

## POULTRY, MEAT, FISH AND EGG SCAN

Antibiotics are used in animal husbandry for the treatment and prevention of diseases. To avoid undesired residues of these substances in edible tissues, maximum residue limits (MRL's) have been established in the EU. Products produced in the EU have to fulfill these residue requirements. Self-monitoring by the meat-industry, farmers and retailers is an important tool to ensure the consumer safety with regard to antibiotic residues.

For this purpose RIKILT developed the SCAN tests (SCAN = SCreening ANTibiotics). The SCAN tests are novel microbiological screening methods for the detection of antibiotic residues in fresh meat (poultry, pig and bovine), fish and eggs. The tests are ideal for use 'on site'. The tests are:

- fast and reliable; reliable result are obtained in less than 12 hours (overnight incubation)
- able to classify the antibiotic residue: The tests enable class identification (e.g. tetracyclines, penicillines, quinolones etc).
- suited for on-site application: easy handling; only an incubator and a pipette is needed to conduct the test, no apparatus is needed to interpret the results
- cheap

Furthermore:

- positive control samples are included; positive control samples guarantee the performance of the tests; comparison with the positive control samples gives a first insight in the level of contamination (= semi-quantitative test)



Figure 1: an example of the POULTRY SCAN test

The SCAN test allows farmers, slaughterhouses, processors and retailers to cost-effectively screen on the presence of residues of antibiotics in poultry, meat, fish and eggs. The SCAN tests are extremely sensitive, and are detecting a large variety of antibiotic residues in these products. The detection limits are at or below the maximum residue levels set by the European Union.

The SCAN tests are based on the direct application of meat fluid (meat, poultry or fish drip) or homogenized egg onto a microbiological plate system. The system comprises a separate test plate for each class of antibiotic (tetracyclines, quinolones, sulphonamides, macrolides/ $\beta$ -lactams). The detection of  $\beta$ -lactams and macrolides is combined in one test.

Meat fluid can be obtained in several ways:

- freezing and defrosting
- gentle heating (60°C)
- pressing with a garlic press

The meat juice (or homogenized egg) is pipetted into holes in the test plate. Each test plate contains 9 holes, allowing the simultaneous analysis of 8 samples and 1 positive control sample. After the holes are filled, a drop of a specific reagent is added to each hole and the test plates are incubated overnight at 30°C.

The presence of antibiotic-residues is shown by the formation of zones of inhibition around the holes. (see Figure 1). The test plate showing the largest inhibition zone reveals the class identity of the antibiotic present. Confirmation by instrumental analytical methods (LC-MS) is necessary to verify whether EU MRLs are exceeded.

The positive control sample should give a zone of inhibition. If this is not the case, the analysis is not valid and has to be repeated.

Absence of inhibition zones indicates the absence of residues of veterinary drugs exceeding the legal limits in meat or eggs (see appendix).

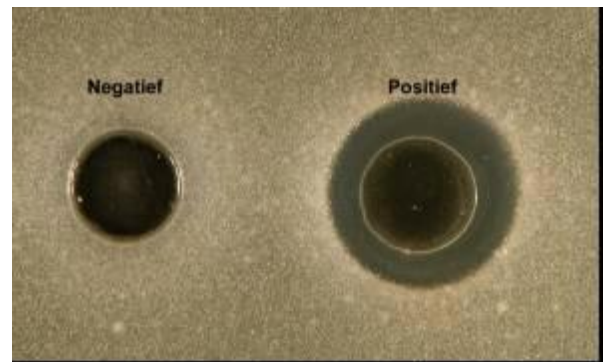


Figure 1: A sample without inhibition zone (negative) and a sample with inhibition zone (positive)

The POULTRY and MEAT SCAN tests are validated according to EU guideline 2002/657/EC. The detection capacities (95% limits of detection) of the methods are given in the Appendix.

### History and current use

The Poultry SCAN test is developed and validated in 2002/2003 and commercially available since 2004. Currently the test is recommended by the Dutch Poultry processing industry for their self-control program on antibiotic residues. In the 2005 approximately 25.000 chicken breasts were screened with the POULTRY SCAN test for tetracyclines or quinolones.

Since the introduction of the Poultry SCAN test in 2002, new tests have been developed, respectively Meat SCAN (2003), Fish SCAN (2004) and Egg Scan (2005). The production of these tests for the Dutch market is growing.

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## Appendix

Table 1 : Overview of EU MRL's and detection limits of **POULTRY-SCAN**.

Drug	EU MRL (µg/kg)	95% limit of detection (µg/g meat)	Drug	EU MRL (µg/kg)	95% limit of detection (µg/kg meat)
<b>Quinolones (test 1)</b>			<b>Macrolides (test 4)</b>		
Flumequine	0.4	0.1	Tylosin	0.1	0.1
Enrofloxacin	0.1	0.025	Lincomycin	0.1	0.1
Difloxacin	0.3	0.05	Tiamulin	0.1	0.1
Oxolinic acid	0.1	0.05	Josamycin	0.2	0.1
<b>Tetracyclines (test 2)</b>			Spiramycin	0.2	0.1
Oxytetracycline	0.1	0.05	Tilmicosin	0.075	0.05
Tetracycline	0.1	0.05	Erythromycin	0.2	0.05
Doxycycline	0.1	0.025	<b>Penicillines (test 4)</b>		
Chlortetracycline	0.1	0.025	Penicillin	0.05	0.025
<b>Sulphonamides + diamino-pyrimidines (test 3)</b>			Amoxicillin	0.05	0.05
Sulphamethazin	0.1	<0.1*	Ampicillin	0.05	0.025
Sulphadiazin	0.1	<0.1*	<b>Aminoglycosides (test 5)**</b>		
Sulphamethoxazole	0.1	<0.1*	Aminosidin	0.5	0.1
Trimethoprim	0,05	<0.05*	DH-streptomycin	0.5	0.1
			Neomycin	0.5	0.1
			Kanamycin	0.1	0.1-0.2

\* = not yet fully validated

\*\* requires additional sample preparation, therefore not commercially available

Table 2 : Overview of EU MRL's for pork and meat and detection limits of **MEAT-SCAN**.

Drug	EU MRL (µg/g meat)	95% limit of detection (µg/g meat)	Drug	EU MRL (µg/g meat)	95% limit of detection (µg/g meat)
<b>Quinolones (test 1)</b>			<b>Macrolides (test 4)</b>		
Flumequine	0,2	0.1	Lincomycin	0.1	0.1
Enrofloxacin	0,1	0.025	Erythromycin	0.4	0.05
Difloxacin	0,4	0.05	Tilmicosin	0.05	0.05
Danofloxacin	0,1 <sup>B</sup> /0,2 <sup>P</sup>	0.05	Tylosin	0.1	0.1
Oxolinic acid	0,1	0.05	Josamycin	0.2	0.1
Marbofloxacin	0,15	0.02	Spiramycin	0.2 <sup>B</sup> /0.25 <sup>P</sup>	0.1
<b>Tetracyclines (test 2)</b>			Pirlimycin	0.1	0.05
Oxytetracycline	0,1	0.05	<b>Penicillines (test 4)</b>		
Doxycycline	0,1	0.025	Ampicillin	0.05	0.025
Chlortetracycline	0,1	0.025	Penicillin	0.05	0.025
Tetracycline	0,1	0.05	Amoxicillin	0.05	0.05
<b>Sulphonamiden + diamino-pyrimidines (test 3)</b>			Cloxacillin	0.3	0.6
Sulphadiazine	0,1	<0.1*	Oxacillin	0.3	0.1
Sulphamethazine	0,1	<0.1*	Nafcillin	0.3	0.05
Sulphamethoxazole	0,1	<0.1*	<b>Aminoglycosides (test 5)**</b>		
Sulphadimethoxine	0,1	<0.1*	Neomycin	0.5	0.25
Trimethoprim	0,05	<0.05*	Aminosidin	0.5	0.25
Baquiloprim		<0.1*	Kanamycin	0.1	0.1-0.2
<b>Cephalosporines (test 4)</b>			DH-streptomycin	0.5	0.25
Cephquinom	0,05	0.05	Apramycin	1	0.5
Ceftiofur	1	1	Gentamicin	0.05	0.05
Cephapirin	0,05	0.1			

\* = not yet fully validated

\*\* requires additional sample preparation, therefore not commercially available

<sup>B</sup>= bovine, <sup>P</sup> = pork

Table 3: Overview of EU MRL's and detection limits of **FISH-SCAN**.

Drug	EU MRL (µg/g fish)	95% limit of detection (µg/g fish)
<b>Quinolones (test 1)</b>		
Flumequine	0.6	0.2
Enrofloxacin	-	0.005
Sarafloxacin	0.03	0.03
Oxolinic acid	0.1	0.1
<b>Tetracyclines (test 2)</b>		
Oxytetracycline	-	0.075
Doxycycline	-	0.05
Chlortetracycline	-	0.05
Tetracycline	-	0.075

 Table 4 : Overview of EU MRL's and detection limits of **EGG-SCAN**.

Drug	EU MRL (µg/g egg)	95%-limit of detection (µg/g egg)	Stof	EU MRL (µg/g egg)	95%-limit of detection (µg/g egg)
<b>Quinolones (test 1)</b>			<b>Macrolides (test 4)</b>		
Oxolinic acid	-	0.05	Erythromycin	0.15	0.02
Flumequine	-	0.15	Tylosin	0.2	0.1
Enrofloxacin	-	0.015	Josamycin	0.2	0.3
Danofloxacin	-	0.015	Spiramycin	0.2	0.1
Difloxacin	-	0.05	Lincomycin	0.05	0.1
			Tiamulin	1	0.75
			Tilmicosin	-	0.05
<b>Tetracyclines (test 2)</b>			<b>Penicillines (test 4)</b>		
Chlortetracycline	0.2	0.05	Amoxicilline	-	0.02
Tetracycline	0.2	0.1	Penicilline	-	0.02
Oxytetracycline	0.2	0.1	Ampicilline	-	0.02
Doxycycline	-	0.05			
<b>Sulphonamides (test 3)*</b>			<b>Aminoglycosides (test 5)*</b>		
Sulphaclozin	-	0.075	Neomycin	0.5	0.25
Sulphacl.pyridazin	-	0.1	DH streptomycin	-	0.5
Sulphamethoxazol	-	0.05			
Sulphadiazin	-	0.05	<b>Polypeptides (test 6)*</b>		
Sulphaquinoxalin	-	0.05	Colistin	0.3	0.15
Sulphamethazin	-	0.075			
<b>Cephalosporines (test 4)</b>					
Ceftiofur	-	0.1			
Cephapirin	-	0.1			
Cephalexin	-	0.75			
Cephquinom	-	0.075			

- requires additional sample preparation therefore not commercially available