CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

112401

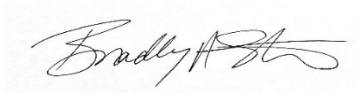
The AOAC Research Institute hereby certifies the method known as:

11+Myco MS-PREP® with LC-MS/MS

manufactured by

R-Biopharm Rhône Ltd.
Block 10 Todd Campus
West of Scotland Science Park, Acre Rd.
Glasgow G20 0XA Scotland

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*[SM] Program. 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.



Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date	November 11, 2024
Expiration Date	December 31, 2025

METHOD NAME	CATALOG NUMBER	ORIGINAL CERTIFICATION DATE
11+Myco MS-PREP® with LC-MS/MS	RBRP128 (10 columns), RBRP128B (50 columns)	November 01, 2024

PRINCIPLE OF THE METHOD

The 11⁺Myco MS-PREP Immunoaffinity Column contains a gel suspension of monoclonal antibodies specific to the toxins of interest. Following extraction of the toxins, the extract is filtered, diluted, and passed slowly through the immunoaffinity column. Any toxins present in the sample are retained by the antibodies within the gel suspension. The column is washed to remove unbound material, and the toxins are then released from the column following elution with solvent. The eluate is collected prior to analysis by LC-MS/MS.

The total extraction and clean up takes approximately 60 minutes to perform and multiple samples can be processed simultaneously. The result is improved clean up and concentration of the toxins from food and feed samples, reducing ion suppression and removing the need for matrix-matched standards. This provides cleaner chromatography, improved sensitivity, and greater accuracy. The columns also have the added advantage that they can be automated for large scale analysis of samples.

A 5 g test portion of ground sample is subjected to a 50% acetonitrile extraction by shaking under controlled conditions. Following filtration and dilution in aqueous buffer, the diluted extract may be further filtered before being added directly to the 11⁺Myco MS-PREP IAC. The prepared extract is then allowed to pass through the IAC at approximately 2 mL/min where any toxins present in the sample are retained by the antibodies within. Unbound material is then washed from the column using an ammonium acetate buffer. Finally, the captured toxins are released from their antibodies by an elution process using 100% methanol which is completed by an equivalent volume of deionized water.

The LC-MS/MS analysis employs a gradient LC method with Electrospray Ionization in positive polarity using Scheduled Multiple Reaction Monitoring (sMRM). For each batch run, a calibration curve is analyzed before and after the sample injections. A mid-point standard is injected several times (≥6) before the first calibration curve and at regular intervals amongst the samples. Diluent injections are included at the start of the sequence as well as before and after the calibration curves to check for carryover.

CERTIFIED CLAIM STATEMENT: The 11+Myco MS-PREP with LC-MS/MS method is certified for the determination and confirmation of Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1, Aflatoxin G_2, Aflatoxin M_1, Deoxynivalenol, Fumonisin B_1, Fumonisin B_2, Fumonisin B_3, Ochratoxin A, T-2, HT-2, and Zearalenone within the scope of Tables 1, 2 and 3.

***Certification includes**:*

1. Agilent 1260 Infinity II series LC, G7112B Binary pump, G7129A Vial sampler and G7116A MCT column oven with QTRAP 5500 MS/MS (SCIEX, Framingham, MA), Analyst software version 1.7.2, Analyst Device Driver, and MultiQuant version 3.0.3 (Method Developer).
2. Waters Acquity UPLC with Waters Xevo TQ-S Instrument (Waters Limited, Stamford Avenue, Altrincham Road, Wilmslow, SK9 4AX) (Independent Laboratory).
3. Luna Omega 3 µm Polar C18 column, 100 x 3 mm, Phenomenex (Torrance, CA) Cat. No. 00D-4760-Y0.

Table 1. Method Performance Claims

Matrix[a]	Test Portion	Extraction Solvent	Range, µg/kg	Performance supporting certification			
				LOD[b], µg/kg	LOQ[b], µg/kg	Recovery, %	RSD_r, %
Aflatoxin B_1							
Corn	5 g	50% ACN	0.5-5.9	0.013-0.023	0.040-0.070	93-122	3.5-9.7
Wheat	5 g	50% ACN	1.4-12	0.013	0.040	105-115	2.0-38
Cereal-based infant food with dairy	5 g	50% ACN	0.06-0.20	0.008	0.024	99-116	3.1-6.0
Cereal-based infant food without dairy	5 g	50% ACN	0.05-0.21	0.006	0.018	94-103	2.5-6.8
Animal feed (cereal-based)	5 g	50% ACN	1.9-33.4	0.046-0.047	0.14	57-123	1.1-33
Paprika	5 g	50% ACN	1.2-4.4	0.024	0.073	76-96	5.5-12
Chili powder	5 g	50% ACN	1.2-5.2	0.11	0.32	95-103	3.4-8.0
Aflatoxin B_2							
Corn	5 g	50% ACN	0.44-6.1	0.013-0.023	0.070	106-128	3.1-7.5
Wheat	5 g	50% ACN	0.83-5.7	0.023	0.070	99-119	4.0-35
Cereal-based infant food with dairy	5 g	50% ACN	0.05-0.20	0.012	0.037	100-108	4.1-8.3
Cereal-based infant food without dairy	5 g	50% ACN	0.06-0.22	0.013	0.039	102-115	2.4-7.7
Animal feed (cereal-based)	5 g	50% ACN	0.83-33	0.043-0.093	0.13-0.28	75-121	3.6-33
Paprika	5 g	50% ACN	0.15-4.8	0.047	0.14	96-101	4.3-11
Chili powder	5 g	50% ACN	1.2-4.9	0.047	0.14	92-99	2.4-6.2
Aflatoxin G_1							
Corn	5 g	50% ACN	0.5-6.1	0.013-0.023	0.040-0.070	101-126	3.3-8.6
Wheat	5 g	50% ACN	0.10-5.6	0.010	0.040	104-116	2.0-32
Cereal-based infant food with dairy	5 g	50% ACN	0.05-0.20	0.006	0.019	99-103	3.5-5.3
Cereal-based infant food without dairy	5 g	50% ACN	0.05-0.21	0.013	0.038	93-104	2.5-4.8
Animal feed (cereal-based)	5 g	50% ACN	0.22-33	0.047	0.14	82-109	3.1-30
Paprika	5 g	50% ACN	1.3-4.7	0.023	0.070	83-101	5.6-13
Chili powder	5 g	50% ACN	1.2-5.0	0.023	0.070	94-100	2.5-9.1
Aflatoxin G_2							
Corn	5 g	50% ACN	0.43-6.1	0.023	0.070	106-128	2.9-5.8
Wheat	5 g	50% ACN	1.0-5.7	0.023	0.070	109-124	1.6-4.6
Cereal-based infant food with dairy	5 g	50% ACN	0.05-0.20	0.012	0.036	99-103	2.8-7.0
Cereal-based infant food without dairy	5 g	50% ACN	0.05-0.21	0.013	0.038	97-107	3.6-9.2
Animal feed (cereal-based)	5 g	50% ACN	2.1-33	0.047-0.093	0.14-0.28	83-110	2.5-29
Paprika	5 g	50% ACN	0.80-4.9	0.047	0.14	99-103	4.6-11
Chili powder	5 g	50% ACN	0.88-4.9	0.047	0.14	91-100	2.3-8.3
Total Aflatoxins (B_1 + B_2 + G_1 + G_2)							
Corn	5 g	50% ACN	1.9-24	NA[b]	NA	100-126	2.5-7.6
Wheat	5 g	50% ACN	5.9-22	NA	NA	104-118	2.4-38
Cereal-based infant food with dairy	5 g	50% ACN	0.2-0.8	NA	NA	100-107	2.0-4.5
Cereal-based infant food without dairy	5 g	50% ACN	0.2-0.85	NA	NA	87-110	1.6-5.1
Animal feed (cereal-based)	5 g	50% ACN	8.0-130	NA	NA	58-109	1.1-31
Paprika	5 g	50% ACN	4.8-19	NA	NA	94-100	4.2-11.5

Chili powder	5 g	50% ACN	4.7-20	NA	NA	93-99	2.4-8.0
Aflatoxin M_1							
Cereal-based infant food with dairy	5 g	50% ACN	0.05-0.20	0.012	0.036	99-106	3.0-7.6
Deoxynivalenol							
Corn	5 g	50% ACN	329-1670	1.3-6.0	4.0-18	106-123	1.4-15.4
Wheat	5 g	50% ACN	433-1833	3.7	11	104-141	1.9-3.4
Cereal-based infant food with dairy	5 g	50% ACN	98-380	2.1	6.3	95-98	1.5-2.5
Cereal-based infant food without dairy	5 g	50% ACN	103-409	2.4	7.1	95-118	2.4-3.6
Animal feed (cereal-based)	5 g	50% ACN	410-5710	25-26	75-77	79-133	1.4-65
Paprika	5 g	50% ACN	55-227	2.7	8.0	109-115	1.8-4.3
Chili powder	5 g	50% ACN	46-206	2.7	8.0	92-103	2.5-4.3
Fumonisin B_1							
Corn	5 g	50% ACN	111-1320	3.3-5.4	10-16	84-115	3.6-8.4
Wheat	5 g	50% ACN	264-1030	3.3	10	90-112	1.9-11
Cereal-based infant food with dairy	5 g	50% ACN	57-228	1.7	5.0	98-103	0.5-2.4
Cereal-based infant food without dairy	5 g	50% ACN	52-220	1.6	4.9	87-100	1.6-4.3
Animal feed (cereal-based)	5 g	50% ACN	161-9662	12	35-37	70-115	1.9-13
Paprika	5 g	50% ACN	53-211	6.7	20	92-98	4.2-7.6
Chili powder	5 g	50% ACN	56-233	6.7	20	81-95	3.2-6.4
Fumonisin B_2							
Corn	5 g	50% ACN	111-665	2.7-2.9	8.0-8.6	84-114	3.2-8.8
Wheat	5 g	50% ACN	138-535	2.7	8.0	85-94	3.5-7.8
Cereal-based infant food with dairy	5 g	50% ACN	27-117	1.3	4.0	98-100	1.7-4.7
Cereal-based infant food without dairy	5 g	50% ACN	28-119	1.3	4.0	90-101	2.5-3.6
Animal feed (cereal-based)	5 g	50% ACN	174-3420	2.8-11	8.2-32	76-110	2.5-11
Paprika	5 g	50% ACN	26-107	5.3	16	90-95	2.6-9.4
Chili powder	5 g	50% ACN	28-110	5.3	16	93-96	7.6-8.5
Fumonisin B_3							
Corn	5 g	50% ACN	14-300	1.3	3.8-4.0	98-114	4.5-9.9
Wheat	5 g	50% ACN	63-243	1.3	4.0	88-93	3.6-11
Cereal-based infant food with dairy	5 g	50% ACN	11-46	0.67	2.0	84-86	2.4-3.1
Cereal-based infant food without dairy	5 g	50% ACN	13-53	0.67	2.0	89-100	2.7-3.9
Animal feed (cereal-based)	5 g	50% ACN	109-1580	2.6-5.3	7.7-16	81-120	2.0-7.1
Paprika	5 g	50% ACN	13-52	2.7	8.0	96-99	4.3-8.1
Chili powder	5 g	50% ACN	11-45	2.7	8.0	82-98	2.2-6.9
Total Fumonisins ($B_1 + B_2 + B_3$)							
Corn	5 g	50% ACN	237-2280	NA	NA	95-114	2.3-8.7
Wheat	5 g	50% ACN	464-1810	NA	NA	90-102	2.1-10
Cereal-based infant food with dairy	5 g	50% ACN	97-391	NA	NA	97-98	1.1-4.2
Cereal-based infant food without dairy	5 g	50% ACN	93-393	NA	NA	90-98	1.7-3.5
Animal feed (cereal-based)	5 g	50% ACN	848-13,900	NA	NA	81-114	1.3-12
Paprika	5 g	50% ACN	92-370	NA	NA	92-95	2.6-5.4
Chili powder	5 g	50% ACN	95-388	NA	NA	94-97	2.7-6.4

Ochratoxin A							
Corn	5 g	50% ACN	0.50-7.0	0.10-0.13	0.30-0.38	94-122	2.5-7.1
Wheat	5 g	50% ACN	1.9-25	0.10	0.30	115-129	4.0-23
Cereal-based infant food with dairy	5 g	50% ACN	0.35-1.1	0.050	0.15	114-138	2.1-4.0
Cereal-based infant food without dairy	5 g	50% ACN	0.32-1.2	0.050	0.15	100-127	2.6-4.8
Animal feed (cereal-based)	5 g	50% ACN	2.8-164	0.26-0.50	0.77-1.5	83-112	2.6-14
Paprika	5 g	50% ACN	9.3-36	0.72	2.1	91-118	6.2-24
Chili powder	5 g	50% ACN	8.9-37	1.5	4.4	89-97	2.2-18
T-2							
Corn	5 g	50% ACN	4.6-24	0.33-0.64	1.0-1.9	93-120	3.1-9.5
Wheat	5 g	50% ACN	5.6-436	0.33	1.0	98-112	4.5-11
Cereal-based infant food with dairy	5 g	50% ACN	2.6-11	0.17	0.50	104-107	2.6-4.2
Cereal-based infant food without dairy	5 g	50% ACN	2.5-10	0.17	0.50	93-104	2.1-5.5
Animal feed (cereal-based)	5 g	50% ACN	14-837	1.3-2.5	3.8-7.5	75-112	2.3-47
Paprika	5 g	50% ACN	5.2-22	0.67	2.0	103-110	5.3-12
Chili powder	5 g	50% ACN	5.6-22	0.67	2.0	106-111	5.2-7.8
HT-2							
Corn	5 g	50% ACN	5.6-24	0.47-0.64	1.4-1.9	84-123	1.3-19
Wheat	5 g	50% ACN	5.9-253	0.47	1.4	96-128	1.7-8.2
Cereal-based infant food with dairy	5 g	50% ACN	2.9-11	0.23	0.70	107-114	1.9-5.4
Cereal-based infant food without dairy	5 g	50% ACN	2.7-10	0.23	0.70	96-109	1.4-2.5
Animal feed (cereal-based)	5 g	50% ACN	46-790	1.5-2.5	4.4-7.5	72-109	2.7-49
Paprika	5 g	50% ACN	6.5-22	1.0	3.0	110-129	3.1-6.1
Chili powder	5 g	50% ACN	5.7-21	1.0	3.0	106-115	2.8-5.7
Sum T-2 + HT-2							
Corn	5 g	50% ACN	10-47	NA	NA	102-121	1.9-9.8
Wheat	5 g	50% ACN	12-689	NA	NA	104-115	1.6-8.1
Cereal-based infant food with dairy	5 g	50% ACN	5.4-22	NA	NA	107-109	1.8-4.2
Cereal-based infant food without dairy	5 g	50% ACN	5.2-21	NA	NA	94-104	2.2-2.8
Animal feed (cereal-based)	5 g	50% ACN	88-1630	NA	NA	74-110	1.7-48
Paprika	5 g	50% ACN	12-43	NA	NA	109-116	3.9-6.4
Chili powder	5 g	50% ACN	11-43	NA	NA	105-113	3.2-5.5
Zearalenone							
Corn	5 g	50% ACN	30-246	1.3-1.8	4.0-5.4	99-130	2.2-10
Wheat	5 g	50% ACN	51-408	0.83	2.5	90-106	1.7-3.7
Cereal-based infant food with dairy	5 g	50% ACN	9.5-39	0.82	2.5	95-96	1.4-3.1
Cereal-based infant food without dairy	5 g	50% ACN	8.7-37	0.42	1.3	82-91	1.6-2.1
Animal feed (cereal-based)	5 g	50% ACN	35-581	2.3-2.4	7.0-7.2	66-109	2.3-40
Paprika	5 g	50% ACN	23-81	1.7	5.0	81-90	7.1-13
Chili powder	5 g	50% ACN	18-80	2.1	6.3	73-80	4.4-14.5

[a] All matrixes were tested by spiking. In addition, data include commercial QC materials for corn, wheat, cereal-based infant food without dairy, animal feed (DDGS and dog kibble), paprika, and chili powder for a variety of analytes. All spiked and QC materials for corn and animal feed were tested by both the method developer and the independent

laboratory. All other matrixes were tested by the method developer alone.

[b] Where a range is given, LOD and LOQ differed between the method developer and the independent laboratory for corn and/or animal feed.

[c] NA = Not applicable.

Table 2. Method Selectivity in Ground Corn

		No. Compounds Interfering	
Compounds	Concentration	Blank	Method Standard 7
4 non-target mycotoxins[a]	60-750 ng/mL	0	0

[a] Comprising sterigmatocystin, nivalenol, neosolaniol, and α–zearalenol.

Table 3. Column Capacity

Mycotoxin	Capacity (ng)
Total Aflatoxin (B_1:B_2:G_1:G_2 at 1:1:1:1)	450
Deoxynivalenol	2000
Total Fumonisins (B_1 + B_2 + B_3 at 4:2:1)	6000
Ochratoxin A	1400
Sum T-2 + HT-2 (1:1)	1050
Zearalenone	800

Table 4. Method History

No.	Date	Summary	Supporting Data
1	November 2024	Original Certification.	Certification Report