Premi®Test – fast & easy antibiotic screening

Speaker: Gilbert Garrido
Date: 29.09.2011
Place: Almaty, Kasachstan
Introduction

Technology

Premi®Test

Detection Limits

Instructions

Business

Summary

Premi®Test

08.04.2011

Introduction

- diseases
- antibiotics
- too short withdrawal time
- antibiotic residues in animal products
- Health risk for consumers
- Surveillance programs: NOEL -> ADI -> MRLs

How to test?
LEGISLATION

**Permitted substances**

- **NOEL**
  - No Observed Effect Level
- **ADI**
  - Acceptable Daily Intake
- **MRL**
  - Maximum Residue Limit

**Tests systems**

- **Validation**
- **LOD**
  - Limit of Detection

**Prohibited substances**

- **Prohibition**
- **MRPL**
  - Minimum Residue Performance Limit

Monitoring of compliance by NRKP and ERKP.
## EC 37/2010 example: Acetylsalicylic acid

<table>
<thead>
<tr>
<th>Animal species</th>
<th>MRL</th>
<th>Target Tissue</th>
<th>Other provisions 14(7) EC 470/2009</th>
<th>Therapeutic classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>All food producing species except fin fish</td>
<td>No MRL required</td>
<td>Not applicable</td>
<td>Not for use in animals from which milk or eggs are produced for human consumption.</td>
<td>No entry</td>
</tr>
</tbody>
</table>

MRL = Maximum Residue Limit
EC 37/2010 example: Ampicillin

<table>
<thead>
<tr>
<th>Animal species</th>
<th>MRL</th>
<th>Target Tissue</th>
<th>Other provisions 14(7) EC 470/2009</th>
<th>Therapeutic classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>All food producing</td>
<td>50 µg/kg</td>
<td>Muscle</td>
<td>For fin fish the muscle MRL relates to ‘muscle and skin in natural proportions’. MRLs for fat, liver and kidney do not apply to fin fish. For porcine and poultry species the fat MRL relates to ‘skin and fat in natural proportions’. Not for use in animals from which eggs are produced for human consumption.</td>
<td>Anti-infectious agents/ Antibiotics</td>
</tr>
<tr>
<td></td>
<td>50 µg/kg</td>
<td>Fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 µg/kg</td>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 µg/kg</td>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 µg/kg</td>
<td>Milk</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MRL = Maximum Residue Limit
EC 37/2010 Prohibited substances

<table>
<thead>
<tr>
<th>Pharmacologically active substance</th>
<th>MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aristolochia spp.</em> and preparations thereof</td>
<td>MRL can not be established</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform</td>
<td>-</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>-</td>
</tr>
<tr>
<td>Colchicine</td>
<td>-</td>
</tr>
<tr>
<td>Dapsone</td>
<td>-</td>
</tr>
<tr>
<td>Dimetridazole</td>
<td>-</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>-</td>
</tr>
<tr>
<td>Nitrofurans (including furazolidone)</td>
<td>-</td>
</tr>
<tr>
<td>Ronidazole</td>
<td>-</td>
</tr>
</tbody>
</table>

-> MRPL etablished
Premi® Test

screening test for the detection of antibiotic residues in animal products in less than 4 hours
Premi®Test technology

**NEGATIVE**
- pH-Indicator Bromocresol
- Bacillus stearothermophilus

**POSITIVE**
- antibiotic residues
Premi®Test consists of:

- Polystyrene package
- 25 Test-Ampoules
- Perforated foil
- 1 Syringe
- 30 Tipps
- Manual in 6 languages
- Colour chart
**Premi® Test**

**Test Date:** 08.04.2011

**Performance Tested by AOAC**

**RBP 31/02 – 04/11**

**ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS**

**Certified by AFNOR Certification**

**www.afnor-validation.com**

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### Detection Limits

<table>
<thead>
<tr>
<th>Substance</th>
<th>Poultry</th>
<th>Pork</th>
<th>Beef</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-lactams</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracyclins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrolides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulphonamides</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**µg / kg = ppb**

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**Instructions**

**Business Summary**

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**Introduction Technology Premi® Test**

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**www.r-biopharm.com**

**R-Biopharm Group**

Argentina • Australia • Austria • Brazil • Canada
China • France • Germany • Italy • Netherlands
Spain • Switzerland • UK • USA

**R-BioPharm Rhone LTD**

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**β-lactams**

**Penicillin**

---

**Macrolides**

**Tetracyclins**

**Sulphonamides**
# Premi®Test

## Detection Limits

<table>
<thead>
<tr>
<th>Substance</th>
<th>Poultry</th>
<th>Pork</th>
<th>Beef</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polypeptides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virgintamycin</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Zn-bacitracin</td>
<td>1250</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Colistin</td>
<td>&gt;1000</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td><strong>Ionophores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salinomycin</td>
<td>1000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monensin</td>
<td>1250</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>Oligosaccharides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avilamycin</td>
<td>&gt;5000</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florphenicol</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Chloramphenicol**</td>
<td>2500</td>
<td>0</td>
<td>2500</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Narasin</td>
<td>1250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amprolull</td>
<td>&gt;2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphomycin</td>
<td>&gt;1500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furazolidone**</td>
<td>&gt;1500</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamycin</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1500</td>
<td>1500</td>
<td>500</td>
</tr>
<tr>
<td>Neomycin</td>
<td>300</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td><strong>Quinolones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxolinic acid</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>&gt;600</td>
<td>100</td>
<td>&gt;600</td>
</tr>
<tr>
<td>Flumequine</td>
<td>&gt;100</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Danofoxacin</td>
<td>200</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

**Note:** µg / kg = ppb
Workflow

Sample Preparation

Matrices:
- Meat (beef, pork, poultry)

Incubation
- Kidney & Liver
- Egg
- Feed (pork/poultry)

Colour Reading
- Plasma/Scrum
What you need for Premi®Test:

- Starter Kit
  - Briefcase
  - Incubator
  - Meatpress
  - Scissors
  - Lab-Timer
Sample preparation

Cut off the required number of ampoules (number of samples + negative control)

Essential:
Negative control = sample of the same matrix/species which contains no antibiotics
Sample preparation

Take approx. 2 cm³ of meat and use the meatpress to extract meatjuice.
Sample preparation

In case you have to analyze a large number of samples, the Multipress allows to squeeze 12 samples at once.
Instructions for use

Pipette 100 μl of the sample slowly onto the agar in the ampoule.
Instructions for use

Allow to stand at room temperature for 20 minutes for pre diffusion of the antibiotic residues into the agar.
Instructions for use

Flush the sample away by gently washing the test twice with demineralised water and remove the water from the test carefully.
Close the test ampoule with the supplied foil to avoid evaporation and start incubation.
Sample preparation: Meat, fish, shrimps

1. Squeeze the sample with the meatpress or the multipress to obtain juice
2. Freeze and thaw the sample and collect the released juice
3. Incubate the samples in a ziplock bag in a waterbath at 64°C for 10 / 5 min and collect the released juice (preservatives?)

Sample preparation: Egg

1. Homogenize whole egg
Sample preparation: Kidney

1. Cut the kidney horizontally.
2. Cut a piece of around 1-2 cm³ out of the medulla.
3. Squeeze the kidney sample with the meatpress or Multipress to obtain ‘kidney’ juice

Sample preparation: Liver

1. Grind the liver to obtain a paste
2. Dilute the liver-paste 1:1 with demineralised water
3. Mix well to a homogeneous mixture
Sample preparation: poultry/pig feed

1. Grind the feed.
2. Mix 10 g of the grinded feed with 30 ml of demineralized water
3. Stir for half an hour at room temperature

problems with complex matrices (rice bran)

Sample preparation: Honey

protocol in validation process
Sample preparation – Urine, Plasma

Premi®Test Urine
20 ampoules for the pretreatment of cattle/pork urine or serum/plasma
workflow

1. Sample Preparation
2. Incubation
3. Colour Reading
Incubation

1-Step Incubation
meat, fish, liver, urine, serum
approx. 3 h at 64 °C

2-Step Incubation
shrimps, egg, feed, kidney
10 min at 80 °C
approx. 3 h at 64°C
Workflow

- Sample Preparation
- Incubation
- Colour Reading
Withdraw the sample ampoules from the incubator when the negative control changed its colour into yellow (approx. 3 h).
Determination of results: by eye

- **positive**
  - conc. > LOD

- **pos/neg**
  - conc. = LOD

- **negative**
  - conc. < LOD
Determination of results: Premi®Scan
Software for interpretation & documentation of results

Premi®Scan
• Premi®Scan Software
• 2 templates
• Kodak colour chart for calibration
• Black Cloth
• not supplied: Scanner
Determination of results: Premi®Scan

<table>
<thead>
<tr>
<th>Position</th>
<th>Description</th>
<th>z-value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Sample 1</td>
<td>-12.35</td>
<td>NEG</td>
</tr>
<tr>
<td>B1</td>
<td>Sample 2</td>
<td>11.84</td>
<td>POS</td>
</tr>
</tbody>
</table>
## Premi®Test publications

<table>
<thead>
<tr>
<th>Title</th>
<th>Authors(s)</th>
<th>Journal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of Premi®Test for the detection of sulphonamide residues in chicken eggs</td>
<td>Hussein K.a, Marcinc(ak S.a, Máté D.a, Kozárová Ivona, Sokol J.a, Zdolec N.b</td>
<td>Acta veterinaria 2005, vol. 55, iss. 5-6, pp. 493-500.</td>
</tr>
</tbody>
</table>
R-Biopharm product portfolio for antibiotic testing

Premi®Test:
broad spectrum antibiotic screening test

RIDASCREEN®:
ELISAs for specific antibiotic screening

EASY EXTRACT® Immunoaffinity columns for use in conjunction with an HPLC or LC-MS/MS system
Telivago Premi® Test antibiotic screening for all matrices excluding dairy

Delvo® Test antibiotic screening for dairy products

Premi® Test Salmonella Salmonella serotyping

Production

Distribution

Situation until 31st Dec 2010

Premi® Test

Delvo® Test

Premi® Test

Business Summary
Premi®Test products

Premi®Test 25 R3925

Premi®Test 4x25 R3900

Premi®Test Urine R3920

Starter Kit ZPT2000

Multipress ZPT2012

Premi®Scan ZPT2010
SUMMARY

Premi®Test is

- **FAST** < 4 hours (test implementation: ½ h + incubation: 3 h)
- **EASY** simple handling and no cost intensive equipment needed
- **SENSITIVE** detects antibiotic residues in line with MRL’s
- **RELIABLE**
Thank you for your Attention