



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
060601

The AOAC Research Institute hereby certifies the method known as:

Premi[®] Test
manufactured by
DSM PREMI[®] Test B. V.
P. O. Box 6500
6401 JH HEERLEN
The Netherlands

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*SM Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date	December 19, 2022
Expiration Date	December 31, 2023

AUTHORS Angelique de Rijk	MANUFACTURER DSM Nutritional Products DSM Premi® Test BV P. O. Box 6500 6401 JH Heerlen The Netherlands	CERTIFICATION MARK LICENSEE R-Biopharm AG An der Neuen Bergstraße 17, 64297 Darmstadt, Germany
METHOD NAME Premi® Test	CATALOG NUMBERS R3925, R3900	
INDEPENDENT LABORATORY Central Science Laboratory (CSL) York YO41 1LZ, England USDA Agricultural Research Service Eastern Regional Research Center Microbial Biophysics and Residue Chemistry Research Unit 600 East Mermaid Lane Wyndmoor, PA 19038, USA	AOAC EXPERTS AND PEER REVIEWERS Joe Boison ¹ , Michael Murphy ² ¹ Canadian Food Inspection Agency, Saskatchewan, CANADA ² University of Minnesota, Minnesota, USA	
APPLICABILITY OF METHOD Target analyte – Penicillin G		
Matrix – Bovine muscle tissue		
Performance claims – See Table 1		
ORIGINAL CERTIFICATION DATE September 27, 2006	CERTIFICATION RENEWAL RECORD Renewed annually through December 2023.	
METHOD MODIFICATION RECORD 1. November 2019 Level 1 2. December 2022 Level 1	SUMMARY OF MODIFICATION 1. Editorial/clerical changes. 2. Editorial/clerical changes.	
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PRINCIPLE OF THE METHOD (1)

The Premi®test is based on the inhibition of the growth of *Bacillus Stearothermophilus*, a bacterium which is very sensitive to many antibiotics and sulfa compounds. A standardized number of spores are imbedded in an agar medium with selected nutrients.

A small amount of meat juice is added to the test tube. After a pre-incubation period of 20 minutes at room temperature (18-22 °C i.e. 64.4-71.6 °F), the test tube is washed and incubated for approximately 3 hours at 64°C (147°F).

At this temperature, the spores will start to grow (germinate) and multiply with the production of acid if no antimicrobial (non-inhibitory) substances are present in the meat juice. This will be visible by a color change in the test tube from purple to yellow. When antimicrobial (inhibitory) substances are present (at or above the detection sensitivity), no growth will occur and the color in the test tube will remain purple.

DISCUSSION OF THE VALIDATION STUDY (1)

The results show that PremiTest met all the requirements for this AOAC validation study for the detection of penicillin G residues in bovine muscle tissue. The results also show that there is cross reactivity with many other antimicrobial compounds. PremiTest is a broad-spectrum screening test which can detect many relevant antimicrobials in meat.

Table 1: Summary of performance parameters of Premi®Test for penicillin G obtained in the study (1)

Laboratory	Study	Performance Pen G (ppb)	Remarks
CSL	Part 1: 6 out of 6	10	A range of 5 different concentrations from 0 – 50 ppb was tested. At 10 ppb, all 6 of 6 samples analysed tested positive.
USDA	Part 2: 30 out of 30	10	Results confirmed that the detection sensitivity of the PremiTest for pen G was 10 ppb with 95% confidence level
DSM	Part 3: Ruggedness, interference, cross-reactivity	10	The presence of other antimicrobial drugs did not interfere with the PremiTest kits ability to detect pen G residues at a concentration of 10 ppb added to bovine muscle tissue.
USDA	Part 4: Incurred tissue	10	The detection sensitivity determined using fortified tissue samples was further confirmed using incurred bovine muscle tissues. PremiTest was able to detect all pen G residues in incurred samples containing pen G at concentrations greater than or equal to 12 ppb .

Table 9: Scanner results to confirm the Premi®Test’s detection sensitivity as 10 ppb for penicillin G (1)

Zero control			
Sample	Interpreted Scanner Results	Sample	Interpreted Scanner Results
	NEG		
1	NEG	9	
2	NEG	10	
3	NEG	11	NEG
4	NEG	12	NEG
5	NEG	13	NEG
6	POS	14	NEG
7	NEG	15	NEG
8			NEG
Penicillin G 10 ppb			
Sample	Interpreted Scanner Results	Sample	Interpreted Scanner results
	POS		POS
1	POS	16	POS
2	POS	17	POS
3	POS	18	POS
4	POS	19	POS
5	POS	20	POS
6	POS	21	POS
7	POS	22	POS
8	POS	23	POS
9	POS	24	POS
10	POS	25	POS
11	POS	26	POS
12	POS	27	POS
13	POS	28	POS
14		29	
15		30	

REFERENCE CITED

- de Rijk, Angelique., Validation Study Report Premi®Test, AOAC Performance Tested MethodsSM certification number 060601.