

Veterinary drug residue monitoring – practical aspects (e.g. sampling) and analytical tools available

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Scope of presentation

- Residue plans (sampling)
  - EU vs Third Country
  - Populations to be tested
- Compliance with 96/23/EC or Codex?
- Method validation EU vs Third Country
- Sources of information /training

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Sampling levels and frequencies required in EU Member States

- Sampling levels and frequencies are laid down in Council Directive 96/23/EC and Commission Decision 97/747/EC
- Based on (previous year) annual national production figures.
- Every EU Member State is obliged to observe these sampling levels
- e.g.....




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## Council Directive 96/23/EC (example sampling rates)



Species	Commodity	Frequency
Bovine / Ovine / Caprine	Milk	One per 15000 tonnes of annual production - minimum 300 samples
Porcine	Meat	0.05 % of the animals slaughtered the previous year
Poultry	Meat	One per 200 tonnes of annual production (deadweight)
Poultry	Eggs	One per 1000 tonnes of annual production for human consumption - minimum 200 samples
Bees	Honey	10 per 300 tonnes of annual production for human consumption for the first 3000 tonnes + 1 sample for every 300 tonnes thereafter

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## Sampling – Third countries (1)



### Equivalent guarantees must be offered

- For third countries, the number of samples to be taken depends on the structure of the relevant industry
- Where animals and products from any farm are eligible to be exported to the EU, the proportion of animals sampled should be taken relative to the annual national production figures

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## Sampling – Third countries (2)



- Where only a defined population of animals are eligible for export to the EU (i.e from regions/zones), and where there is a system in place guaranteeing that only those animals from those farms are eligible for export, it is permissible that the proportion of animals sampled is relative to that defined population rather than the national population

- Each sample can be analysed for detecting the presence of one or more substances within a substance group. The use of multi-residue analytical methods is to be encouraged

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### Codex vs Council Directive 96/23/EC (1)



- Codex Alimentarius (Volume 3, 1995)
  - Random, statistically based sampling
  - Selection of substances - risk based
  - Assumes homogeneity in animal population
  - 300 samples should detect 1% violation prevalence with 95% Confidence Interval
  - Model used by many trading partners
    - Canada, USA, New Zealand, Brazil etc

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### Codex vs Council Directive 96/23/EC (2)



- Council Directive 96/23/EC
  - Targeted, prescriptive – proportion of national production
  - Mandatory substance groups – aims to detect illegal use
  - Little scope for risk assessment of substances
  - Can result in higher sample numbers vs. Codex

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### Third countries: Laboratories



- No specific requirement for third country laboratories to be accredited to ISO 17025
  - *If not, can equivalence be guaranteed?*
- Must be able to demonstrate 'fitness for purpose' otherwise certification requirements can not be met
- Must be capable of meeting Community MRLs and MRPLs (and, in the future, RPAs).

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### Third countries: Laboratories (1)



- Third country laboratories are not obliged to validate methods according to EU standards (Commission Decision 2002/657/EC)
- Should have validated methods (e.g. using other validation models Codex Alimentarius Volume 3)



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### Third countries: Laboratories (2)



- No specific requirement for third country to use national laboratories
- Work can be outsourced to another country
- Laboratories should be accredited to ISO 17025  
- If not, how can authorities have confidence in the results?

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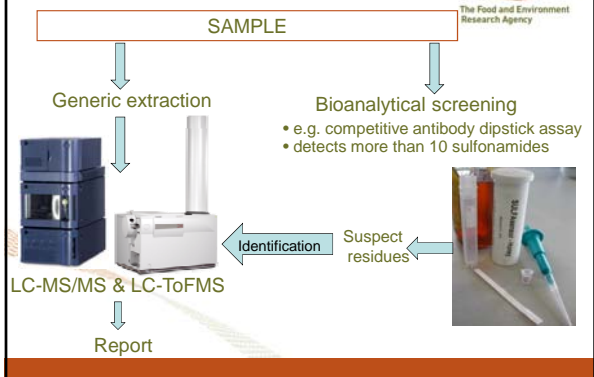
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### Analytical strategy for drugs



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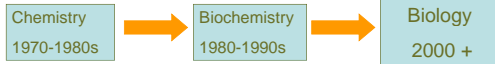
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## The need for rapid biological screening assays?



The requirement to rapidly test chemical contaminants has become a necessity

↑↑↑ Chemical residues, natural contaminants, public health, consumer confidence...



### Emerging life science technologies:

- ✓ Measurement of total biological activity
- ✓ Biomarker fingerprinting
- ✓ "omics" technologies

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## The need to validate?



Residue analysis answers 2 questions;

1. Is an analyte present in the sample?

### Analyte Identification

2. Is its concentration > Action Limit?

### Analytical Limits

(measurement uncertainty, detection capability etc.)

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## EU rules on analyte identification & validation



Commission Decision 2002/657/EC

- Method Validation
- Rules for selectivity
- Rules for identification



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Typical assay validation

2002/657/EC

Qualitative screening method



- “Methods used to detect the presence of a substance at the level of interest” (2002/657/EC)
- High sample through-put and used to screen large numbers of samples for potential non-compliant results
- **Low false compliant ('negative') rate at the MRL**

1. Repeatability (r)
2. Detection capability (**CC $\beta$** )
3. Specificity (analyte and matrix)
4. Ruggedness (applicability)
5. Analyte storage stability (solution and matrix)

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Typical assay validation 2002/657/EC

Quantitative, confirmatory assays



1. Familiarisation (analyst & second analyst)
2. Specificity (analyte and matrix)
3. Calibration curve
4. Recovery or trueness
5. Repeatability (r)
6. Within-laboratory reproducibility
7. Reproducibility (R)
8. Decision limit (**CC $\alpha$** ) (similar, but not the same as an LOD)
9. Detection capability (**CC $\beta$** ) (similar, but not the same as an LOQ)
10. Ruggedness (applicability)
11. Analyte storage stability (solution and matrix)

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:221:0008:0036:EN:PDF>

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EU rules on identification & validation - specificity

*Too many words*



A method shall be able to distinguish between the analyte and the other substances under the experimental conditions. An estimate to which extent this is possible has to be provided. Strategies to overcome any foreseeable interference with substances when the described measuring technique is used, e.g. homologues, analogues, metabolic products of the residue of interest have to be employed. It is of prime importance that interference, which might arise from matrix components, is investigated

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**EU rules on identification & validation specificity cont.**



A method shall be able to **distinguish** between the **analyte** and the **other substances** under the experimental conditions. An estimate to which extent this is possible has to be provided. Strategies to overcome any foreseeable interference with substances when the described measuring technique is used, e.g. **homologues, analogues, metabolic products** of the residue of interest have to be employed. It is of prime importance that interference, which might arise from **matrix components**, is investigated

The Key Words!

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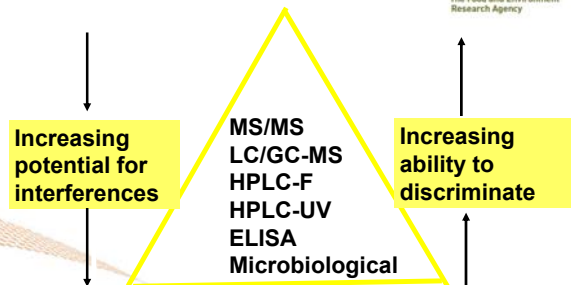
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**Discrimination depends on method**




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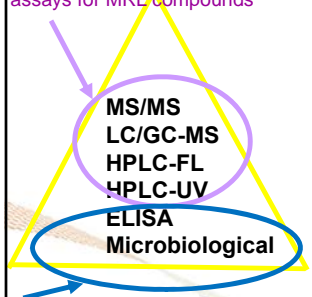
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**Method Discrimination (2)**



Suitable for use as confirmatory assays for MRL compounds



HPLC -UV/visible: Single wavelength

- only suitable for Group B (MRL) compounds in Annex 1 of 96/23/EC
- if 2 different chromatographic systems are employed,
- or 2 independent detection methods are used

Suitable for screening assays only

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### Discrimination of methods (3)



#### HPLC with fluorescence detection

- Group B compounds (Annex 1 of 96/23/EC) only
- Only for naturally fluorescent molecules and fluorescent products of derivatisation and/or transformation

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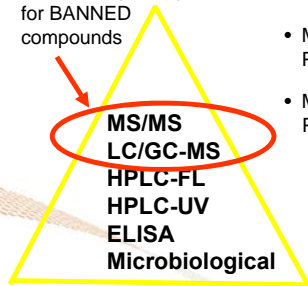
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### Method Discrimination (4)



Suitable for use as confirmatory assays for BANNED compounds



- Must score  $\geq 3$  Identification Points (Group B compounds)
- Must score  $\geq 4$  Identification Points (Group A compounds)

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### Relationship between mass fragment classes & identification points:



MS Technique	IPs earned per ion
Low resolution (LR) MS	1.0
LR-MS <sup>n</sup> Precursor ion	1.0
LR-MS <sup>n</sup> Transition products	1.5
High resolution (HR) MS	2.0
HR-MS <sup>n</sup> Precursor ion	2.0
HR-MS <sup>n</sup> Transition products	2.5

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## EU Contact points at CRLs



### BVL-CRL

Beta-agonists  
Anthelmintics  
Anticoccidials including Nitroimidazoles  
Non-steroidal anti-inflammatory drugs (NSAIDs)

Germany

<http://fis-vl.bund.de/Public/irc/fis-vl/Home/main?index>

### ISS-CRL

Chemical elements

Italy

<http://www.iss.it/lcdr>

### RIVM-CRL

Stilbenes, stilbene derivatives  
and their salts and esters  
Antithyroid agents  
Steroids  
Resorcylic Acid Lactones  
(RALs) including zeranol  
Sedatives  
Mycotoxines

The Netherlands

<http://www.rivm.nl/residues>

### AFSSA-ERMVD-CRL

Antibacterial substances,  
including sulphonamides and  
quinolones  
Dyes  
Carbadox and olaquinox  
Chloramphenicol  
Dapsone  
Nitrofuranes

France

<http://crl.fougeres.afssa.fr>

## Other possible sources of help



- EU National Reference Laboratories
- Consolidated list of NRLs from:

<http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1998D0536:20070101:EN:PDF>

## Further training / proficiency testing schemes



School for Advanced Residue Analysis in Food – France  
<http://www.saraf-educ.org/>

Training in residue methods - UK  
<http://www.fera.defra.gov.uk/training/>

Proficiency Testing schemes – essential for ISO17025 accreditation

[www.fapas.com](http://www.fapas.com)

<http://www.progettotrieste.com/>