MYCOTOXINS IN FEED

New York State
Department of Agriculture and Markets
Food Laboratory Division

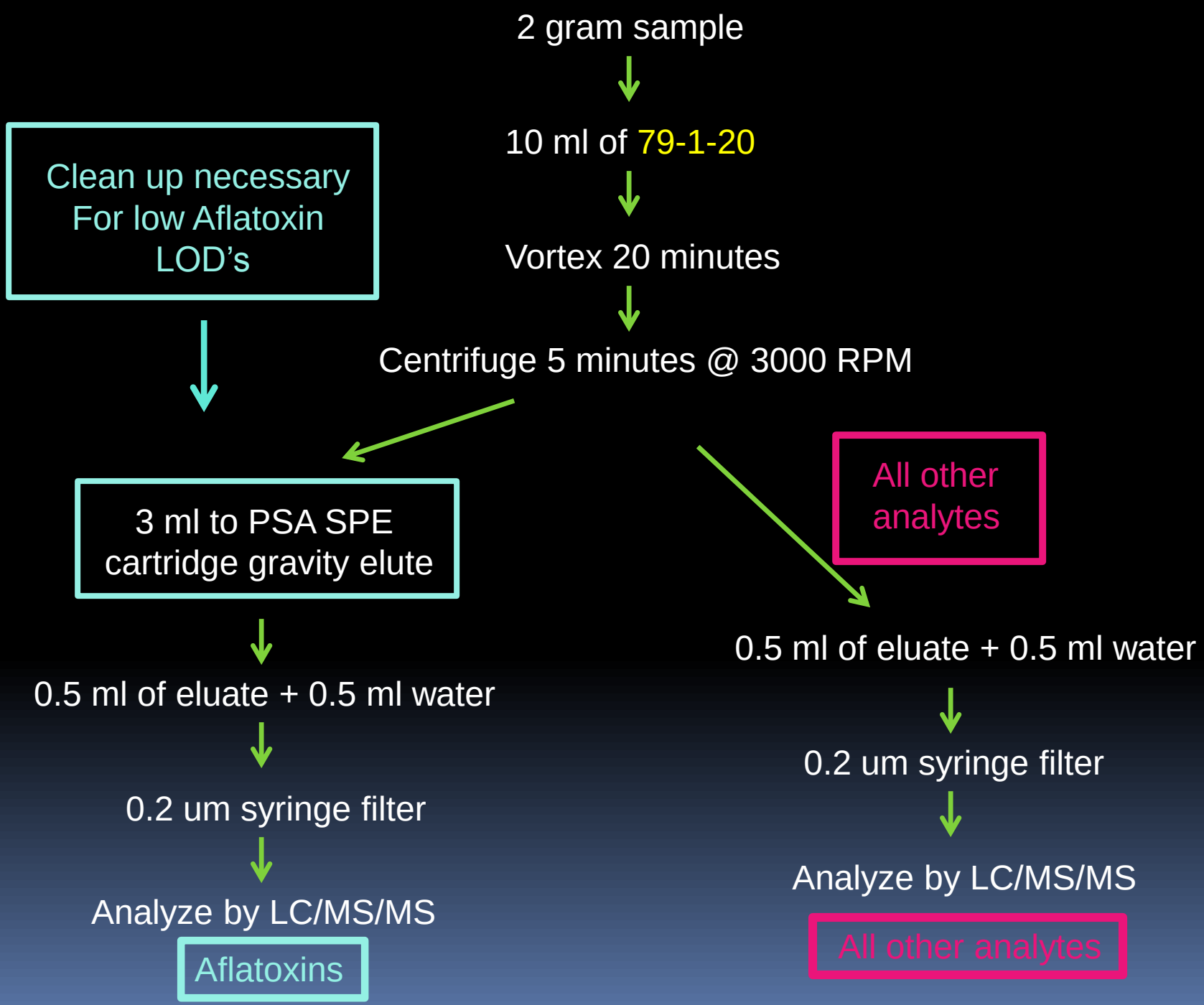
Multi-analyte method

- Latest work from Romer Labs
  - Extraction solvent
    - 79% acetonitrile
    - 1% acetic acid
    - 20% water
  - Extracts lipophylics as well as hydrophylic Fumonisins
  - This replaces previous Romer method of 2 extractions to recover all 11 analytes

79-1-20 mixture

- AAFCO methods needs statement
  - Aflatoxin B1
  - Aflatoxin B2
  - Aflatoxin G1
  - Aflatoxin G2
  - Ochratoxin A
  - Fumonisin B1
  - Fumonisin B2
  - Fumonisin B3
  - Zearalenone
  - Deoxynivalenol
  - T-2

2nd extraction no longer needed


2 gram sample
10 ml of 79-1-20
Vortex 20 minutes
Centrifuge 5 minutes @ 3000 RPM
Clean up necessary For low Aflatoxin LOD's
3 ml to PSA SPE cartridge gravity elute
0.5 ml of eluate + 0.5 ml water
0.2 um syringe filter
Analyze by LC/MS/MS
Aflatoxins
All other analytes
0.5 ml of eluate + 0.5 ml water
0.2 um syringe filter
Analyze by LC/MS/MS
All other analytes

Multi-analyte methods

Romer Labs

- Standards in solvent
  - ^{13}C Internal standards added after extraction
  - Accounts for matrix suppression/enhancement not loss during extraction
- 25 gram sample-100 ml solvent
- No clean-up

NY variation

- Standards in feed matrix
- 2 grams sample-10 ml solvent
- PSA clean-up for aflatoxins
  - Necessary for achieving low detection limits

5 AAFCO check samples analyzed 3 times with updated method

201461	Poultry Feed
201462	Swine Feed
201463	Cattle Feed
201464	Dog Food
201561	Dairy Feed

AAFCO 142061 ave. n=3 poultry feed

	result	Assigned value
Afla B1	19.2	18.5
Afla B2	2.1	1.6
Afla G1	0	0
Afla G2	0	0
DON	1175	1847
FB1	2162	2839
FB2	807	917
FB3	370	500
Ochra A	264	239
T2	81	95
ZON	221	328

AAFCO 142062 ave. n=3
swine feed

	result	Assigned value
Afla B1	25.3	20
Afla B2	1.9	1.5
Afla G1	0	0
Afla G2	0	0
DON	649	830
FB1	1404	1500
FB2	417	400
FB3	205	200
Ochra A	85	85
T2	BDL	47
ZON	99	184

Target LOQ 100ppb

AAFCO 142063 ave. n=3
cattle feed

	result	Assigned value
Afla B1	45.5	40.2
Afla B2	2.3	1.7
Afla G1	0	0
Afla G2	0	0
DON	2120	2700
FB1	1666	1400
FB2	476	270
FB3	323	140
Ochra A	125	189
T2	142	244
ZON	65	250

AAFCO 142064 ave. n=3

Dog food

	result	Assigned value
Afla B1	37.0	30.1
Afla B2	3.4	2.8
Afla G1	0	0
Afla G2	0	0
DON	1208	1288
FB1	1894	1437
FB2	663	476
FB3	274	323
Ochra A	163	125
T2	247	254
ZON	235	328

AAFCO 152061 ave. n=3

Dairy Feed

	result	Assigned value	
Afla B1	12.4	12.0	
Afla B2	0.96	1.4	Below our LOQ
Afla G1	0	0	
Afla G2	0	0	
DON	1175	1847	
FB1	2162	2839	
FB2	807	917	
FB3	370	500	
Ochra A	264	239	
T2	81	95	
ZON	205	328	

Check sample material

201461	Poultry Feed
201462	Swine Feed
201463	Cattle Feed
201464	Dog Food
201561	Dairy Feed

Participating labs

- 7 to 10 labs have expressed interest in round robin study
- Amount of sample will depend on final method
  - Romer method : 25 grams
  - NY method : 2 grams (same ratio of sample to solvent)
- Several labs are waiting for a method

Round robin

- Should both versions of multiresidue method be considered equivalent ?
  - 25 grams vs 2 grams
  - ^{13}C internal standards vs matrix matched external standards
  - Same extraction solvent (79-1-20)
  - Same analysis method (LC/MS/MS two transitions per analyte for confirmation)

Next steps

- We propose sending portions of check sample material to all interested labs to allow them to get comfortable with this method
- We can provide sources for standards and consumables necessary as well as fielding questions about the extraction or analysis
- We will use an e-mail group to keep all participants in the loop with progress
  - Possibly exchange extracts, standards
- When all labs are ready we can begin formal study