



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

060601

The AOAC Research Institute hereby certifies that the performance of the test kit 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[®] *Performance Tested MethodsSM* Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance TestedSM* certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (November 12, 2018 – December 31, 2019). Renewal may be granted at the end of one year 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

November 12, 2018

Date

METHOD AUTHORS
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SUBMITTING COMPANY
DSM Nutritional Products
DSM Premi® Test BV
P. O. Box 6500
6401 JH Heerlen
The Netherlands

CURRENT SPONSOR
R-Biopharm AG
An der Neuen Bergstraße 17, 64297
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KIT NAME(S)
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
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AOAC EXPERTS AND PEER REVIEWERS
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APPLICABILITY OF METHOD
Target analyte – Penicillin G

Matrices – Bovine muscle tissue

Performance claims – See Table 1

ORIGINAL CERTIFICATION DATE
September 27, 2006

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2019

METHOD MODIFICATION RECORD
NONE

SUMMARY OF MODIFICATION
NONE

Under this AOAC® *Performance Tested*SM License Number, 060601 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*SM License Number, 060601 this method is distributed as:
NONE

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 Premi® Test 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. Premi® Test 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 TestedSM certification number 060601.