs should be specified in the appropriate national regulations.

28. To mitigate potential risk, restrictions may be imposed on:
(a) formulations;
(b) criteria of use (e.g. time, species) and route of administration;
(c) indications for use; and
(d) withdrawal time/withholding time/food harvest restriction.

National register

29. All formulations of veterinary drugs approved in a country should be recorded in a national register.

Information on veterinary drugs

30. Information and/or education programmes on suitable use to provide effective treatment while affording protection of consumers should be provided for each approved veterinary product formulation.

Sale and use

31. National/regional regulations should establish which veterinary drugs may be sold domestically and how these may be used. Formulations not recorded in the national register should not be used and sanctions should be in place to act as a deterrent against such use.

32. It may be appropriate, where justified by a relevant risk profile for competent authorities, to impose additional conditions on the sale and use of certain veterinary drugs to ensure appropriate use and to prevent misuse or abuse.

33. Sale and use conditions may include:
(a) requiring all sales to be subject to a prescription from a veterinarian or other professional with approved competencies;
(b) restricting administration to individuals or professionals with approved competencies;
(c) requiring all treated animals/production systems to be identified in specified ways;
(d) requiring all uses to be recorded and/or notified to a unified database(s).

34. Efficacy and the necessity of use conditions should be regularly reviewed against the local risk profile. In doing this, it should be considered that the non-availability of necessary treatments may encourage use of non-approved veterinary drugs or prohibited substances.

35. Competent authorities may establish legislation/regulation that allows, as an exception, the use of non-approved veterinary drugs off-label/extra label in accordance with direct and written veterinary advice and oversight. Such legislation should be consistent with national and/or international guidance and technical information on this issue.

36. In animals from which milk, eggs or honey, respectively, are collected for human consumption, only veterinary drugs specifically approved for use in lactating animals, laying birds and honey bees should be used. Specific exemptions may be made for off-label/extra label use.

Responsibilities of business operators (best practice guidance)

37. Producers should only use veterinary drugs that have been approved for use in food-producing animals. Non-approved veterinary drugs should not be used. Veterinary drugs should be used strictly in accordance with the officially approved/recognized instructions. Off-label use of veterinary drugs should only be permitted in accordance with direct and written advice from a veterinarian in accordance with national authorities' laws and regulations. Such advice should be consistent with national and/or international guidance documents and technical information on this issue.

38. Producers should be encouraged to seek the advice of veterinarians or other competent professionals on the application of the correct withdrawal time where the label direction for use may not be available or may not be clear.

39. Records should be kept of all details of the treatment and the withdrawal time/withholding time required before the animal or product from the animal can be harvested for human consumption.

40. Business operators (whether primary producers or others) should be required to communicate food harvesting restrictions (withdrawal/withholding times) still in place on the animal or animal product at the time of sale to subsequent purchasers of the animal(s).

41. Processors should be required to ensure that they only purchase and/or process animals and/or animal products from suppliers (whether primary producers or others) that can credibly attest to the suitability/safety of the animal or animal product for the purpose intended.

42. Producers should have appropriate on-farm food safety assurance measures in place with respect to the use of, and/or exposure of food-producing animals to, veterinary drugs. All workers directly involved with the animals should be familiar with these measures.

43. Producers should be able to identify all food-producing animals, or lots of these animals, that have been treated with or exposed to veterinary drugs to ensure compliance with withdrawal/withholding times.

44. Continuous food safety assurance measures such as record-keeping should ensure that products (e.g. milk, eggs, honey) are harvested only if appropriate withdrawal/withholding times have been followed.

45. Treated or exposed animals for which the withdrawal time/withholding time has not elapsed should be kept separate from animals that have not been treated, or be positively identified to reduce the potential for mistakes.

46. Products from animals under harvest restrictions should be handled in such a way that ensures their product does not mix with that being harvested for human consumption. Any equipment likely to be contaminated should be adequately cleaned prior to being used on other animals.

VERIFICATION PROGRAMMES

Purpose

47. A verification programme that combines audits/inspection of various control points and point-of-harvest testing should be implemented. This approach will reduce reliance on chemical analyses and provide a higher degree of assurance.

48. The overall objective of the verification programme is to provide an appropriate degree of confidence that the practices and controls in place are adequate and being applied to the extent necessary to ensure the health of consumers of animal products. It will therefore attempt to ensure that exposure to residues in excess of the acceptable daily intake (ADI) rarely occurs.

49. Verification programmes may contribute to the:
(a) verification of assumptions made in the registration process;
(b) identification of unacceptable production, marketing and/or chains of advice;
(c) evaluation of the effectiveness of veterinary drug label information as it relates to food safety;
(d) evaluation of the effectiveness of education or risk reduction programmes;
(e) evaluation of quality management systems;
(f) verification of implementation and effectiveness of corrective actions.

General design principles

50. Verification programmes should cover, as appropriate, the entire food chain. A combined system of inspection/audits and sampling/laboratory analysis should be implemented. To provide the most effective control, the frequency, point and type of activity should be based on an assessment of the risk.

51. Verification programmes can be classified as follows according to objective and criteria applied to the sample selection:
(a) system verification programmes;
(b) risk-targeted verification programmes;
(c) surveys;
(d) port-of-entry testing programmes.

52. Verification programmes may focus on assessing the:
(a) effectiveness of a control system; and/or
(b) compliance by individuals or groups.

System and targeted verification programme design

53. Verification programmes should:
(a) define their purpose;
(b) identify the population being sampled;
(c) state whether the sampling is non-biased or targeted (directed), and
- base the number of samples for non-biased sampling protocols on statistics,
- pre-determine targeting criteria to direct sampling;

(d) pre-determine the criteria to be applied to the analysis of the results;
(e) define sampling and identification procedures that allow tracing each sample back to its origin and independent confirmation of the finding in case of dispute.

Risk profiling

54. It is the responsibility of the competent authorities to determine the risk profiles for their country and/or production system.

55. The frequency and intensity of verification or inspection/audit of each drug residue chosen to be monitored under the system verification programme should depend on the veterinary drug and use profile.

56. Risk profile considerations concerning veterinary drugs include:
(a) the type of hazard presented;
(b) the class and severity of the adverse human health effect associated with the residue (e.g. chronic toxicity, acute pharmacological, allergic reaction or microbiological disturbance);
(c) the use and/or production circumstances required to produce residues and the likelihood of these occurring in foods derived from the production system at concentrations and in frequencies presenting a risk to consumer health;
(d) the dietary consumption required for the residue to give rise to a realistic consumer health risk.

57. Competent authorities should attempt to make realistic estimates of the types, quantities and use patterns of veterinary drugs in their jurisdiction.

58. Subsequently, the following should be considered:
(a) circumstances required for each veterinary drug to cause an adverse health impact on consumers;
(b) likelihood of such circumstances occurring.

59. When considering and ranking the residues associated with the veterinary drugs likely to be present at some stage in the production system, potential sources and exposure pathways should be described.

60. The following sources of veterinary drug residues should be considered:
(a) veterinary drugs authorized in the jurisdiction of the competent authority;
(b) veterinary drugs that are known to be, or suspected of being, misused.

61. The exposure pathways of veterinary drug residues should be considered:
(a) intended, e.g. direct administration to the animals;
(b) indirect administration to the animals through addition to feed or water;
(c) unintended contamination via e.g. feed, water or the environment.

62. Competent authorities should, as appropriate to the risk profiles in the country and/or production system, consider the following potential pre-harvest control points for audit/inspection in the verification programme:
(a) the sellers and purchasers of veterinary drugs, to verify what is being sold and how it is being marketed;
(b) the users of veterinary drugs (including farmers, veterinarians and feed compounders), to verify how drugs are actually being used in the production systems, e.g. according to label, what records are being kept and how the treatment status of animals is identified;

(c) the animal and animal product distributors, to verify that any food harvest restrictions associated with the animal or product are effectively communicated;
(d) the assurance systems used by processors and/or producers, to ensure the suitability of the animals or product they are being supplied with for the purposes they intend using it for.

CHOICE OF VERIFICATION PROGRAMME

System verification programmes

63. In setting up system verification programmes, the following should be considered:
(a) examination of the relevant control points of the control system;
(b) non-biased sampling of a specified population with broadly similar attributes so that the results can be used to derive a statistical confidence as to the extent of control present in that population as a whole.

64. System verification programmes can focus on the degree of application of specific controls in the process or can focus on monitoring the residues in the animals/products at or close to the point of harvest.

65. Non-biased sampling programmes should be used in order to find out whether one of the controls within the system needs adjusting. They should not be relied upon for product evaluation.

66. Where the competent authority has linked the approval of a veterinary drug to particular use conditions/restrictions in order to avoid misuse or abuse, the appropriateness of the use conditions/use restrictions should be regularly verified with risk-targeted verification programmes as to their efficacy and necessity to manage the risk posed by the use of the veterinary drug.

67. Generally, non-biased sampling protocols are not efficient in detecting low incidences of non-compliance. Where such incidences are a potential significant risk to human health, other assurance programmes should be employed.

Risk-targeted verification programmes

68. In setting up risk-targeted verification programmes the following should be considered:
(a) previous performance, history of non-compliance;
(b) the quality management components usually relied on;
(c) potential risk factors that may be correlated with an increased use of veterinary drugs such as:
- high somatic cell counts in milk, or
- significant ante- or post-mortem findings, e.g. injection site lesions or resolving pathology;

(d) any other information linked to non-compliance and drug use.

69. Competent authorities may complement the risk-targeted pre-harvest verification programmes with established risk-targeted post-harvest verification programmes.

Surveys

70. Surveys may be performed to:
(a) assess the initial situation before a verification programme is started;
(b) evaluate the efficiency and appropriateness of specific aspects of control programmes;
(c) monitor the impact that variables, such as location, season or age, may have on the presence, absence or concentration of a residue.

Review

71. Control and verification programmes should be regularly reviewed to ensure their continued efficacy and/or necessity, as well as to review the potential impact of changes to the risk profiles.

72. Where a significant incidence of non-compliance is identified in any one year and consequent changes to the control programme implemented, a higher standard of verification may be appropriate until the effectiveness of the corrective actions has been demonstrated. Some of the selected lower risk profile veterinary drugs should be considered for rotation in and out of the programme based on history of compliance to ensure that the scope is as wide as possible.

SAMPLE TAKING

General principles

73. Appropriate mechanisms to prevent possible bias occurring in both the selection and taking of samples should be put in place.

74. Ideally, samples should be taken before animals and/or products are commingled with animals or product from other suppliers.

Traceability/product tracing

75. Competent authorities should ensure that all samples can, throughout the sampling, storing, shipping, analysis and reporting, be traced back to their origin.

76. Each sample needs to be clearly identified so that appropriate follow-on actions can be applied in case of non-compliant results.

77. If subunits of a consignment are sampled, care should be taken to identify those subunits clearly. Sufficient samples should be taken to allow for unprocessed subunits to be retained, allowing possible independent confirmation of the findings.

STATISTICAL CONSIDERATIONS

General

78. The number of samples for system verification programmes can be statistically predetermined (see Appendix A for additional guidance).

79. In designing a sampling protocol, it is essential to define both the purpose of the programme and the population of interest. It is also important to define the criteria to be applied when analysing the results with respect to the need/desirability for any further action, and especially how such criteria and actions directly relate to the protection of human health.

80. Ultimately, "a population" made up of "units of food consumed" is the most relevant to human health. However, as it is the application of appropriate pre-harvest practices and controls that ensures food safety, a sampling strategy that verifies both the appropriateness and extent of compliance of these pre-harvest practices and controls can be used to provide appropriate assurances that the health of consumers is unlikely to be negatively affected. Generally, the population of interest for targeting pre-harvest compliance/appropriateness verification information will be those population units to which common practices and controls should be applied such as:
(a) the seller of the veterinary drug input into the production system;
(b) the producer;
(c) the supplier of the animals or animal product to the processor; or
(d) the processor.

81. However, because the potential consequences to human health are much larger when large production units (farms) are out of control, the usual pre-harvest population randomly sampled is a standardized unit of production sold at any one time, e.g. individual animal, vat of milk, barrel of honey, or defined weight of aquaculture product. In this way, the larger producers/suppliers should effectively have a greater probability of being sampled while still maintaining the randomness of the sampling protocol.

82. Generally, conclusions will be drawn from the prevalence, or lack thereof, of non-complying results in the units sampled during the production season or calendar year. However, where problems are found during the course of the production season, corrective actions may have already been applied and have started to have a positive effect well before the end of production season or calendar year. For small populations, or for either low risk or reasonably stable exposure scenarios, several production seasons or calendar years may be used/needed to collect the number of samples statistically determined to give the required confidence.

83. Where it is possible to further refine and describe the affected population associated with defined risk factors such as season, region or specific type of production, then a correlation of the sampling protocol to such a co-variable may be justified.

84. The point at which a sample is taken depends on the objective of the specific programme. Where the objective is to verify the effectiveness of controls at the supplier stage, samples are generally taken at the point of sale/harvest in order to correlate the unit sampled with a supplier or producer.

85. On-farm sampling may also be used as part of a pre-harvest quality assurance programme or where there are concerns associated with the possible use of substances prohibited by the competent authority.

86. Where the objective is to verify the overall effectiveness of a system at ensuring the general population's exposure is less than the ADI, then multiple sample units can be combined before analysis, or commingled product sampled and analysed.

87. Where the objective is to verify the credibility and effectiveness of the control and verification programmes present in an exporting country, samples may be taken from standardized units of export at the port of entry. Such secondary verification programmes have quite different design considerations with respect to their objective, the population of interest and the type of response to any identified incidence of non-compliance. The statistical tables in Appendix A are not relevant to such programmes and the number of samples should reflect the importing country's confidence in the performance of the exporting country.

Retention of consignments during laboratory analysis

88. Competent authorities should not routinely retain lots of production associated with randomly selected samples pending the availability of the analytical results. Competent authorities may routinely retain lots of production where it is considered likely that a risk-targeted test will produce non-compliant results that present a potential risk for consumer health.

Result interpretation

89. A greater degree of assurance is achieved if verification programmes such as statistically based systems involving non-biased sampling and risk-targeted verification programmes (e.g. specific suppliers or products) are operated in parallel.

90. The results of risk-targeted verification programmes alone do not allow conclusions on the exposure of the general population with residues of veterinary drugs.

91. Conclusions on the exposure of the general population can be drawn from the combining the results of:
(a) statistically based system verification programmes involving non-biased sampling; and
(b) risk-targeted verification programmes.

Port-of-entry testing programmes (specific requirements)

92. Competent authorities should consider port-of-entry testing programmes only as a secondary system verification tool.

93. The matrices used in port-of-entry programmes may vary from those used for national verification programmes.

94. Except where a risk to health is suspected or detected, certified product should be subjected to non-biased sampling and release programmes at a frequency determined by the importing country based on the exporting country's record of compliance. Consignments of animal products tend to be heterogeneous by nature and will often be made up from a variety of animals, farms and processing dates. Results will reflect

the performance of the national control and verification system as a whole and should not be extrapolated to specific judgements on other units within the consignment except where a common pre-harvest risk factor is shared and a direct health threat is indicated.

95. The application of directed or targeted sampling in port-of-entry sampling programmes is only appropriate where it is known or suspected that products share the same risk profile.

96. However, following the detection of non-compliant results during port-of-entry programmes, importing countries may increase the overall frequency of testing of directly related food of animal origin from the exporting country for a period as an added verification of the effectiveness of any additional controls being implemented by the exporting country.

97. In the interpretation of laboratory results of consignments of animal products, it should be considered that these are made up of commingled product from a variety of animals, farms and processing dates and, therefore, heterogeneous. Because of this, results should not be taken to judge other units of a consignment except where units share a common pre-harvest risk factor and where a direct risk to health is suspected or detected.

98. Results of port-of-entry testing programmes should only be communicated if confirmed with methods fully validated for the specific matrix and analyte.

99. Laboratory reports on non-compliant results should include:
(a) a description of the method used;
(b) performance characteristics of the method of analysis (including the confidence interval of the result).

100. Laboratory reports on non-compliant results should be distributed to all parties affected by the result (e.g. the owner of the consignment and the certifying competent authority of the exporting country).

101. Competent authorities of importing countries should regularly provide exporting countries with the results of their verification programmes, including information for purposes of traceability/product tracing.

102. In cases of non-compliance with the food safety parameters, competent authorities from the exporting country should conduct a trace-back, apply appropriate corrective actions and then provide a summary of these to the importing country.

103. Where the type, incidence and/or frequency of non-compliance detected raises concerns as to whether the imports are meeting the standard of human health protection required by the importing country, then additional assurances may be requested.

104. The importing country may also choose to increase the frequency of port-of-entry verification to confirm that the assurances given are in fact addressing the problem.

105. Where residues of substances that should not be used in food-producing animals in either the exporting or the importing country are detected in port-of-entry testing, both competent authorities should cooperate in order to identify potentially similarly affected food of animal origin and to resolve any potential wider control problem.

106. Resolution of such problems will require the originating country to conduct an analysis to determine the possible source of such residues, the identification of deficiencies within the country's own control and monitoring system, and subsequent application of appropriate additional controls and measures to address the situation.

107. In cases where the exporting country is a less-developed country, consideration should be given by the importing country to the provision of technical assistance to help resolve the issue.

108. The application of new sampling and testing methods may reveal the presence of types and concentrations of residues previously unknown to exist by one or both parties. The determination of the source of such residues and their significance may take some time.

109. Where the presence of such residues is associated with previously accepted production practices, the implementation of changes, should these be deemed necessary, may require an extended period of time for capacity building.

REGULATORY ACTION

Investigation of non-compliances

110. Competent authorities should investigate each non-compliant result to ascertain the contributing factors that led to its occurrence and the systemic significance of the identified case.

111. An attempt should be made to identify the substances and the consumer health significance of their occurrence in food.

112. When an animal tissue/food contains residues in excess of the relevant MRL at the point of harvest, the following possibilities should be considered:

(a) the veterinary drug was not used according to label or prescription instructions;
(b) a non-authorized veterinary drug or formulation was used;
(c) the recommended withholding time was not observed or is not appropriate;
(d) treated and non-treated animals were commingled;
(e) unintended exposure to feed, water or contaminated environment occurred;
(f) the food is part of the statistically predictable small percentage of animals with residues in excess of the MRL even when the required withdrawal period has elapsed;
(g) sample contamination, analytical method problems or analytical error.

113. Laboratories should report all suspect positive samples that they have not been able to confirm positively using established confirmation criteria. This will allow the competent authority to identify possible patterns of non-compliance.

Measures in case of non-compliance: conduct

114. Competent authorities should adjust the scale and type of response to identified non-compliances to the relative importance that the respective hazard has for consumer health protection.

115. Competent authorities should take proportionate action when considering whether the non-compliance is the result of negligence or intent.

116. Competent authorities should, in cases of isolated mistakes due to ignorance or negligence, require that appropriate advice and training measures be followed.

117. In the case of proven negligence or intent, punitive measures in line with the Codex member's penal system should be considered (e.g. condemnations, fines, movement controls, etc.) to act as a deterrent.

118. Competent authorities should, in cases of widespread non-compliance, advise stakeholders and motivate the respective business sector to initiate the necessary changes.

119. Competent authorities should verify that appropriate corrective action is taken and monitor the success of these measures through inspection/audits and/or sampling/ laboratory analysis.

Measures in case of non-compliance: product

120. Unsafe product should not be passed as fit for human consumption and should be disposed of appropriately.

121. Where the results of samples taken on-farm for risk-targeted verification programmes do not provide the necessary confidence that the rest of the lot has been produced using appropriate practices and controls, the lot should not be passed for human consumption until sufficient information can be generated to provide the required degree of assurance as to its safety.

122. Where the results indicate there is a direct risk to consumer health, an attempt should be made to trace and remove all similarly affected products.

123. In non-biased sampling programmes, the unidentified proportion may represent a much greater potential threat to consumers than the identified proportion. Accordingly, any actions taken with respect to the identified non-compliant lot are less significant than the actions taken on the system as a whole.

124. When pre-harvest controls are not carried out or are unreliable owing to a high incidence of misuse of veterinary drugs, more frequent post-harvest verification may be appropriate to provide the required degree of consumer assurance. This should be regarded as an interim measure only until the appropriate corrective actions to the control programme have been put in place and subsequently demonstrated to be effective.

Corrective action in cases of non-compliance

125. Depending on the results of such investigations, local and/or systemic corrective actions may be considered appropriate to prevent reoccurrence.

126. Where the investigation of non-compliances indicates that use and distribution provisions for the substance(s) are inappropriate, competent authorities should take appropriate corrective action by modifying approval and distribution rules.

127. Where the investigation of non-compliances identifies local or systemic control failures, competent authorities should ensure that appropriate corrective action is taken at the relevant points.

128. The competent authority should verify that the measures are taken. Respective action should be proportionate in time and intensity to the consumer health hazard, scale and frequency of the non-compliance.

129. In cases where the failure lies outside the direct control of the business operator, the competent authority should prevent repetition of the failure by applying appropriate measures at the relevant control point.

INTERACTION BETWEEN THE CONTROL PROGRAMMES OF TWO COMPETENT AUTHORITIES

130. Competent authorities should cooperate to ensure that consumer health in all countries is protected.

131. This cooperation aims at achieving greater assurance than can be achieved through sole reliance on port-of-entry inspection programmes.

132. Trading countries should exchange copies of their control and verification programmes along with the results of these programmes from preceding years on a regular basis.

133. In order to facilitate trade from developing countries, longer transition periods and technical assistance regarding all aspects of a residue control programme should be considered.

ANALYTICAL METHODS FOR RESIDUE CONTROL

GENERAL CONSIDERATIONS ON ANALYTICAL METHODS FOR RESIDUE CONTROL

Introduction

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

135. Methods may also be required in regulatory control programmes for the detection of residues of substances for which ADIs and MRLVDs have not been established by the Codex Alimentarius Commission. For some substances, the toxicological evaluation leads to the conclusion that an ADI or MRLVD should not be established. For such substances, the determination of the lowest concentration at which the residue can be detected and the identity confirmed in a food is a primary concern in the method validation. Performance characteristics related to quantitative analyses may be less critical for such substances, where detection and confirmation of the presence of the substance as a residue is the major issue. Confirmation of identity of a residue is generally based on the comparison of a set of characteristics of a detected substance with those of a known standard of the suspected residue.

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

Integrating analytical methods for residue control

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

138. The principal performance attributes of analytical methods used in residue control programmes are dependent on whether a method is intended simply to detect, to quantify or to confirm the presence of a target residue. Completion of a full collaborative study[2] is not a requirement for recognition of a method to be placed in one of these three categories.

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

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

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

142. These three categories of methods – screening, quantitative and confirmatory – often share some performance characteristics. In addition, each category has other specific

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

considerations. Understanding the relationship between these three categories of methods is important in the development and operation of a balanced residue control programme. These three categories of methods may be applied sequentially in a residue control programme.

143. Samples that test "positive" with the screening method are considered suspect and are usually designated for further laboratory testing using more definitive methods. This could include repeat testing of replicate test portions with a screening method, but typically quantitative and/or confirmatory methods are used in the laboratory to establish that the sample does contain residues in excess of the regulatory limit. Such tests should be conducted on new test portions of the sample material used in the initial screening test to confirm that the analyte detected in the initial test is definitely the suspected compound and that the MRLVD (or other regulatory action limit established by the competent authority) has indeed been exceeded. The performance attributes, or characteristics, that must be determined during method validation for each type of method – screening, quantitative, confirmatory – are presented in the section "Attributes of analytical methods for residues of veterinary drugs in foods" (below).

Consideration for selection and validation of analytical methods

Identification of method requirements

Method scope

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

Marker residue

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

Target tissue

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

Implementing other Codex Alimentarius Commission guidelines

147. The Codex Alimentarius Commission has issued guidelines for laboratories involved in the import/export testing of foods,[3] which recommend that such laboratories should:

(a) use internal quality control procedures, such as those described in the "Harmonized guidelines for internal quality control in analytical chemistry laboratories";[4]
(b) participate in appropriate proficiency testing schemes for food analysis that conform to the requirement laid out in "The international harmonized protocol for proficiency testing of chemical analytical laboratories";[5]
(c) Comply with the general criteria for testing laboratories laid down in ISO/IEC Guide 17025:2005 "General requirements for the competence of calibration and testing laboratories"; and
(d) Whenever available, use methods that have been validated according to the principles laid down by the Codex Alimentarius Commission.

148. Methods used for analyses of veterinary drug residues in foods should be capable of detecting the compounds included in the residue control programme. The analytical recovery and precision for the target foodstuffs should meet the criteria stated elsewhere in this document. The methods should be used within an established laboratory quality management system that is consistent with the principles in the document on internal quality control referenced above. When methods that have not been subjected to a multilaboratory performance trial are used in a regulatory programme for control of veterinary drug residues in foods, the quality control and

[3] *Guidelines for the assessment of the competence of testing laboratories involved in the import and export control of food* (CAC/GL 27-1997).

[4] Thompson, M. & Wood, R. 1995. Harmonized guidelines for internal quality control in analytical chemistry laboratories. *Pure and Applied Chemistry*, 67(4): 649–666.

[5] Thompson, M., Ellison, S.L.R. & Wood, R. 2006. The international harmonized protocol for proficiency testing of chemical analytical laboratories. *Pure and Applied Chemistry*, 78(1): 145–196.

quality assurance procedures applied with these methods require careful definition, implementation and monitoring. In the case of methods that have been through multilaboratory trials, performance characteristics, such as recovery and precision, are defined through the results obtained during the study. For a method validated within a single laboratory, data must be generated to define the performance characteristics expected of the method when used by analysts within that laboratory. The ongoing performance must be monitored through the quality management system in place in the laboratory.

Method validation and fitness for purpose

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

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

151. Quantitative methods studied collaboratively according to the revised harmonized protocol adopted in 1995 by AOAC International, the International Union of Pure and Applied Chemistry (IUPAC) and the International Organization for Standardization (ISO) have been evaluated in a minimum of eight laboratories, unless highly complex equipment or other unusual requirements were identified (in such cases, a minimum of five participating laboratories is required).[5] Collaborative studies of qualitative methods currently require a minimum of ten participating laboratories. Collaborative studies conducted prior to 1995 completed method evaluation in a minimum of six laboratories in an acceptable, statistically designed study. These multilaboratory method performance studies generally satisfy the analytical requirements for use in a regulatory programme, as information on method performance in the hands of different analysts in different laboratories is obtained through these studies. However, relatively few of the analytical methods currently used in residue control programmes for veterinary drug residues in foods have been validated by such a multilaboratory study. Collaborative study designs are based on the analyses of coded duplicate test materials that represent the combinations of analytes, matrices and concentrations included in the scope of the method and include an independent peer review of both the study design and the results. In some situations, multilaboratory studies may be conducted that do not have the minimum number of laboratories required to qualify as a collaborative study. Such studies, when conducted using the same scientific

principles of design, evaluation and review as are applied in collaborative studies, can provide useful information on method performance in the hands of multiple analysts in different laboratories, but do not provide the same degree of statistical confidence obtained from the results of a collaborative study.

152. Multilaboratory and collaborative studies of methods usually do not encompass all possible combinations of residue, tissue and species to which the method may subsequently be applied. Methods may be extended to include related analytes, additional tissues, species or products (or combinations of these not included in the original multilaboratory study) by completing additional within-laboratory studies. Analytical results from method extension studies may require additional review before use in a regulatory programme. Whenever possible, analytical results obtained using methods that have not been validated by traditional interlaboratory study should be compared with results obtained using a method that has been validated through a collaborative or multilaboratory study or tested using sample materials from a recognized proficiency programme. The comparison should be based on a statistically acceptable study design using portions of the same (homogeneous) samples. The data from such studies should be independently reviewed by a qualified third party (such as a quality assurance [QA] unit, a peer group of regulatory scientists, auditors of national accreditation body) to determine the comparability of method performance.

153. Some residue control methods that have been demonstrated to be suitable for determining compliance with MRLVDs have a history of use in one or more expert laboratories, but have not been subjected to a formal multilaboratory study. These methods were demonstrated to be suitable at the time of initial regulatory use and have continued in use over an extended period of time either in the absence of alternative validated methods, or because they remain a preferred choice for reasons that may include use of available technology, cost, reliability and suitability for use within the constraints of a national programme. Although evidence of a formal collaborative or multilaboratory method trial is lacking, the method performance has been demonstrated through successful use and from quality control data in one or more laboratories over time.

154. Most regulatory laboratories rely on the use of veterinary drug residue methods that have not have been subjected to a multilaboratory study. Factors that have contributed to this situation include a requirement for specialized expertise or equipment, cost of such studies, lack of suitable collaborating laboratories, analyte and/or sample instability and rapidly changing technologies. While for many years the focus on equivalency of analytical results was based on the use of standardized methods that had performance characteristics defined on the basis of collaborative study, accredited laboratories now operate in an environment where it is the responsibility of the individual laboratory to demonstrate that the methods used and the analytical results produced meet performance criteria established in consultation with a client. In the absence of methods validated through interlaboratory method trials, regulatory laboratories must frequently use analytical methods that have been subjected to studies conducted within their own laboratory to characterize the method performance.

Single laboratory validation – the criteria approach

155. A guidance document on single laboratory validation of methods, "Harmonized guidelines for single-laboratory validation of methods of analysis", has been published as a technical report by the IUPAC.[6] The *Procedural Manual*[7] recognizes that interlaboratory validated methods are not always available or applicable, particularly for multianalyte/multisubstrate methods and new analytes. In such cases, methods may be validated in a single laboratory to meet the general criteria for the selection of methods of analysis, as well as the additional criteria:

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

(b) use of the method is embedded in a quality management system in compliance with the ISO/IEC 17025:2005 standard or with the principles of good laboratory practice;

(c) the method should be complemented with information on accuracy, demonstrated for example by:

- regular participation in proficiency schemes, where available,
- calibration using certified reference materials, where applicable,
- recovery studies performed at the expected concentration of the analytes,
- verification of result with other validated method, where available.

156. The criteria approach, which combines a single laboratory validation model with a requirement that methods meet specific performance specifications, has been adopted by some regulatory authorities.

ATTRIBUTES OF ANALYTICAL METHODS FOR RESIDUES OF VETERINARY DRUGS IN FOODS

Introduction

157. The performance characteristics of analytical methods used to determine 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. The section "General considerations of analytical methods for residue control" (above) presents a discussion of general types or categories of regulatory methods, and provides a scheme for using these analytical methods based upon their intended purpose in a regulatory framework. In the discussion below, attributes common to the three categories of methods (referred to as confirmatory, quantitative and screening methods) for determining compliance with Codex MRLVDs are presented. The additional attributes that are applicable to only one or two categories of methods are also discussed.

Method development considerations

158. The development of an analytical method requires analysts experienced in the analytical techniques to be used, as well as appropriate laboratory space, equipment and financial

[6] Thompson, M., Ellison, S.L.R. & Wood, R. 2002. Harmonized guidelines for single-laboratory validation of methods of analysis. *Pure and Applied Chemistry* 74(5): 835–855.

[7] FAO/WHO Codex Alimentarius Commission *Procedural Manual*.

support. Before initiating method development activities, the intended use and need for a method in a residue control programme should be established, including the required performance parameters. Other considerations include the required scope of the method (compound or class of compounds of interest and types of sample materials), potential interfering substances, the required performance characteristic of the measurements system, the pertinent physical and chemical properties that may influence method performance, the specificity of the desired testing system and how it will be determined, analyte and reagent stability data and purity of reagents, the acceptable operating conditions for meeting method performance factors, sample preparation guidelines, environmental factors that may influence method performance, safety considerations, and any other specific information pertinent to programme needs. In particular, stability of standards, both under normal conditions of storage and use and during processing of samples, should be assessed. Analyte stability in samples during typical conditions of sample storage prior to analysis should also be determined, including any period for which a sample may be held pending a potential re-analysis for confirmatory purposes.

159. Establishing method performance attributes is essential, as these provide the necessary information for food safety agencies to develop and manage their public health programmes. Performance attributes for analytical methods also provide a basis for good management decisions in future planning, evaluation and product disposition. For the animal health care industry, it provides a guideline for knowing exactly what performance must be achieved in developing analytical procedures. All will benefit by having well-defined analytical method performance factors. Method performance requirements will vary depending on whether the method is used for the screening, quantification or confirmation of a residue for which MRLs have been established, or for residues of a drug for which an ADI and MRLVDs have not been recommended. In the latter case, the competent authority may establish a minimum performance standard that must be met by analytical methods used for regulatory control purposes. However, when no safe concentrations of these compounds in foods have been established, the competent authority may review such limits periodically to ensure they reflect improvements in technology and analytical capability. When such limits have not been formally established by the competent authority, they are usually established de facto by the detection capabilities of the methods used in the regulatory laboratories.

Analytical performance characteristics

Performance characteristics of screening methods

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

161. For screening tests, particularly those involving test kit technologies, the term "*sensitivity*" refers to the lowest concentration at which the target analyte may be reliably detected within defined statistical limits. In the AOAC Performance Tested Methods℠ Program for test kits, this is determined experimentally by testing a minimum of 30 residue-free sample materials fortified with the analyte at the target concentration. The sample materials should be from at least six different sources (that is, at least five replicates from each of at least six sources), all of which should yield a positive result when fortified at the target concentration. Three or more negative results constitute a failure of the sensitivity test. If one or two of the results are negative, the experiment should be repeated and two negative results would then constitute failure. The experiment should be repeated with known incurred material at the target concentration, if such material is available.

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

163. The "cut-off" or threshold for the test for a particular compound is established by conducting concentration-response experiments, typically using 30 replicates (from at least six sources) fortified at each of a series of increasing concentrations. Once the concentrations have been established where all 30 replicates give a negative response and all 30 replicates give a positive response, the experiment is repeated using the blank matrix materials fortified at four evenly spaced concentrations between the "all negative" and "all positive" concentrations. An additional set is tested at a concentration 20 percent above the "all positive" concentration. Statistical analysis of the results enables the user to establish a reliable detection concentration at the required confidence level (usually 95 percent).[8]

[8] Finney, D.J. 1978. *Statistical method in biological assay.* 3rd edition. New York, USA, MacMillan Publishing Co.

Performance characteristics for quantitative methods

164. *Selectivity*, the ability of an analytical method to detect and discriminate the signal response from a compound in the presence of other compounds that may be present in the sample material, is of particular importance in defining the performance characteristics of methods used in regulatory control programmes for veterinary drug residues in foods. There are two aspects that must be considered – the ability of the method to provide a signal response that is free from interferences from other compounds that may be present in a sample or sample extract, and the ability of the method to identify unequivocally a signal response as being exclusively related to a specific compound. For a quantitative method, the requirement is that the signal used for quantification should relate only to the target analyte and not contain contributions for coextracted materials. Chromatographic analyses based on peaks that are not fully resolved provide less reliable quantitative results. Use of element-specific detectors or detection wavelengths or mass-selective detectors that are more specific to a particular compound or structure, combined with chromatographic separation, improves the selectivity of quantitative methods for veterinary drug residues in foods.

165. In addition to the selectivity of a method, the ability of the method to provide a quantitative result that is reliable must be demonstrated. This consists of two factors:
(a) the closeness of the result to the true or accepted value for the concentration of analyte present in the sample material, expressed in terms of *accuracy*, *trueness* or *bias*; and
(b) the ability of the method to provide consistent results on replicate determinations, expressed in terms of *precision* (*repeatability* and *reproducibility*).

166. It is recommended that methods used to support Codex MRLVDs should meet the performance standards for trueness and precision listed in Table 1, where CV_A refers to the coefficient of variation determined by test portions of blank matrix fortified prior to extraction and CV_L is the overall laboratory variability, which includes a 10 percent estimate for variability of sample processing.[9]

[9] Alder, L., Holland, P.T., Lantos, J., Lee, M., MacNeil, J.D., O'Rangers, J., van Zoonen, P. & Ambrus, A. 2000. *Guidelines for single-laboratory validation of analytical methods for trace-level concentrations of organic chemicals* (available at http://www.iaea.org/trc/pest-qa_val2.htm).

TABLE 1

Performance criteria that should be met by methods suitable for use as quantitative analytical methods to support MRLVDs for residues of veterinary drugs in foods[10]

Concentration	Coefficient of variation (CV)				Trueness
	Repeatability (within-laboratory, CV_A)	Repeatability (within-laboratory, CV_L)	Reproducibility (between-laboratory, CV_A)	Reproducibility (between-laboratory, CV_L)	Range of mean % recovery
(µg/kg)	*Percentage*				
≤ 1	35	36	53	54	50–120
1 to 10	30	32	45	46	60–120
10 to 100	20	22	32	34	70–120
100 to 1 000	15	18	23	25	70–110
≥ 1 000	10	14	16	19	70–110

167. The *accuracy* of a method may be determined by analysis of a certified reference material, by comparison of results with those obtained using another method for which the performance parameters have previously been rigorously established (typically, a collaboratively studied method) or, in the absence of reference materials or methods validated by interlaboratory trial, by determination of the *recovery* of analyte fortified into known blank sample material. The determination of accuracy as recovery is frequently used in validation of methods for veterinary drug residues in foods, as both certified reference materials and methods validated by interlaboratory trial are often not available. The accuracy of a measurement is closely related to *systematic error* (analytical method bias) and analyte recovery (measured as percent recovery). The accuracy requirements of methods will vary depending upon the planned regulatory use of the results. The accuracy should be carefully characterized at concentrations near the MRLVD or target concentration for regulatory action (typically at concentrations from 0.5 to 2.0 times the target concentration) to ensure that regulatory action is only taken on samples containing residues that can be demonstrated to exceed the regulatory action limit with a defined statistical confidence.

168. *Recovery* is usually expressed as the percentage of analyte experimentally determined after fortification of sample material at a known concentration and should be assessed over concentrations that cover the analytical range of the method. In interpreting recoveries, it is necessary to recognize that analyte added to a sample may not behave in the same manner as the same biologically incurred analyte (veterinary drug residue). In many situations, the amount of an incurred residue that is extracted (the yield or recovered fraction) is less than the total incurred residues present. This may be due to losses during extraction, intracellular binding of residues, the presence of conjugates, or other factors that are not fully represented by recovery experiments conducted with analyte-fortified blank tissues. At relatively high concentrations, analytical recoveries

[10] *Harmonized IUPAC Guidelines for the use of recovery information in analytical measurement* (CAC/GL 37-2001); see also Thompson, M., Ellison, S.L.R., Fajgelj, A., Willetts, P. & Wood, R. 1999. Harmonized guidelines for the use of recovery information in analytical measurement. *Pure Applied Chemistry*, 71(2): 337–348.

are expected to approach 100 percent. At lower concentrations, particularly with methods involving extensive extraction, isolation and concentration steps, recoveries may be lower. Regardless of what average recoveries are observed, recovery with low variability is desirable so that a reliable correction for recovery can be made to the final result, when required. Recovery corrections should be made consistent with the guidance provided by the Codex Alimentarius Commission.[10]

169. *Precision*, which quantifies the variation between replicated measurements on test portions from the same sample material, is also an important consideration in determining when a residue in a sample should be considered to exceed an MRLVD or other regulatory action limit. Precision of a method is usually expressed in terms of the within-laboratory variation (*repeatability*) and the between-laboratory variability (*reproducibility*) when the method has been subjected to a multilaboratory trial. For a single laboratory method validation, precision should be determined from experiments conducted on different days, using a minimum of six different tissue pools, different reagent batches, preferably different equipment, etc. and preferably by different analysts. Precision of a method is usually expressed as the standard deviation. Another useful term is relative standard deviation, or coefficient of variation (the standard deviation divided by the absolute value of the arithmetic mean). It may be reported as a percentage by multiplying by 100.

170. Method variability, achieved in a laboratory developing a method, is usually less than the variability achieved by another laboratory that may later use the method. If a method cannot achieve a suitable standard of performance in the laboratory where it was developed, it cannot be expected to do any better in other laboratories.

171. Quantitative methods are usually based on a comparison of the response from an analyte in a sample with the response from standards of the analyte in solution at known concentrations. In method development and validation, the calibration curve should first be determined to assess the detector response to standards over a range of concentrations. These concentrations (a minimum of five, plus blank) should cover the full range of analytical interest and the resultant curve should be statistically expressed. However, although it is recommended practice to include a suitable blank with the calibration samples, this does not imply that it is acceptable to extrapolate into the region of the curve below the low standard to obtain a quantitative result. The analytical function relates the response for the analyte recovered from sample material at various concentrations throughout the range of analytical interest. For analytes for which an MRLVD or regulatory action limit has been established in a particular sample material (matrix), response is typically determined for known blank sample material and for blank sample material fortified at a range of concentration above and below the MRLVD (use of six different sources of blank materials is recommended).

172. The analytical function experiment data can also be used to calculate the analytical recovery at each concentration and are of particular importance when the presence of matrix coextractives modifies the response of the analyte as compared with analytical

standards. The *linearity* is determined from the analytical function experiments and is the statistical expression of the curve obtained for the analysis of sample materials fortified at the target concentrations. It is typically determined from a linear regression analysis of the data, assuming there is a linear response. It is increasingly common in methods for veterinary drug residues in foods to base the quantitative determination on a standard curve prepared by addition of standard to known blank representative matrix material at a range of appropriate concentrations that bracket the target value (the analytical function). Use of such a "tissue standard curve" for calibration incorporates a recovery correction into the analytical results obtained.

173. It is also necessary to establish the lower limits at which reliable detection, quantification or confirmation of the presence of an analyte may be performed using a particular analytical method. The *detection limit* may be described in practical terms as the lowest concentration where the analyte can be identified in a sample. It can be estimated using the standard deviation ($s_{y/x}$) from the linear regression analysis of the standard curve generated in the analytical function experiment described above.[11] Using this approach, the limit of detection is calculated using the y-intercept (assuming a positive value) of the curve plus three times $s_{y/x}$. This approach provides a conservative estimate of the detection limit. The detection limit can also be estimated by measurements on representative test materials as the weakest relevant response of the analyte in the blank plus three times its standard deviation. It is often necessary to fortify test materials at a concentration resulting in a barely detectable response to obtain an approximation of the standard deviation of the blank when using this approach.

174. The *limit of quantification* (LOQ), also referred to as quantification limit, may be established from the same experiments using the y-intercept of the curve plus ten times $s_{y/x}$. For methods used to support MRLVDs established by the Codex Alimentarius Commission, the LOQ should meet the criteria for precision and accuracy (recovery) in Table 1 and should be equal to or less than one-half the MRLVD. However, when the LOQ of a method is lower than the actual concentrations monitored for compliance with an MRLVD, the validation and subsequent application of the method should be based on a *lowest calibrated level* (LCL), which is typically 0.5 × the MRLVD. For use in a regulatory programme, the limits of detection and quantification are important parameters when the method will be applied to estimate exposures to residues, where there may be an interest in monitoring residues at concentrations below the MRLVD, or when conducting residue analyses for substances that do not have ADIs or MRLVDs. For monitoring compliance with an MRLVD, it is important that an LCL be included in the analysis that adequately demonstrates that the MRL concentration may be reliably determined. The LCL of a method used to support an MRLVD should not be less than the LOQ. The *Procedural Manual* recommends the term *determination limit* under "Terms to be used in the criteria approach".[7]

[11] Miller, J.C. & Miller, J.N. 1993. *Statistics for analytical chemistry*. 3rd Edition. Chichester, UK, Ellis Horwood Ltd.

Performance characteristics for confirmatory methods

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

176. Typically, a minimum of four identification points is required to meet accepted performance criteria for regulatory methods. Methods based on high-resolution mass spectrometry are considered to give a higher reliability through more precise measurement of mass than can be obtained using low-resolution mass spectrometry techniques. Method performance requirements for confirmatory methods based on low resolution gas chromatography mass/spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS), as recently published by an international expert body,[12] are given in Table 2.

TABLE 2
Performance requirements for relative ion intensities (sample compared to standard) using various mass spectrometric analytical techniques[9]

Relative ion intensity (% of base peak)	GC-MS (EI) (relative)	GC-MS (CI), GC-MS/MS LC-MS, LC-MS/MS (relative)
Percentage	*Percentage*	*Percentage*
> 50	≤ 10	≤ 20
20–50	≤ 15	≤ 25
10–20	≤ 20	≤ 30

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

178. Additional confidence is provided when high-resolution mass spectrometers are used in a confirmatory method, as the high resolution provides more precise identification of the mass and may be used to predict the elemental composition of each fragment. For a single high-resolution mass spectrometer, each structurally significant fragment detected is

[12] Bethem, R., Boison, J.O., Gale, J., Heller, D., Lehotay, S., Loo, J., Musser, S., Price, P. & Stein, S. 2003. Establishing the fitness for purpose of mass spectrometric methods. *Journal of the American Society for Mass Spectrometry*, 14(5): 528–541.

assigned a value of 2 identification points, while product ions generated in high-resolution MS/MS experiments are assigned an identification point value of 2.5 each. In addition, at least one ion ratio must also be measured to eliminate the potential for fragments of the same mass arising from isobaric compounds of similar structure.

179. Other techniques, when they are used in combination, may be capable of achieving a comparable degree of selectivity as confirmatory techniques. For example, identification may be verified by combinations of methods such as:
(a) thin layer chromatography;
(b) element-specific gas-liquid chromatography and accompanying detection systems;
(c) formation of characteristic derivatives followed by additional chromatography; or
(d) determining compound-specific relative retention times using several chromatographic systems of differing polarity.

180. Such procedures must be applicable at the designated MRLVD of the analyte. When a confirmatory method such as mass spectrometry is not available, information on the selectivity associated with the analysis of a particular veterinary drug residue in a sample may be developed from various sources.[13] This information may be captured in a structured logging document of all the information that leads to the conclusion a method has detected a particular compound in a sample, at a measured concentration as reported. While no single measurement or analysis may provide the unequivocal proof of compound identity and/or quantity present that is desired, the combined information that has been compiled provides evidence that the analyst has made a conscientious effort to arrive at a logical result consistent with the data and other information available. Examples of analytical techniques that may be suitable to meet criteria for confirmatory analytical methods are summarized in Table 3.

TABLE 3
Examples of detection methods suitable for the confirmatory analysis of substances, as recommended by the Miskolc Consultation[9]

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

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

[13] Stephany, R.W. 2003. *SPECLOG – the specificity log*. CRD-9, Codex Committee on Residues of Veterinary Drugs in Foods, 14th Session, Arlington, USA, 4–7 March.

181. Although confirmatory 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 confirmatory method, if it has sufficient sensitivity and precision.

General performance characteristics for methods for use in a regulatory control programme

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

183. *Ruggedness* testing should be conducted using the standard factorial design approach to determine any critical control points.[14] Typical factors to include in a design include variations in reagent volumes or concentrations, pH, incubation or reaction time and temperature, reagent quality, and different batch or source of a reagent or chromatographic material. Ruggedness testing of a confirmatory method may be required if the method differs significantly from the quantitative method previously validated (if the method uses different extraction or derivatization procedures than are used in the quantitative method).

184. *Cost-effectiveness* is the use of reagents and supplies that are readily available in the required purity from local suppliers and equipment for which parts and service are also readily available. The *method efficiency* is increased when multiple samples can be analysed at the same time. This reduces the analytical time requirements per sample and usually reduces the cost per sample, as there are certain fixed costs associated with the analysis of samples whether done singly or in larger sets. The ability of a method to accommodate multiple samples in a batch is important when large numbers of samples must be analysed in short or fixed time frames. *Portability* is the analytical method characteristic that enables it to be transferred from one location to another without loss of established analytical performance characteristics.

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

[14] Youden, W.J. & Steiner, E.H. 1975. *Statistical Manual of the Association of Official Analytical Chemists*. Gaithersburg, USA, AOAC International.

Method development and validation considerations for residue control methods

Selection of appropriate test material for validation

186. Laboratories must demonstrate that the methods in use for analysis of regulatory samples have been suitably validated. Traditionally, the multilaboratory method validation study has been the preferred approach to provide analytical data to define method performance characteristics. However, other models have been developed that include multilaboratory trials with smaller numbers of laboratories than are required to conduct a full collaborative study and single laboratory validation based on rigorous in-house evaluation of method performance, supported by a quality management system, independent audits and analysis of proficiency or reference materials, when available.

187. In developing and validating a residue control method, data should be derived from three types of sample material. Control test material from non-treated animals provides information about analytical background and matrix interferences. Fortified test material, containing known amounts of the analyte added to the control material, yields information about the method's ability to recover the analyte of interest under controlled conditions. Tissues should be obtained from multiple sources to cover the variations resulting from factors such as different diets, husbandry practices, sex and breed of animals. A minimum of six different sources of material is recommended.

188. In some instances, known drug-free sample materials may not be available for use in residue control laboratories. In these instances, an equivalent sample material may be used. Equivalent sample materials may consist of either the same matrix as the test sample matrix from an unknown source, or a different matrix from a known drug-free source that closely matches the sample matrix. In all cases, the residue control laboratory must demonstrate that the equivalent sample material is free from interferences for the drug and exhibits satisfactory recovery for fortified samples. Additionally, when a material is used from an unknown source for quantitative or screening methods, it is recommended that a second method be used to demonstrate that the matrix does not contain residues of the drug. It is the responsibility of the residue control laboratory to demonstrate fitness for purpose of the equivalent sample material.

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

Measurement uncertainty

190. Laboratories should provide their customers on request with information on the measurement uncertainty or statement of confidence associated with the quantitative results produced by each quantitative method. Guidance on estimation of measurement uncertainty is being developed by the IUPAC and has been published by other independent scientific bodies.[15]

[15] Ellison, S.L.R., Roslein, M. & Williams, A. 2000. *Quantifying uncertainty in analytical measurement*. EURACHEM/CITAC Guide CG 4 (available at http://www.measurementuncertainty.org/mu/QUAM2000-1.pdf).

Use of internal standards

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

Environmental considerations

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

Choice of validation model

193. An analytical method developed and used in only one laboratory may have limited use in a residue control programme unless care is taken to meet the rigorous expectations for single laboratory method validation associated with accreditation under ISO/IEC 17025 or equivalent accreditation procedures for testing laboratories. The reliability of reported values may be a concern even though strong quality control procedures may have been employed, unless supported by data from an ongoing proficiency programme, comparison with a suitable method validated in an interlaboratory trial or other forms of interlaboratory comparison of results. Ideally, a method should be validated by at least three laboratories. Methods that have been carefully validated in a single laboratory with inclusion of properly designed ruggedness tests should be able to undergo successfully a collaborative study involving at least eight different laboratories.

194. The principles for conducting a single laboratory method validation, a multilaboratory method trial or a collaborative study of a residue control method are the same. Samples for evaluating method performance should be unknown to the analyst, in randomized replicates, containing the residue near the MRLVD or other target concentration, as well as samples with the analyte above and below the concentration of interest, and test material blanks. A minimum of three individual datasets should be generated over three analysis periods, on at least three separate occasions (at least one day apart), preferably with replicate analysis, to improve statistical evaluation of method performance and provide an estimate of interday variability. It should be noted that these are only minimal requirements. The establishment of statistically based performance standards for methods is enhanced by increasing the number of independent analysts and laboratories testing the method, as well as by the number of samples tested. In a single laboratory validation, it is recommended that the method should be tested by multiple analysts to provide appropriate measures of within-laboratory performance. Expanding the validation to include other laboratories, preferably to the number required for a collaborative study, is recommended. Analyses of blind duplicates, as required in the collaborative study protocol,[7] in only eight laboratories, with one or two animal species and tissues, yields limited quality estimates for overall repeatability and reproducibility. The validation of a collaboratively studied method can be extended to include additional tissues and species in a subsequent study conducted by a single expert laboratory, as required.

Quality management systems

195. A quality management system is an essential component of residue analysis. It both monitors those factors associated with the analysis of a sample by an analyst and provides the oversight by independent reviewers to ensure that the analytical programme is performing in an acceptable manner. The use of an accredited quality management system is invaluable to support decision-making for residue control agencies, improving the reliability of analytical results, and providing quality data for residue control programmes to demonstrate food safety to consumers, producers and law-making bodies regarding residues of veterinary drugs in food. The establishment of quality measures consistent with the principles published by the IUPAC is recommended for regulatory control laboratories.

APPENDIX A
SAMPLING STRATEGIES

NON-BIASED SAMPLING

Purpose

1. Non-biased sampling is designed to provide profile information, especially as to the extent of application or performance of a control or assurance system for a specified animal/food population over a defined period.

Statistical considerations on sampling population size

2. The number of samples for non-biased sampling protocols should be statistically based and may be influenced by the size of the population (where less than 5 000), the prevalence of non-compliance determined to be significant, the confidence to be placed in the results as well as economic considerations.

3. The number of samples based on the binomial distribution will always be equal to or greater than the required number of samples based on the hypergeometric distribution.[1]

4. If the size of the population is small, the effect of sampling without replacement is significant and the sampling distribution should be based on the hypergeometric distribution.

5. In populations larger than 5 000 units, the effect of sampling without replacement is negligible. Thus, the binomial distribution can be used to determine an appropriate number of samples.

6. The number of samples for a defined confidence will be effectively constant for populations exceeding 5 000 units.

Sampling confidence reporting

7. Where non-compliant results are detected, it is possible to derive a crude estimate of the likely prevalence in the general population.

8. However, where no non-compliant results are found, then any statements about prevalence need to be stated with a defined confidence that the prevalence of non-compliant results does not exceed a specified percentage.

9. The number of samples required to give a required statistical assurance can be read from Table 1. Other scientifically based statistical protocols may also be used.

[1] In probability theory and statistics, the *hypergeometric distribution* is a discrete (consisting of unconnected distinct parts) probability distribution that describes the number of successes in a sequence of *n* draws from a finite population without replacement.

TABLE 1

Number of samples required to detect at least one non-compliant result with pre-defined probabilities (90, 95 and 99 percent) in a population having a known non-compliance prevalence

Non-compliant prevalence (% in a population)	Minimum number of samples required to detect a non-compliant result 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	2 302	2 995	4 603

TABLE 2

Probability of failing to detect a non-compliance

Prevalence (%)	Number of animals/units of product in sample tested									
	5	10	25	50	75	100	200	250	500	1 000
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	0.590	0.389	0.095	0.009	0.001					
10	0.528	0.349	0.072	0.005	0.000					
12	0.470	0.279	0.041	0.002						
14	0.418	0.221	0.023	0.001						
16	0.371	0.175	0.013	0.000						
18	0.328	0.137	0.007							
20	0.254	0.107	0.004							
24	0.193	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								

10. The probability of failing to detect a specified prevalence of non-compliant results associated with a specified targeting mechanism can be read from Table 2. Because of the low efficacy of sampling protocols in detecting low prevalences of non-compliance, other assurance mechanisms are more important where a low prevalence of non-compliance is expected.

DIRECTED OR TARGETED SAMPLING

Purpose

11. Directed or targeted sampling protocols are designed to place a greater intensity of inspection/audit on suppliers or product considered to have possibly a greater potential than the general population of being non-compliant.

12. It is not possible to extrapolate from non-compliant results to draw conclusions about the general population because a subpopulation that is considered to have greater chance of non-compliance is being sampled (biased sampling).

13. However, if compliant results confirm non-biased programme results, they provide increased assurance that the system is working effectively.

APPENDIX B

SAMPLING OF COMMODITIES

SCOPE

1. This Appendix applies to the following commodities: primary food commodities of animal origin and processed products of animal origin made from primary food appearing in Table A and Table B of this Appendix, and honey of the following origins and/or processing methods:
   (a) blossom or nectar honey that comes mainly from nectaries of flowers;
   (b) honeydew honey that comes mainly from secretions of or on living parts of plants;
   (c) comb honey stored by bees in the cells of freshly built broodless combs, and sold in sealed whole combs or sections of such combs;
   (d) extracted honey obtained by centrifuging decapped broodless combs;
   (e) pressed honey obtained by pressing broodless combs with or without the application of moderate heat.

DEFINITIONS

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

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

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

Bulk sample means the combined total of all the primary samples taken from the same lot.

Final laboratory sample means the primary or bulk sample, or a representative portion of the primary or bulk sample, intended for laboratory analysis.

Final laboratory test portion means the representative portion of the final laboratory sample on which an analysis is conducted. The entire laboratory sample may be used for analysis in some cases but typically will be subdivided into representative test portions for analysis. It is prepared by combining and thoroughly mixing the primary samples.

Lot of honey means a discrete quantity of 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.

Consignment of honey means a discrete quantity of honey as described on a particular contractor's shipping document. A consignment may be made up of different lots.

Primary honey sample means 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.

SAMPLING PROCEDURES

2. Samples must be collected by those officially authorized for this purpose.

3. Each lot to be examined must be sampled separately.

4. During collection and processing, care must be taken to prevent contamination or other changes in the samples that would alter the residue, affect the analytical determination, or make the laboratory test portion not representative of the bulk or laboratory sample.

5. Guidance on sample type and quantity for different commodities is provided in Table A (meat and poultry products) and Table B (milk, eggs and dairy products). The following are general instructions:
   (a) Each primary sample should be taken from a single animal (or group of animals) or unit in a lot, and when possible, be selected randomly.
   (b) When several animals are required for adequate sample size of the primary sample (e.g. poultry liver), the samples should be collected consecutively after initial random selection.
   (c) Frozen product should not be thawed before sampling.
   (d) Canned or packaged product should not be opened for sampling unless the unit size is at least twice the amount required for the final laboratory sample. The final laboratory sample should contain a representative portion of juices surrounding the product.
   (e) Unopened cans or packages that constitute a final laboratory sample should be sent unopened and intact to the laboratory for analysis.
   (f) The contents of cans or packages opened by the authorized inspector should be frozen as described in paragraph 23(d) (below) before dispatch to the laboratory for analysis.
   (g) Large, bone-containing units of product (i.e. prime cuts) should be sampled by collecting edible product only as the primary sample.
   (h) When portions of single unit are less than described as a primary sample, additional sample units need to be taken to satisfy bulk sample requirements.
   (i) Portions remaining of final laboratory samples should be frozen and stored in conditions that will maintain the sample integrity.

6. The number of primary samples collected will depend on whether a lot is considered suspect.

7. A lot is suspect if there is:
   (a) a history of non-compliance with the maximum residue limit for veterinary drugs (MRLVD);
   (b) evidence of contamination during transport;
   (c) signs of toxicosis (systemic poisoning) observed during ante- or post-mortem inspection; or
   (d) other relevant information available to the authorized inspection official.

8. A minimum of 6 to a maximum of 30 primary samples should be collected from a suspect lot. When the suspected residues are expected to occur throughout the lot, the smaller number of samples is sufficient.

9. Imports from countries that do not run verification programmes for compliance with MRLVDs should be sampled as suspect lots.

SPECIFIC SAMPLE PREPARATION INSTRUCTIONS FOR HONEY

   (a) Collect 250 ml of liquid or strained honey after the following preparations as applicable.
   (b) Liquidize 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 openings of 0.500 mm by 0.500 mm (ISO 565:1990).[1]
   (c) 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.

10. When a sample is free from granulation, mix thoroughly by stirring or shaking; if granulated, place closed container in water-bath without submerging, and heat for 30 minutes at 60 °C; then, if necessary, heat at 65 °C until liquefied. Occasional shaking is essential. Mix thoroughly and cool rapidly as soon as the sample liquefies.

STATISTICAL CONCERNS

11. For non-suspect lots, a statistically based, non-biased sampling programme is recommended. Any of the following types of sampling can be used.

Stratified random sampling

12. Where consignments are commingled, simple random criteria cannot be applied and stratified random sampling should be considered.

13. In stratified random sampling, the consignment is divided into non-overlapping groups or strata, e.g. geographical origin, genders, time. A sample is taken from each stratum.

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

14. Homogeneity within each stratum is better than in the whole population. Countries or geographic regions are considered natural strata based on uniformity in agricultural practices.

15. Time strata (e.g. month, quarter) are commonly used for convenience, efficiency and detection of seasonal variability. Random number tables[2] 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.

Systematic sampling

16. In systematic sampling, units are selected from the population at a regular interval (e.g. once an hour, every other lot, etc.).

17. It may be applied when there is reliable information on product volumes to determine the sampling interval that will provide the desired number of samples over time. However:
(a) If the sampling system is too predictable, it may be abused.
(b) Consignments need to be homogeneous, because systematic sample units are uniformly distributed over the population.

Biased or estimated worst case sampling

18. In biased or estimated worst case sampling, investigators use their judgement and experience regarding the population, lot or sampling frame to decide which primary samples to select.

19. The population group anticipated to be at greatest risk may be identified, but no general conclusion should be made about the population sampled from the data collected (non-random samples).

PREPARATION OF LABORATORY SAMPLES

20. The final laboratory sample is sent for analysis.

21. Some national/regional legislation/regulation may require that the final laboratory sample is subdivided into two or more portions for separate analyses. Each portion should be representative of the final laboratory sample. Precautions indicated under *sampling procedures* should be observed.

22. The laboratory test portion should be prepared from the final laboratory sample by an appropriate method of reduction.

[2] Random number tables consist of a randomly generated series of digits (0–9). To improve readability, there are spaces e.g. after every fourth digit and after every tenth row. Reading can begin anywhere (at random), but having started, has to continue across the line or down a column and NOT jump about. Example: extract from a table of random sampling numbers: 3680 2231 8846 5418 0498 5245 7071 2597.

SHIPMENT OF LABORATORY SAMPLES

23. Final laboratory samples should be prepared as follows:
   (a) Each sample should be placed in a clean, thermally insulating, chemically inert container to protect the sample from contamination, defrosting and damage in shipping.
   (b) The container should be sealed so that unauthorized opening is detectable.
   (c) The container should be sent to the laboratory as soon as possible, after taking precautions against leakage and spoilage.
   (d) For shipping, all perishable samples should be frozen to minus 20 °C immediately after collection and packed in a suitable container that retards thawing. Freezer packs or other suitable refrigerants should be used to maintain freezer temperatures during shipment. Samples and freezer packs should be fully frozen to minus 20 °C prior to dispatch.
   (e) Replicate portions of the final laboratory sample that may be retained as required by national/regional legislation or as an administrative policy should be placed in a clean, chemically inert container to protect the sample from contamination, sealed so that unauthorized opening is detectable and stored under suitable conditions to prevent a change in the product or any residues it may contain in case future analysis is required for comparison with analytical results obtained on the sample material submitted to the laboratory.

RESULT INTERPRETATION IN THE LABORATORY

24. For purposes of control, the MRLVD is applied to the residue concentration found in each laboratory sample taken from a lot.

25. Lot compliance with an MRLVD is achieved when the mean result for analysis of the laboratory test portions does not indicate the presence of a residue that exceeds the MRLVD.

SAMPLING RECORDS

26. Each primary or bulk sample and each final laboratory sample should be uniquely linked to a record with the type of sample, analyses required, its origin (e.g. country, state or town), its location of collection, date of sampling, and additional information required for follow-up action if necessary.

27. If there is a deviation from recommended sampling procedures, records accompanying the sample should describe procedures actually followed in detail.

GUIDANCE ON SAMPLE TYPE AND QUANTITY FOR DIFFERENT COMMODITIES

TABLE A

Meat and poultry products

Commodity	Instructions for collection	Minimum quantity required for laboratory sample
I. Group 030 (Mammalian meats)		
A. Whole carcass or side, unit weight normally 10 kg or more	Collect diaphragm muscle, supplement with cervical muscle, if necessary, from one animal.	500 g
B. Small carcass (e.g. rabbit)		500 g after removal of skin and bone
C. Fresh/chilled parts		
1. Unit minimum weight of 0.5 kg, excluding bone (e.g. quarters, 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 expressed in carcass fat)		
A. Animals sampled at slaughter	See instructions under II. Group 031.	
B. Other meat parts	Collect 500 g of visible fat, or sufficient product to yield 50–100 g of fat for analysis. (Normally, 1.5–2.0 kg of product is required for cuts without trimmable fat.)	Sufficient to yield 50–100 g of fat
II. Group 031 (Mammalian fats)		
A. Large animals sampled at slaughter, usually weighing at least 10 kg	Collect kidney, abdominal or subcutaneous fat from one animal.	500 g
B. Small animals sampled at slaughter[1]	Collect abdominal and subcutaneous fat from one or more animals.	500 g
C. Bulk fat tissue	Collect equal size portions from 3 locations in container.	500 g
III. Group 032 (Mammalian edible offal)		
A. Liver	Collect whole liver(s) or portion sufficient to meet laboratory sample size requirements.	400–500 g

(continued)

TABLE A (continued)

Meat and poultry products

Commodity	Instructions for collection	Minimum quantity required for laboratory sample
B. Kidney	Collect one or both kidneys, or kidneys from more than one animal, sufficient to meet laboratory sample size requirement. Do not collect from more than one animal if size meets the low range for sample size.	250–500 g
C. Heart	Collect whole heart or ventricle portion sufficient to meet laboratory sample size requirement.	400–500 g
D. Other fresh/chilled 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
D. Fresh/chilled or frozen parts		
1. Wholesale package		
a. Large parts	Collect an interior unit from a selected container.	500 g after removal of skin and bone
b. Small parts	Collect sufficient parts from a selected layer in the container	500 g after removal of skin and bone
2. Retail packaged	Collect a number of units from selected container to meet laboratory sample size requirement.	500 g after removal of skin and bone
IVa. Group 036 (Poultry meats where MRLVD is expressed in carcass fat)		
A. Birds sampled at slaughter	See instructions under V. Group 037	
B. Other poultry meat	Collect 500 g of fat or sufficient product to yield 50–100 g of fat. (Normally, 1.5–2.0 kg is required.)	500 g of fat or enough tissue to yield 50–100 g of fat
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

(continued)

TABLE A (continued)

Meat and poultry products

Commodity	Instructions for collection	Minimum quantity required for laboratory sample
VI. Group 038 (Poultry edible offal)		
A. Liver	Collect 6 whole livers or a sufficient number to meet laboratory sample requirement.	250–500 g
B. Other fresh/chilled or frozen edible offal product	Collect appropriate parts from 6 birds. If bulk frozen, take a cross-section from container.	250–500 g
VII. Class E – Type 16 (Secondary meat and poultry products)		
A. Fresh/chilled or frozen comminuted product of single species origin	Collect a representative fresh or frozen cross-section from selected container or packaged unit.	500 g
B. Group 080 (Dried meat products)	Collect a number of packaged units in a selected container sufficient to meet laboratory sample size requirements.	500 g, unless fat content is less than 5% and MRLVD is expressed on a fat basis. Then, 1.5–2.0 kg is required.
VIII. Class E – Type 18 (Manufactured, single ingredient product of animal origin)		
A. Canned product (e.g. ham, beef, chicken), unit size of 1 kg or more	Collect one can from a lot. When unit size is large (greater than 2 kg), a 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

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

TABLE B

Milk, eggs, dairy 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. Bulk containers (barrels, drums): Mix the contents carefully and scrape adhering material from the sides and bottom of the container. Remove 2–3 litres, repeat the stirring and take a 500 ml sample.	500 ml
B. Evaporated milk – evaporated full-cream & skimmed milk	Small retail containers: Take sufficient units to meet laboratory sample size requirements.	500 ml
C. Milk powders		
1. Whole	Bulk containers: Pass a dry borer tube steadily through the powder at an even rate of penetration. Remove sufficient bores to make up a sample of 500 g. Small retail containers: Take sufficient units to meet laboratory sample size requirements.	500 g
2. Low-fat	As for whole milk powders.	500 g
III. Group 087 (Derived milk products)		
A. Cream – fresh, frozen & UHT; single, whipping, whipped, double & clotted	Bulk containers: Plunge to ensure thorough mixing, moving the plunger from place to place, avoiding foaming, whipping and churning. Take a 200 ml sample by means of a dipper. Small containers: Take sufficient units to meet laboratory sample size requirements.	200 ml
B. Butter – including whey butter and low-fat spreads containing butterfat	In bulk: Take two cores or more of butter so that the minimum total sample weight is not less than 200 g. In pats or rolls: For units weighing over 250 g, divide into four and take opposite quarters. For units weighing less than 250 g, take one unit as sample.	200 g
C. Butter oil – including anhydrous butteroil and anhydrous milk fat	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

(continued)

TABLE B (continued)
Milk, eggs, dairy products

Commodity	Instructions for collection	Minimum quantity required for laboratory sample
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)		
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 250 ml liquid or 500 ml packed shavings from aseptic drillings into containers.	500 g
B. Dried egg products	Use sample schedule. For containers of 500 g or less or 25 ml or less, collect a minimum of 2 units per subsample. For containers of 500 g to 10 kg, select 1 unit per subsample. For containers of 10 kg or more, collect 1 kg from each unit sampled. Collect with aseptic technique.	500 g
C. Shell eggs		
1. Retail packages	Use sample schedule. Subsample size is 12 eggs.	500 g or 10 whole eggs
2. Commercial cases	For 15 cases or less, collect 12 eggs from each case, minimum of 24 eggs. For 16 or more cases, collect 12 eggs from 15 random cases.	500 g or 10 whole eggs