

# PREMI®TEST

## BROAD SPECTRUM SCREENING TEST

### FOR THE DETECTION OF ANTIBACTERIAL COMPOUNDS IN MEAT, FISH AND EGGS

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#### Introduction

Medication of farm animals to cure or prevent diseases will lead to residues of the medicine in meat for a certain period of time. Growing concerns over health related issues and over drug resistant bacteria have led to an increased demand for a simple and reliable test system. DSM has developed a new test, especially for detection of anti microbial compounds in meat, and meat products: **Premi®Test**.

#### Test principle

Premi®Test is based on inhibition of the growth of *Bacillus stearothermophilus*, a thermophilic bacterium very sensitive to many antibiotics and sulpha compounds. A standardised number of spores is imbedded 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 in case no inhibitory substances are present. This will be visible by a colour change from purple to yellow of the indicator Bromocresol purple, added to the agar medium. When antibacterial compounds are present in sufficient amount (above the detection level) no growth will obtain and the colour remains purple.



**Colour range of the Premi® Test.**



#### Validation of the Premi®Test

Premi®Test is validated both in our laboratory as well as at several certificated external institutes. Presented are 3 external studies executed by the national reference laboratory of the UK, Central Science Laboratory, the University of Utrecht (NL) and TNO-Voeding (NL). In a study at Central Science Laboratory in York, the sensitivity of Premi®Test for a wide range of relevant anti microbial compounds is examined in spiked samples. Premi®Test is validated at the University of Utrecht for its suitability to screen fish from sea- and freshwater, cold and warm-water species with low and high fat content on the presence of anti-microbial agents commonly used in aquaculture. DSM validated Premi®Test for its suitability to screen eggs on the presence of anti microbial compounds commonly used in the poultry industry. DSM has developed a special patented sampling procedure to screen eggs. Premi®Test together with the egg sample is heated 10 minutes at 80°C to inactivate natural inhibiting compounds. In a study executed at TNO-Voeding, Premi®Test is validated with samples of tissue from pigs treated with relevant anti microbial compounds. After slaughtering muscle tissue is collected and analysed with the Premi®Test and the Four-Plate Test (FPT). The results of these validation studies are presented.

#### Spiked samples

Table 1. Minimum detection levels of Premi®Test (µg/l)

Antibiotic		LOD in pork Muscle	MRL in muscle tissue
<i>β-lactams</i>	Amoxicillin	5	50
	Ampicillin	5	50
	β-Penicillin	<2.5	50
	Oxacillin	100	300
<i>Tetracycline's</i>	Chlortetracycline	>100	100
	Oxytetracycline	75-100	100
	Doxycycline	50	100
	Tetracycline	50	100
	Demecocycline	50	-
<i>Sulphonamides</i>	Sulfadimethoxine	25-50	100
	Sulfapyridine	50	100
	Sulfamethizole	50-100	100
	Sulfamethoxypropyridine	25	100
	Sulfisoxazole	25	100
	Sulfathiazole	25	100
	Sulfadiazine	50	100
	Sulfachloropyridazine	25	100
	Sulfamerazine	25	100
	Sulfanilamide	150	100
	Sulfaquinaxaline	50	100
	Sulphamethazine	50-100	100
	<i>Other</i>	Spiramycin	>200
Tylosin		<25	100
Bacitracin		150-200	150*
Streptomycin		1000-1500	500
	Erythromycin	50-75	200

\* MRL in Milk  
S. Stead, M. Sharman, Central Science Laboratory, York, United Kingdom

#### Fish trial

As a screening test the Premi®Test offers a first tool to assay fish for the presence of relevant residues of antibacterial substances.

The Premi®Test showed successfully the presence of incurred Sulfadiazine in fillets containing the residues near or above the limit of detection.

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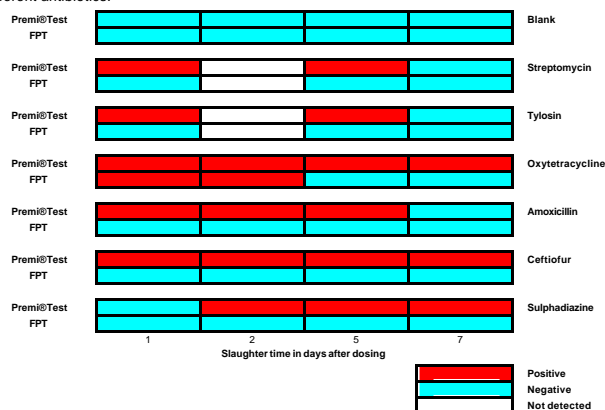
#### Egg trial

Premi®Test can detect a large range of anti microbial compounds in egg at or around the MRL. The test is very sensitive for β-lactams, macrolides, sulphonamides and amino glycosides. The sensitivity of the test for tetracyclines is above the MRL.

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#### Pig trial

Figure 1. Comparison of Premi®Test and the FPT in samples of muscle from animals treated with different antibiotics.



*C. Arts, R. Wilkamp, TNO-Voeding, The Netherlands*

#### Conclusion

The results demonstrate that:  
Premi®Test detects a broad spectrum of anti microbial compounds at or below the EC Maximum Residue Level  
Premi®Test detects anti microbial compounds at or below the EC Maximum Residue Level in a broad range of food products (meat, fish, eggs)