Proficiency Testing – Are we fully utilising a valuable resource ?

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Introduction

- What do participants want from PT
- What does the analytical community want from PT

Limitations of proficiency testing

- Choice of assigned value
- Bump hunting
- Selection of σ–value

Method performance – examples of insights from PT data

- Tin in vegetable puree
- Chloramphenicol in prawns
- Acrylamide in cooked foods
- Deoxynivalenol in wheat

Conculsions

What do participants want from PT

Satisfactory z-score based on:-

- Accurate assigned value without method dependence
- σ-value decided on best practice
- Relevant test material
- Relevant analyte at relevant concentration

What does the analytical community want from PT?

- Progressive improvement in performance of laboratories globally
- Insight into any analyte method dependence
- Insight into method performance

Assigned values and target σ values

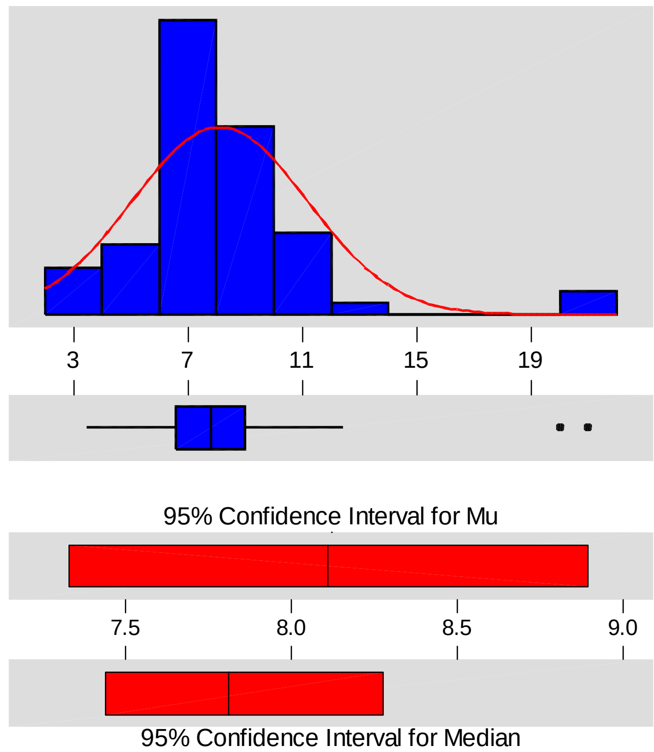
- ‘True’ value and ‘assigned value’
  - the ‘true’ value is an ideal
  - the ‘assigned value’ is the best estimate of the determinand
- Target Standard Deviation
  - The target standard deviation sets the limits of satisfactory performance in the PT

The Assigned Value

- FAPAS® derives the assigned value from the most appropriate measure of central tendency:
  - robust mean
  - median
  - mode

Simple Mean v Robust Mean

Descriptive Statistics



Variable: ass. value

Anderson-Darling Normality Test

A-Squared:	2.450	Robust Mean 7.82879
P-Value:	0.000	

Mean	8.11113
StDev	3.06012
Variance	9.36431
Skewness	2.08479
Kurtosis	7.53429
N	61
Minimum	3.4700
1st Quartile	6.5900
Median	7.8100
3rd Quartile	9.0000
Maximum	21.0000

95% Confidence Interval for Mu

7.3274 8.8949

95% Confidence Interval for Sigma

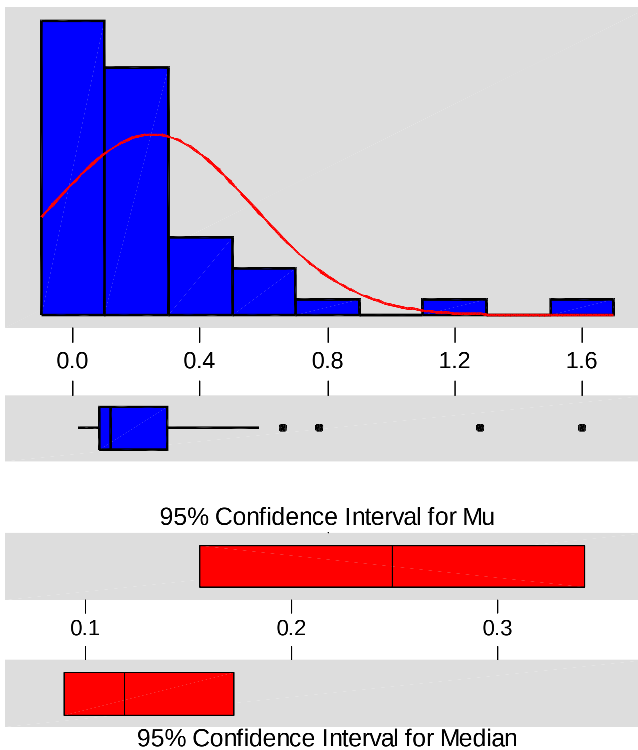
2.5972 3.7255

95% Confidence Interval for Median

7.4405 8.2757

Robust Mean and Median Limitations

Descriptive Statistics



Variable: afm1

Anderson-Darling Normality Test

A-Squared:	5.339	Robust Mean 0.184686
P-Value:	0.000	
Mean	0.248691	
StDev	0.314717	
Variance	9.90E-02	
Skewness	2.78245	
Kurtosis	8.74819	
N	46	
Minimum	0.01700	
1st Quartile	0.08100	
Median	0.11900	
3rd Quartile	0.29500	
Maximum	1.60000	

95% Confidence Interval for Mu

0.15523 0.34215

95% Confidence Interval for Sigma

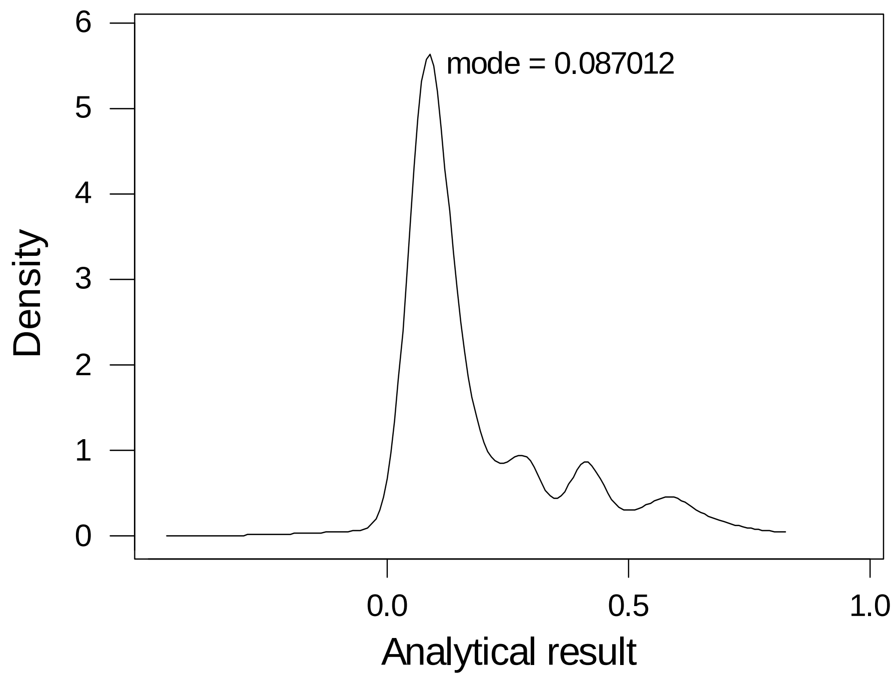
0.26104 0.39639

95% Confidence Interval for Median

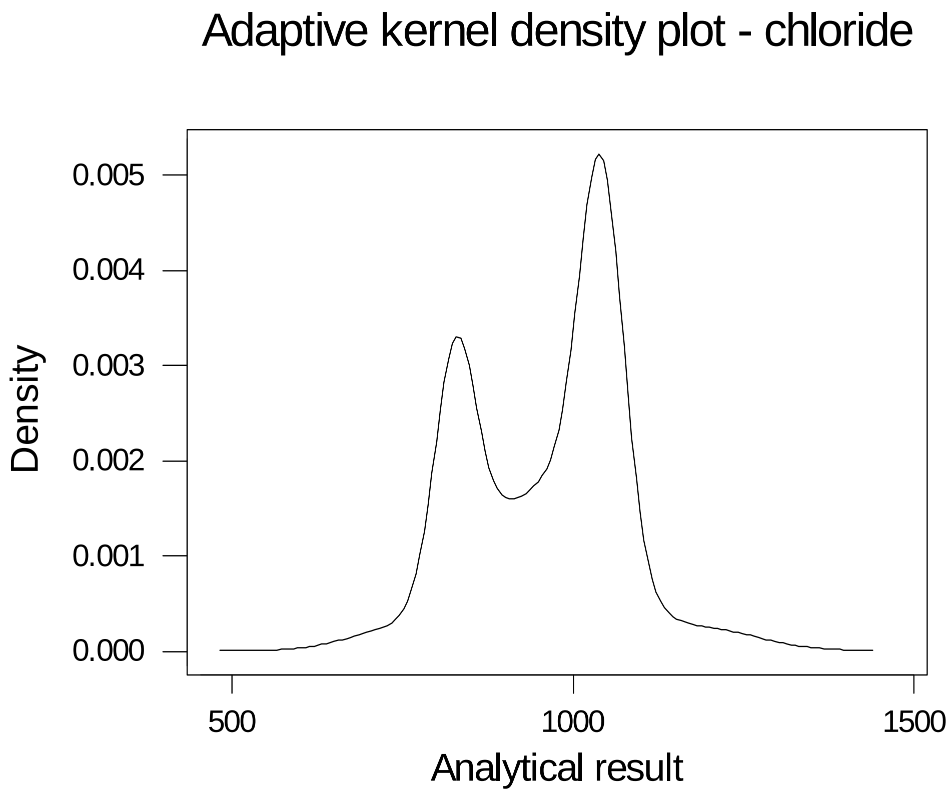
0.08998 0.17190

‘Bump-hunting’ for the Mode(s)

Adaptive kernel density plot – aflatoxin M_1



Bimodal Results – Poor Methodology?



Influence of σ-values on z-scores

Total aflatoxins in peanut meal - mean 25.4 ppb

	Acceptable range ppb	Numbers of participants		% Satisfactory
		Satisfactory	Unsatisfactory	
Horwitz	11.3 - 39.6	102	19	84
95% ±2 z-score	2.4 - 48.4	114	7	95
Best practice	15.8 - 35.1	86	35	71

To satisfy PT Participants organisers MUST:-

- Be confident in assigned value
- Be confident that there is no method dependence in dataset
  - bump-hunting
- Need to set target standard deviation at realistic value
- Be confident in homogeneity and in stability of test material

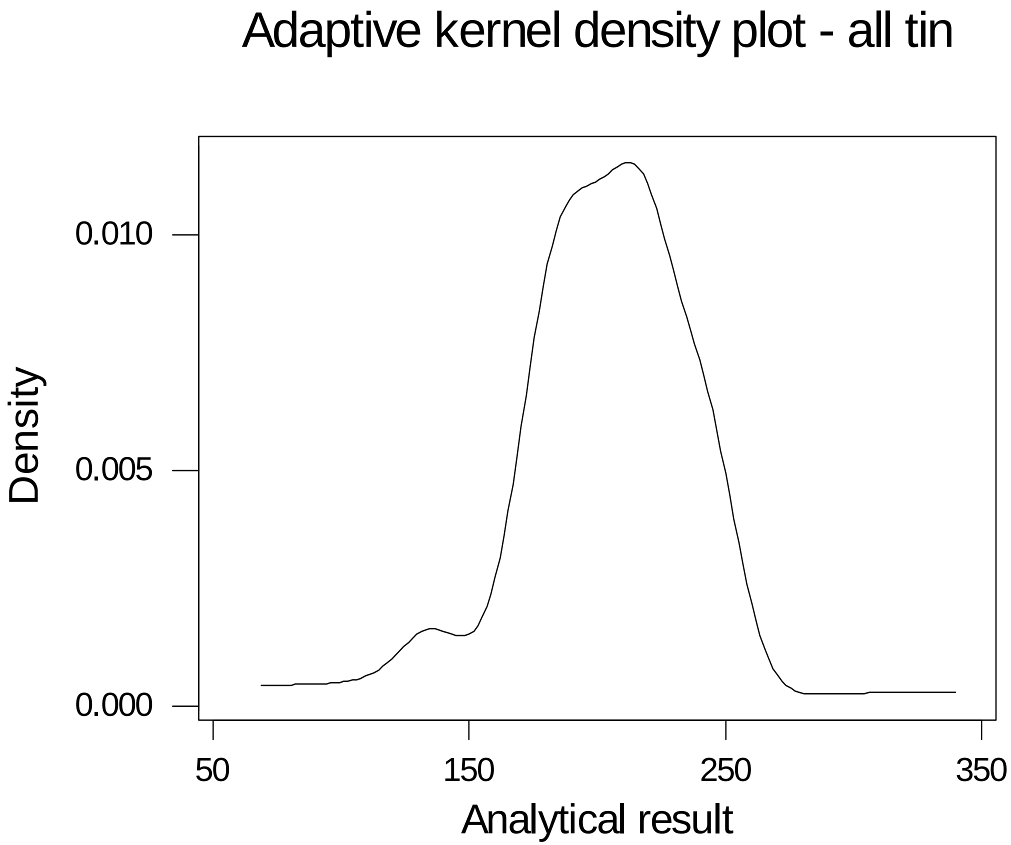
Method performance – examples of insights from PT data

- Tin in vegetable puree
- Chloramphenicol in prawns
- Acrylamide in cooked foods
- Deoxynivalenol in wheat

FAPAS 0738 – Tin in Tomato Paste

- Preparation
  - Spiked at 250 mg/kg
  - Homogeneity mean was 251 mg/kg
- Results from participants
  - Consensus of results was 204 mg/kg
  - Consensus was 20% lower than the spike
  - Homogeneity mean would have resulted in a z-score of 3.2
  - Using ICP-IDMS result was 247.8 mg/kg

0738 – Tin in Tomato Paste



Issues with PT for Tin Analysis

- No correlation between methods used and the results received
- FAPAS protocol indicates that we should use the consensus value not the spike value
- Complaints from participants that obtained results that correlated with the spike value, but they received unsatisfactory z-scores

FAPAS 0741 – Tin in Vegetable Puree

- Preparation
  - Spiked at 200 mg/kg
  - Homogeneity mean was 204 mg/kg
- Results from participants
  - Consensus of results was 179 mg/kg
  - Consensus was 10% lower than the spike
  - Homogeneity mean would have resulted in a z-score of 1.9

FAPAS 0741 – Tin in Vegetable Puree (continued)

- Report production was delayed until discussed with Advisory Committee
- Comments from the Committee:
  - ‘Expert labs’ and LGC ICP-IDMS give good agreement with spike (0738 round)
  - No trends in method information seen
  - For tin analysis only, FAPAS would deviate from the protocol i.e. the assigned value would not be set as the consensus if this deviated from the spike value

Use of Reference Value rather than Consensus of Participants' Results 0745 – Tin in Tomato Puree

Summary

– Homogeneity mean was 282 mg/kg
– Consensus of results was 255.9 mg/kg
– Consensus was 10% lower than the spike
– Thus, assigned value was set at ICP-IDMS result provided by LGC (286.5 mg/kg)
– Homogeneity mean would have resulted in a z-score of –0.2

Consensus and Reference Values 0745 – Tin in Tomato Puree

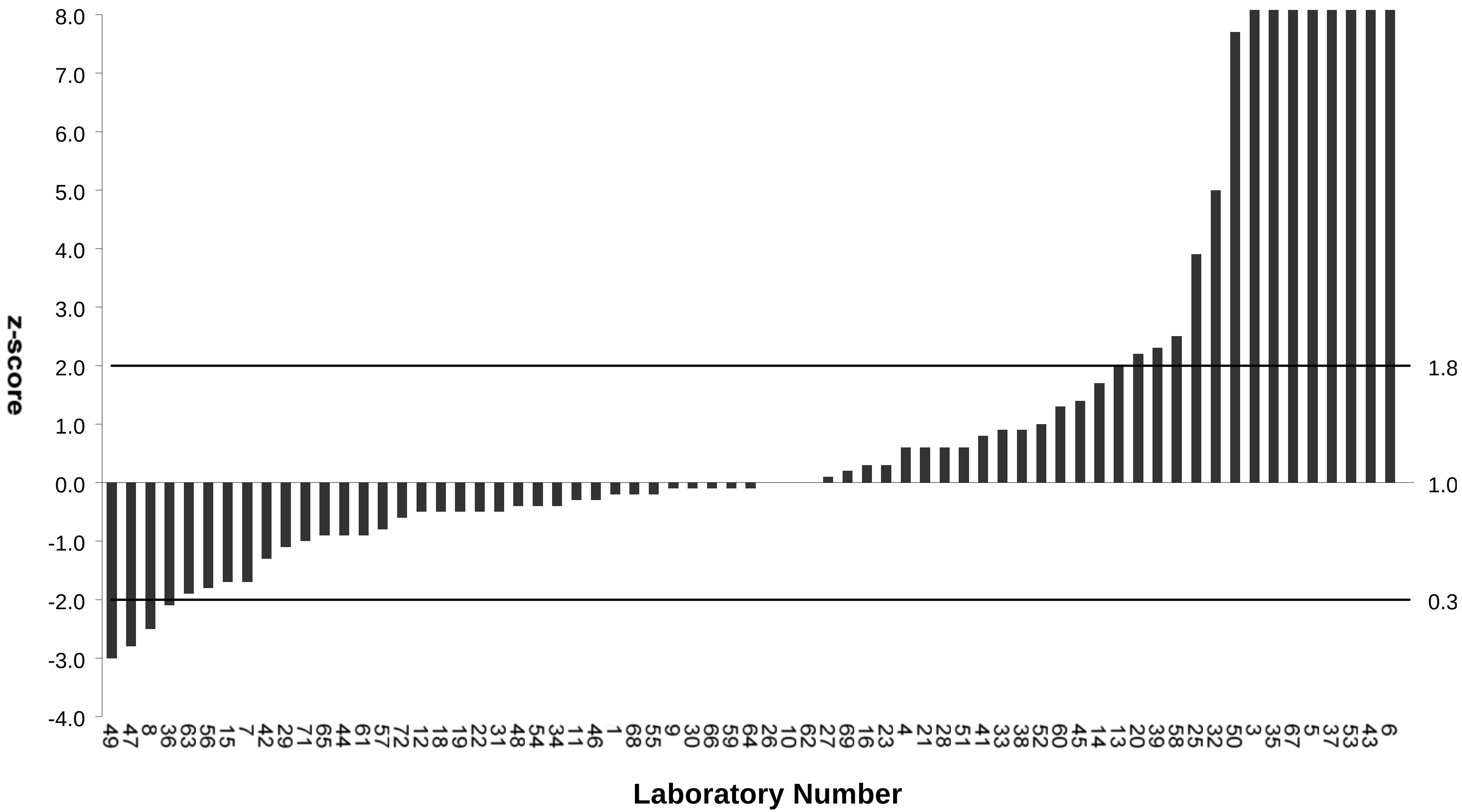
- Reduction in % satisfactory z-scores when reference value was used:
  - Using reference value – 60% satisfactory
  - Using consensus – 72% satisfactory
- Contacted participants to inform them of the problem
- On request, provided information on method used by the homogeneity laboratory

Most Recent Round of Tin in Vegetable Puree

- Under reporting not a problem (consensus agreed with the spike)
- Consensus used as the assigned value
- Using a reference laboratory pushed participants towards addressing problems with their methodology

Results from recent FAPAS® *Rounds*

Round 41 (November 2002) chloramphenicol in prawns (assigned value 0.9 ppb)



Choice of methods and FAPAS® scores (chloramphenicol in prawns)

Method	No. of Labs	No. Satisfactory	% Satisfactory
GC & GC/MS	13	13	100
LC/MS	10	10	100
LC	27	16	60
ELISA	21	15	71
Overall	67	49	73

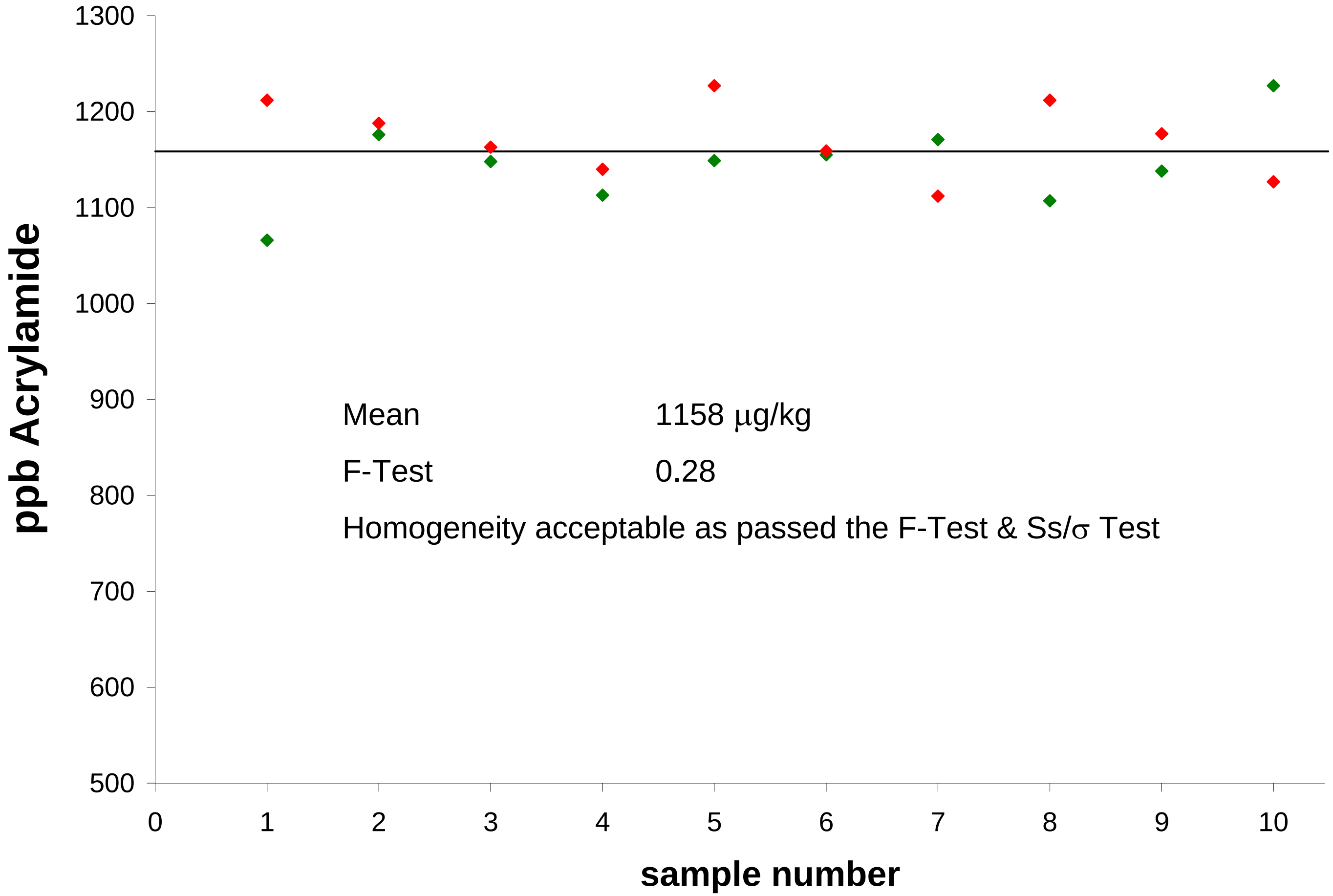
Indications of problem areas

- Lack of specificity in HPLC detection
- High proportion of labs with unsatisfactory z-scores (70% - 7 out of 10) used UV detection in HPLC
- ELISA more appropriate for screening than quantification

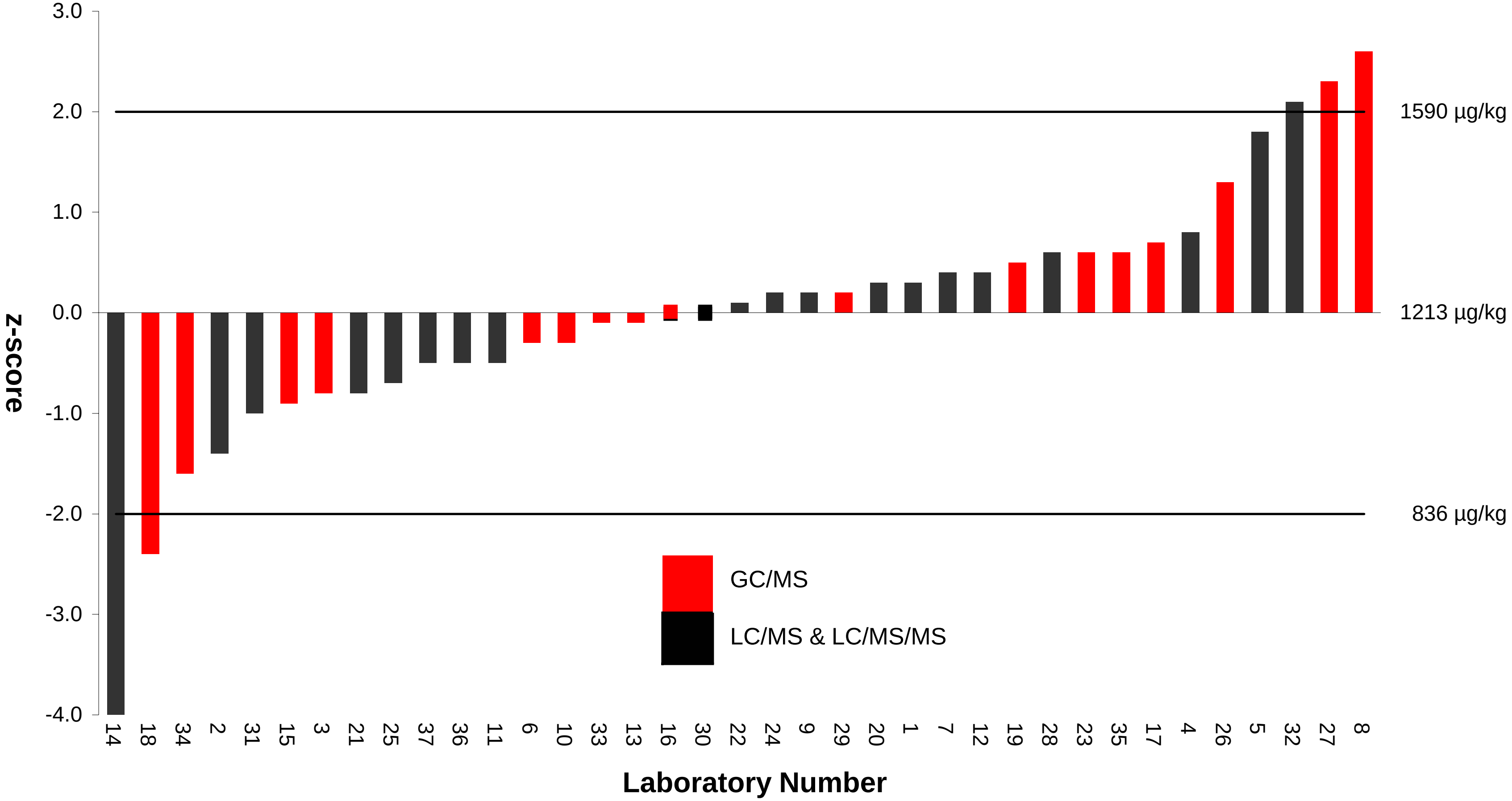
Acrylamide
FAPAS® Rounds 2002-2003

Round No.	Material	Fat	Carbohydrate	Protein	Acrylamide ppb
1	Crispbread	2%	63%	9%	1200
2	Potato crisps	34%	49%	6%	170
3	Breakfast cereal	3%	68%	11%	100-300
4	Ground roasted coffee	12%	35%	3%	400-600

FAPAS® Homogeneity Data for Acrylamide in Crispbread



Z-scores for Acrylamide (1213 μg/kg) in Crispbread Test Material



Information Available on Methodology from FAPAS®

- Literature reference to method if available
- Sample weight taken
- Internal standard used
- Extraction solvent used

Information Available on Methodology from FAPAS® (continued)

- Treatment of extract – filtered, centrifuged etc.
- Clean-up – SPE etc.
- Determination step GC/MS; LC/MS; LC/MS/MS etc.
- GC/LC – column, length, temperature programme etc.
- MS – ions monitored, scan mode etc.

Correlating Performance with Methodology

- Sample size taken
- Use of internal standard
- Choice of extraction solvent
- Clean-up
- GC/MS or LC/MS

Sample Size Taken for Analysis

	Nos of unsatisfactory Labs		
	1g – 2g	2g – 5g	5g – 35g
Round 1	0	3	2
Round 2	2	2	1
Round 3	3	5	3
No evidence of influence of sample size			

Use of Internal Standards

Nos of unsatisfactory Labs				
	None	d_3-acrylamide	^{13}C-acrylamide	other
Round 1	1 (50%)	2 (11%)	-	2 (20%)
Round 2	1 (50%)	3 (13%)	1 (14%)	-
Round 3	1 (50%)	4 (20%)	2 (30%)	4 (50%)

High proportion of Labs not using an internal standard or not using a labelled internal standard were unsatisfactory

Choice of Extraction Solvent

	Nos of unsatisfactory Labs		
	Water	Aqueous Organic	Organic
Round 1	1 (4.5%)	-	4 (40%)
Round 2	3 (13%)	-	2 (40%)
Round 3	8 (33%)	1 (20%)	2 (33%)
High proportion of Labs using organic solvents were unsatisfactory			

Choice of Sample Clean-up

	Nos of unsatisfactory Labs			
	None	Liquid/Liquid	SPE	Other
Round 1	1	1	-	-
Round 2	1	1	1	-
Round 3	1	-	8	2

No trend or patterns between clean-up and satisfactory performance

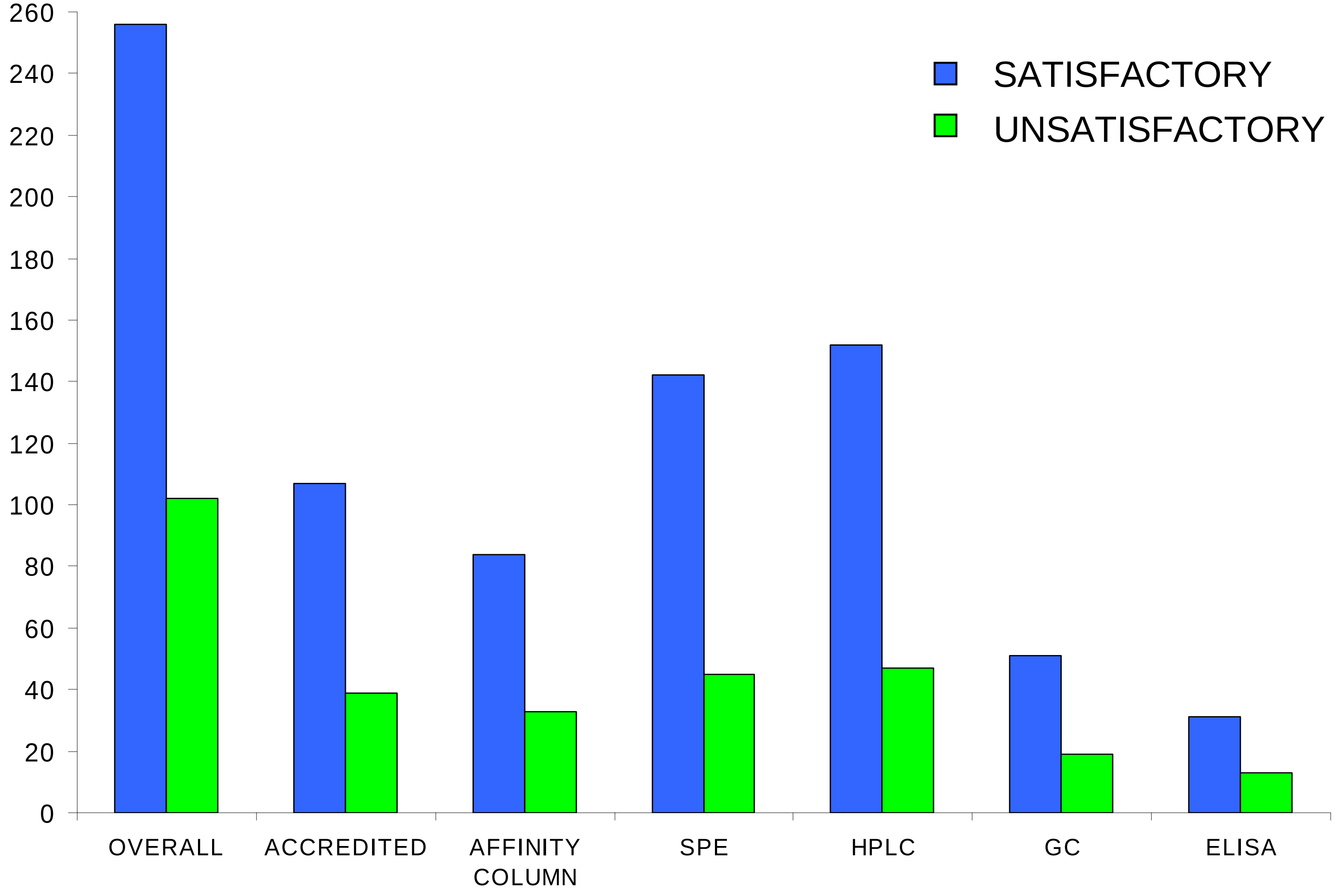
GC/MS or LC/MS or LC/MS/MS

	Nos of unsatisfactory Labs		
	GC/MS	LC/MS	LC/MS/MS
Round 1	3 (15%)	2 (14%)	-
Round 2	2 (11%)	2 (25%)	-
Round 3	3 (21%)	4 (44%)	4 (31%)
No evidence of GC/MS, LC/MS or LC/MS/MS influencing performance			

Acrylamide – Proficiency Testing

- Variety of methods used
- Methods using organic solvents and not using labelled internal standards gave poorer performance
- No evidence of better results by GC/MS or LC/MS or LC/MS/MS

Method Performance for Deoxynivalenol in Wheat - combined results for several PT rounds



Conclusions

- Participation in proficiency testing is leading to improved laboratory performance

    Leads to sounder compliance decisions

- PT data can be used to identify problems with methodology

- PT data can provide insights into method validation

    BUT limitation in extent to which methods are 'customised'