



Improvements to the Screening of Antimicrobial Drug Residues in Food by the use of the Premi®Test

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The Need

Improved antimicrobial screening because of the large amount of residue screening required and the limitations of existing chemical and biological methods.

The Solution

Combination of multi-residue solvent based extraction and the microbial inhibition test, Premi®Test.

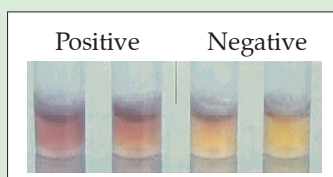
Capabilities & Developments

- Proven to reliably detect a wide range of tetracyclines, sulphonamides, β -lactams and macrolides at or below half MRLs in muscle and kidney.
- Offers high daily sample throughput (only 3-4 hour incubation) and inexpensive.
- Other matrices including egg, milk, fish muscle, processed meats and honey have been evaluated using this validated integrated strategy.

Test Principle

Premi®Test is based on inhibition of the growth *Bacillus stearothermophilus*, a thermophilic bacteria very sensitive to many antibiotics and sulpha compounds. A standardised number of spores are embedded in an agar medium with selected nutrients. When Premi®Test is heated at 64°C, the spores will germinate. The germinated spores will multiply with the production of acid when no inhibitory substances are present. This is visible by a colour change from purple to yellow mediated by the indicator Bromocresol purple, added to the agar medium. When antibacterial compounds are present above the Limit of Detection (LOD), no growth occurs and the colour remains purple.

Premi®Test Colour Responses



Extraction Protocol for Muscle Tissue

Sample (2g)

- ↓ 1. Homogenise in acetone/nitrate/acetone (70:30%).
2. Centrifuge.
3. Remove supernatant, evaporate to near dryness under nitrogen at 40-45°C.

Extract

- ↓ Resuspend residue using 200 μ L broth medium. Incubate ~3.5 hours at 64°C until negative control changes colour.

Results

Compound	Limit of Detection in pig muscle (ug / kg)	MRL in muscle (ug / kg)
Beta-Lactams		
Penicillin G	<2.5	50
Ampicillin	<2.5	50
Amoxicillin	<2.5	50
Oxacillin	<50	300
Cloxacillin	<50	300
Cefazolin	100	1000
Cefacetrile	<100	1000
Cephalonium	<100	1000
Ceftiofur	<100	1000
Cephapirin	<100	1000
Cephalexin	100	1000
Cefoperazone	<100	1000
Tetracyclines		
Chlortetracycline	50	100*
Oxytetracycline	50	100*
Tetracycline	25-50	100*
Demecocycline	25-50	100*
Doxycycline	25-50	100*
Sulphonamides		
Sulfaguanidin	100	100**
Sulfadimethoxine	<25	100**
Sulfapyridine	100	100**
Sulfamethizole	<50	100**
Sulfamethoxypridine	<50	100**
Sulfisoxazole	<25	100**
Sulfathiazole	<25	100**
Sulfadiazine	<50	100**
Sulfachloropyridazine	<50	100**
Sulfamerazine	<50	100**
Sulfanilamide	100	100**
Sulfaquinoxaline	<50	100**
Sulfamethazine	50-75	100**
Others		
Tylosin	12.5	100
Chloramphenicol	500-1000	Annex IV
Dapsone	<2.5	Annex IV
Erythromycin	<100	200
Lincomycin	<100	100

Table 1. Minimum detection levels for some of the compounds tested on Premi®Test in spiked Porcine muscle

Bold indicates LOD is at or below half MRL
 * Sum of parent drug and its epimer.
 ** Combined total residues of all substances within the sulfonamide group should not exceed 100 ug/kg.

Conclusions

The results demonstrate that :-

- Premi®Test in combination with the multi-residue solvent extraction detects a broad spectrum of anti-microbial compounds at or below the EC Maximum residue limits.
- Premi®Test integrated strategy detects anti-microbial compounds at or below the EC MRL in a broad range of food products including meat, eggs, fish and honey.