JIFSAN Acrylamide in Foods Workshop
April 14-15, 2004
Report of the Analytical Methods Working Group

Working Group Participants:

John Yen	Pepsi Beverages and Foods
Steve Lehotay	USDA/ARS
Paul LaLuzerne	Covance
Dan Tallmadge	Procter & Gamble
John Roach	FDA
Karl-Erik Hellenas	Swedish NFA
George Haas	Kraft Foods
Marvin L. Hopper	FDA
Yeonhwa Park	Univ. of Wisconsin
Reto Battaglia	Swiss Quality Testing Services
Forrest Bayer	The Coca-Cola Co.
Lars Reimann	Eurofins US
Richard Cantrill	AOCS
Elke Anklam	European Commission, JRC
Laurence Castle	CSL
I-Pin Ho	NFPA
Henry Chin	NFPA

Henry Chin welcomed the members of the Working Group and asked that they introduce themselves.

I-Pin Ho led the discussion about results from the testing round. The WG suggested changes in the presentation of information to facilitate the review of the data. There was some conjecturing about the methodology used by Lab #7 because the reported values appeared to be consistently high. After adjustment for statistical outliers, participants thought that the results from the testing appeared to be quite good. The results are attached.

Following this presentation some WG members commented that the state of the analytical methodology seemed to be quite advanced. A question was asked about the effect of the age of the samples on the analytical results. Acrylamide levels in coffee have been observed to decrease with the age of the sample.

John Roach commented that an important component of the identification of chromatographic peaks as acrylamide was to ensure that the relative abundance of ions in the sample matched that expected from a standard. Steve Lehotay suggested that in some samples a peak belonging to what might be a carboxylic acid could be misidentified as acrylamide.

To compare the results from this testing round to the results experienced by other groups, Laurence Castle presented a summary of the FAPAS proficiency testing rounds and Elke Anklam presented a summary of the ring test organized by the European Commission's JRC. Their presentations are attached to this report.

Anklam indicated that JRC will be certifying a crispbread that can be used as a reference material. The stability of acrylamide is being checked. Another proficiency round is being to be finalised and another being planned. In addition, the JRC is planning to validate two analytical methods. The JRC also hosts a European monitoring database.

Castle said that 6 testing rounds have already been completed and that the seventh round was sent to participants in March. He commented that the FAPAS rounds appeared to indicate that underivatized GC/MS methods were giving higher results.

Richard Cantrill gave an oral briefing on the proficiency program being conducted by AOCS.

In a general discussion, a question was raised about the confidence that all of the available acrylamide in a food was being extracted. The WG was not able to point to anything that demonstrated definitively that all of the acrylamide was extracted, but did say that the preponderance of the evidence from different extraction studies seemed to indicate that all of the acrylamide was extracted. A comment was made that the studies using radiolabeled precursors seemed to show that all of the labeled materials could be accounted for.

Steve Lehotay gave a presentation on a method to simplify the extraction of acrylamide and the clean-up of the extracts.

Following these discussions, the WG addressed the questions that were posed to the group. The WG decided that the first question would be better restated as five separate questions. The Questions and the consensus response of the WG are shown below.

1. What conclusions can be drawn about the general capability of laboratories to analyze foods for acrylamide?
    a. What is the status of analytical methods for acrylamide in foods?
        i. While there is limited information about the performance of methods across a wide range of food products from proficiency programs (the data is mostly limited to crisp bread, cookies, etc.), experience indicates all matrices of concern are reasonably covered.
        ii. Need precise description of foods tested (e.g., food preparation technique, canning, etc.)
    b. Do methods have adequate sensitivity of methods? Do laboratories have the ability to measure levels of interest?
        i. Available methods can determine 20-50 ppb, depending upon method and matrix (the Horwitz criteria can be used to estimate precision).

ii. Adequate for major food groups, may not meet the needs for some populations and some food groups (that would need to be identified by risk assessors).

c. Is there confidence in the identification of detected compounds as acrylamide?
    i. Yes. There are generally accepted criteria for identification of compounds by mass spectrometry. If these are followed, there can be confidence in the identification.
    ii. Current methods (the set of LC/MS/MS, GC/MS methods) meet those criteria needs.

d. Is there confidence in the numerical results of the analysis?
    i. Generally satisfactory, depending upon concentration and matrix (include variability from proficiency tests).

e. Are we measuring all of the acrylamide present in foods?
    i. The best data say yes.

2. What is the availability of proficiency testing programs and are there other similar needs?
    a. There are regular ongoing rounds, about 10 per annum, for the use with common matrices. The organizing groups include:
        i. FAPAS
        ii. AOCS
        iii. JRC
    b. Need "certified" reference materials for a variety of matrices (recognizing instability of acrylamide in many matrices)
3. What are critical elements to ensure methodology is properly applied?
    a. Participation in proficiency testing
    b. Use of isotopic labeled internal standards
    c. Avoid artifact formation, e.g., during extraction and underivatized GC analysis.
    d. Use reagent blanks.
    e. Record multiple ions and relative abundance to distinguish from possible interferences.
    f. The retention time and peak shape must match a contemporaneously run standard.
4. Remaining analytical needs
    a. Interlaboratory validation of reference methods. The WG encouraged laboratories to participate in the JRC effort to validate methods for acrylamide in foods.
    b. Validated Biomarker assays
    c. Validated methods for precursors (3APA) at low concentrations (raw materials)
5. Possibility of other methods
    a. New derivatization techniques for non-MS analysis, (e.g., Rxn w/thiobenzoate acid, other strong fluorescent derivative)
    b. Streamlined underivatized MS method
6. Other Recommendations

a. Develop methods for precursors at low levels.

The results from the check samples are on the following pages.

NFPA_AA_1: Cereal

Lab ID	Result #1 (ppb)	Result #2 (ppb)	Ave. (ppb)	Z-value
1	21.0	22.0	21.5	-0.03
2	No Report	No Report		
3	15.9	16.7	16.3	-0.39
4	59.0	68.0	63.5	2.84
5	16.0	16.0	16.0	-0.41
6	46.0	(60, 33)	47.0	1.71
7	17.0	17.0	17.0	-0.34
8	<30	<30	<30	
9	<20	<20	<20	
10	ND (LOD<20 ppb)	ND (LOD<20 ppb)	<20	
11	12.7	14.0	13.4	-0.59
12	<20 (15)	<20 (15)	15.0	-0.48
13	17.0	14.0	15.5	-0.44
14	14.0	14.0	14.0	-0.55
15	No Report	No Report		
16	16.3	18.6	17.5	-0.31
17	n/a	n/a	386.0	24.92
18	16.0	18.0	17.0	-0.34
19	21.0	18.0	19.5	-0.17
20	16.7	13.0	14.9	-0.49

Ave.	22.0
Std. Dev.	14.6

Results from Lab #17 are not included in calculations.
Results with "<" are not included in calculations.

Excluding results from Lab 4 and 6.

Ave. *	16.5
Std. Dev. *	2.3
Rel. Std. Dev. (%) *	13.9

NFPA_AA_2: Peanut Butter

Lab ID	Result #1 (ppb)	Result #2 (ppb)	Ave. (ppb)	Z-value
1	102.0	101.0	101.5	-0.02
2	No Report	No Report		
3	88.0	85.2	86.6	-0.23
4	101.0	104.0	102.5	-0.01
5	86.0	84.0	85.0	-0.25
6	77.0	(77, 77)	77.0	-0.35
7	330.0	445.0	387.5	3.82
8	100.0	90.0	95.0	-0.11
9	77.0	83.0	80.0	-0.31
10	45.0	48.0	46.5	-0.76
11	82.1	81.1	81.6	-0.29
12	80.0	78.0	79.0	-0.33
13	95.0	90.0	92.5	-0.15
14	91.2	87.5	89.4	-0.19
15	No Report	No Report		
16	97.1	91.0	94.1	-0.13
17	n/a	n/a	150.0	0.63
18	90.0	89.0	89.5	-0.19
19	93.0	87.0	90.0	-0.18
20	75.5	82.8	79.2	-0.33

Ave.	103.3
Std. Dev.	74.3

Excluding results from Lab 7.

Ave. *	85.6
Std. Dev. *	13.0
Rel. Std. Dev. (%) *	15.2

NFPA_AA-3: Chocolate

Lab ID	Result #1 (ppb)	Result #2 (ppb)	Ave. (ppb)	Z-value
1	222.0	211.0	216.5	1.93
2	No Report	No Report		
3	140.9	146.8	143.9	0.47
4	73.0	78.0	75.5	-0.90
5	118.0	157.0	137.5	0.34
6	48.0	(38, 57)	48.0	-1.46
7	17.0	25.0	21.0	-2.00
8	165.0	160.0	162.5	0.84
9	147.0	139.0	143.0	0.45
10	103.0	101.0	102.0	-0.37
11	122.3	127.1	124.7	0.08
12	126.0	126.0	126.0	0.11
13	144.0	153.0	148.5	0.56
14	140.0	152.0	146.0	0.51
15	No Report	No Report		
16	178.0	Not Reported	178.0	1.16
17	n/a	n/a	140.0	0.39
18	120.0	139.0	129.5	0.18
19	64.0	53.0	58.5	-1.25
20	94.4	80.3	87.4	-0.67
Ave.			120.5	
Std. Dev.			49.8	

NFPA_AA-4: Coffee

Lab ID	Result #1 (ppb)	Result #2 (ppb)	Ave. (ppb)	Z-value
1	147.0	142.0	144.5	-0.32
2	No Report	No Report		
3	140.0	142.7	141.4	-0.39
4	191.0	168.0	179.5	0.52
5	142.0	149.0	145.5	-0.29
6	130.0	(136, 131)	132.0	-0.61
7	207.0	221.0	214.0	1.34
8	150.0	150.0	150.0	-0.19
9	135.0	131.0	133.0	-0.59
10	89.0	74.0	81.5	-1.82
11	146.5	143.6	145.1	-0.30
12	292.0	277.0	284.5	3.02
13	155.0	157.0	156.0	-0.04
14	143.0	145.0	144.0	-0.33
15	No Report	No Report		
16	158.5	150.0	154.3	-0.08
17	n/a	n/a	1557.0	33.33
18	148.0	139.0	143.5	-0.34
19	161.0	170.0	165.5	0.18
20	144.3	192.6	168.5	0.25

Ave.	157.8
Std. Dev.	42.0

Excluding results from Lab 10 and 12.

Ave. *	154.4
Std. Dev. *	21.0
Rel. Std. Dev. (%) *	13.6

NFPA_AA-5: Cereal
(Duplicate of NFPA_AA-1)

Lab ID	Result #1 (ppb)	Result #2 (ppb)	Ave. (ppb)	Z-value
1	22.0	22.0	22.0	-0.24
2	No Report	No Report		
3	15.4	14.3	14.9	-0.47
4	20.0	24.0	22.0	-0.24
5	13.0	14.0	13.5	-0.52
6	30.0	(23, 17)	25.0	-0.14
7	100.0	101.0	100.5	2.29
8	<30	<30	<30	
9	<20	<20	<20	
10	110.0	Not Enough Sample	110.0	2.59
11	12.4	13.0	12.7	-0.54
12	<20 (16)	<20 (15)	15.5	-0.45
13	14.0	13.0	13.5	-0.52
14	15.0	14.5	14.8	-0.48
15	No Report	No Report		
16	15.2	21.1	18.2	-0.37
17	n/a	n/a	1201.0	37.76
18	19.0	21.0	20.0	-0.31
19	17.0	22.0	19.5	-0.32
20	19.7	21.1	20.4	-0.29

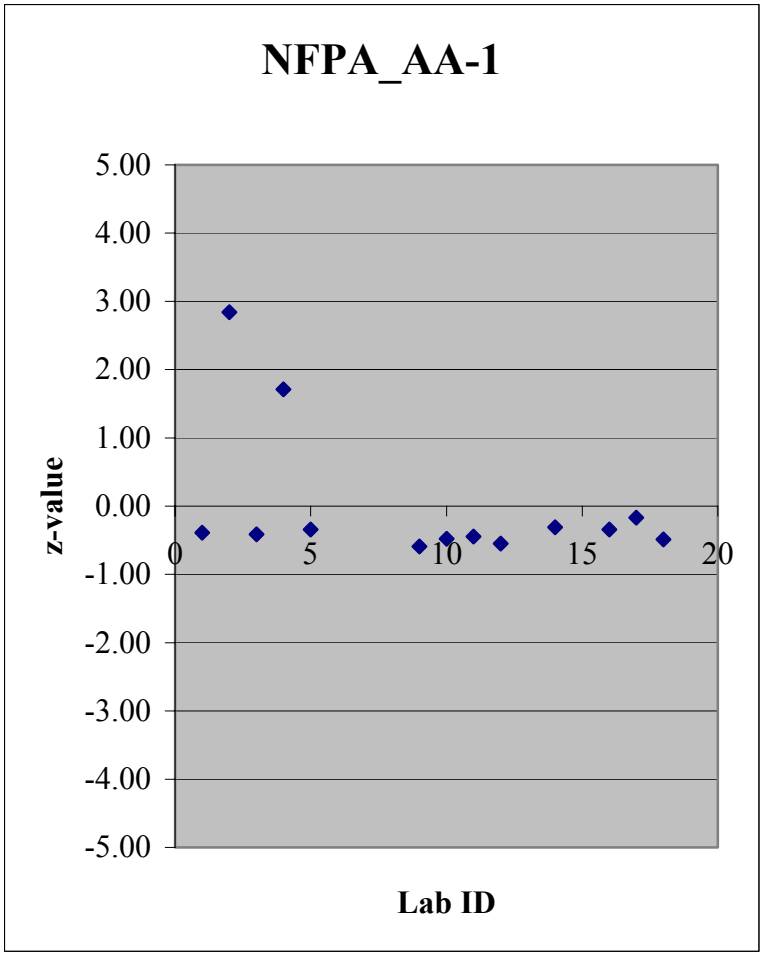
Ave.	29.5
Std. Dev.	31.0

Excluding results from Lab 7 and 10.

Ave. *	18.2
Std. Dev. *	5.5
Rel. Std. Dev. (%) *	30.0

NFPA_AA-6: Water
(QC; 20 ppb)

Lab ID	Result #1 (ppb)	Result #2 (ppb)	Ave. (ppb)	Z-value
1	19.0	19.0	19.0	-0.42
2	No Report	No Report		
3	20.0	19.0	19.5	-0.28
4	27.0	19.0	23.0	0.69
5	<5	<5	<5	
6	Can not do	Can not do		-5.74
7	19.0	18.0	18.5	-0.56
8	17.0	17.0	17.0	-0.98
9	29.0	23.0	26.0	1.53
10	ND (LOD<20 ppb)	ND (LOD<20 ppb)	<20	
11	16.4	15.6	16.0	-1.26
12	<20 (18)	<20 (19)	19.5	-0.28
13	20.0	19.0	19.5	-0.28
14	20.6	19.9	20.3	-0.07
15	No Report	No Report		
16	23.1	n/a	23.1	0.72
17	n/a	n/a	ND (<50)	
18	18.0	18.0	18.0	-0.70
19	32.0	26.0	29.0	2.37
20	18.0	19.8	18.9	-0.45
Ave.			20.5	
Std. Dev.			3.6	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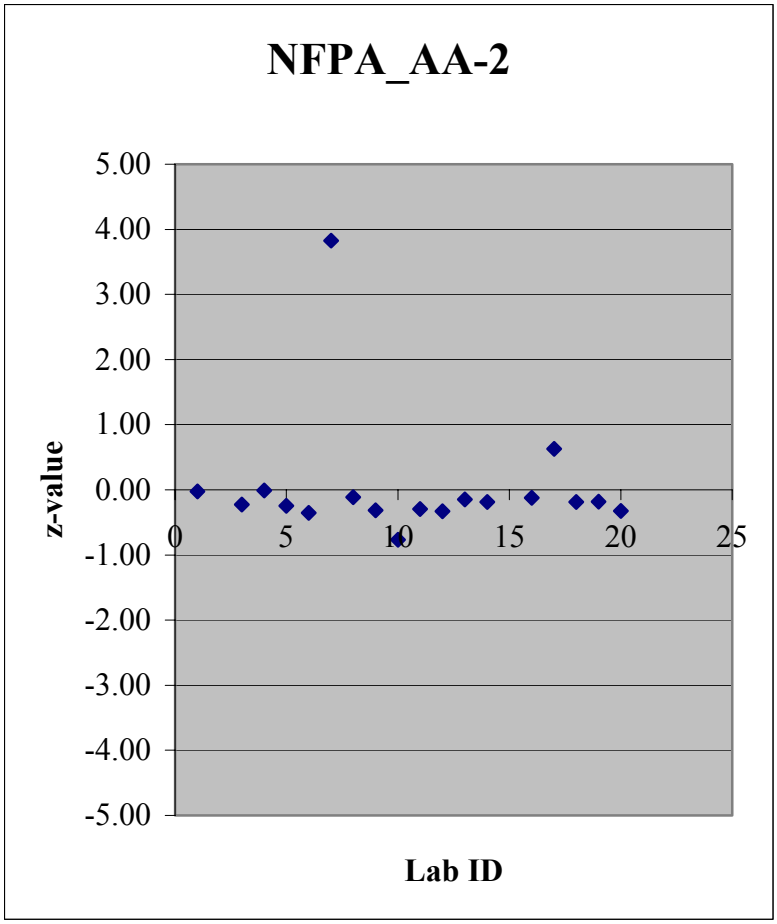
Lab ID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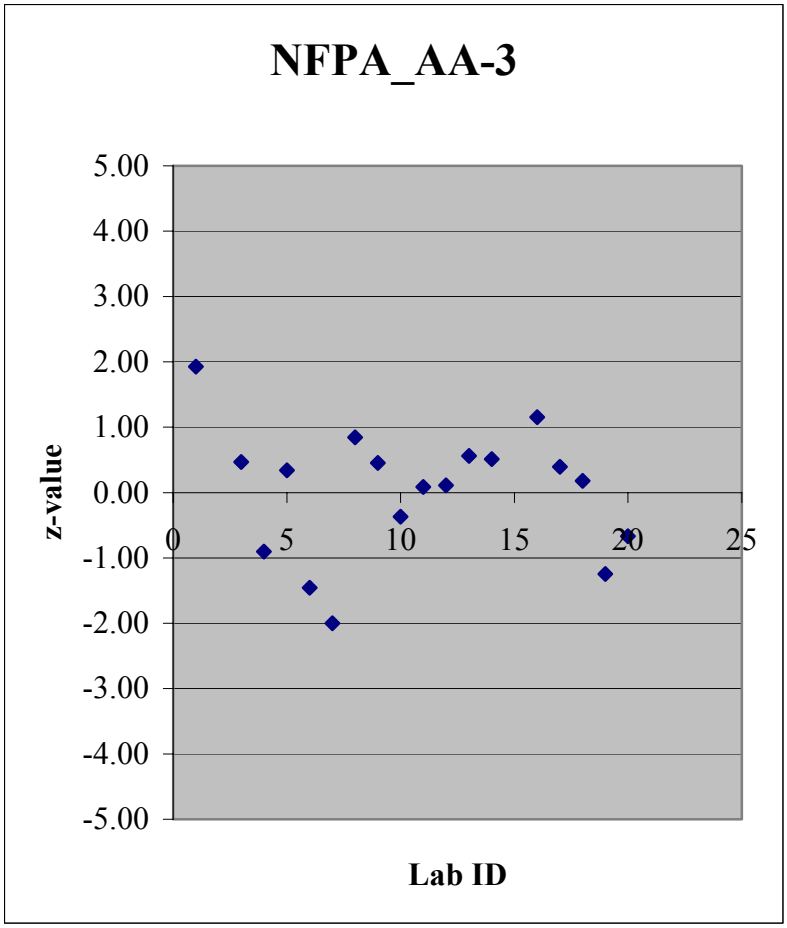
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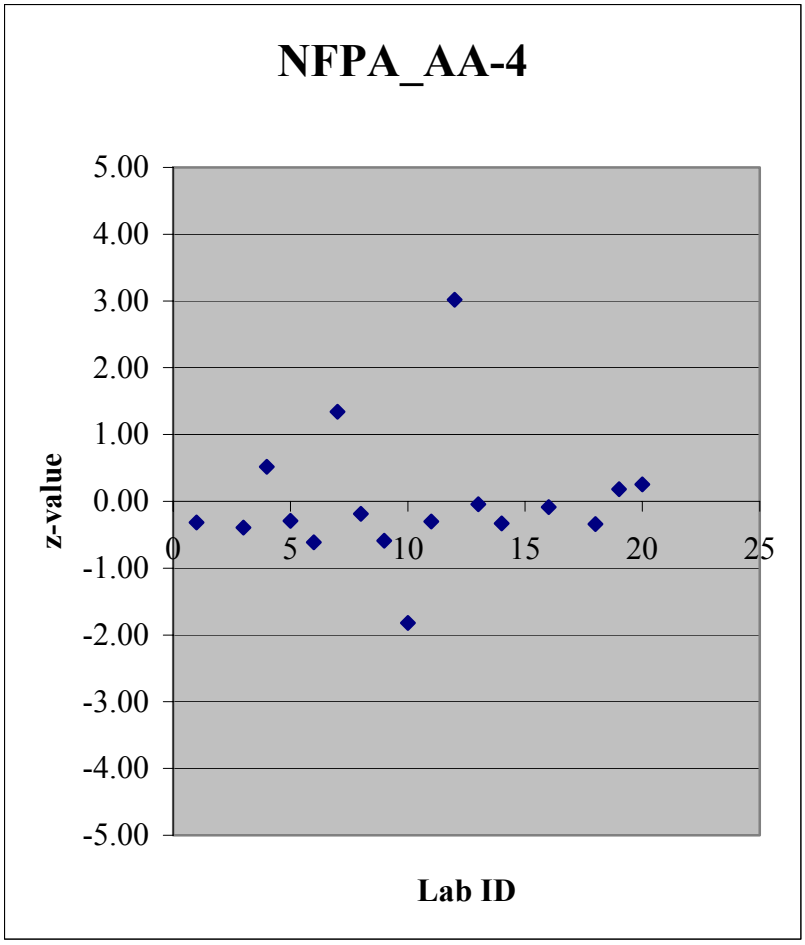
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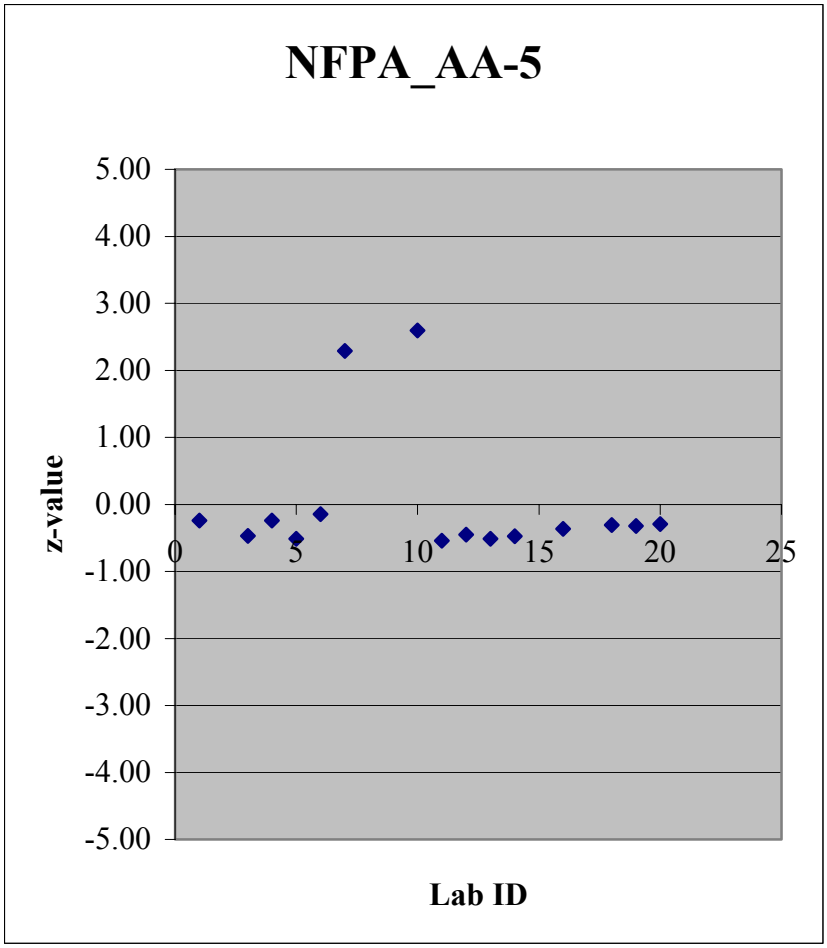
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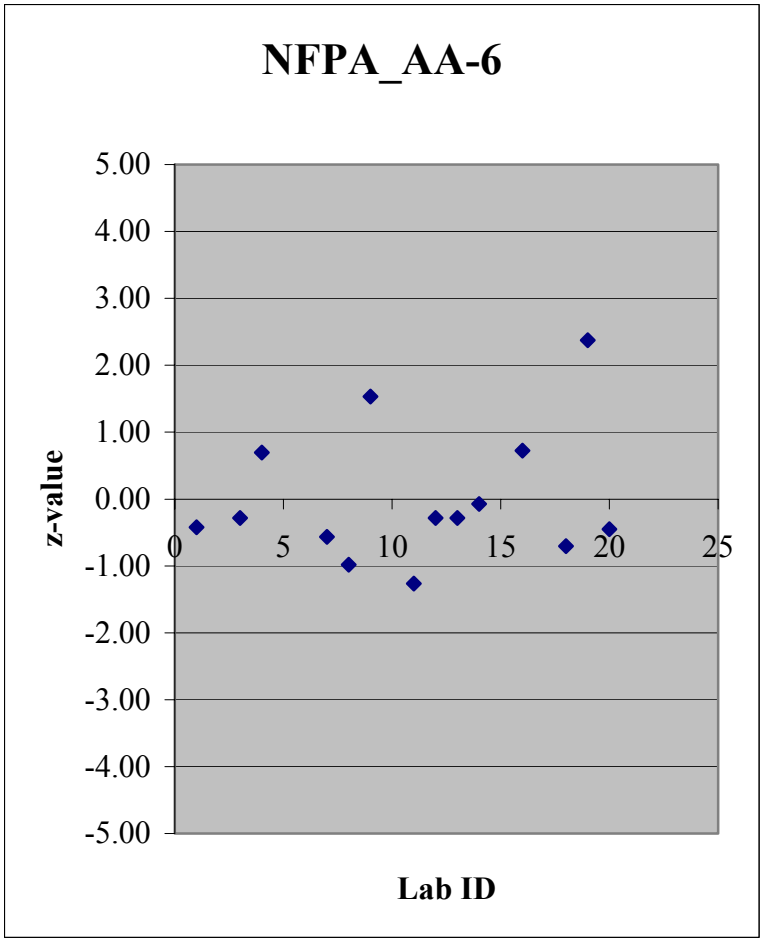
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