

Verification of Randox e- Investigator for Myco-7

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Timeline & Background Randox

- 2019, AAFCO LSMC approached to ask labs to participate in collaborative study for mycotoxins using e-Investigator
- Via lots of working together, came up with list, schedule, & samples
- 2019 results presented & met AOAC's requirements for collaborative study
- Several labs began purchasing, installation & training put on hold because of Covid
- UKDRS purchased, installation, & trained fall of 2021
- Learning curve
- Verified Myco-7
- Began verification of coccidiostats
- Learning curve
- Experienced: some delays, some internal, some shipping---Covid

Process of Verification, UKDRS

- We are ISO 17025:2017 Accredited (13 methods; 31 analytes)
- Verify instrument suitability & robustness (manufacturer, upon installation)
- Pre-approval of Method Validation/Verification Form: Customer's expectations written out, chemical & instrumentation method requirements
- Reference SOP vs UKDRS SOP
- Analytical Selectivity is met
- PTP data
- Calibration requirements, curve, R^2
- Accuracy & Bias using CRMs or QRMs
- LODs & LOQs and M_{us}
- Recoveries, Repeatability, Reproducibility
- Control Charts
- SOP Reviewer Checklist

Validation/Verification Process, continued

- Meet all ISO 17025:2017 requirements
- 6.2.6a: Development, modification, verification, & validation of methods
- Determination method meets performance requirements of Official Method
- Meets internal QMS
- Meets ISO requirements
- Signage of DOQ & LAD

Statistical Requirements

Recovery

- 1000 ppm, 90 – 108
- 100 ppm, 85 – 110
- 10 ppm, 80 – 115
- 1 ppm, 75 – 120
- 10 ppb, 70 - 125

Repeatability

- 1000 ppm, 2.80
- 100 ppm, 4.00
- 10 ppm, 5.60
- 1 ppm, 7.90
- 10 ppb, 15.80

Statistical Requirements

Reproducibility

- 1000 ppm, 5.60
- 100 ppm, 8.00
- 10 ppm, 11.20
- 1 ppm, 15.90
- 10 ppb, 31.70

Mycotoxins in Myco-7

- AB1
- AG1
- Fumonisin
- Ochratoxin
- Deoxynivalenol (DON) (Vomitoxin)
- T2 (Trichothecene mycotoxin)
- Zearalenone

Recovery AB1

Run	Sample type	UK Sample ID	UKDRS Found, ppb	Consensus Value, ppb	% Recovery
11/05/21	AAFCCO	2020-61	43.91	38.52	114.0
11/05/21	AAFCCO	2020-63	22.69	22.97	98.8
11/05/21	AAFCCO	2021-62	25.71	20.37	126.2
10/28/21	Trilogy CRM	A-C-2261	28.38	29.30	96.9
09/22/21	Trilogy CRM	A-C-2253	17.93	16.20	110.7
09/22/21	Trilogy CRM	MT-C-9999M	18.52	18.60	99.6

Recovery is calculated as per UK-QU-006 using:

$$\% \text{ Rec} = (\text{Found}) / (\text{Consensus PT value OR actual value}) * 100$$

$$\% \text{ Rec} = (\text{UKRDS Found, \%}) / (\text{Consensus Value, \%}) * 100$$

Acceptability: 70 - 125%

Recovery AG1

Run	Sample type	Sample ID	Found, ppb	Consensus Value, ppb	% Recovery
10/28/21	AAFCO	2020-61	5.13	2.743	186.9
10/28/21	AAFCO	2020-63	1.34	1.733	77.2
10/28/21	AAFCO	2021-61	9.57	8.348	114.7
11/05/21	AAFCO	2021-61	8.40	8.348	100.6

Recovery is calculated as per UK-QU-006 using:

$$\% \text{ Rec} = (\text{Found}) / (\text{Consensus PT value OR actual value}) * 100$$

$$\% \text{ Rec} = (\text{UKRDS Found, \%}) / (\text{Consensus Value, \%}) * 100$$

Acceptability: 70 - 125%

Recovery Fumonisin

Run	Sample type	Sample ID	Found, ppb	Consensus Value, ppb	% Recovery
10/28/21	AAFCO	2020-61	4727	4536	104
10/28/21	AAFCO	2020-63	967	1018	95
10/28/21	AAFCO	2021-61	3454	3291	105
09/22/21	AAFCO	2021-62	5471	5823	94
11/05/21	Trilogy CRM	F-C-478	1910	1900	101
09/22/21	Trilogy CRM	MT-C-9999M	3887	3100	125
Recovery is calculated as per UK-QU-006 using:					
$\% \text{ Rec} = (\text{Found}) / (\text{Consensus PT value OR actual value}) * 100$					
$\% \text{ Rec} = (\text{UKRDS Found, \%}) / (\text{Consensus Value, \%}) * 100$					
Acceptability: 90 -108%					

Recovery Ochratoxin

Run	Sample type	Sample ID	Found, ppb	Consensus Value, ppb	% Recovery
11/05/21	AAFCO	2020-61	143.54	151.4	94.8
09/22/21	AAFCO	2020-63	42.24	41.3	102.4
10/28/21	AAFCO	2021-61	8.56	8.6	99.6
11/05/21	AAFCO	2021-62	122.67	100.6	121.9
10/28/21	Trilogy CRM	O-C-875	11.80	11.4	103.5
10/28/21	Trilogy CRM	MT-C-9999M	14.00	14.1	99.3

Recovery is calculated as per UK-QU-006 using:

$$\% \text{ Rec} = (\text{Found}) / (\text{Consensus PT value OR actual value}) * 100$$

$$\% \text{ Rec} = (\text{UKRDS Found, \%}) / (\text{Consensus Value, \%}) * 100$$

Acceptability: 70-125%

Recovery DON

Run	Sample type	Sample ID	Found, ppb	Consensus Value, ppb	% Recovery
10/28/21	Trilogy	D-C-653	2839	2600	109.2
09/22/21	Trilogy	MT-C-9999M	1575	1700	92.6
11/05/21	AAFCO	2020-61	5587	5354	104.4
09/22/21	AAFCO	2020-63	1884	1879	100.3
11/05/21	AAFCO	2021-61	10376	8192	126.7
11/05/21	AAFCO	2021-62	1405	1475	95.2

Recovery is calculated as per UK-QU-006 using:

$$\% \text{ Rec} = (\text{Found}) / (\text{Consensus PT value OR actual value}) * 100$$

$$\% \text{ Rec} = (\text{UKRDS Found, \%}) / (\text{Consensus Value, \%}) * 100$$

Acceptability: 90-108

Recovery T2

Run	Sample type	Sample ID	Found, ppb	Consensus Value, ppb	% Recovery
11/05/21	AAFCO	2020-61	98.26	96.68	101.6
11/05/21	AAFCO	2020-63	63.91	60.57	105.5
11/05/21	AAFCO	2021-61	200.47	116.90	171.5
09/22/21	AAFCO	2021-62	159.86	146.10	109.4

Recovery is calculated as per UK-QU-006 using:

$$\% \text{ Rec} = (\text{Found}) / (\text{Consensus PT value OR actual value}) * 100$$

$$\% \text{ Rec} = (\text{UKRDS Found, \%}) / (\text{Consensus Value, \%}) * 100$$

Acceptability: 70-125%

Recovery Zearalenone

Run	Sample type	Sample ID	Found, ppb	Consensus Value, ppb	% Recovery
11/05/2021	Trilogy	Z-C-340	87.89	90.5	97.1
11/05/2021	Trilogy	MT-C-9999M	290.36	296.1	98.1
11/05/2021	AAFCO	2020-61	911.40	884.9	103.0
10/28/2021	AAFCO	2021-62	361.46	300.1	120.4

Recovery is calculated as per UK-QU-006 using:

$$\% \text{ Rec} = (\text{Found}) / (\text{Consensus PT value OR actual value}) * 100$$

$$\% \text{ Rec} = (\text{UKRDS Found, \%}) / (\text{Consensus Value, \%}) * 100$$

Acceptability: 70-125%

Repeatability AB1

Set	Sample type	Sample ID	Found, ppb	Average, ppb	RSDr, %	Horwitz RSDr, %	HORRATr
10/28/21	Trilogy RM	A-C-2261	28.38	25.95	13.24	13.74	0.96
11/05/21			23.52				
10/28/21	Trilogy RM	A-C-2253	17.93	16.82	9.34	14.66	0.64
11/05/21			15.71				
09/22/21	Trilogy RM	C-9999M	18.52	18.52	0.03	14.45	0.00
11/05/21			18.51				
10/28/21	AAFCO	2020-63	17.93	18.53	4.60	14.45	0.32
11/05/21			19.14				
10/28/21	AAFCO	2021-62	26.95	26.33	3.33	13.71	0.24
11/05/21			25.71				
10/28/21	AAFCO	2021-61	20.70	19.92	5.54	14.29	0.39
11/05/21			19.14				

Repeatability AB1, continued

RSD_r, %	$=(\sigma(\text{Found Values})/\text{Avg} * 100)$			C*	=Average/X
Horwitz RSD_r, %	$= C^{(-0.15)}$			HORR ATr	<math>=(\text{RSD}_{r, \%} / \text{Horwitz RSD}_{r, \%})</math>

*** C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.**

	X =	1000000000					
Acceptability: 15.80%							

Repeatability AG1

Set	Sample type	Sample ID	Found, ppb	Average, ppb	RSDr, %	Horwitz RSDr, %	HORRATr
10/28/21	AAFCO	2020-61	5.13	5.68	13.74	17.25	0.80
11/05/21			6.23				
10/28/21	AAFCO	2020-63	3.30	2.92	18.70	19.07	0.98
11/05/21			2.53				
10/28/21	AAFCO	2021-61	9.57	8.99	9.23	16.10	0.57
11/05/21			8.40				
09/22/21	AAFCO	2021-62	7.66	8.03	9.94	17.44	0.57
11/05/21			8.40				

Repeatability AG1, continued

$RSD_r, \% = (\sigma(\text{Found Values}) / \text{Avg} * 100)$	$C^* = \text{Average} / X$
Horwitz $RSD_r, \% = C^{(-0.15)}$	HORRATr $= (RSD_r, \% / \text{Horwitz } RSD_r, \%)$

* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.

X =	1000000000						
Acceptability:	15.80%						

Repeatability Fumonisin

Set	Sample type	Sample ID	Found, ppb	Average, ppb	RSDr, %	Horwitz RSDr, %	HORRAT r
11/05/21	Trilogy CRM	O-C-875	3387	3317	3.02	6.64	0.45
11/23/21			3246				
09/22/21	Trilogy CRM	A-C-2261	249	245	2.31	9.81	0.24
11/23/21			241				
09/22/21	Trilogy CRM	F-C-478	2236	2194	2.72	7.06	0.39
10/28/21			2152				
09/22/21	Trilogy CRM	Z-C-340	716	724	1.63	8.34	0.20
11/05/21			733				
10/28/21	Trilogy CRM	D-C-653	2230	2252	1.36	7.03	0.19
11/23/21			2273				
10/28/21	Trilogy CRM	A-C-2253	347	343	1.68	9.33	0.18
11/23/21			339				
09/22/21	Trilogy CRM	MT-C-9999M	3887	3758	4.87	6.51	0.75
10/28/21			3628				
09/22/21	AAFCO	2020-61	3959	3979	0.70	6.46	0.11
11/23/21			3999				

Repeatability Fumonisin, continued

Set	Sample type	Sample ID	Found, ppb	Average, ppb	RSDr, %	Horwitz RSDr, %	HORRATr
09/22/21	AAFCO	2021-61	4547	4615	2.07	6.32	0.33
10/28/21			4682				
11/05/21	AAFCO	2021-62	5493	5426	1.75	6.16	0.28
11/23/21			5359				
RSDr, %	=($\sigma(\text{Found Values})/\text{Avg} * 100$)			C* = Average/X			
Horwitz RSDr, %	= C ^(-0.15)			HORRATr = (RSDr, % / Horowitz RSDr, %)			
* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.							
	X =	1000000000					
Acceptability (1000 ppb): 7.90							
Acceptability (100 ppb): <15.80>7.90							

Repeatability Ochratoxin

Set	Sample type	Sample ID	Found, ppb	Average, ppb	RSD _r , %	Horwitz RSD _r , %	HORRAT _r
10/28/21	Trilogy CRM	O-C-875	11.80	10.84	12.62	15.66	0.81
11/05/21			9.87				
10/28/21	Trilogy CRM	MT-C-9999M	14.00	15.32	12.19	14.87	0.82
11/05/21			16.64				
10/28/21	AAFCO	2020-61	189.12	192.64	2.58	10.17	0.25
11/05/21			196.15				
09/22/21	AAFCO	2020-63	42.24	40.12	7.46	12.87	0.58
11/05/21			38.01				
09/22/21	AAFCO	2021-61	7.67	8.12	7.84	16.35	0.48
11/05/21			8.56				
09/22/21	AAFCO	2021-62	135.66	129.16	7.11	10.80	0.66
11/05/21			122.67				

Repeatability Ochratoxin, continued

RSD _r , %	$= (\sigma(\text{Found Values}) / \text{Avg} * 100)$	C*	= Average/X
Horwitz RSD _r , %	$= C^{(-0.15)}$	HORRAT _r	$= (\text{RSD}_{r, \%} / \text{Horwitz RSD}_{r, \%})$

* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.

X =	1000000000						
Acceptability:	15.80%						

Repeatability DON

Set	Sample type	Sample ID	Found, ppb	Average, ppb	RSDr, %	Horwitz RSDr, %	HORRATr
10/28/21	Trilogy	D-C-653	2839.43	2902.08	3.05	6.77	0.45
11/23/21			2964.73				
10/28/21	Trilogy	MT-C-9999M	1882.88	1821.58	4.76	7.26	0.66
11/05/21			1760.28				
09/22/21	AAFCO	2020-61	5382.68	5149.32	6.41	6.21	1.03
10/28/21			4915.97				
09/22/21	AAFCO	2020-63	1946.16	1924.64	1.58	7.20	0.22
10/28/21			1903.11				
09/22/21	AAFCO	2021-61	13351.56	12915.57	4.77	5.41	0.88
10/28/21			12479.58				
09/22/21	AAFCO	2021-62	1297.67	1351.29	5.61	7.59	0.74
10/28/21			1404.90				

Repeatability DON, continued

$RSD_r, \% = (\sigma(\text{Found Values}) / \text{Avg} * 100)$	$C^* = \text{Average} / X$
Horwitz $RSD_r, \% = C^{(-0.15)}$	HORRATr $= (RSD_r, \% / \text{Horwitz } RSD_r, \%)$

* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.

X =	1000000000						
Acceptability:	2.80%						

Repeatability T2

Set	Sample type	Sample ID	Found, ppb	Average, ppb	RSD _r , %	Horwitz RSD _r , %	HORRAT _r
09/22/21	Trilogy	MT-C-9999M	97	89.54	11.92	11.41	1.05
10/28/21			82				
10/28/21	AAFCO	2020-61	120	109.13	14.09	11.07	1.27
11/05/21			98				
09/22/21	AAFCO	2020-63	74	68.47	10.37	11.88	0.87
10/28/21			63				
10/28/21	AAFCO	2021-61	233	216.81	10.65	9.99	1.07
11/05/21			200				
09/22/21	AAFCO	2021-62	160	151.79	7.51	10.54	0.71
10/28/21			140				

Repeatability T2, continued

$RSD_r, \% = (\sigma(\text{Found Values}) / \text{Avg} * 100)$	$C^* = \text{Average} / X$
Horwitz $RSD_r, \% = C^{(-0.15)}$	HORRATr $= (RSD_r, \% / \text{Horwitz } RSD_r, \%)$

* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.

X =	1000000000						
Acceptability:	15.80%						

Repeatability Zearalenone

Set	Sample type	UK Sample ID	UKDRS Found, ppb	Average, ppb	RSDr, %	Horwitz RSDr, %	HORRA Tr
09/22/21	Trilogy	Z-C-340	132.90	134.45	1.63	10.73	0.15
10/28/21			136.00				
10/28/21	Trilogy	MT-C-9999M	290.36	315.99	11.47	9.44	1.21
11/05/21			341.61				
10/28/21	AAFCO	2020-61	911.40	1039.53	17.43	7.90	2.21
11/05/21			1167.65				
10/28/21	AAFCO	2021-61	2099.84	2182.02	5.33	7.07	0.75
11/05/21			2264.19				
09/22/21	AAFCO	2021-62	321.70	341.58	8.23	9.33	0.88
10/28/21			361.46				

Repeatability Zearalenone, continued

$RSD_r, \% = (\sigma(\text{Found Values}) / \text{Avg} * 100)$	$C^* = \text{Average} / X$
Horwitz $RSD_r, \% = C^{(-0.15)}$	HORRATr $= (RSD_r, \% / \text{Horwitz } RSD_r, \%)$

* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.

X =	1000000000						
Acceptability:	15.80%						

Reproducibility AB 1

Set	Sample type	UK Sample ID	Found, ppb	Avg, ppb	RSDR, %	Horwitz RSDR, %	HORRATR
09/22/2021	Trilogy RM	A-C-2261	36.316	29.41	21.96	26.96	0.81
10/28/2021	Trilogy RM	A-C-2261	28.382				
11/05/2021	Trilogy RM	A-C-2261	23.523				

Reproducibility is calculated as per UK-QU-006 using:

$$\text{RSDR, \%} = (\sigma (\text{Found, \%}) / (\text{Avg, \%})) * 100$$

$$C = \text{Average} / X$$

$$\text{Horwitz RSDR, \%} = \text{RSDR} = (2 * (C)^{-0.15})$$

$$\text{HORRATR} = (\text{RSDR, \%} / \text{Horwitz RSDR, \%})$$

* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.

$$X = 1000000000$$

Acceptability: 31.70%

Reproducibility AG1

Set	Sample type	Sample ID	Found, ppb	Avg, ppb	RSDR, %	Horwitz RSDR, %	HORRAT R
09/22/2021	AAFCO	2021-62	7.663	8.55	11.28	32.45	0.35
10/28/2021		2021-62	9.575				
11/05/2021		2021-62	8.402				

Reproducibility is calculated as per UK-QU-006 using:

$$\text{RSDR, \%} = (\sigma (\text{Found, \%}) / (\text{Avg, \%})) * 100$$

$$C = \text{Average} / X$$

$$\text{Horwitz RSDR, \%} = \text{RSDR} = (2 * (C)^{-0.15})$$

$$\text{HORRATR} = (\text{RSDR, \%} / \text{Horwitz RSDR, \%})$$

* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.

$$X = 1000000000$$

Acceptability: 31.70%

Reproducibility Fumonisin

Set	Sample type	Sample ID	Found, ppb	Avg, ppb	RSDR, %	Horwitz RSDR, %	HORRATR
10/28/21	Trilogy RM	F-C-478	2151.864	2040.78	5.97	14.27	0.42
11/05/21	Trilogy RM	F-C-478	1910.429				
11/23/21	Trilogy RM	F-C-478	2060.043				

Reproducibility is calculated as per UK-QU-006 using:

$$\text{RSDR, \%} = (\sigma(\text{Found, \%}) / (\text{Avg, \%})) * 100$$

$$C = \text{Average} / X$$

$$\text{Horwitz RSDR, \%} = \text{RSDR} = (2 * (C)^{-0.15})$$

$$\text{HORRATR} = (\text{RSDR, \%} / \text{Horowitz RSDR, \%})$$

* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.

$$X = 1000000000$$

Acceptability:

5.60

Reproducibility Ochratoxin

Set	Sample type	UK Sample ID	Found, ppb	Avg, ppb	RSDR, %	Horwitz RSDR, %	HORRATR
09/22/2021	Trilogy RM	O-C-875	7.341	9.67	23.14	31.86	0.73
10/28/2021	Trilogy RM	O-C-875	11.802				
11/05/2021	Trilogy RM	O-C-875	9.869				

Reproducibility is calculated as per UK-QU-006 using:

$$\text{RSDR, \%} = (\sigma (\text{Found, \%}) / (\text{Avg, \%})) * 100$$

$$C = \text{Average} / X$$

$$\text{Horwitz RSDR, \%} = \text{RSDR} = (2 * (C)^{-0.15})$$

$$\text{HORRATR} = (\text{RSDR, \%} / \text{Horowitz RSDR, \%})$$

* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.

$$X = 10000000000$$

Acceptability: 31.70%

Reproducibility DON

Set	Sample type	Sample ID	Found, ppb	Avg, ppb	RSDR, %	Horwitz RSDR, %	HORRAT R
09/22/21	Trilogy CRM	D-C-653	2218	2230	3.30	14.09	0.23
10/28/21			2162				
11/05/21			2308				

Reproducibility is calculated as per UK-QU-006 using:

RSDR, % = $(\sigma \text{ (Found, \%)} / (\text{Avg, \%})) * 100$							
C = Average/X							
Horwitz RSDR, % = $\text{RSDR} = (2 * (C)^{-0.15})$							
HORRATR = $(\text{RSDR, \%} / \text{Horowitz RSDR, \%})$							

* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.

X = 1000000000							
Acceptability: 5.60%							

Reproducibility T2

Set	Sample type	Sample ID	Found, ppb	Avg, ppb	RSDR, %	Horwitz RSDR, %	HORRAT R
09/22/21	Trilogy CRM	MT-C-9999M	97.1	94.26	11.80	22.64	0.52
10/28/21			103.7				
11/05/21			82.0				

Reproducibility is calculated as per UK-QU-006 using:

$$\text{RSDR, \%} = (\sigma (\text{Found, \%}) / (\text{Avg, \%})) * 100$$

$$C = \text{Average} / X$$

$$\text{Horwitz RSDR, \%} = \text{RSDR} = (2 * (C)^{-0.15})$$

$$\text{HORRATR} = (\text{RSDR, \%} / \text{Horowitz RSDR, \%})$$

* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.

$$X = 1000000000$$

Acceptability: 31.70%

Reproducibility Zearalone

Set	Sample type	UK Sample ID	Found, ppb	Avg, ppb	RSDR, %	Horwitz RSDR, %	HORRATR
09/22/2021	Trilogy CRM	A-C-2253	12.0	12.34	18.91	30.72	0.62
10/28/2021			11.3				
11/05/2021			10.4				
11/23/2021			15.7				

Reproducibility is calculated as per UK-QU-006 using:

$$\text{RSDR, \%} = (\sigma (\text{Found, \%}) / (\text{Avg, \%})) * 100$$

$$C = \text{Average} / X$$

$$\text{Horwitz RSDR, \%} = \text{RSDR} = (2 * (C)^{-0.15})$$

$$\text{HORRATR} = (\text{RSDR, \%} / \text{Horowitz RSDR, \%})$$

* C is a mass fraction see UK-QU-006, Table 2 for value of dividend in relation to concentration.

$$X = 1000000000$$

Acceptability: 31.70%

Conclusions

- Determined that Myco-7 using e-Investigator meets UKDRS' customers needs
- Also have analyzed samples using SOA method (LC/MS/MS), numbers are in agreement
- Still working on coccidiostat verification
- Timeline: End of February complete
- Will analyze samples analyzed via Official method
- Not complicated method but must PAY ATTENTION!
- Learning from you at this meeting too!

Next Steps

- Move forward with verification of Coccidiostats
- Next mycotoxin sampling season, analyze mycotoxins via Radox e-Investigator

Thanks to:

- Lauren Smith, Laboratory Accreditation Specialist & Microbiologist
- Ryan Baldwin, Research Analyst & Safety Committee Chair
- Kristin Brock, Supervisor Milk & Specialty Feeds
- Jenny Combs, AFRPS Specialist, Jack of all Trades

Questions?