



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

060601

The AOAC Research Institute hereby certifies the performance of 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[®] 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 (October 23, 2021 – December 31, 2022). 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

October 23, 2021

Date

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|---|--|---|
| METHOD 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, Saskatoon, SK, Canada ² University of Minnesota, Minneapolis, MN, 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 2022. | |
| METHOD MODIFICATION RECORD 1. November 2019 Level 1 | SUMMARY OF MODIFICATION 1. Editorial changes to inserts. | |
| Under this AOAC® Performance TestedSM License Number, 060601 this method is distributed by: NONE | Under this AOAC® Performance TestedSM 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 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

1. de Rijk, Angelique., Validation Study Report Premi®Test, AOAC® Performance TestedSM certification number 060601.