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Ministerie van Landbouw, Natuur en Voedselkwaliteit

Screening for antibiotic residues in slaughter animals:

comparison of the EU-four plate method, Nouws Antibiotic Test and Premi®Test

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Introduction

Microbial growth inhibition tests are widely used as the primary screening approach for the detection of antibiotic residues in slaughter animals and as such form the foundation of the residue monitoring system. Within the European Union, no harmonized approach exists with respect to screening methods for antibiotic residues. As a consequence, detection capabilities of the methods used vary widely, and the effectiveness of monitoring and surveillance therefore highly depends on the applied method. In this study we evaluated and compared the performance of the EU-four plate method (EU4pt), the Nouws Antibiotic Test (NAT), and Premi®Test applied to both muscle and kidney, by parallel analysis of 735 slaughter animals.

Procedure

The NAT comprises five test plates, specific for either tetracyclines (T), macrolides & β-lactam antibiotics (B&M), quinolones (Q), sulfonamides & diaminopyrimidines (S) or aminoglycosides (A)[1]. The analysis is performed on paper disks impregnated with renal pelvis fluid. EU4pt consists of three *B. subtilis* based test plates (pH 6, 7.2 (+TMP) and 8) and a *K. rhizophila* pH 8 plate[2]. The analysis is performed using meat disks. Premi®Test was performed essentially according to the manufacturers instructions, using meat or kidney fluid as matrix. Suspect samples were analyzed with physicochemical methods like HRLC-ToF-MS and LC-MS/MS.

Results: non-compliant

Non-compliant results	Matrix	MRL (μg kg^{-1})	EU4pt	NAT	Premi®Test	
					muscle	kidney
Sulfamethazine (1100 μg kg^{-1})	muscle	100	-	+	+	+
Doxycycline (305 μg kg^{-1})	muscle	100	+	+	-	+
Tulathromycin* (648 μg kg^{-1})	muscle	-	+	+	-	+
Gentamicin (870 μg kg^{-1})	kidney	750	-	-	-	+
Neomycin (5207 μg kg^{-1})	kidney	5000	-	+	-	+

**Technically this is not a non-compliant result, since no MRL has been established for tulathromycin in muscle, but since the other carcass MRL (skin plus fat) is set at 100 μg kg^{-1} we consider this sample non-compliant.*

Conclusions

- EU4pt: very limited sensitivity
- Premi®Test-muscle: limited sensitivity, high false-positive rate

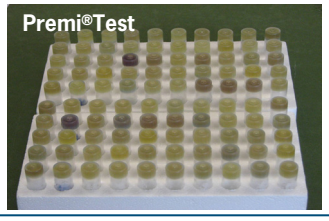
→ *EU4pt and Premi®Test muscle are considered inappropriate for screening purposes*

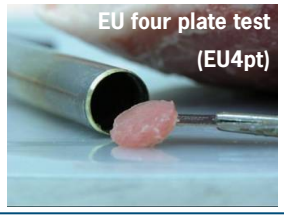
- NAT: excellent sensitivity for tetracycline residues, aminoglycoside detection variable, due to matrix (renal pelvis fluid) variation. Antibiotic group identification results in reduced confirmatory efforts.
- Premi®Test-kidney: satisfactory sensitivity for aminoglycosides, sensitivity for tetracyclines remains questionable, will be subject of additional research.

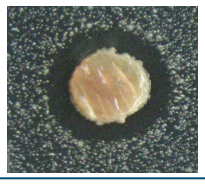
Results: overview

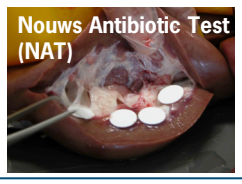
	EU4pt				NAT				PremiTest (muscle)				PremiTest (kidney)			
	suspect	false-positive	<MRL	>MRL	suspect	false-positive	<MRL	>MRL	suspect	false-positive	<MRL	>MRL	suspect	false-positive	<MRL	>MRL
total	2				36[I]				9	8			30	13		
tetracyclines	1			1	24	9[II]	14[III]	1							1	1
aminoglycosides					10[IV]	1	8	1[V]							11[IV]	2[V]
sulfonamides					1			1				1				1
macrolides	1			1	1			1								1
beta-lactams																
quinolones																

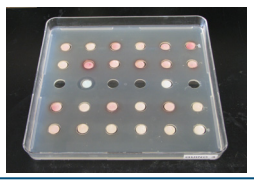
[I]NAT results from initial renal pelvis-fluid analysis, [II]negative kidney post-screening results, no physicochemical data, [III]in routine analysis only 8 out of these 14 would be forwarded to chemical confirmation (kidney post-screening result > control: 600 μg/kg oxytetracycline), [IV]overlap between NAT and Premi®Test aminoglycoside containing samples is limited to 4 animals, [V]chemical confirmation is performed on muscle, except for aminoglycosides

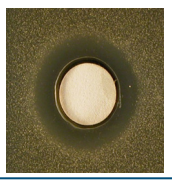












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References

[1] Pikkemaat et al. (2008) Food Control 19, p781
[2] Bogaerts & Wolf (1980) Fleischwirtschaft 60, p667

Acknowledgement

This research was (partly) financed by the Dutch Ministry of Agriculture, Nature and Food Quality