Study on the analytical capability and readiness of selected laboratories to analyze fortified food

July 2017

Table of Contents

Acknowledgements

This study was financed by GAIN supported by a grant provided by The Government of The Netherlands.

The author acknowledges the inputs of David Morgan (GAIN), Gerhard Rimkus (Intertek Food Services) and Phillip Makhumula (Fortification Consultants) into the experimental design.

The following laboratories participated in this study and their co-operation under tight time constraints is gratefully acknowledged:

- Bangladesh Small and Cottage Industries Corporation in collaboration with CIDD (Control of Iodine Deficiency Disorders) Chittagong
- Bangladesh Small and Cottage Industries Corporation in collaboration with CIDD (Control of Iodine Deficiency Disorders) Dhaka
- Bangladesh Standards & Testing Institute Chittagong
- Bangladesh Standards & Testing Institute Dhaka
- Germany BioAnalyt
- Germany Intertek Food Services
- Germany SGS
- Kenya Bureau of Standards
- Malawi Bureau of Standards
- Malawi Community Health Service Unit
- Mozambique Laboratorio Nacional de Higiene, Aguas e Alimentos
- Nigeria - Standards Organisation of Nigeria
- Nigeria Agency for Food and Drug Administration and Control
- South Africa Southern African Grains Laboratory
- Uganda Bureau of Standards
- Zambia Bureau of Standards

Acronyms

ANOVA	Analysis of variance
CAPA	Corrective and Preventative Action(s)
CV	Coefficient of variation (specified at a particular confidence percentage)
ECSA-HC	East, Central and Southern Africa – Health Community
g	gram
Kg	Kilogram
MAD	Median absolute difference
mg	milligram
RE	retinol equivalent
U	Measurement uncertainty at 95% confidence using robust standard deviation and median in this study

Executive Summary

This study set out to quantify measurement uncertainty in analysis of typical indicators used in food fortification monitoring such as iodine in salt, vitamin A in edible oils and, in some countries cereals and sugar, plus iron in cereals. It was intended to also look to see if this uncertainty could be quantified by methodology and/or vehicle.

Samples of salt, edible oil, maize meal, and wheat flour were spiked with known levels of iodine, vitamin A and iron as appropriate and sent to participating laboratories on three separate occasions. Each round of samples were unique and each laboratory received three different levels and blind replicates.

The laboratories participating in this study use a variety of different techniques such as international reference methods, regional (ECSA-HC) reference methods and rapid test kits (iCheck). Almost all of the laboratories involved play a role in regulatory monitoring and enabling regulators to make decisions based on their analytical reports. Very few of the laboratories participate in any form of proficiency schemes (and they recognise this as a weakness) due to the perceived high cost of such schemes and find themselves unable to motivate to management for participation. Available schemes, however, related either to a wheat flour sample with known values vitamins, minerals and some proximate parameters or to breakfast cereals and infant formulas. There appear to be, currently, no proficiency schemes targeting fortified food vehicles.

It is not unknown for regulator monitoring results to be in conflict (for a variety of other reasons such as sample collection and extended turnaround time of analysis) with one another creating situations of mistrust between regulator and industry, even regulators in trading countries.

During the course of the investigation, which was carried out over the period April to June 2017, the objectives moved towards noting areas worthy of investigation away from focussing on quantification unless that quantification was evident.

It should be noted that the robust standard deviation and median were used in calculating the Z-score for all participating laboratories and the only deviation from this policy was for the baseline vitamin A in oil as the data was clearly bi-modal and not normally distributed as required by robust statistics.

In comparing the spike value with the assigned value the latter is higher in every case except for the vitamin A in oil. For iodine in salt there is no clear explanation whereas for vitamin A in oil Reference laboratory 1 was clearly closer to the spiked value than Reference laboratory 2. The apparent over recovery of nutrients in the grains is probably due to overages in the premix for vitamin A and for iron the overages plus the intrinsic content.

With vitamin A in oil the baseline clearly shows average recoveries of < 90% though clear differences between the two HPLC methods with Laboratory 1 closer to theoretical than Laboratory 2. This indicates that the choice of reference method within a technique could be critical plus it is of importance to note that few industries use HPLC – the instrument of choice amongst regulators – but use iCheck and/or UV/Vis spectrophotometers

With vitamin A in maize meal – which has higher CV and U at low assigned values than wheat flour – this may be due to the apparent lack of homogeneity identified.

In terms of analytical methods used the iCheck provided slightly less of half the data (46%) but provided over two thirds (68%) of the Z-Scores <±2 and 72% of those <±3. In terms of missing data iCheck accounted for 19% of that total and official reference methodology the rest which is very concerning as the laboratories knew well in advance this study was being planned. That 40% of the data was not reported is also a clear message that laboratory capacity needs to be investigated in depth and laboratories be honest with themselves over the constraints they are obviously facing.

The lack of reference method analysis of vitamin A in cereal products from the regulatory authority laboratories is of particular concern.

All of the laboratories measured TOTAL iron as the intrinsic level was not provided – nor would it be likely to be available in a regulatory situation. The intrinsic iron content of maize meal and wheat flour was measured by one of the reference laboratories on two separate occasions.

The values for iron range from 10% to 100% greater than the addition level with the low iron addition having the higher recovery. It would be on the basis of these values that the regulatory authorities would determine if fortification had been complied with or, as is the concern of many regulators, fortification had been over dosed.

Using the assigned value and subtracting the intrinsic iron content determined by one of those laboratories a significantly different picture emerges. By deducting the intrinsic value the baseline laboratories indicate that iron was actually under-recovered with the data indicating recoveries of between 70% and 95% with the low iron addition having a lower recovery.

This data starts to indicate that having a fixed correction value for intrinsic iron can be questioned that the correction value could, and this would need further investigation, be dependent on the addition level of the iron.

Most of the laboratories have reason to investigate at least part of their systems and a couple are in need of extensive technical support. Factors affecting the quality of the laboratory analysis included failure to strictly follow method protocol, use of differing calibration techniques, apparent failure to use control samples, equipment instability and use of different correction factors. The latter applied particularly to the use of iCheck technology where some laboratories would use a correction factor for vitamin A in grains to correct for background and others would not. Correction for intrinsic iron content also varied by laboratory with others reporting only total content.

From discussions in the duration of this exercise it has become clear that some form of regular proficiency testing is not only required but would be welcomed.

It is recommended that the derivation of such an exercise be organised by - at least within the ECSA Region – bringing in support from the ECSA Laboratory Working Group. Further that support be given to the working group especially as the question of validation of the ECSA methodologies themselves is being openly questioned by some and vehemently rejected by others (who feel it unnecessary). On balance the ECSA methodologies did give more reason for concern.

If such a proficiency scheme is launched it is strongly recommended that the participant base be widened to include all regulatory authorities, 3rd party laboratories and industry. It is recommended such an initiative set itself a goal to be self-funding in a short period of time for sustainability issues (previous attempts have failed due to slow delivery by laboratories). Fee for service maybe an option to explore but whether a subscription system would speed up laboratory performance is open to debate.

Background

Recent workshops have brought to the fore several issues which impact on programme management of fortification and potentially lead to decisions being made based on false quantified data:

1. Material submitted to laboratories for analysis do not take cognisance of internationally acceptable sampling protocols and/or
2. Material is taken at point of final sale with no scientific data of possible losses of micronutrients during that particular distribution chain.
    a. Additionally arguments prevail regarding which entity or entities are responsible for monitoring all points between production and point of final sale.
3. Analytical results are taken as being absolute without:
    a. Analytical data regarding measurement uncertainty
    b. Analytical data regarding inter-method and/or inter-technique comparison

Bullet point 1 deals with sampling uncertainty (believed to be highly significant) which cannot be estimated without prior knowledge of measurement uncertainty and bullet point 2 similarly requires prior knowledge of measurement uncertainty. Both of these bullets are outside the scope of this assessment

Before attempting to address bullet 3 it is necessary to see how individual laboratories, known to be using varying analytical techniques, actually perform when provided with the same samples and if remedial action is required.

Objectives

The laboratories participating in this study use a variety of different techniques such as international reference methods, regional (ECSA-HC) reference methods and rapid test kits (predominantly iCheck). Each laboratory was sent spiked food vehicles so that data could be gathered to quantify:

1. Analytical data quantifying measurement uncertainty
2. Analytical data regarding inter-method and/or inter-technique comparison
    a. Identify and record the specific methodology used by individual laboratories

    b. Quantify difference (if any) between methodology and/or techniques and estimate if they are due to the methodology and/or technique or due to laboratory performance
3. Use statistical analysis to analyse the collected data over, ideally, three (3) separate sets of samples (a set being samples of each matrix that is relevant to that laboratory) with regard to inter and intra laboratory analysis and measurement uncertainty.
4. Report on the findings of the above ring trials
    a. At an ECSA meeting circa June 2017 – completed and report updated information at a future ECSA meeting (date to be decided)
    b. Seek to publish a technical article
5. Draw a conclusion, with justification, if funding needs to be sought to expand such an exercise to all food laboratories conducting analysis of micronutrients in food fortification programmes

From bullets 1-3 an indication of how reliable and/or variable individual laboratories can be estimated.

During the course of the investigation the objectives moved towards noting areas worthy of investigation (discussed in Statistics later) away from focussing on quantification unless that quantification was evident.

Important notes & considerations

1. Blind coded samples to be sent to each laboratory for analysis
2. Three unique separate rounds of samples to each participating laboratory
3. Precise instructions to accompany each sample set
4. Laboratories to use their existing analytical protocols and identify what these protocols were
5. Results within 30 days of receipt
6. All results to remain confidential
7. Individual laboratories to be informed of their own data analysis at the conclusion of all 3 rounds by all participants

Sample Preparation

Spiked samples were prepared by 3rd parties namely BioAnalyt and BASF.

It is recognised that neither party is ISO 17043 accredited but both parties have extensive experience of preparing spiked samples for clients over a number of years

Samples were prepared on three (3) separate occasions for

1. Edible oil[1] with vitamin A
2. Maize meal with vitamin A and iron (NaFeEDTA)
3. Salt with iodine
4. Wheat flour with vitamin A and iron (NaFeEDTA)

BioAnalyt prepared the above samples.

BASF prepared sugar samples which will be reported on in an update of this document if a suitable method to statistically analyse the data can be identified [all 180 samples are different as they were individually prepared].

The original intention was to supply samples February, March and April however, for various logistical reasons, the samples were only issued April and May with a significantly shorter time period between the samples than anticipated. Materials were sourced in Germany by BioAnalyt and Denmark by BASF.

For spiking salt, potassium iodate was sourced from Merck Millipore Certipur® 99.76% ±0.05 k=2; Number 1.02404.0100 and Lot 162404M. Retinyl palmitate was sourced from Fluka Analytical 99.9% ±0.6 k=2; Number PHR1235 and Lot LRAA5743. Rapeseed oil from Brassica was sourced from Sigma-Aldrich Density D20/4 0.918, Refractive Index N20/D 1.473; Number 83450 and Lot BCBT0168

The maize meal and wheat was spiked with commercial premix [ELCOvit 10678] sourced from Mülhenchemie containing vitamin A, B_1, B_2, B_3, B_9, B_{12}, iron and zinc. The vitamin A was in the form of vitamin A palmitate sourced from BASF and the iron as NaFeEDTA sourced from AkzoNobel.

The BioAnalyt samples were made up in circa 1 Kg batches and aliquoted whereas the samples from BASF were individually prepared due to the nature of sugar fortification. A preblend was prepared and this was added and mixed to pre-weighed aliquots of unfortified sugar.

The homogeneity was checked by taking three sub samples from each batch and testing them using iCheck. Average coefficient of variation @ 95% (U) in analysis of micronutrient per concentrations was:

[1] Only one type of edible oil (rapeseed/canola) was used though it is recognised oil type could be an influencing factor to an unknown extent

Table 1 – Homogeneity Data on spiked food vehicles

Iodine in Salt	Target Concentration. mg/Kg	**15**	**50**	**90**
	CV @ 95%	7%	7%	3%
	1 x SD. mg/Kg	0.6	1.7	1.4
	1.96 x SD. mg/Kg	1.1	3.4	2.8
Vitamin A in Oil	Target Concentration. mg/Kg	**5**	**15**	**30**
	CV @ 95%	22%	11%	11%
	1 x SD. mg/Kg	0.5	0.9	1.5
	1.96 x SD. mg/Kg	1.1	1.7	3.0
Vitamin A in Maize meal	Target Concentration. mg/Kg	**1.25**	**3.75**	**7.5**
	CV @ 95%	45%	19%	9%
	1 x SD. mg/Kg	0.3	0.4	0.3
	1.96 x SD. mg/Kg	0.7	0.8	0.6
Vitamin A in Wheat flour	Target Concentration. mg/Kg	**1.25**	**3.75**	**7.5**
	CV @ 95%	36%	21%	7%
	1 x SD. mg/Kg	0.2	0.4	0.2
	1.96 x SD. mg/Kg	0.5	0.8	0.5
Iron in Maize meal	Target Concentration. mg/Kg	**15**	**45**	**90**
	CV @ 95%	41%	20%	15%
	1 x SD. mg/Kg	4.3	5.2	7.0
	1.96 x SD. mg/Kg	8.5	10.2	13.8
Iron in Wheat meal	Target Concentration. mg/Kg	**15**	**45**	**90**
	CV @ 95%	26%	12%	10%
	1 x SD. mg/Kg	3.0	3.3	4.8
	1.96 x SD. mg/Kg	5.8	6.5	9.4

BioAnalyt prepared the samples as follows:

Salt

The requisite amount of Potassium iodate was dissolved in 100mL deionised water and spray-dried over 1.5 Kg of refined non-iodated table salt.

Table 2 – Potassium iodate addition to salt

Final Concentration mg/Kg	**15**	**30**	**50**	**60**	**90**
Added Potassium iodate [mg]	37.9	75.8	126.5	151.8	227.6
Total final sample weight [g]	1500	1500	1500	1500	1500

Oil

The requisite amount of 3000 mg RE/kg stock solution (retinyl palmitate) was dissolved in rapeseed oil.

Table 3 – Retinyl palmitate addition to oil

Final Concentration mg/Kg	5	10	15	20	30
Added stock solution [mg]	0.8333	1.667	2.5	3.34	5
Total final sample weight [g]	500	500	500	500	500

Maize meal and wheat flour

The requisite amount of commercial premix was added to 1.2 Kg of maize meal and wheat flour

Table 4 – NaFeEDTA and Retinyl palmitate addition to maize meal and wheat flour

Theoretical[2] Concentration Iron [mg/Kg]	15	30	45	60	90
Theoretical Concentration vitamin A [mg RE/Kg]	1.25	2.5	3.75	5.0	7.5
Added Premix [mg]	300	600	900	1200	1800
Total final sample weight [g]	1200	1200	1200	1200	1200

BioAnalyt samples were supplied in plastic zip lock bags and BASF samples in sealed aluminium bags.

Sample were coded using random numbers sourced from "Million Random Digits"[3] [4] Only 4 and 5 digit numbers were used after checking for, and removal of, duplicates.

[2] The concentration is technically theoretical as the calculations are based on the stated values on the Certificate of Analysis (CoA)

[3] https://www.rand.org/pubs/monograph_reports/MR1418/index2.html

[4] https://www.rand.org/pubs/monograph_reports/MR1418.html

Logistical Problems

Though the project inception was June 2016 provisional go ahead was only circa October 2016 when quotes from identified reference laboratories were requested. In November 2016 potential participants (on an expanding list) were approached with requests for specific information [willingness, what techniques/methods they could use, which sample matrices they could analyse etc.] and it would be early February 2017 when that component was finalised.

Samples were sent to the laboratories on April 3rd, April 21st and May 15th 2017 having been significantly delayed on each occasion by Port Health in South Africa [delays of 5 to 10 days each time] though on only 1 occasion were the contents physically inspected.

Samples of sugar were not issued in Round 2 due to staff retirements at BASF and problems of suitable recruitment.

Immediate Issues

Despite the long run up time to commencing the study serious problems arose in the final weeks before commencing.

Laboratories who had indicated willingness to participate suddenly realised they did not have consumables to carry out the necessary analysis and/or indicated they wished to have some additional technical support from BioAnalyt and Phillip Makhumula.

Consumables, mainly iCheck vials but also gasses for HPLC and reference standards, had to be sought and procured which caused a significant portion of the laboratories to start the study late.

This is a clear finding that many laboratories were not ready for analysis. A few other laboratories were to suffer breakdowns in their reference laboratory equipment during the study which may be an indication that maintenance schedules are not adequate.

Reference Laboratories

Intertek Food Services, SAGL and SGS were identified as reference laboratories based on accreditation and/or extensive routine experience to micronutrient analysis. As BioAnalyt prepared some of the samples and to avoid any possible reasons that the findings are discredited at some point in the future because of their involvement in

analysing samples BioAnalyt were not included in the baseline group. This decision was made in retrospect, after extensive discussion within the support team [David Morgan, Gerhard Rimkus, Phillip Makhumula], and was to have unexpected consequences (see Discussion).

Experimental Design

All samples were allocated a 4 or 5 digit random number upon receipt.

Five (5) samples were sent to each laboratory requesting samples in any of the non-shaded areas in table 5 below

Table 5 – Schematic of spiked samples

	Wheat Flour	Maize Meal	Edible Oil	Sugar	Salt
Reference vitamin A					
iCheck vitamin A					
Reference iron					
iCheck iron					
Reference iodine					
iCheck iodine					

For rounds 1 and 2 the samples compromised of Low, Target and High micronutrient content as indicated in table 6 below [units are in mg RE/Kg and mg/Kg as required].

Table 6 – Concentrations of micronutrients per vehicle

	Low	Target	High
Vitamin A in Grain mg RE/Kg	1.25	3.75	7.5
Iron in Grain mg/Kg	15	45	90
Vitamin A in Oil mg RE/Kg	5	15	30
Vitamin A in Sugar mg RE/Kg	7.5	15	25
Iodine in Salt mg/Kg	15	50	90

For round 3 the low and high spiked concentrations were moved closer to the target and the resultant data could not be used (see Discussion) with the exception of the target concentration.

The 5 samples comprised one of each the low, target and high plus any 2 samples randomly picked from the 3 available options. For laboratories identified as using ECSA reference methodology for edible oil a blank (unfortified) oil was provided.

In Rounds 2 and 3 SAGL was additionally provided with unfortified samples of wheat flour and maize meal for intrinsic iron content measurement. Whilst all of the laboratories are measuring total iron regardless of technique or method this number was thought to be probably relevant.

Sugar

The sugar samples are essentially unique samples i.e. every sample was hand made under strictly controlled conditions and to high levels of measurement accuracy. This is currently creating problems in statistical analysis using the techniques being described below. An alternative approach is currently being investigated.

Statistical Analysis

Data was analysed with and without outlier analysis (Dean and Dixon; Grubbs; ANOVA etc.) with the objective of identifying laboratories and/or techniques worthy of investigation.

Routine statistical parameters such as mean; standards deviation and CV[5] @ 95% (expanded measurement uncertainty), Z score and recovery were routinely generated. For the baseline group – the reference points for comparing the participating laboratories – the median/MAD method was used to generate the robust mean and robust standard deviation to mitigate the impact of potential outliers in that baseline data.

When all the data was collated it was then possible to explore potential areas of interest such as bias – possibly identifiable skew, differences (statistically valid differences) between analytical techniques (iCheck x HPLC x Spectrophotometer etc.) and Z score with and without Reference laboratories and straight against Reference laboratories.

All data is reported anonymously i.e. not identifying laboratory or country and unless otherwise indicated all probabilities are assessed at $p < 0.05$

Terminology such as “outlier analysis” needs to be clarified and for that it is necessary to have at least some non-technical knowledge of statistics which is provided in Annex 1

[5] For cases were laboratories provided more than 2 values for a parameter

In this study anomalous data was identified both statistically and subjectively. Statistical identification is clarified in Annex 1 but some data just 'looks wrong' and can be justified as explained by Grubbs bullet 3.

For example. A laboratory is provided with 5 samples with values of 30, 30, 50, 90 and 90 but reports results 25, 24, 30, 40 and 39 (hypothetical case) it is clear that whatever is happening in that laboratory they are failing, for some reason, to adequately distinguish between the data points. Linear regression and statistical analysis of the duplicates would indicate no problems but the slope of the expected and achieved results would be different. Samples at the low end would also be considered statistically valid and, given enough analytical variability it is possible the high end samples could be masked and, therefore, not easily identified statistically. Looking at the group of 5 results as a whole it is, however, clear that something does need investigating. In such cases the data will be deemed justified for exclusion whilst recording that fact.

A preliminary look at the data has identified that many laboratories provided a single[6] number (reportable number) but did duplicate or triplicate analysis. This was asked for in the testing protocol but not all laboratories either did this or provided the data. Looking at the available data it appears that some laboratories had very high differences between replicates and even if the reportable number was statistically valid it appears that some may have variability of a scale that warrants investigation. What is of concern is that some of the replicate analysis may have hidden within it individual data points that they could be statistical outliers. A potential problem is envisaged in that a triplicate analysis with a reportable number of 12 from data points 10, 11 and 15 would statistically concluded 15 was an outlier. In such cases, again, a judgement call will be required and reported.

Establishing a baseline

In order to obtain an estimate of what the analytical response capability of the participating laboratories was a baseline was established using the data obtained from the reference laboratories Intertek Food Services, SAGL and SGS. These laboratories had been chosen on the basis of their laboratory accreditation status, years of experience with

[6] All of the reference laboratories (Intertek Food Services, SAGL and SGS) did this but it is known (Intertek Food Services and SAGL) that each laboratory performed at least duplicate analysis and believed SGS did the same

micronutrient analysis, using 'conventional' international reference methods, in various food matrices.

In the tables relating to the baseline:

1. MAD is the median absolute difference
2. CV 95% - Measurement uncertainty has been calculated from the standard deviation and the mean (arithmetic)
3. U – Measurement uncertainty at 95% k= 1.96 has been calculated from the robust standard deviation and the median.
4. Robust standard deviation = MAD x 1.5
5. Grubbs has been calculated from maximum or minimum value minus the median divided by the robust standard deviation unless otherwise indicated

The baseline data is being used to calculate the assigned or 'true' levels that will be used in the comparison of the participating laboratories and the spiked levels are only theoretical values – especially for iron. The measurement uncertainty, Z score, mean, median, robust mean will be compared using the assigned values so it is important to ensure the baseline data is 'reliable'. This leads to the potential justification of anomalous data to ensure the best assigned values are generated.

Iodine in Salt

The data in Table 7 shows the raw data obtained over 3 rounds.

Laboratory 1 has only 2 rounds of data (rounds 1 and 3) due to an accidental contamination of the round 2 salt samples. All analysis of round 2 salt from Laboratory 1 was aborted.

Reference laboratory 2 has three data points of interest (two at 50 mg/Kg and one at 90 mg/Kg). Looking at Grubbs using the mean and standard deviation (not shown) the data is not indicated as outliers but using Grubbs with median and robust standard deviation statistical outliers are indicated.

The two values at the 50 mg/Kg concentration are almost double the expected result and this is being queried with the laboratory concerned. As the requirement is to generate good assigned values and noting that Grubbs (based on robust statistics) indicate outliers, the mean and median are markedly different and the standard deviation is high these values will be deleted.

The value at 90 mg/Kg is, however, more problematic. Grubbs indicates the value is an outlier but the total number of samples is very small (only 3 data points). The Royal Society of Chemistry Analytical Methods Committee No.6 Apr 2001 "Robust statistics: a method of coping with outliers"[7] note that robust statistics assume roughly normal distribution but that results can be misleading if a large proportion of the data are identical in value. This appears to be the situation with these three values and it was concluded that deletion was not required.

Table 7 - Baseline for salt analysis

	Iodine in Salt		
	Concentration mg/Kg		
Laboratory	**15**	**50**	**90**
Reference Laboratory 1	15.90	54.43	102.93
	15.42	51.56	
		52.79	
		55.95	
Reference Laboratory 2	16.30	49.40	102.00
	16.50	51.50	
	14.70	49.10	95.70
	14.40	47.40	
		95.70	
		97.40	
Count	6	10	3
Mean	15.54	60.52	100.21
Median	15.66	52.18	102.00
MAD	0.74	2.93	0.93
St Dev	0.85	19.16	3.93
Robust St Dev	1.11	4.39	1.40
CV 95%	10.78	62.04	7.69
U	13.89	16.48	2.68
Minimum	14.40	47.40	95.70
Maximum	16.50	97.40	102.93
Grubbs Minimum	1.14	1.09	4.52
Grubbs Maximum	0.76	10.31	0.67
Recovery	103.6	121.0	111.3

[7] http://www.rsc.org/images/robust-statistics-technical-brief-6_tcm18-214850.pdf

The revised data for use in the base line is given Table below.

Table 7 revised Baseline for salt analysis

	Iodine in Salt		
	Concentration mg/Kg		
Laboratory	**15**	**50**	**90**
Reference Laboratory 1	15.90	54.43	102.93
	15.42	51.56	
		52.79	
		55.95	
Reference Laboratory 2	16.30	49.40	102.00
	16.50	51.50	95.70
	14.70	49.10	
	14.40	47.40	
		Deleted	
		Deleted	
Count	6	8	3
Mean	15.54	51.52	100.21
Median	15.66	51.53	102.00
MAD	0.74	2.28	0.93
St Dev	0.85	2.85	3.93
Robust St Dev	1.11	3.42	1.40
CV 95%	10.78	10.86	7.69
U	13.89	13.01	2.68
Minimum	14.40	47.70	95.70
Maximum	16.50	55.95	102.93
Grubbs Minimum	1.14	1.21	4.52
Grubbs Maximum	0.76	1.29	0.67
Recovery	103.6	103.0	111.3
Z score			
Reference Laboratory 1	0.00	0.63	0.67
Reference Laboratory 2	-0.17	-0.64	-2.26

The Z statistic is commonly used in proficiency testing as an indication of laboratory proficiency.

The Z value is calculated as follows:

$$Z = \frac{Median\ value\ at\ concentration - Robust\ mean\ [Median]\ of\ the\ baseline}{Robust\ standard\ deviation\ of\ baseline}$$

Each individual Z statistic represents the decimal number of standard deviations by which an analytical result differs from the estimate of the true value as represented by the average value.

Z statistic < 1 is considered 'outstanding accuracy and precision'; > 1 and < 2 is considered satisfactory accuracy and precision; > 2 and < 3 is considered questionable and indicative of attention being necessary to equipment and/or procedures. Z statistics > 3 are considered unsatisfactory and require urgent investigation.

Out of the three reference laboratories two participated in analysing the salt samples which makes interpretation of Z statistic possibly misleading (with only two data points laboratories could be wildly different but still return a low Z statistics) but are given here for completeness.

Vitamin A in Edible Oil

The data for vitamin A in edible oil (Table 8) may prove contentious as the data is clearly bi-modal and, as such, the "Robust statistics: a method of coping with outliers" monograph notes that robust statistics "… will give misleading results if they are applied to data sets that are markedly skewed or multimodal…". The robust statistics have, nevertheless been generated to demonstrate that point and for possible use against the participating laboratories if that should prove necessary.

Of possible concern, and in need of checking with the participating laboratories data, is the baseline clearly shows average recoveries of < 90% though clear differences between the two HPLC methods with Laboratory 1 closer to theoretical than Laboratory 2. This indicates that the choice of reference method within a technique could be critical.

Table 8 – Vitamin A in Edible Oil

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Laboratory	**5**	**15**	**30**
Reference Laboratory 1	5.01	15.04	30.03
	5.12	15.15	
	4.91	14.61	29.92
	5.01		29.87

		13.68	
		13.73	
Reference Laboratory 2	4.15	12.30	23.60
		10.80	25.60
	3.83	11.50	25.20
	3.81		22.10
		11.90	
		12.40	
Count	7	10	7
Mean	4.55	13.11	26.62
Median	4.91	13.04	25.60
MAD	0.21	1.34	3.50
St Dev	0.59	1.54	3.31
Robust St Dev	0.32	2.01	5.25
CV 95%	25.52	23.05	24.36
U	12.57	30.21	40.20
Minimum	3.81	10.80	22.10
Maximum	5.12	15.15	30.03
Grubbs Minimum using Mean	1.25	1.50	1.37
Grubbs Maximum	0.97	1.32	1.03
Grubbs Minimum using Robust statistics	3.49	1.11	0.67
Grubbs Maximum	0.67	1.05	0.84
Recovery	91.0	87.4	88.7
Z score			
Reference Laboratory 1	0.78	0.86	1.00
Reference Laboratory 2	-1.04	-0.86	-0.75

If robust statistics are used Grubbs sequentially deletes all of reference laboratory 2 data for the 5 mg RE/Kg spiked samples.

Vitamin A in Maize meal

The data in Table 9 shows the results obtained over 3 rounds with two statistically indicated deletions from Laboratory 1 (2.18 and 5.18 for 3.75 and 7.5 mg RE/Kg respectively). Further outlier analysis indicated an additional deletion from Laboratory 2 (9.33 for 7.5 mg RE/Kg).

Table 9 – Vitamin A in Maize meal

Vitamin A in Maize meal			
	Concentration mg RE/Kg		
Laboratory	1.25	3.75	7.5
Reference Laboratory 1	0.82	2.18	7.68
	1.53		5.18
		3.65	6.98
	0.82	3.65	6.98
		4.80	
		3.98	
Reference Laboratory 2	1.27	4.08	9.33
	1.51	4.71	8.29
	0.84	3.92	7.65
	1.11	4.18	7.55
Reference Laboratory 3	1.74	3.67	8.04
	0.99	3.51	7.85
	1.29	4.04	7.05
		3.92	7.50
Count	10	13	12
Mean	1.19	3.87	7.51
Median	1.19	3.92	7.60
MAD	0.33	0.26	0.50
St Dev	0.33	0.64	0.98
Robust St Dev	0.50	0.39	0.74
CV 95%	54.25	32.31	25.59
U	81.53	19.50	19.15
Minimum	0.82	2.18	5.18
Maximum	1.74	4.80	9.33
Grubbs Minimum	0.75	4.46	3.26
Grubbs Maximum	1.11	2.26	2.33
Recovery	119.2	110.5	125.1
Z score			
Reference laboratory 1	-0.27	-0.35	0.01
Reference Laboratory 2	-0.02	0.78	0.81
Reference laboratory 3	0.30	-0.35	0.01

The revised data for use in the base line is given Table below.

Table 9 revised vitamin A in Maize meal

Vitamin A in Maize meal			
	Concentration mg RE/Kg		
Laboratory	1.25	3.75	7.5
Reference Laboratory 1	0.82	Deleted	7.68
	1.53		Deleted
		3.65	6.98
	0.82	3.65	6.98
		4.80	
		3.98	
Reference Laboratory 2	1.27	4.08	Deleted
	1.51	4.71	8.29
	0.84	3.92	7.65
	1.11	4.18	7.55
Reference Laboratory 3	1.74	3.67	8.04
	0.99	3.51	7.85
	1.29	4.04	7.05
		3.92	7.50
Count	10	12	10
Mean	1.19	4.01	7.56
Median	1.19	3.95	7.60
MAD	0.33	0.26	0.35
St Dev	0.33	0.40	0.45
Robust St Dev	0.50	0.38	0.52
CV 95%	54.25	19.72	11.62
U	81.53	18.98	13.35
Minimum	0.82	3.51	6.98
Maximum	1.74	4.80	8.29
Grubbs Minimum	0.75	1.15	1.20
Grubbs Maximum	1.11	2.22	1.33
Recovery	119.2	114.5	126.0
Z score			
Reference laboratory 1	-0.27	-0.43	0.02
Reference Laboratory 2	-0.02	0.71	0.44
Reference laboratory 3	0.30	-0.43	0.02

Of possible concern, and possibly in need of investigation, is that U for the low spiked sample (1 mg RE/Kg) was high at 81.5% between laboratories with a mean/median of

1.2 mg RE/kg and a range of 0.8 mg RE/kg to 1.7 mg RE/kg. This area could be of concern as many countries, adding vitamin A into maize meal do so at levels between 1 and 2 mg RE/Kg levels (see Discussion).

Over recovery may be due to theoretically calculating final concentration of vitamin A from the Certificate of Analysis (CoA) of the premix used.

Vitamin A in Wheat flour

The data in Table 10 shows the results obtained over 3 rounds with one deletion indicated from laboratory 1 (0.6 for 1.25 mg RE/Kg).

Table 10 – vitamin A in Wheat flour

Vitamin A in Wheat flour			
	Concentration mg RE/Kg		
Laboratory	1.25	3.75	7.5
Reference Laboratory 1	1.04	5.23	7.47
	0.60	4.20	6.21
	0.98	3.60	8.07
		3.00	
		4.63	
		2.34	
Reference Laboratory 2	1.37	3.95	8.59
	1.29	4.68	8.09
	1.61	4.71	
	1.16	4.51	
		4.44	
		3.75	
Reference Laboratory 3	1.05	3.30	6.33
	1.21	4.17	8.81
	1.07	3.41	8.50
	1.21	3.86	
Count	11	16	8
Mean	1.14	3.99	7.76
Median	1.16	4.06	8.08
MAD	0.12	0.52	0.56
St Dev	0.25	0.74	1.01
Robust St Dev	0.18	0.77	0.84
CV 95%	43.54	36.43	25.40
U	30.41	37.29	20.38
Minimum	0.60	2.34	6.21

Maximum	1.61	5.23	8.81
Grubbs Minimum	3.11	2.23	2.23
Grubbs Maximum	2.50	1.51	0.87
Recovery	114.5	113.9	129.3
Z score			
Reference laboratory 1	-1.59	0.36	0.31
Reference Laboratory 2	-0.14	-0.49	-0.24
Reference laboratory 3	1.10	0.36	0.31

The revised data for use in the base line is given Table below.

Table 10 revised vitamin A in Wheat flour

	Wheat vitamin A		
	Concentration mg RE/Kg		
Laboratory	1.25	3.75	7.5
Reference laboratory 1	1.04	5.23	7.47
	Deleted	4.20	6.21
	0.98	3.60	8.07
		3.00	
		4.63	
		2.34	
Reference laboratory 2	1.37	3.95	8.59
	1.29	4.68	8.09
	1.61	4.71	
	1.16	4.51	
		4.44	
		3.75	
Reference laboratory 3	1.05	3.30	6.33
	1.21	4.17	8.81
	1.07	3.41	8.50
	1.21	3.86	
Count	10	16	8
Mean	1.20	3.99	7.76
Median	1.19	4.06	8.08
MAD	0.14	0.52	0.56
St Dev	0.19	0.74	1.01
Robust St Dev	0.20	0.77	0.84
CV 95%	30.84	36.43	25.40

U	33.49	37.29	20.38
Minimum	0.98	2.34	6.21
Maximum	1.61	5.23	8.81
Grubbs Minimum	1.01	2.23	2.23
Grubbs Maximum	2.10	1.51	0.87
Recovery	119.9	113.9	129.3
Z score			
Reference laboratory 1	-0.86	-0.29	-0.99
Reference Laboratory 2	0.85	0.36	0.31
Reference laboratory 3	-0.25	-0.49	-0.24

The higher value for U at low concentrations was not repeated in the wheat data set

Again the over recovery may be due to theoretically calculating final concentration of vitamin A from the CoA of the premix used.

Iron in Maize Meal

The preparation for maize and wheat are identical as the premix contained both vitamin A and iron as NaFeEDTA.

The data in Table 11 shows the results obtained over 3 rounds with three statistically indicated outliers. In laboratory 2 (61.0 at 45.0 mg/Kg) and in Laboratory 3 (84.8 in 90 mg/Kg which on deletion indicated laboratory 1 (91.47 in 90 mg/Kg).

Table 11 – Iron in Maize meal

	Iron in Maize meal		
	Concentration mg /Kg		
Laboratory	15	45	90
Reference laboratory 1	21.40	53.22	101.52
	21.08		99.58
		47.88	95.63
	20.18	47.91	91.47
		51.55	
		50.30	
Reference laboratory 2	25.00	52.00	98.00
	25.00	50.00	97.00
	23.00	61.00	98.00
	22.00	57.00	

Reference Laboratory 3	23.50	45.60	97.00
	23.90	52.30	92.60
	23.10	51.40	95.40
		55.80	84.80
Count	10	13	11
Mean	22.82	52.00	95.55
Median	23.05	51.55	97.00
MAD	1.35	1.67	1.60
St Dev	1.63	4.12	4.57
Robust St Dev	2.03	2.51	2.40
CV 95%	14.01	15.55	9.38
U	17.22	9.52	4.85
Minimum	20.18	45.60	84.80
Maximum	25.00	61.00	101.52
Grubbs Minimum	1.42	2.38	5.08
Grubbs Maximum	0.96	3.77	1.88
Recovery	152.1	115.5	106.2
Z score			
Reference laboratory 1	-1.07	-0.55	0.02
Reference Laboratory 2	0.35	1.38	0.28
Reference laboratory 3	0.22	-0.11	-1.90

The revised data for use in the base line is given Table below.

Table 11 revised iron in Maize meal

	Iron in Maize meal		
	Concentration mg /Kg		
Laboratory	15	45	90
Reference laboratory 1	21.40	53.22	101.52
	21.08		99.58
		47.88	95.63
	20.18	47.91	Deleted
		51.55	
		50.30	
Reference laboratory 2	25.00	52.00	98.00
	25.00	50.00	97.00
	23.00	Deleted	98.00
	22.00	57.00	

Reference Laboratory 3	23.50	45.60	97.00
	23.90	52.30	92.60
	23.10	51.40	95.40
		55.80	Deleted
Count	10	12	9
Mean	22.82	51.25	97.19
Median	23.05	51.48	97.00
MAD	1.35	1.75	1.60
St Dev	1.63	3.25	2.56
Robust St Dev	2.03	2.62	2.40
CV 95%	14.01	12.44	5.17
U	17.22	9.97	4.85
Minimum	20.18	45.60	92.60
Maximum	25.00	57.00	101.52
Grubbs Minimum	1.42	2.24	1.83
Grubbs Maximum	0.96	2.11	1.88
Recovery	152.1	113.9	108.0
Z score			
Reference laboratory 1	-1.07	-0.50	0.80
Reference Laboratory 2	0.35	0.58	0.28
Reference laboratory 3	0.22	-0.08	-0.83

Iron in Wheat Flour

The data in Table 12 shows the results obtained over 3 rounds with one statistically indicated deletion in Laboratory 1 (24.35 at 15 mg/Kg).

Table 12 - Iron in Wheat flour

	Iron in Wheat flour		
	Concentration mg /Kg		
Laboratory	15	45	90
Reference laboratory 1	29.67	58.38	109.22
	28.71	57.57	96.96
	24.35	57.13	101.59
		52.06	
		60.33	
		58.34	
Reference laboratory 2	30.00	61.00	107.00
	31.00	58.00	103.00

	28.00	58.00	
		62.00	
		63.00	
		61.00	
Reference Laboratory 3	31.50	55.10	98.30
	30.70	56.10	100.20
	29.00	58.50	104.60
	31.30		
		62.60	
Count	10	16	8
Mean	29.42	58.69	102.61
Median	29.84	58.36	102.30
MAD	1.15	2.26	3.15
St Dev	2.13	2.91	4.22
Robust St Dev	1.72	3.39	4.73
CV 95%	14.18	9.70	8.05
U	11.28	11.39	9.05
Minimum	24.35	52.06	96.96
Maximum	31.50	63.00	109.22
Grubbs Minimum	3.19	1.86	1.13
Grubbs Maximum	0.97	1.37	1.47
Recovery	196.2	130.4	114.0
Z score			
Reference laboratory 1	-1.31	-0.31	0.06
Reference Laboratory 2	-0.10	0.63	0.57
Reference laboratory 3	0.46	-0.08	-0.27

The revised data for use in the base line is given Table below.

Table 12 revised iron in Wheat flour

Iron in Wheat flour			
	Concentration mg /Kg		
Laboratory	**15**	**45**	**90**
Reference Laboratory 1	29.67	58.38	109.22
	28.71	57.57	96.96
	Deleted	57.13	101.59
		52.06	

		60.33	
		58.34	
Reference laboratory 2	30.00	61.00	107.00
	31.00	58.00	103.00
	28.00	58.00	
		62.00	
		63.00	
		61.00	
Reference laboratory 3	31.50	55.10	98.30
	30.70	56.10	100.20
	29.00	58.50	104.60
	31.30	62.60	
Count	9	16	8
Mean	29.99	58.69	102.61
Median	30.00	58.36	102.30
MAD	1.00	2.26	3.15
St Dev	1.23	2.91	4.22
Robust St Dev	1.50	3.39	4.73
CV 95%	8.07	9.70	8.05
U	9.80	11.39	9.05
Minimum	28.00	52.06	96.96
Maximum	31.50	63.00	109.22
Grubbs Minimum	1.33	1.86	1.13
Grubbs Maximum	1.00	1.37	1.47
Recovery	199.9	130.4	114.0
Z score			
Reference laboratory 1	-0.54	-0.31	0.06
Reference Laboratory 2	-0.22	0.63	0.57
Reference laboratory 3	0.42	-0.08	-0.27

Baseline Summary

Table 13a below provides the reference statistics to be used for the participating laboratories.

The assigned value is the median of the baseline values. Robust statistics was used for all parameters except for vitamin A in edible oil which was noted to have a bi-modal distribution as previously described.

Table 13a – Reference statistics for participating laboratory comparison

Analyte	Assigned Value	Robust St Dev	U (Uncertainty %)
Iodine in Salt mg/Kg	15.7	1.1	13.9
	51.5	2.9	13.0
	102.0	1.4	2.7
Vitamin A in Oil mg RE/Kg	4.9	0.3	12.6
	13.0	2.0	30.2
	25.6	5.3	40.2
Vitamin A in Maize meal mg RE/Kg	1.2	0.5	81.5
	4.0	0.4	19.0
	7.6	0.5	13.4
Vitamin A in Wheat flour mg RE/Kg	1.2	0.2	33.5
	4.1	0.8	37.3
	8.1	1.0	20.4
Iron in Maize meal mg/Kg	23.1	1.6	17.2
	51.5	2.6	10.0
	97.0	2.4	4.9
Iron in Wheat flour mg/Kg	30.0	1.5	9.8
	58.4	3.4	11.4
	102.3	4.7	9.1

As has already been mentioned there were only 3 data points for salt at an assigned value of 102 ppm and that Grubb's indicated one of those values to be an outlier. As also previously mentioned results can be misleading if a large proportion of the data are identical in value; which is the case here. When plotting the Z Score for assigned value 102 ppm the results were extremely poor with 11 participating laboratories having a Z Score >±3; 1 reference laboratory with a score between ±2 and ±3 and 2 laboratories (1 of them a participating laboratory) with a score <±2.

After discussion with the support team (David Morgan, Gerhard Rimkus and Phillip Makhumula) it was proposed to recalculate the Z-Scores with a U of 13, like the other two assigned values, and it's corresponding robust standard deviation of 6.7. After rounding the robust SD and recalculating U this became 13.1 as shown in the revised table 13 b below.

Table 13b revised Reference statistics for participating laboratory comparison

Analyte	Assigned Value	Robust St Dev	U (Uncertainty %)
Iodine in Salt mg/Kg	15.7	1.1	13.9
	51.5	2.9	13.0
	102.0	6.7	13.1
Vitamin A in Oil mg RE/Kg	4.9	0.3	12.6
	13.0	2.0	30.2
	25.6	5.3	40.2
Vitamin A in Maize meal mg RE/Kg	1.2	0.5	81.5
	4.0	0.4	19.0
	7.6	0.5	13.4
Vitamin A in Wheat flour mg RE/Kg	1.2	0.2	33.5
	4.1	0.8	37.3
	8.1	1.0	20.4
Iron in Maize meal mg/Kg	23.1	1.6	17.2
	51.5	2.6	10.0
	97.0	2.4	4.9
Iron in Wheat flour mg/Kg	30.0	1.5	9.8
	58.4	3.4	11.4
	102.3	4.7	9.1

This change was significant in that now the laboratories reported 4 participating laboratories >±3, 2 participating laboratories between ±2 and ±3 and 8 laboratories (including the 2 reference laboratories) >±2

Participating Laboratories by vehicle

As stated earlier, to avoid any potential conflict of interest BioAnalyt data was captured as their specific expertise is with iCheck which is widely used, by regulatory authorities, for rapid quantitative analysis of specific micronutrients.

Not all of the laboratories participated in all of the food vehicles. As per agreements with the laboratories the identity of each laboratory has been coded. Table 14 below indicates the samples requested by each laboratory and confirmed before round 1 was issued. [Note: This is not listing samples reported – which could number up to 19]

Table 14 – Classification of Participating Laboratories

Lab Code	Methodology	Salt Iodine	Grain Iron	Oil vitamin A	Grain vitamin A
1	iCheck	x	x	x	x
2	iCheck	x			
3	iCheck	x			
4a	HPLC			x	
4b	iCheck			x	
5a	HPLC			x	
5b	iCheck			x	
6a	ECSA	x	x	x	x
6b	iCheck	x	x	x	x
7a	ECSA	x			
7b	iCheck	x	x	x	x
8	ECSA	x	x	x	x
9	ECSA	x	x	x	x
10	iCheck		x		x
11a	AOAC	x	x	x	x
11b	iCheck	x	x	x	x
12a	ECSA	x		x	
12b	iCheck	x	x	x	x
13a	ECSA	x	x	x	x
13b	iCheck	x	x	x	x
14	ECSA	x	x	x	x
15	ECSA	x	x	x	x
16	ECSA		x	x	x

The data from the participating laboratories was not statistically treated. Deletions were applied when laboratories reported results as "less than X" rather than as a numerical value as per FAPAS protocol[8].

Laboratory Ability to Respond

The ability of a laboratory to respond to samples by delivering the analyses they stated they could carry out and the time to return the data is an important outcome of this study.

[8] http://sid.gsi.co.jp/csl/fapas/fapas_protocol_6th_ed.pdf

A total of 16 laboratories participated in the study (plus 3 reference laboratories).

Using a time frame of 30 days almost all of the data was returned – mostly in the 14 to 30 day period (the original request was for results within 14 days of receipt). A few laboratories missed the 30 day cut off on one occasion. There were four exceptions:

1. One country with multiple laboratories, and this was due to difficulty getting samples into the country and a generally slow response from some of the participating laboratories. Looking at the analytical reports it would appear at least one laboratory waited until it had two rounds of samples before commencing analysis.
2. One country submitted all of the data at the end of the study and was, therefore 30 to 60 days in reporting.
3. Two countries did not report any data but the samples are confirmed (through courier tracking) to have been received.

Looking at the 16 participating laboratories.

Seven laboratories had indicated they wished to participate, for some parameters, using both reference methodology and iCheck. Of these seven:

1. One completed all 3 rounds except for reference method iron and oil.
    a. This was due to equipment malfunction and was reported prior to commencement of this study. For the purpose of the Z Scores they are not included as being a participant for these parameters.
2. Two provided mainly reference method data only
3. One completed reference method iodine but could only provide iCheck data for round 3 due to lack of vials.
4. One had equipment failure and was unable to carry out vitamin A on fortified grains using the reference method but otherwise completed all of the analyses.
5. One only provided iron analysis using reference methodology but otherwise completed all of the analyses.
6. One managed to carry out iron analysis using reference methodology, one round of salt with reference methodology and sporadic iCheck results were also provided [this was initially explained as reagents failing to clear Customs but even after clearance, and some training, data failed to materialize].

In total five laboratories requested only reference laboratory samples and three reported timeously though one of the three laboratories reported inability to carry out vitamin A analysis in grains as the study commenced. Two laboratories requested samples for reference methodology but neither laboratory submitted any results. No explanation has been provided from one laboratory whilst the other responded they were encountering equipment problems which were being resolved.

Four laboratories participated using only iCheck and completed all 3 rounds.

Laboratory Data

Annex 2 gives the instructions to the participating laboratories for reference methods and Annex 3 gives the instructions for the iCheck.

The data presented below is a scatter plot summation of that individual analyses by food vehicle. Where laboratories did not submit data then that Laboratory code appears but with no scatter point(s) above. The reference laboratory data is always provided closest to the Y axis, left of the dotted line

When anomalous data appears in the participating laboratories then two graphs are provided. The first with the 'outlier' and the second without.

The title indicates the assigned value for the sample set i.e. the salt samples below were spiked with 15, 50 and 90 mg/Kg potassium iodate and the baseline laboratories returned a median value of 15.7, 52.2 and 102.0 mg/Kg respectively.

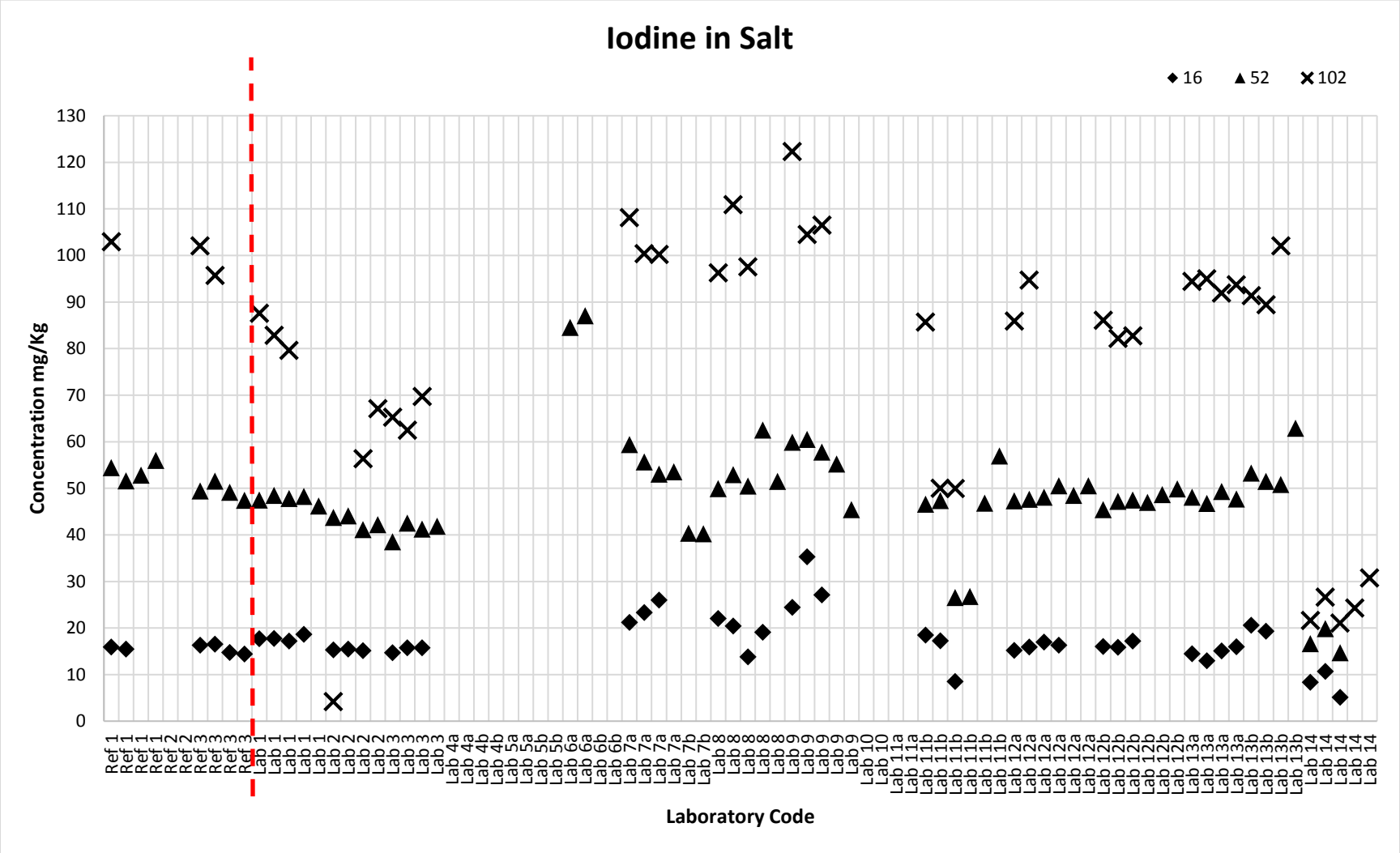
The Z scores calculated from the median (as with the baseline group) are plotted once with a truncated X axis of ±5. Whilst FAPAS warn about over interpretation it is generally considered that ±2 is 'satisfactory' recognising that a laboratory that is performing "fit for purpose" could have a Z score >±2 one in every 20 . Results >±3 do indicate investigation. All laboratory receiving samples, and had not indicated any reasons for not analysing the samples and not submitting results, are separately on the left side of the Y axis. Z scores run left to right negative to positive.

> The vertical dotted line indicates laboratories to the left which did not submit data.

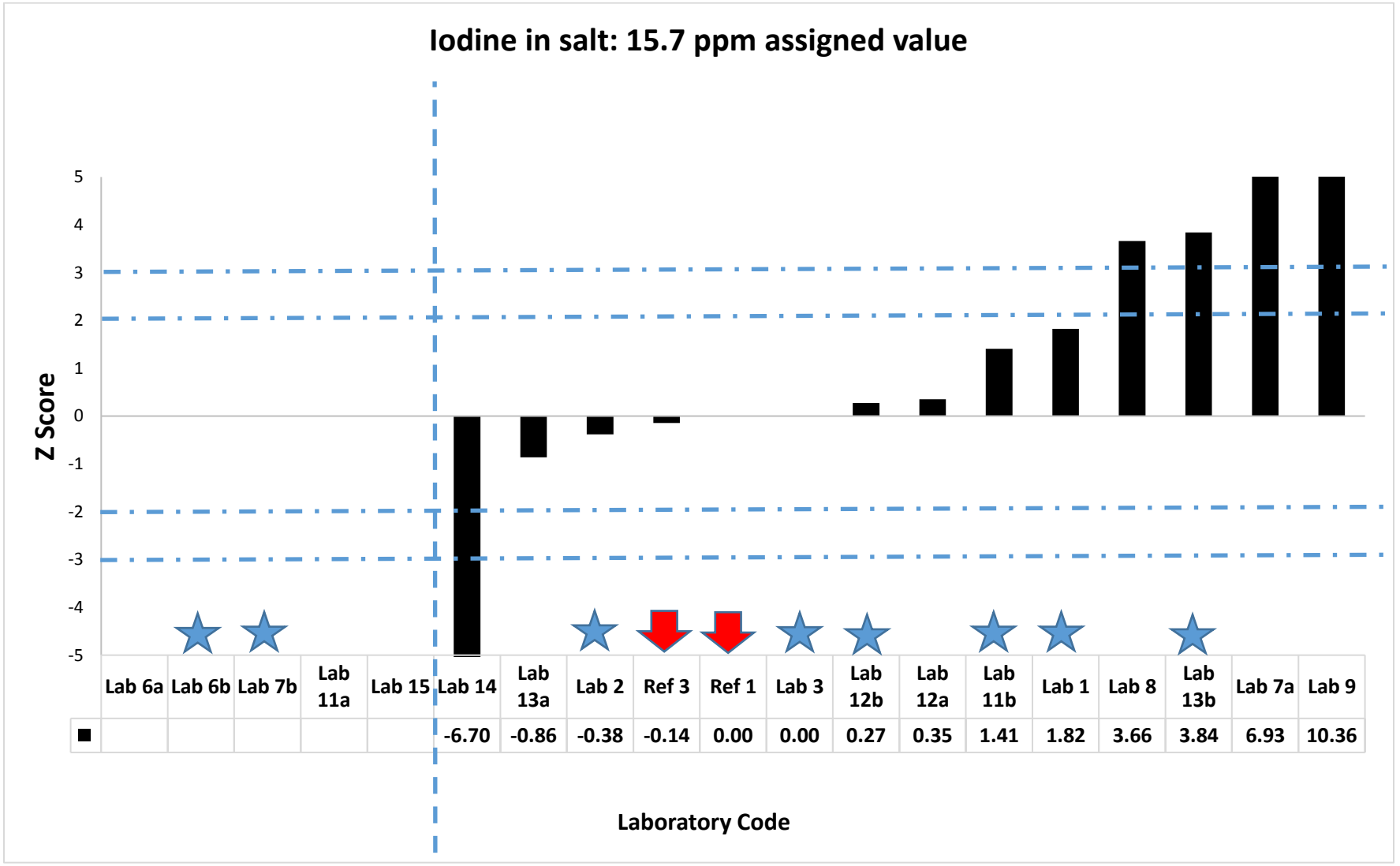
The star symbol (left) over a bar on the histogram indicates the use of the relevant iCheck

Indicates reference laboratory for baseline

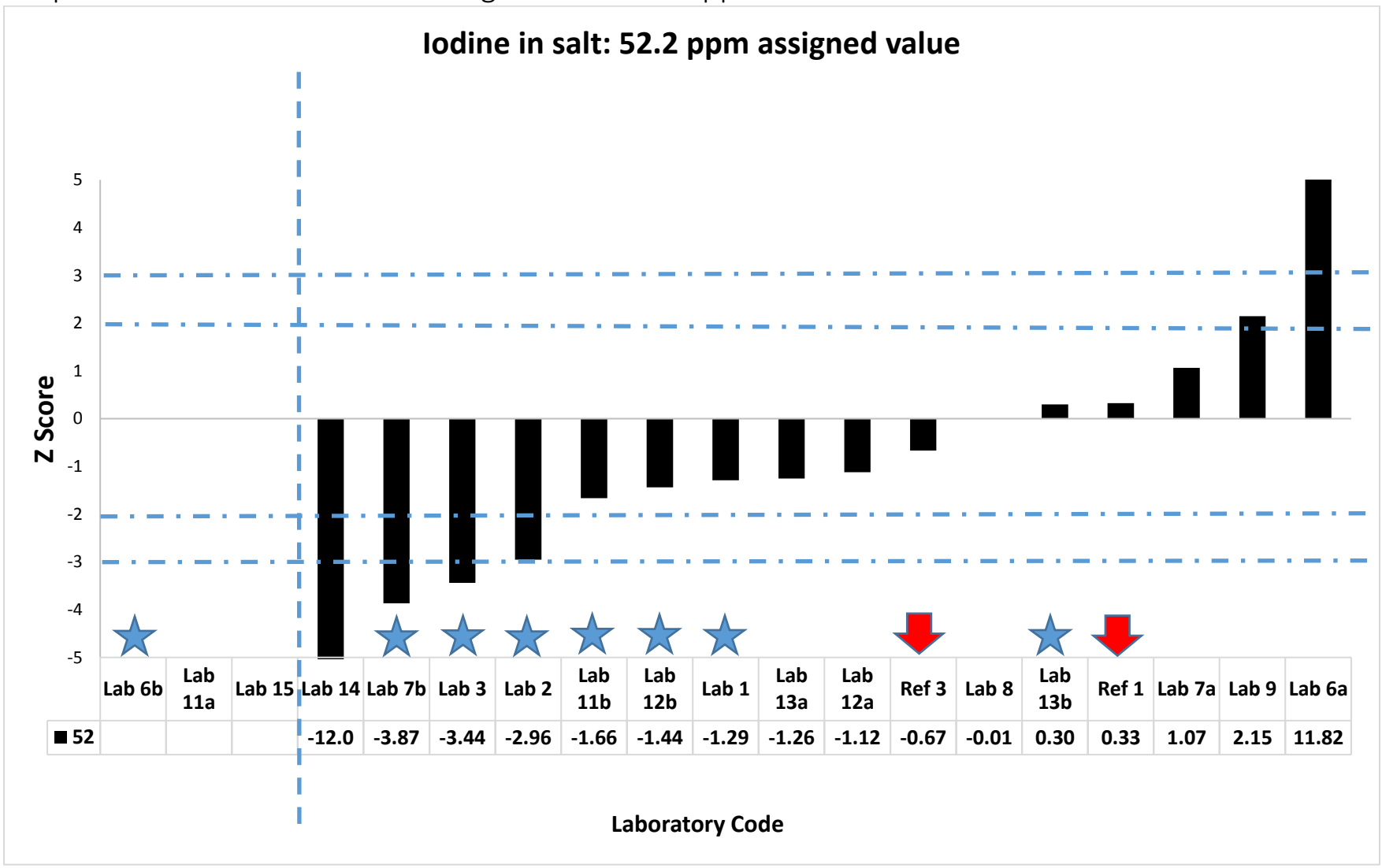
Graph 1 – Scatter Plot Iodine in Salt



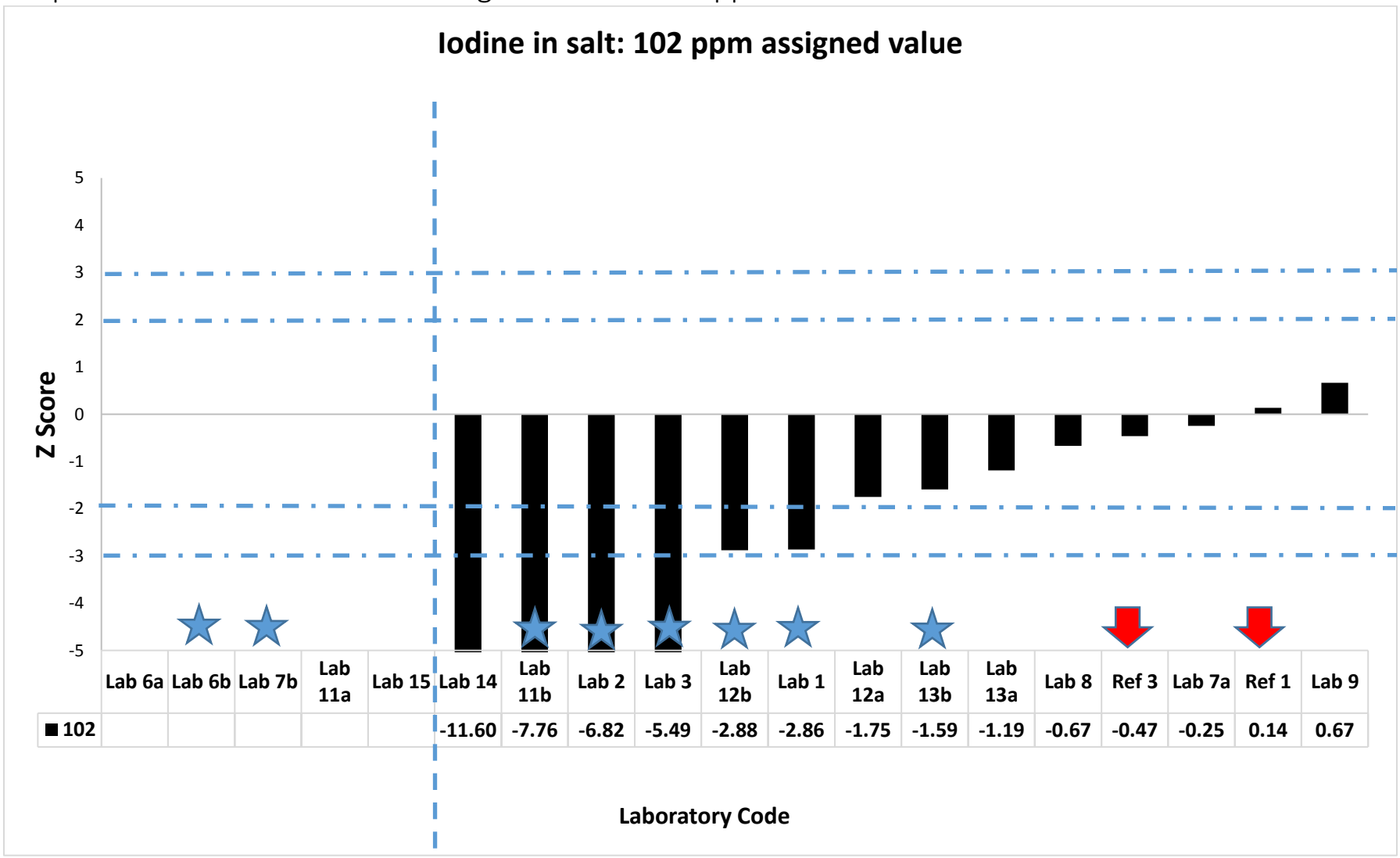
Graph 2 – Z Score Iodine in Salt assigned value 15.7 ppm



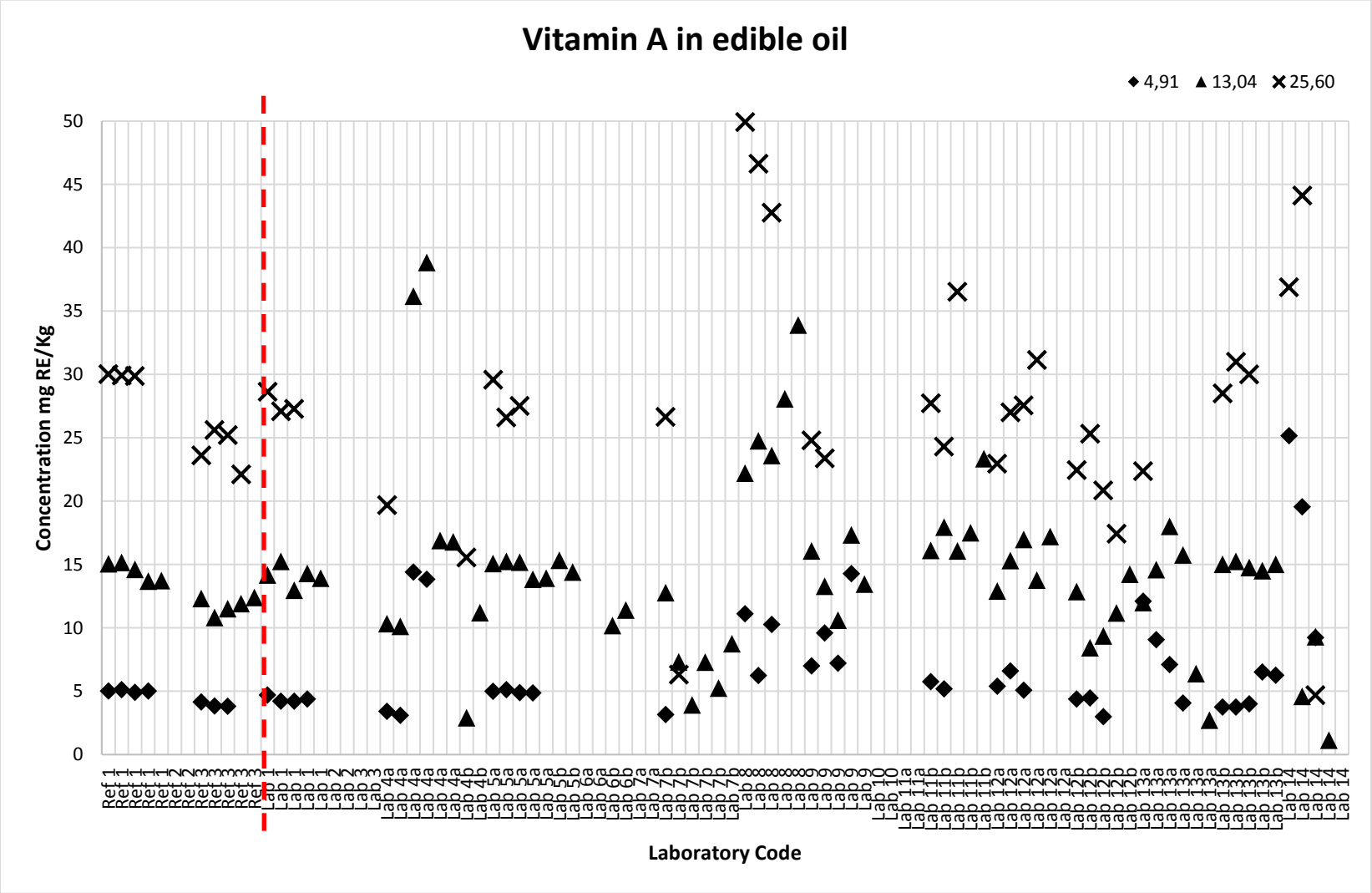
Graph 3 – Z Score Iodine in Salt assigned value 52.2 ppm



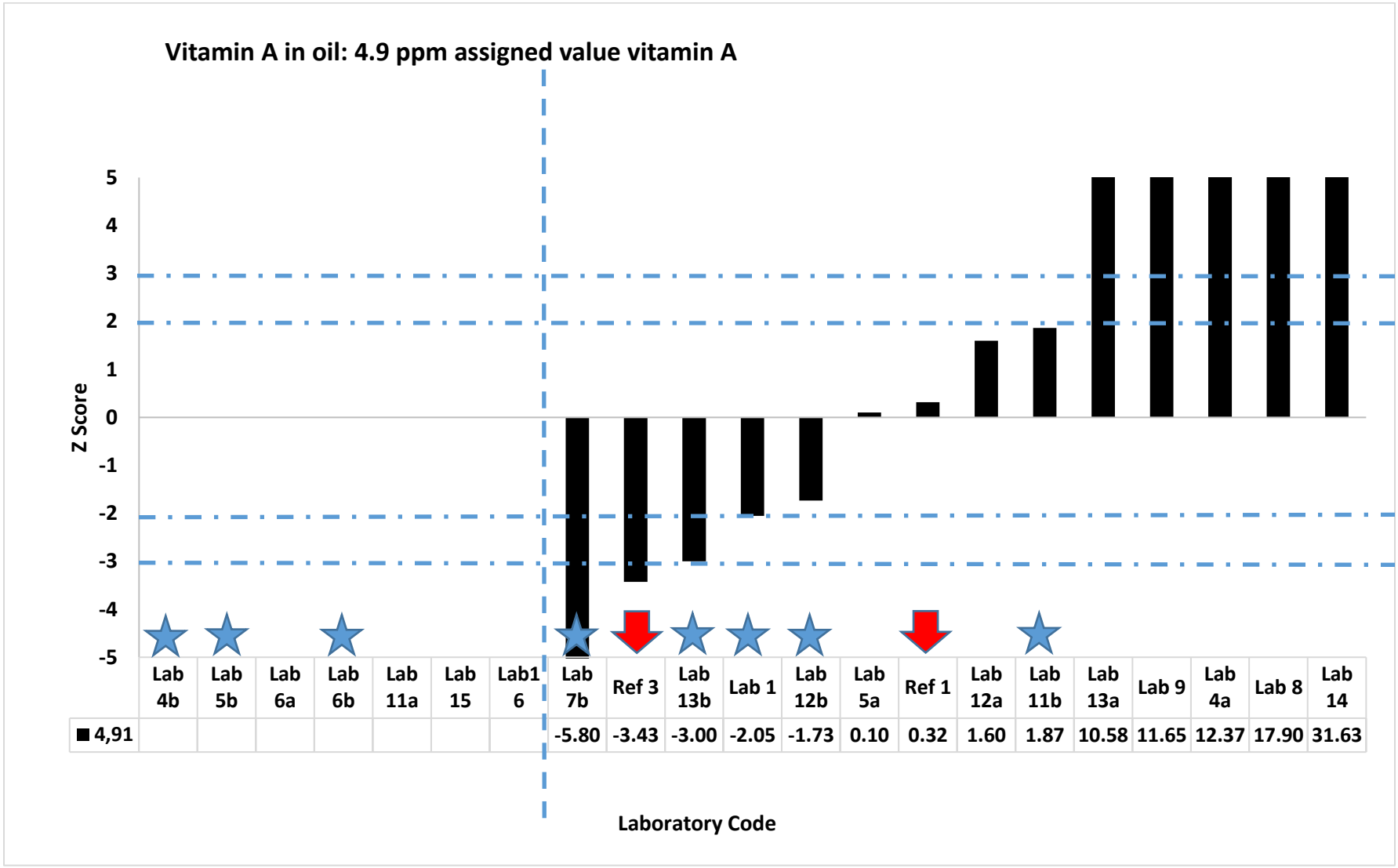
Graph 4 – Z Score Iodine in Salt assigned value 102.0 ppm



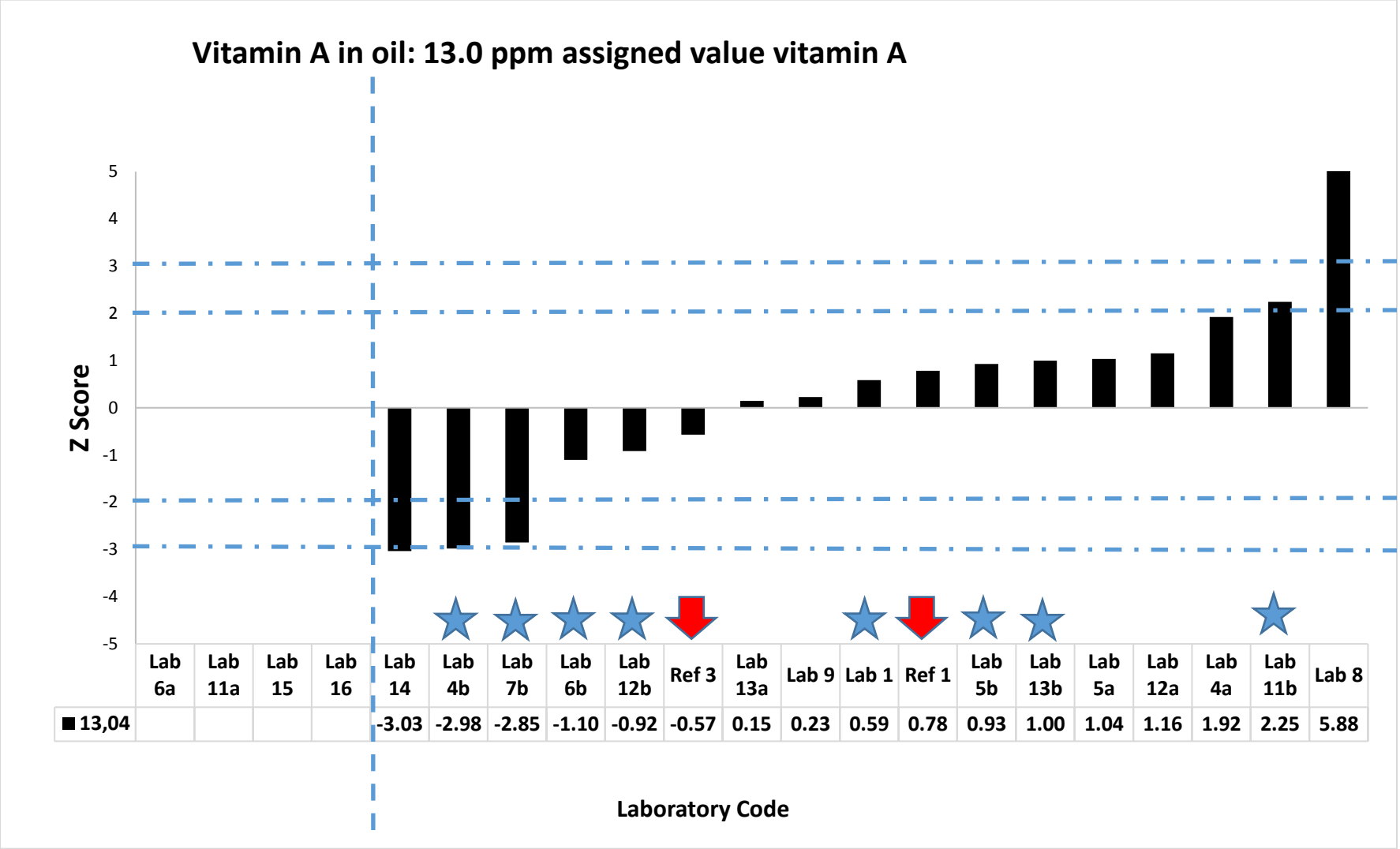
Graph 5 – Scatter Plot Vitamin A in Oil



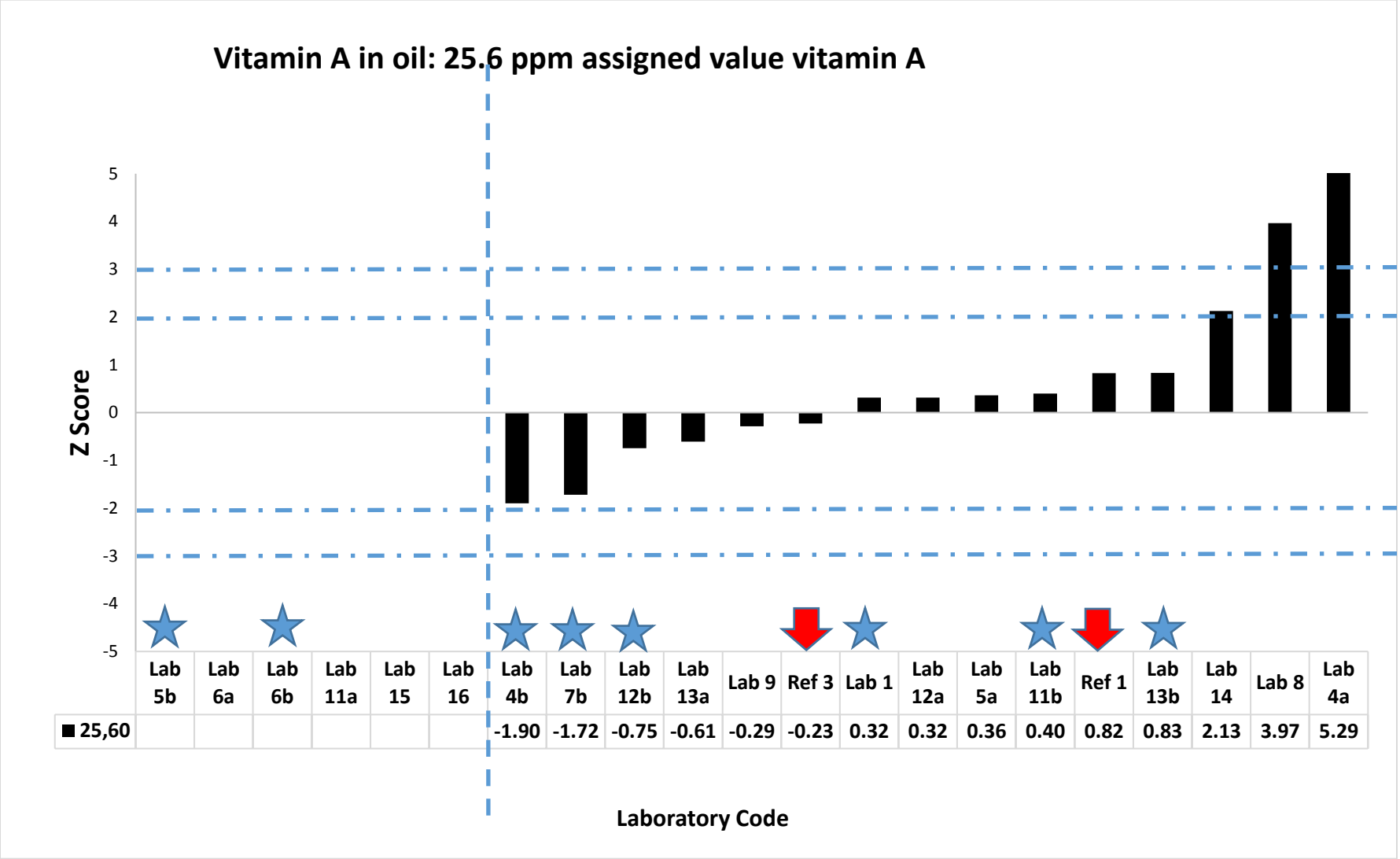
Graph 6 – Z Score Vitamin A in edible Oil assigned value 4.9 ppm



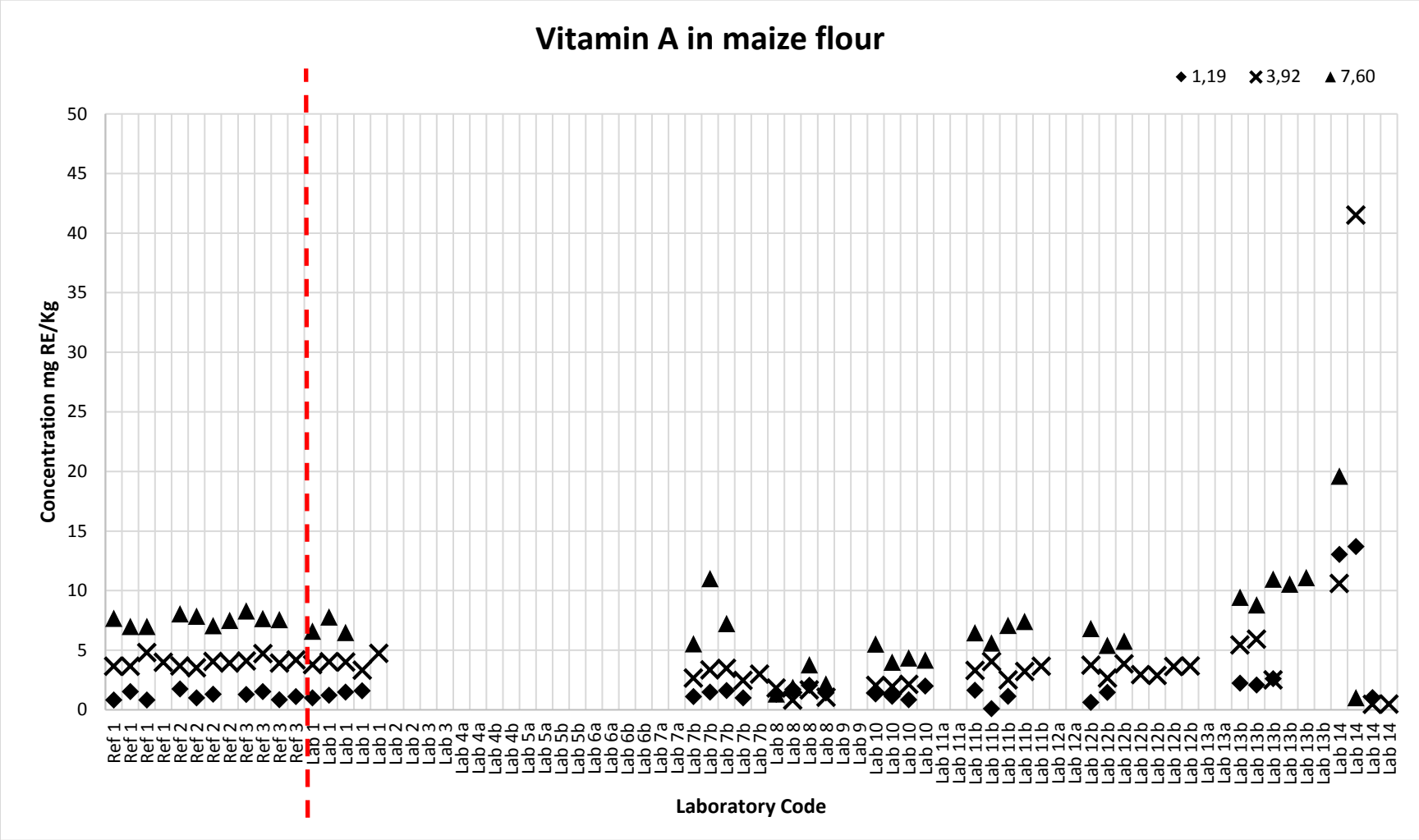
Graph 7 – Z Score Vitamin A in edible Oil assigned value 13.0 ppm



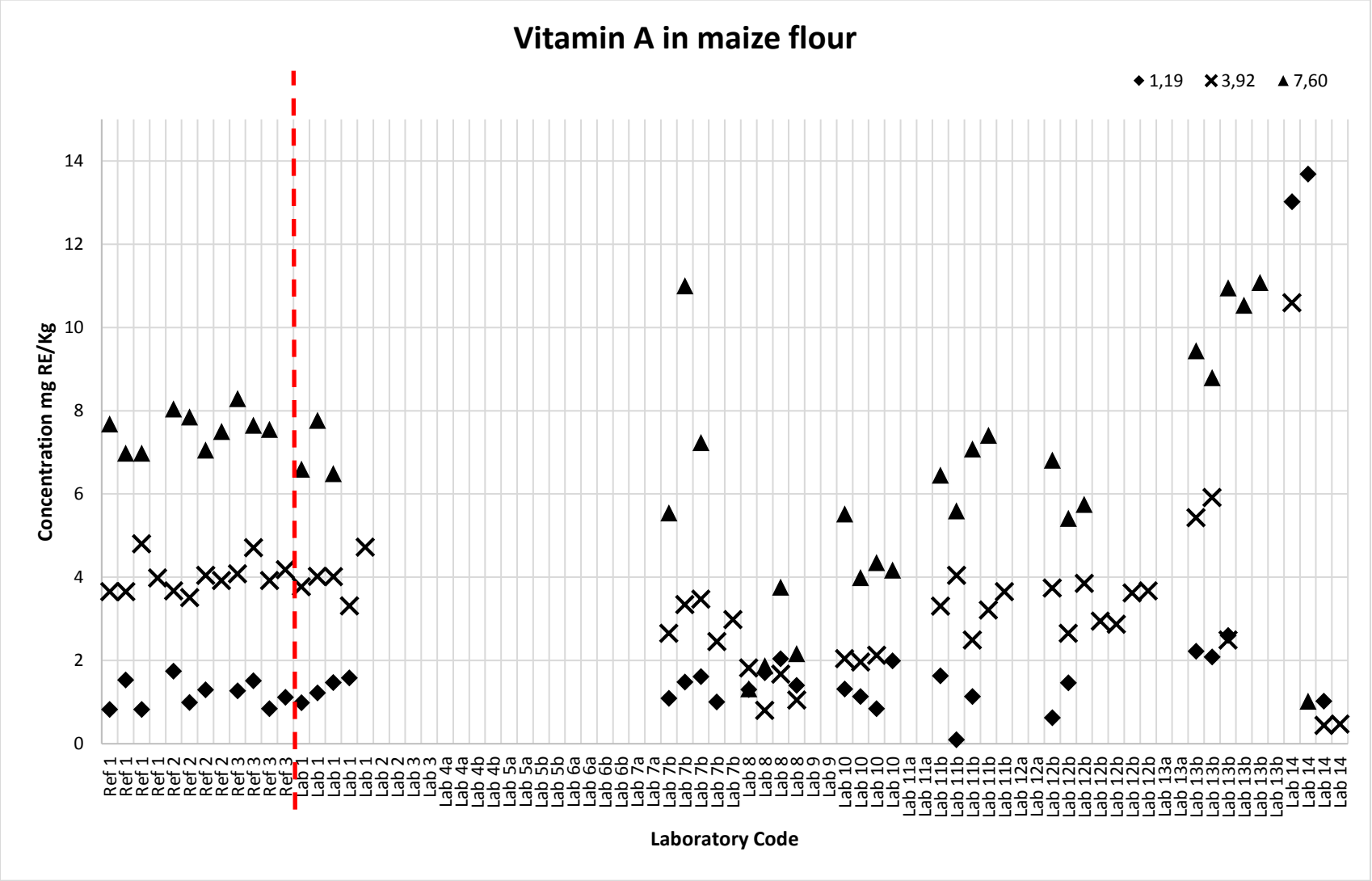
Graph 8 – Z Score Vitamin A in edible Oil assigned value 25.6 ppm



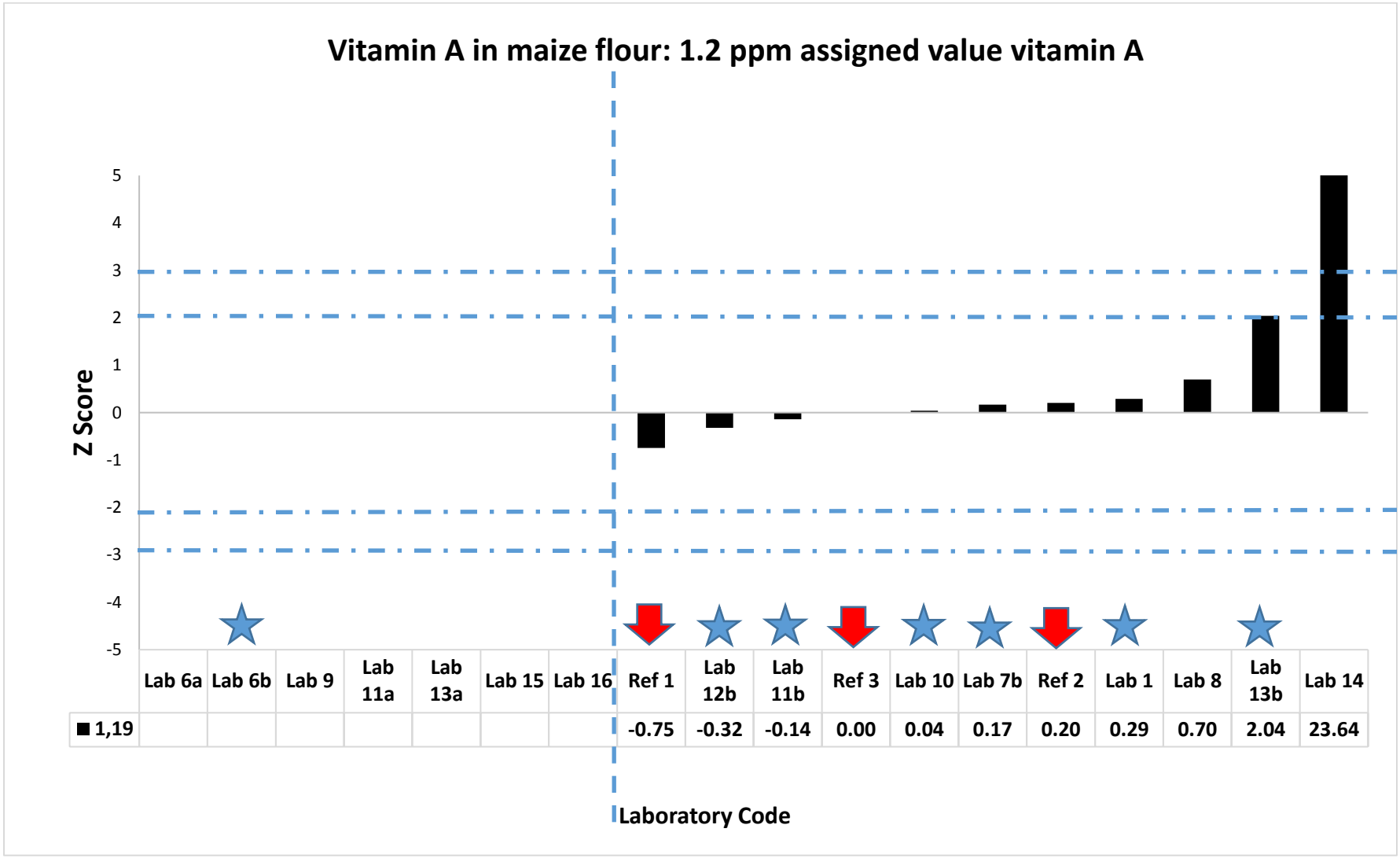
Graph 9a – Scatter Plot Vitamin A in Maize meal



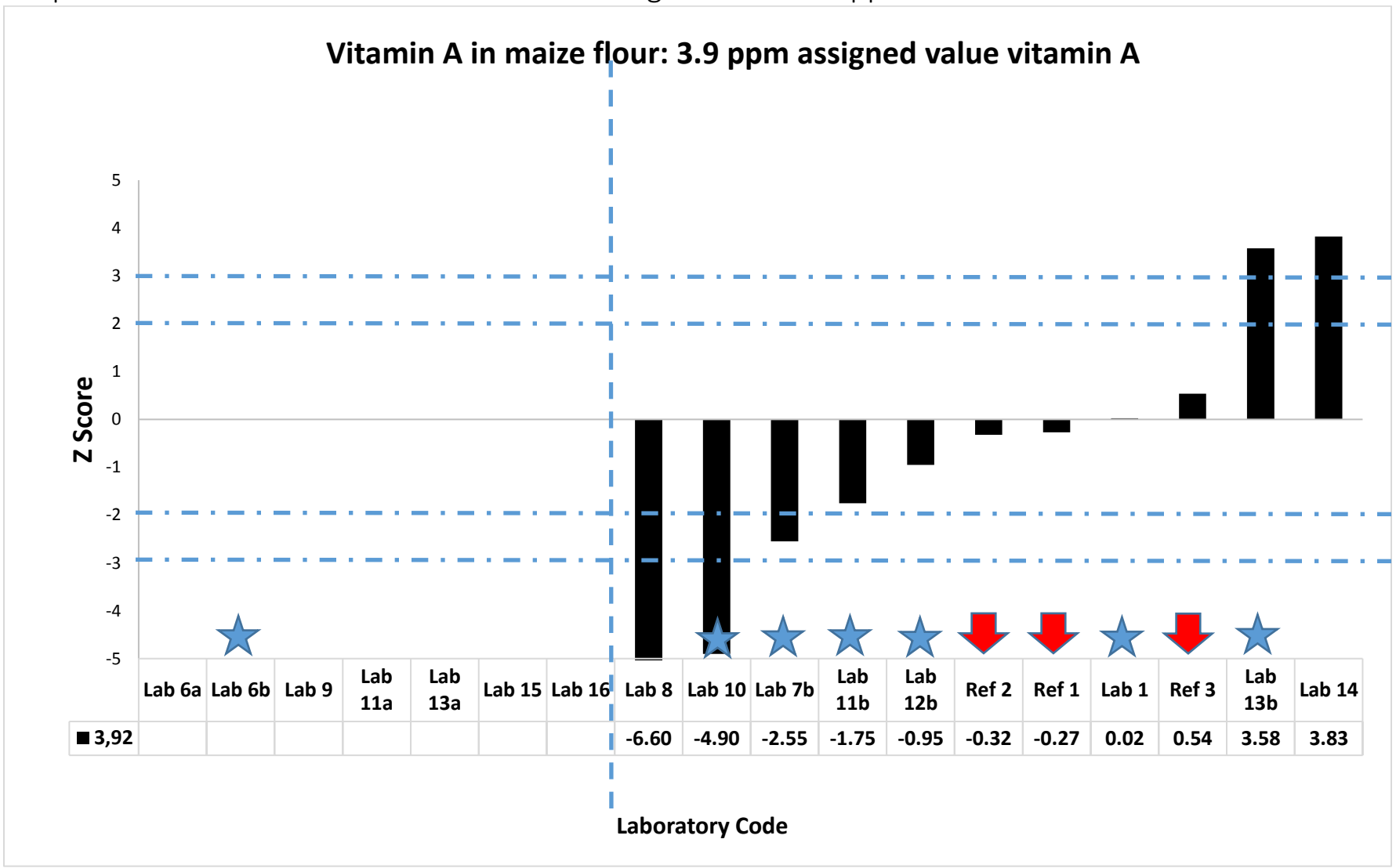
Graph 9b – Scatter Plot revised Vitamin A in Maize meal



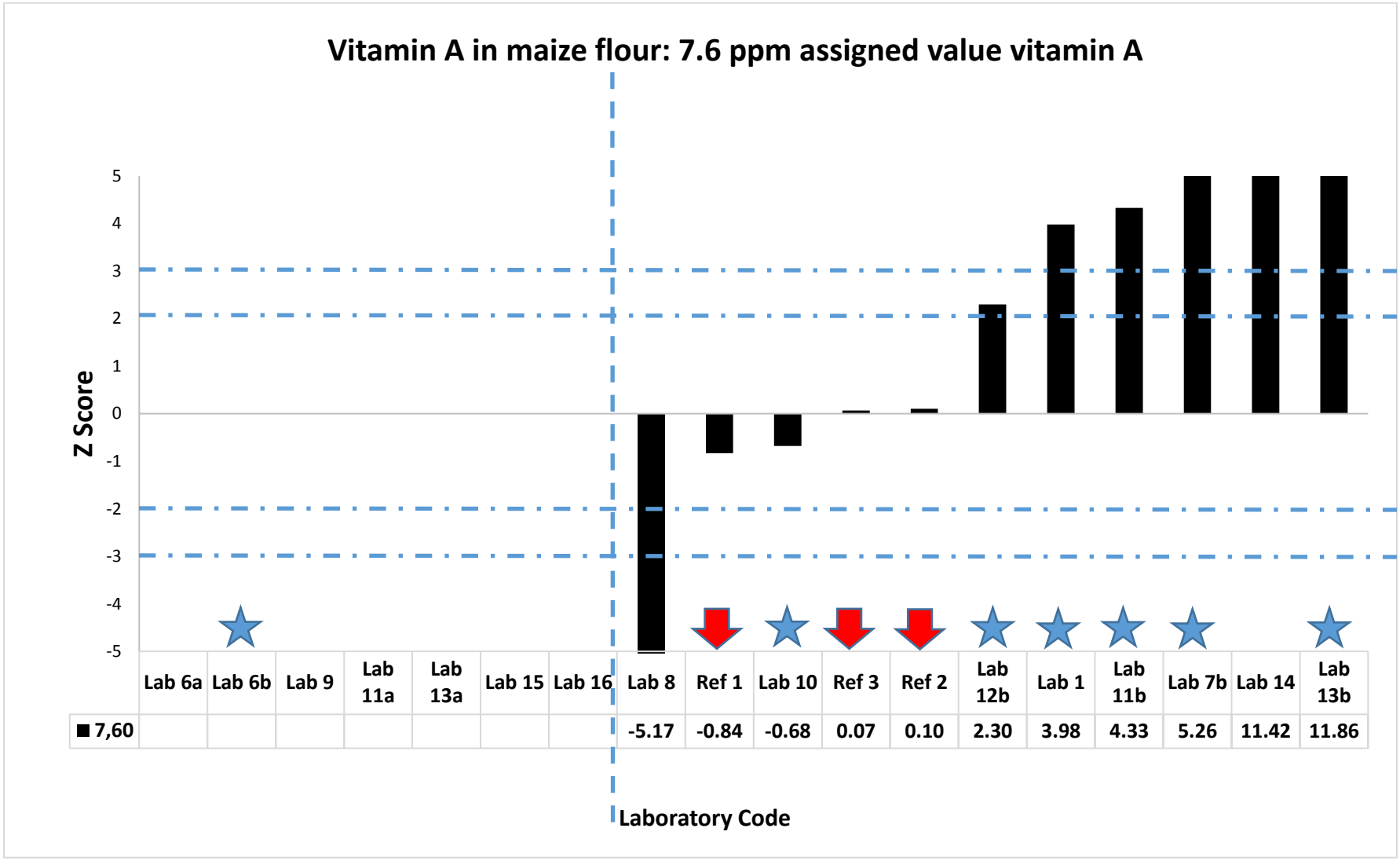
Graph 10 – Z Score Vitamin A in Maize meal assigned value 1.2 ppm



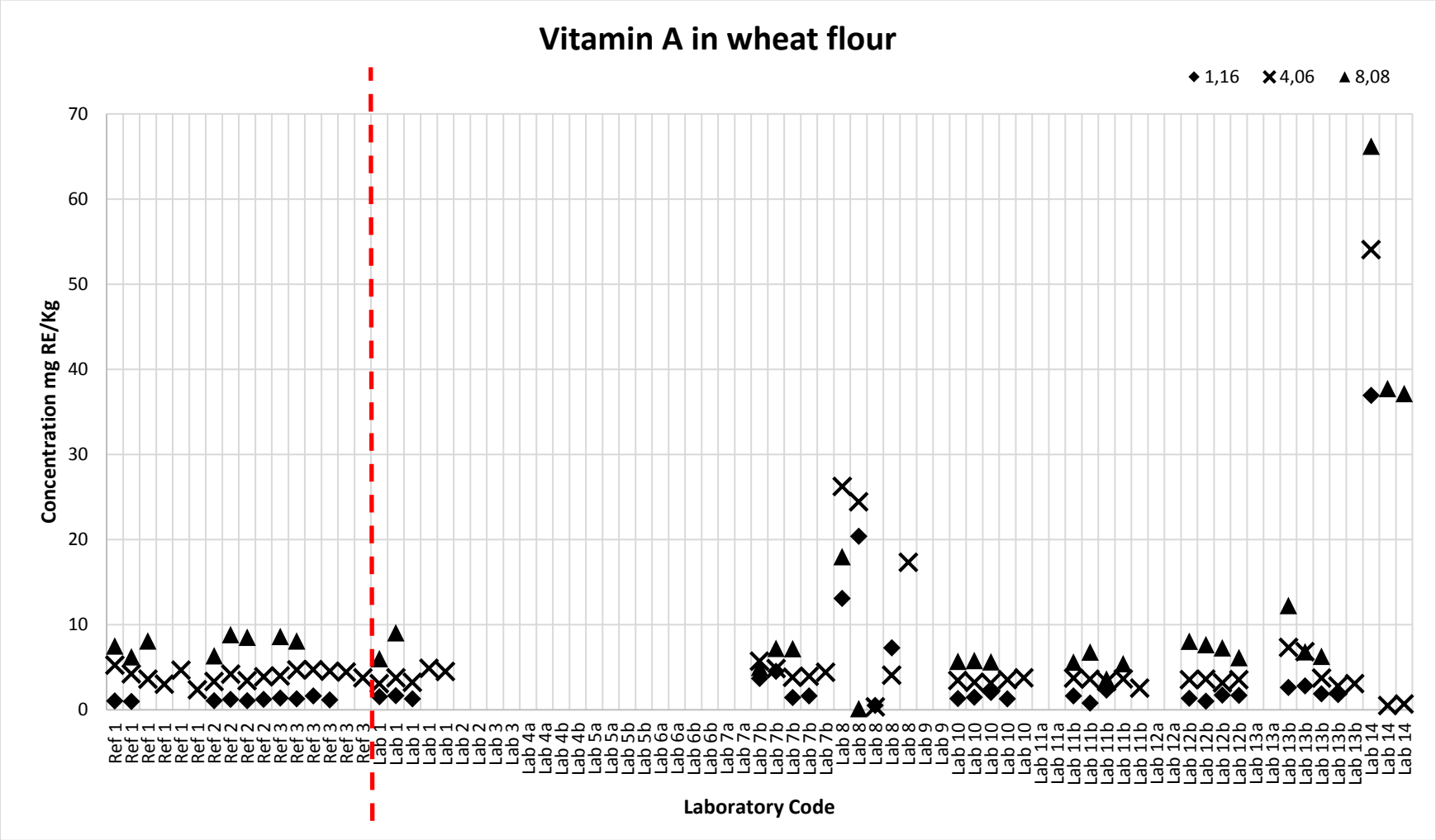
Graph 11 – Z Score Vitamin A in Maize meal assigned value 3.9 ppm



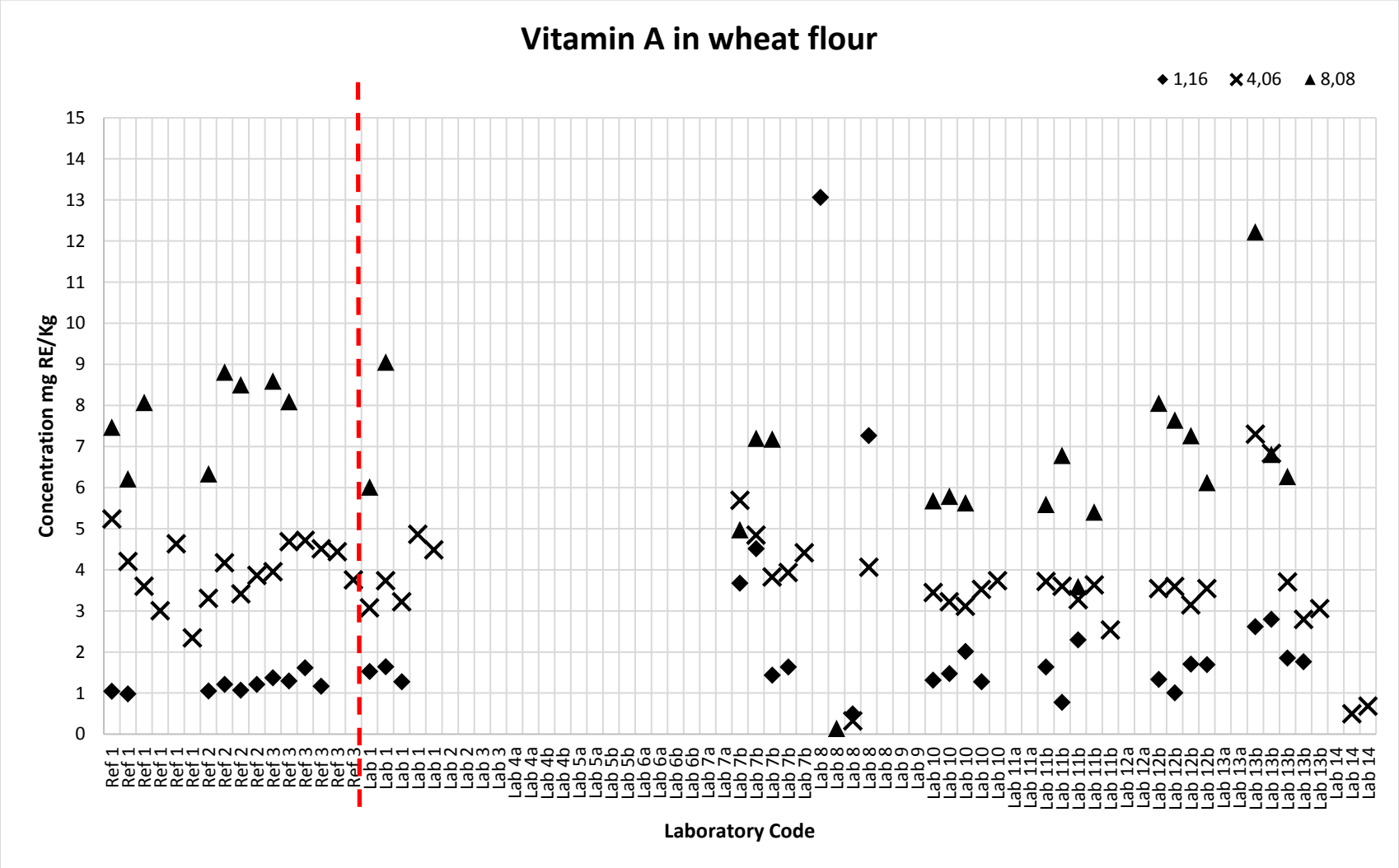
Graph 12 – Z Score Vitamin A in Maize meal assigned value 7.6 ppm



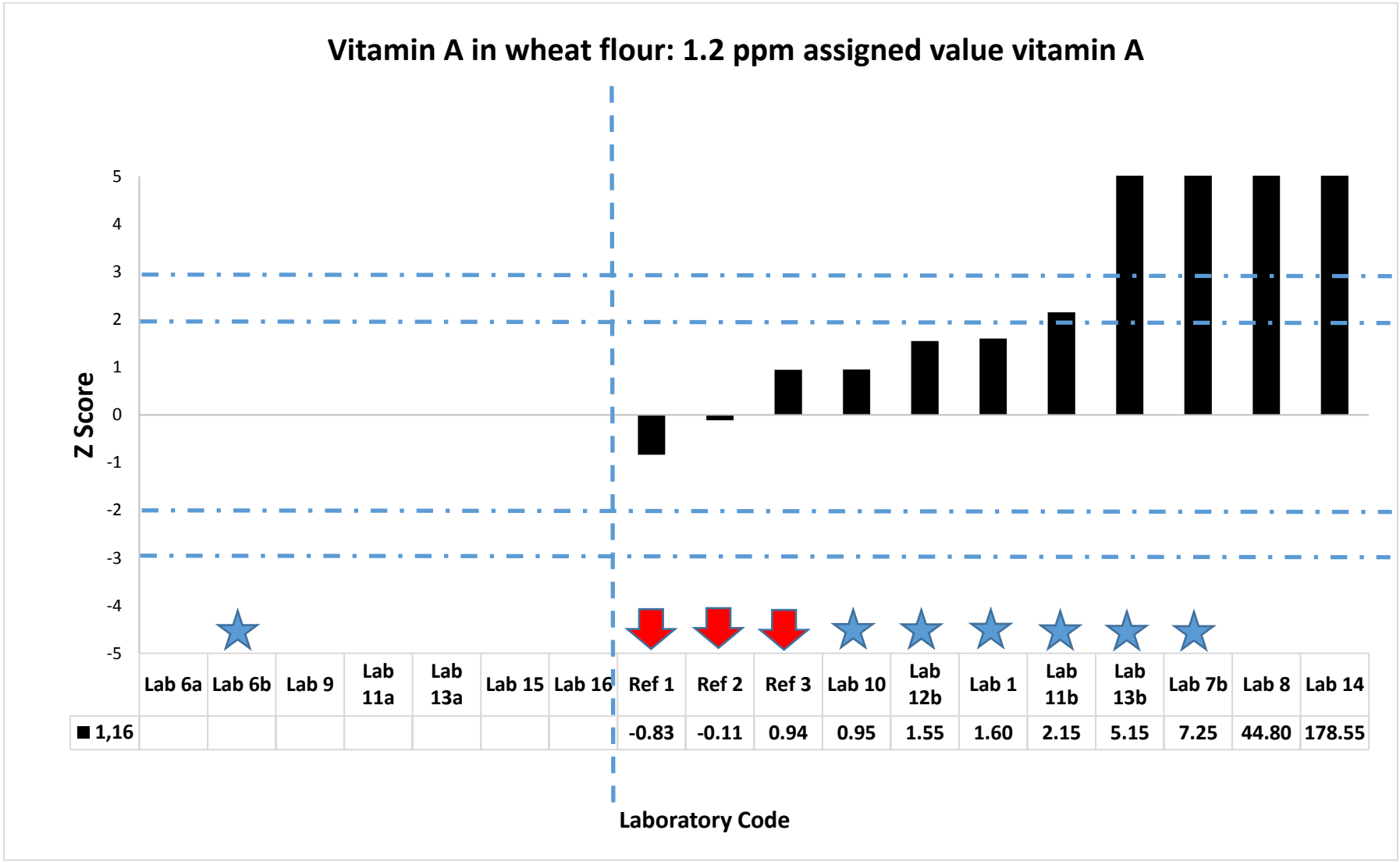
Graph 13a – Scatter Plot Vitamin A in Wheat flour



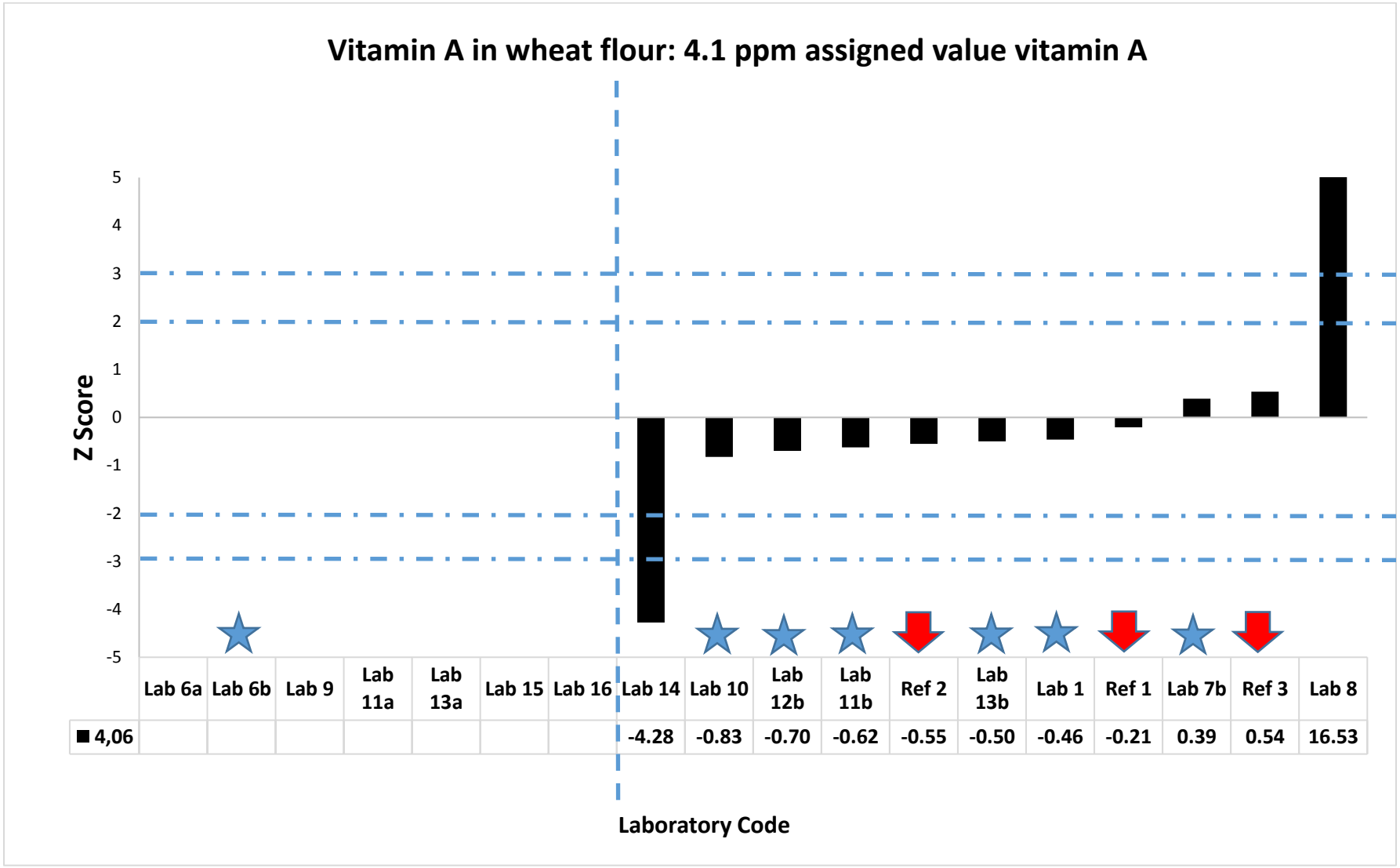
Graph 13b – Scatter Plot revised Vitamin A in Wheat flour



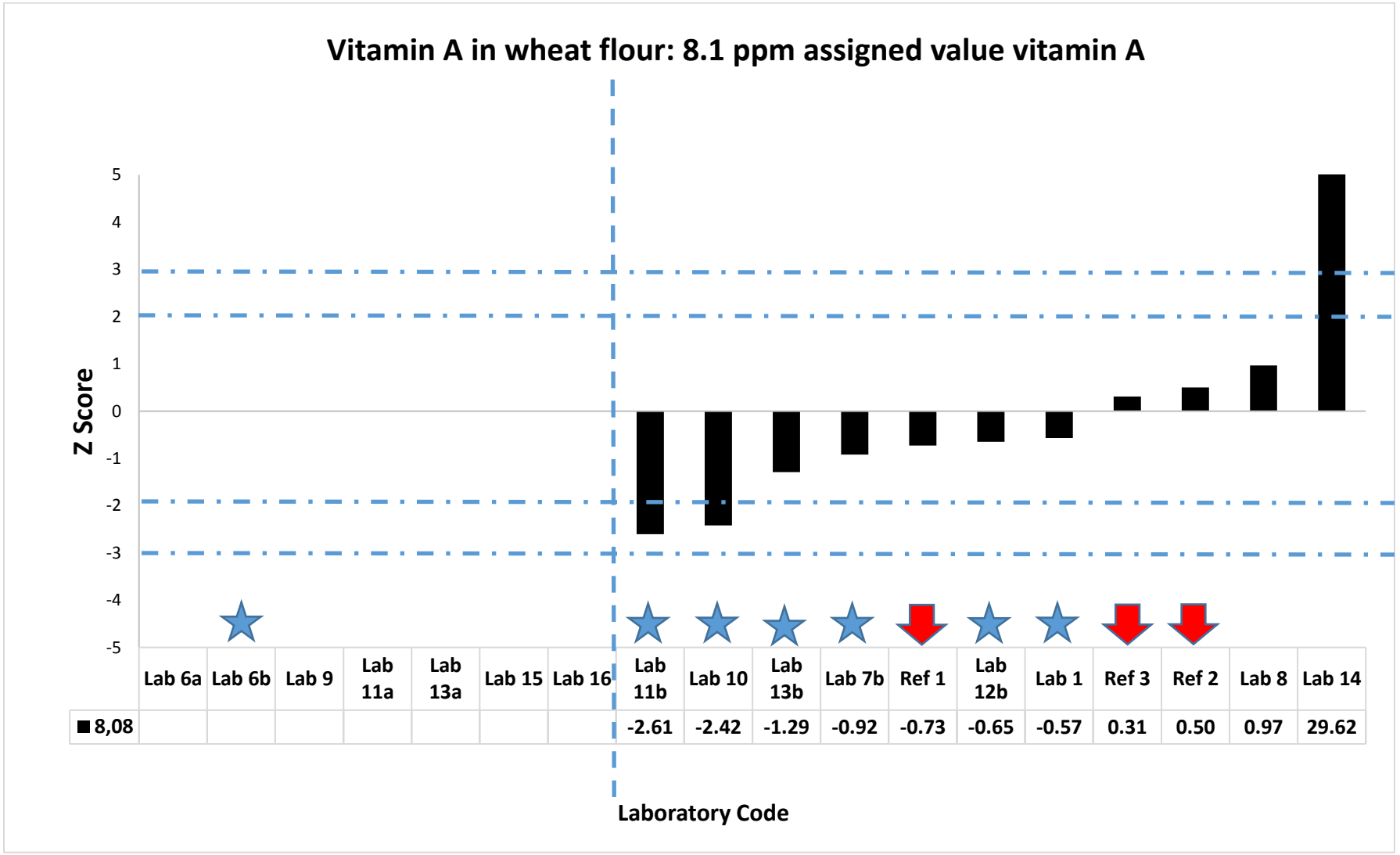
Graph 14 – Z Score Vitamin A in Wheat flour assigned value 1.2 ppm



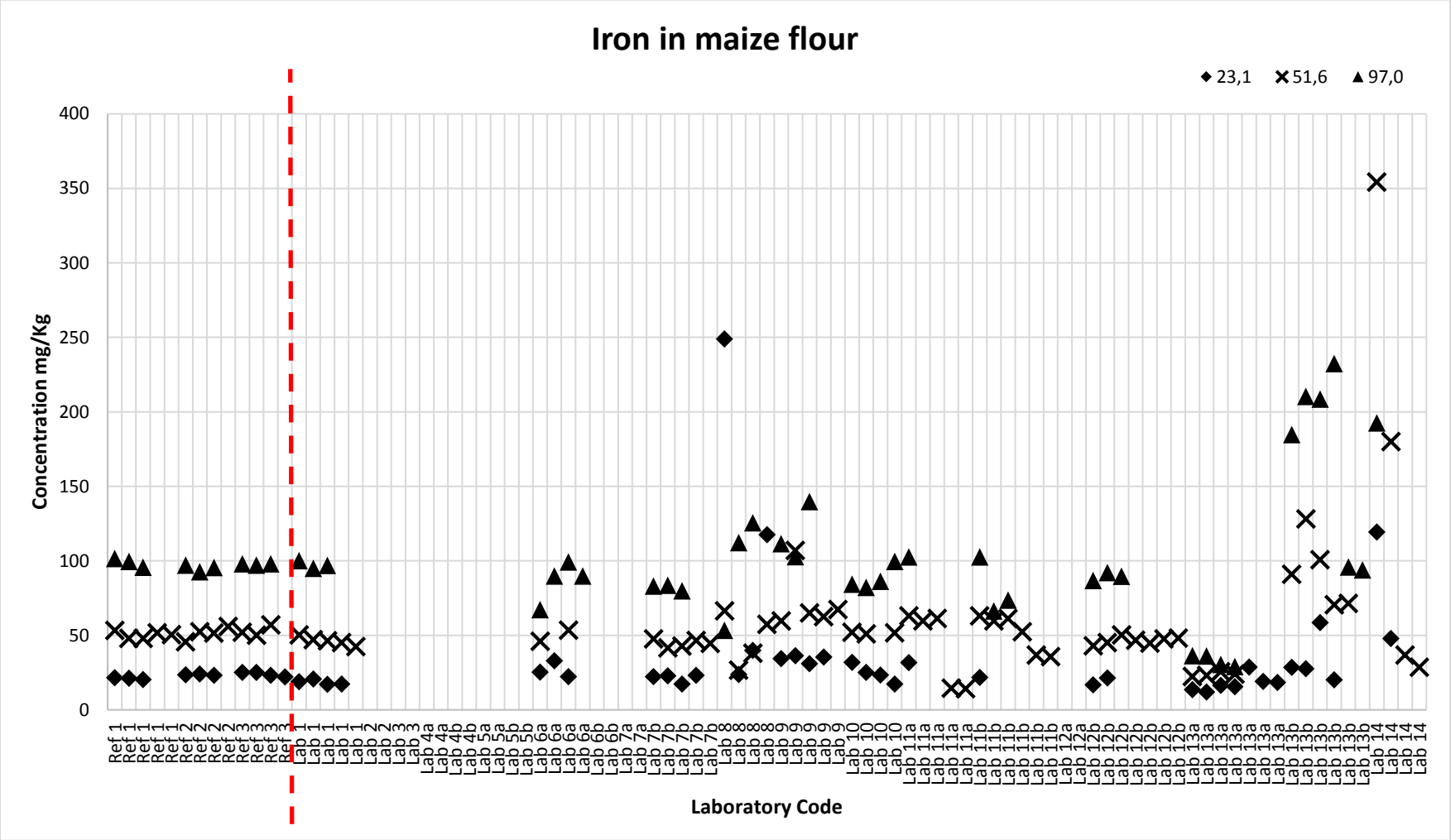
Graph 15 – Z Score Vitamin A in Wheat flour assigned value 4.1 ppm



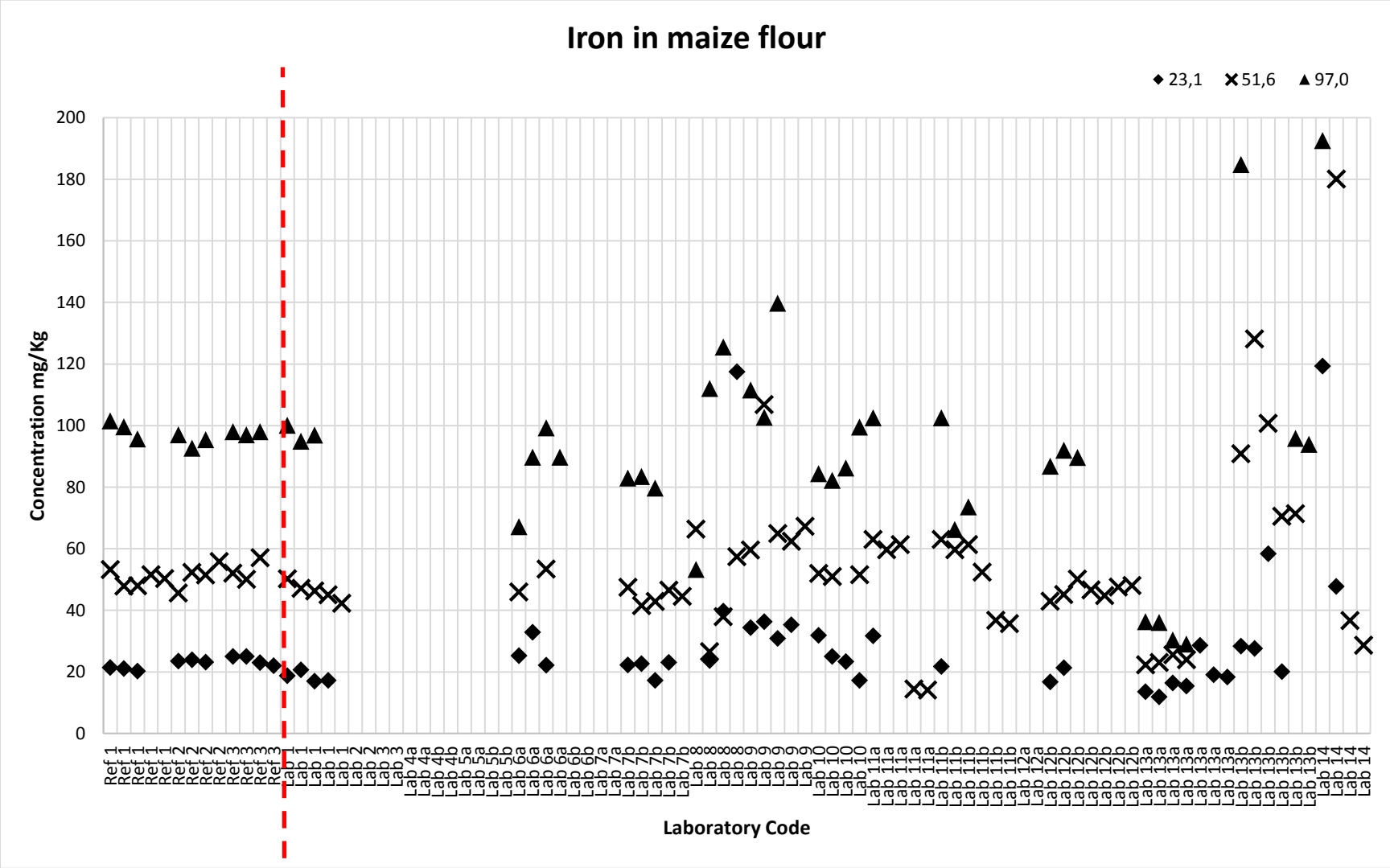
Graph 16 – Z Score Vitamin A in Wheat flour assigned value 8.1 ppm



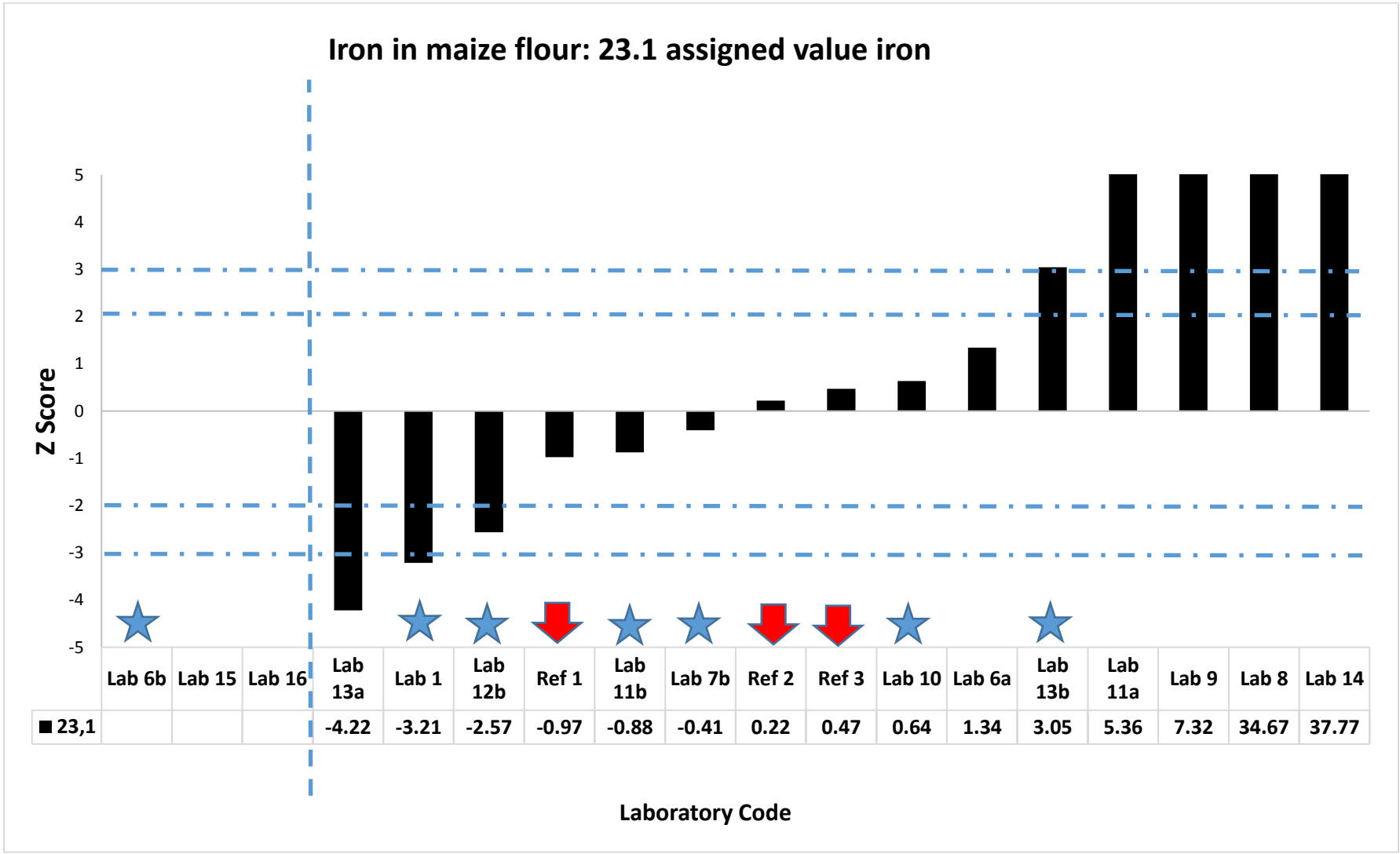
Graph 17a – Scatter Plot Iron in Maize meal



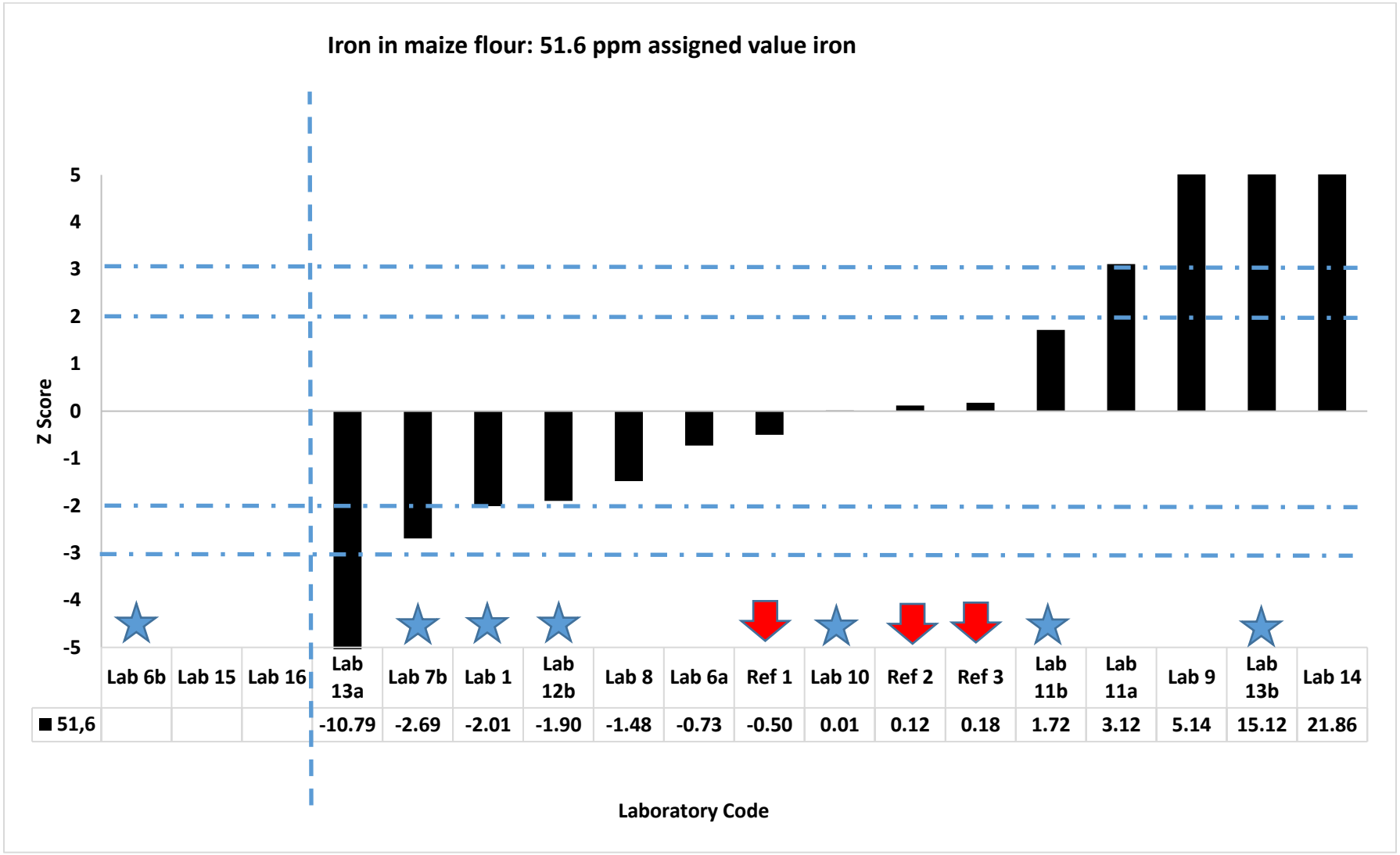
Graph 17b – Scatter Plot revised Iron in Maize meal



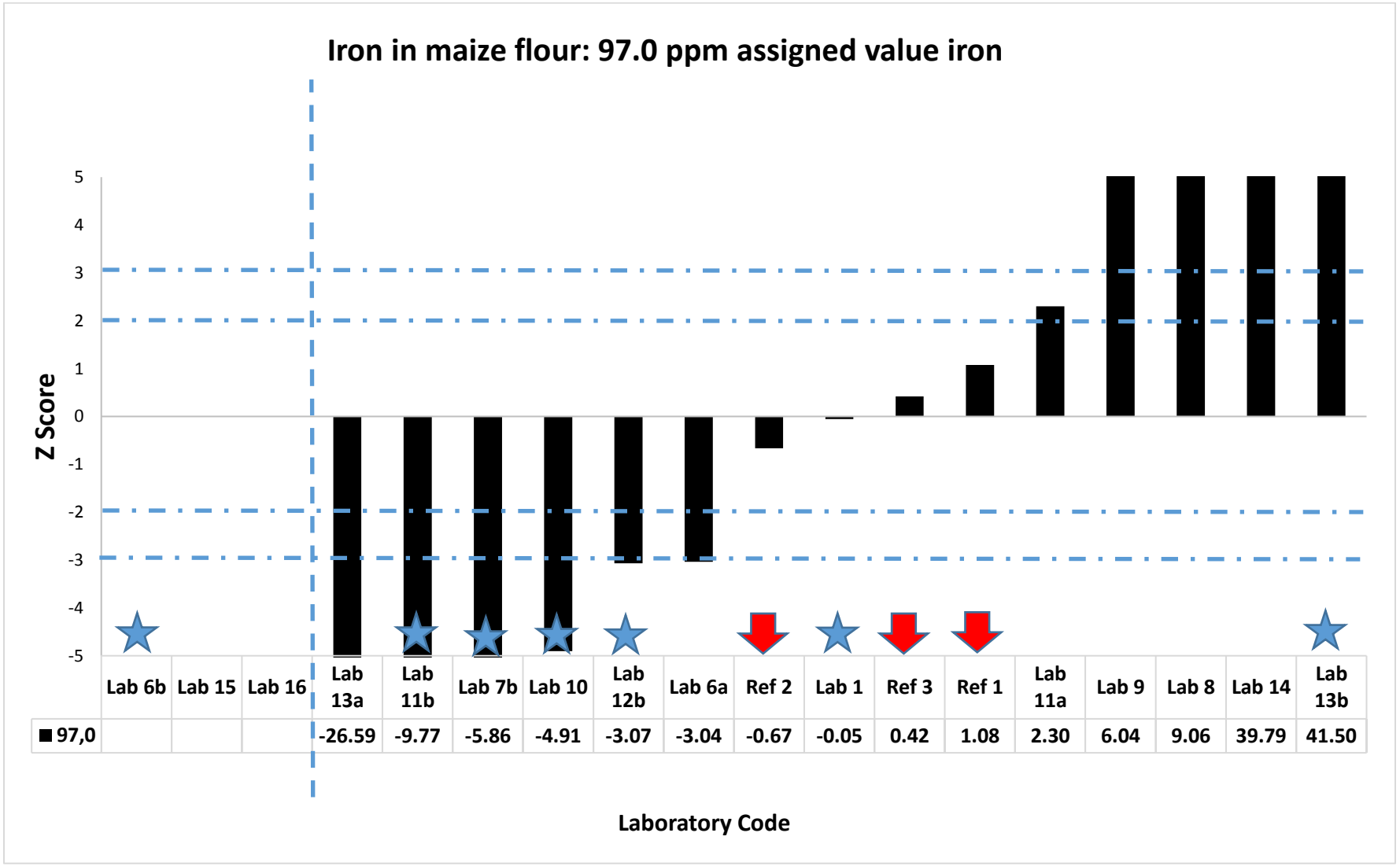
Graph 18 – Z Score Iron in Maize meal assigned value 23.1 ppm



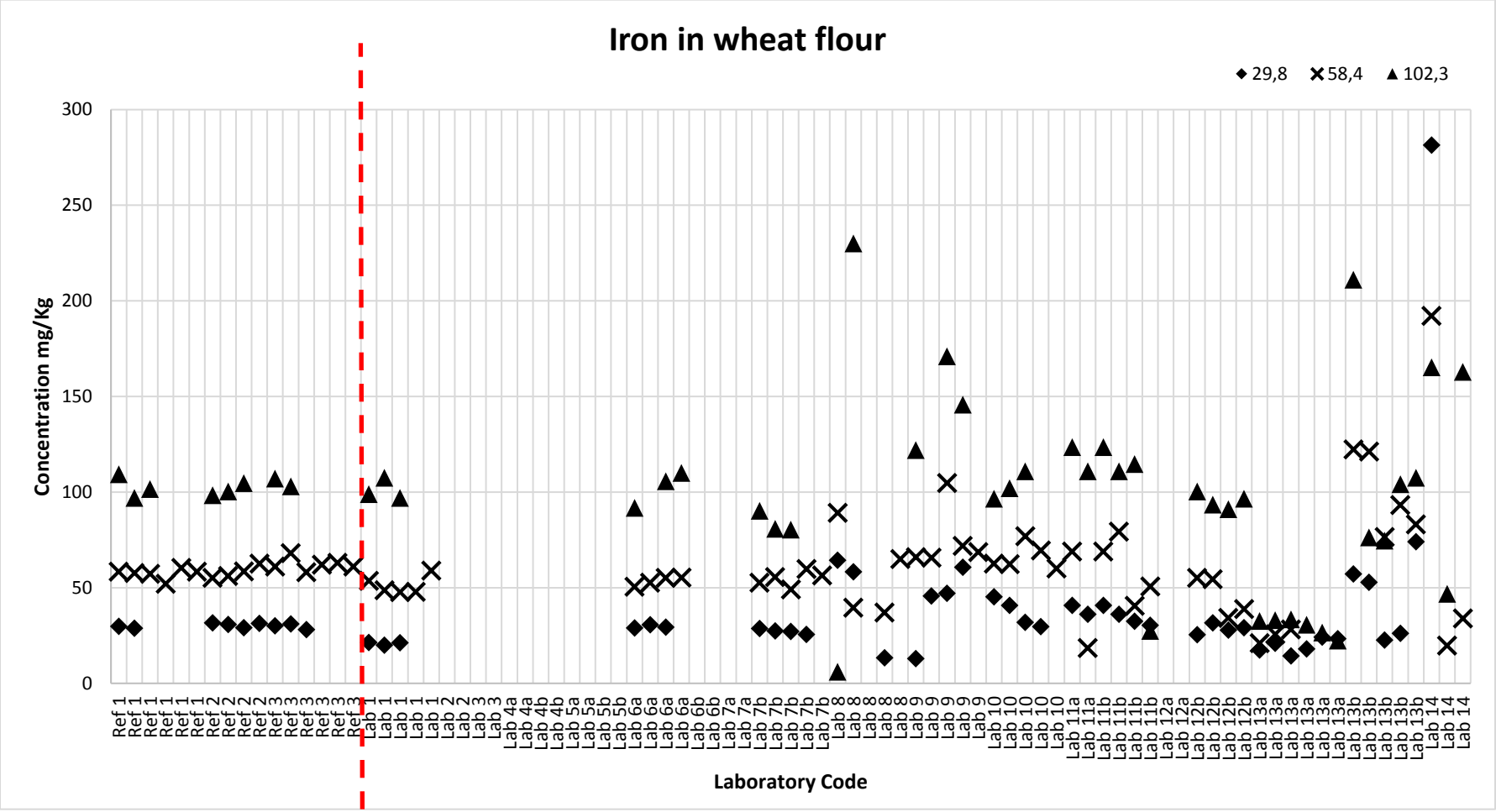
Graph 19 – Z Score Iron in Maize meal assigned value 51.6 ppm



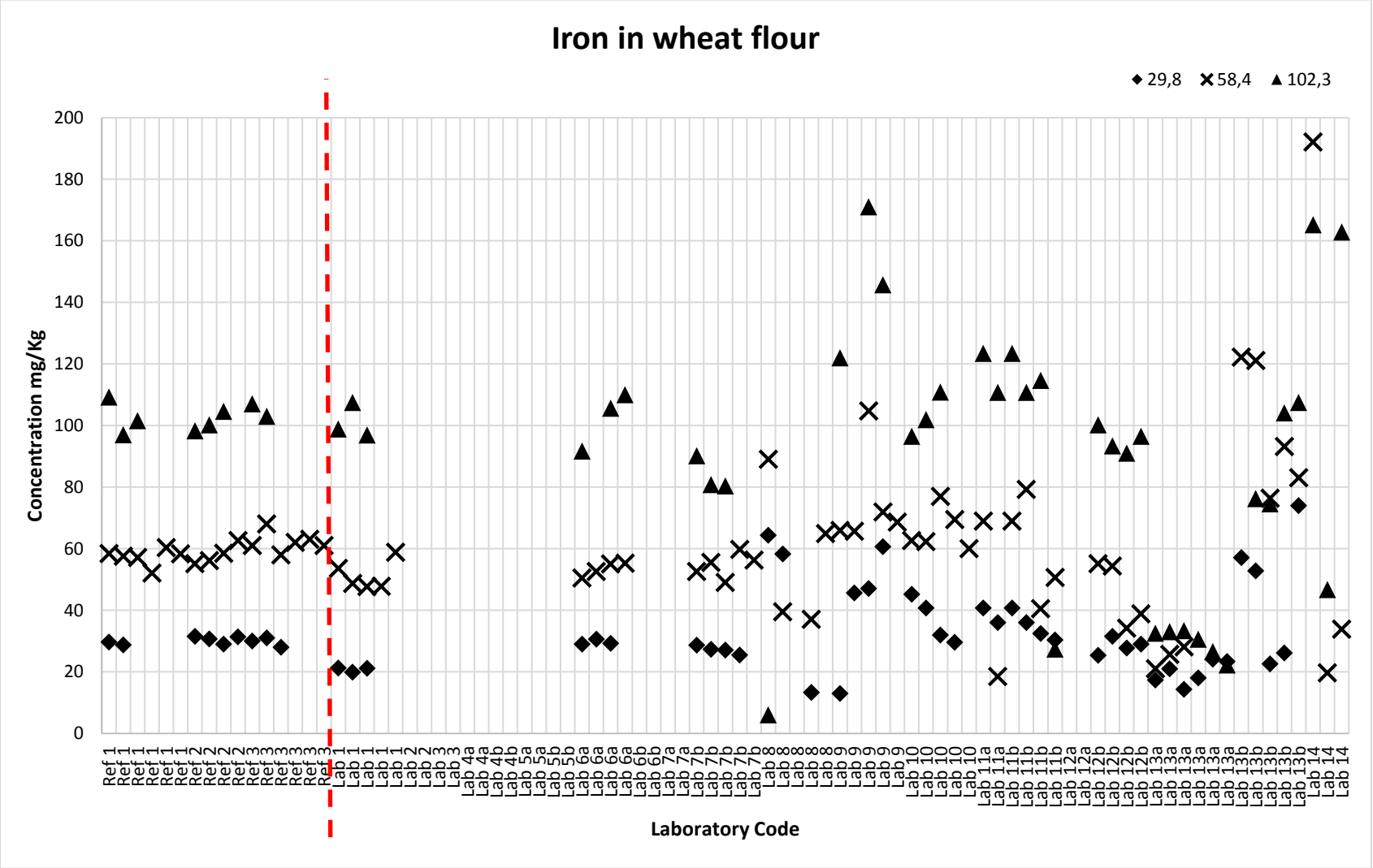
Graph 20 – Z Score Iron in Maize meal assigned value 97.0 ppm



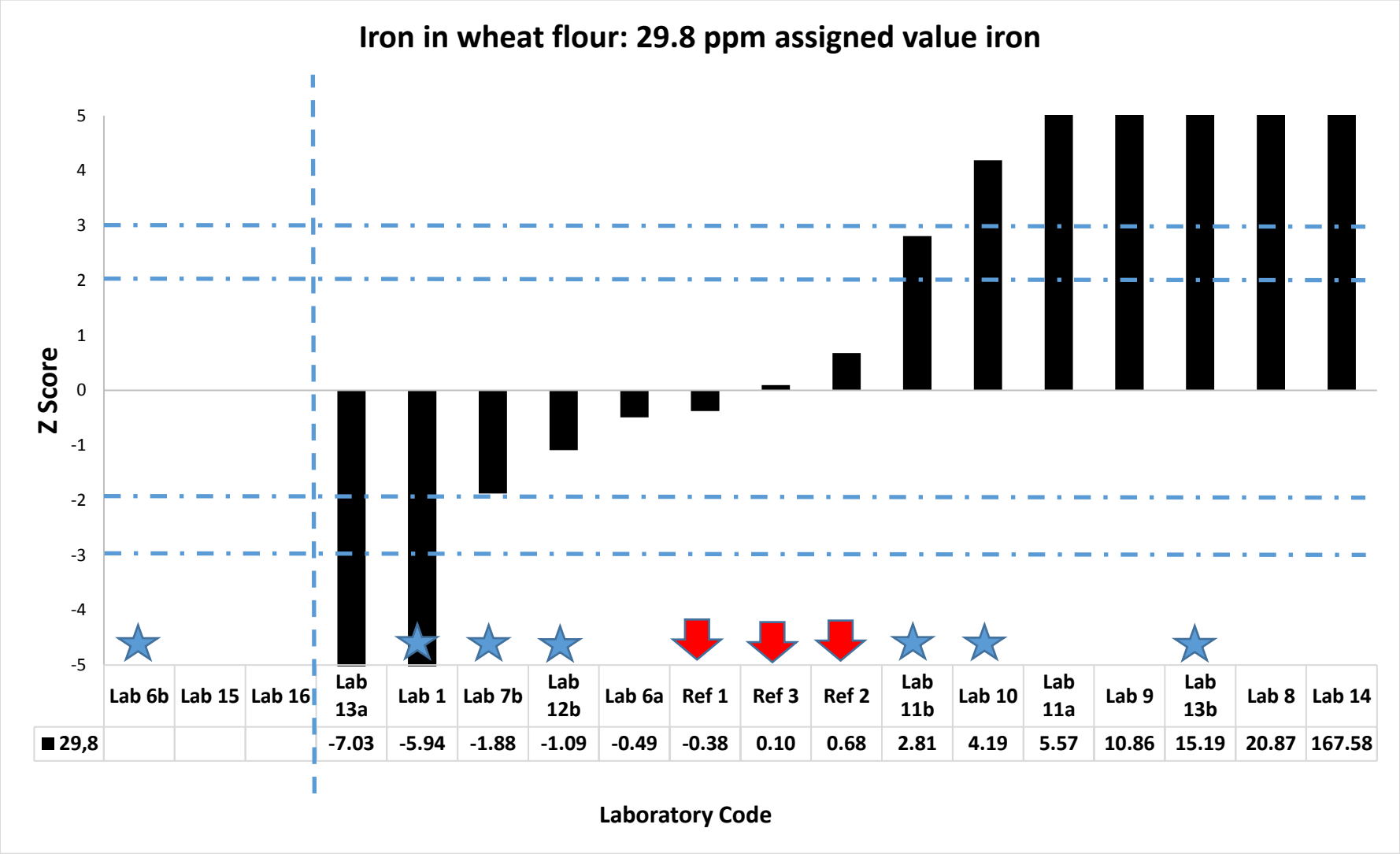
Graph 21a – Scatter Plot Iron in Wheat flour



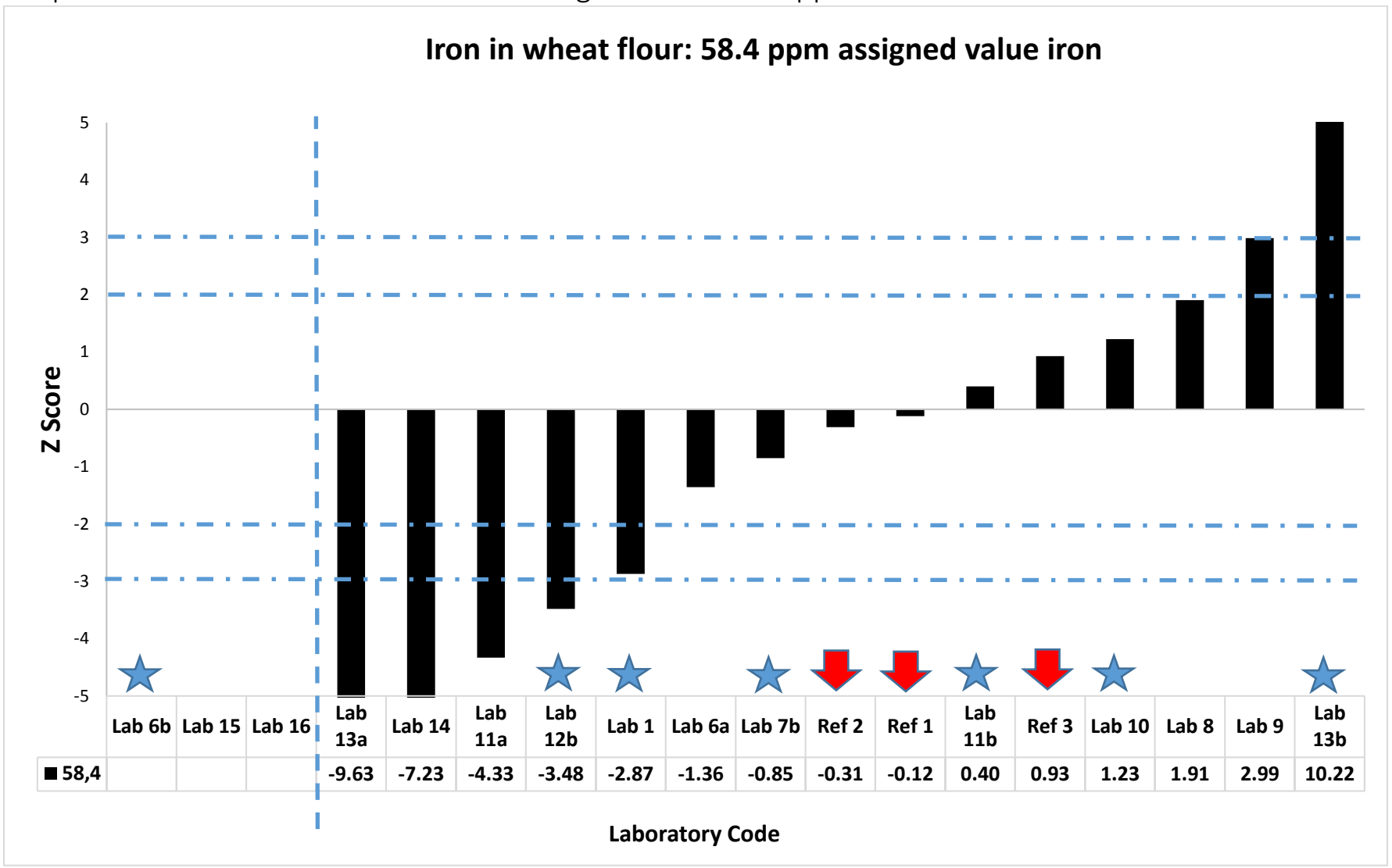
Graph 21b – Scatter Plot revised Iron in Wheat flour



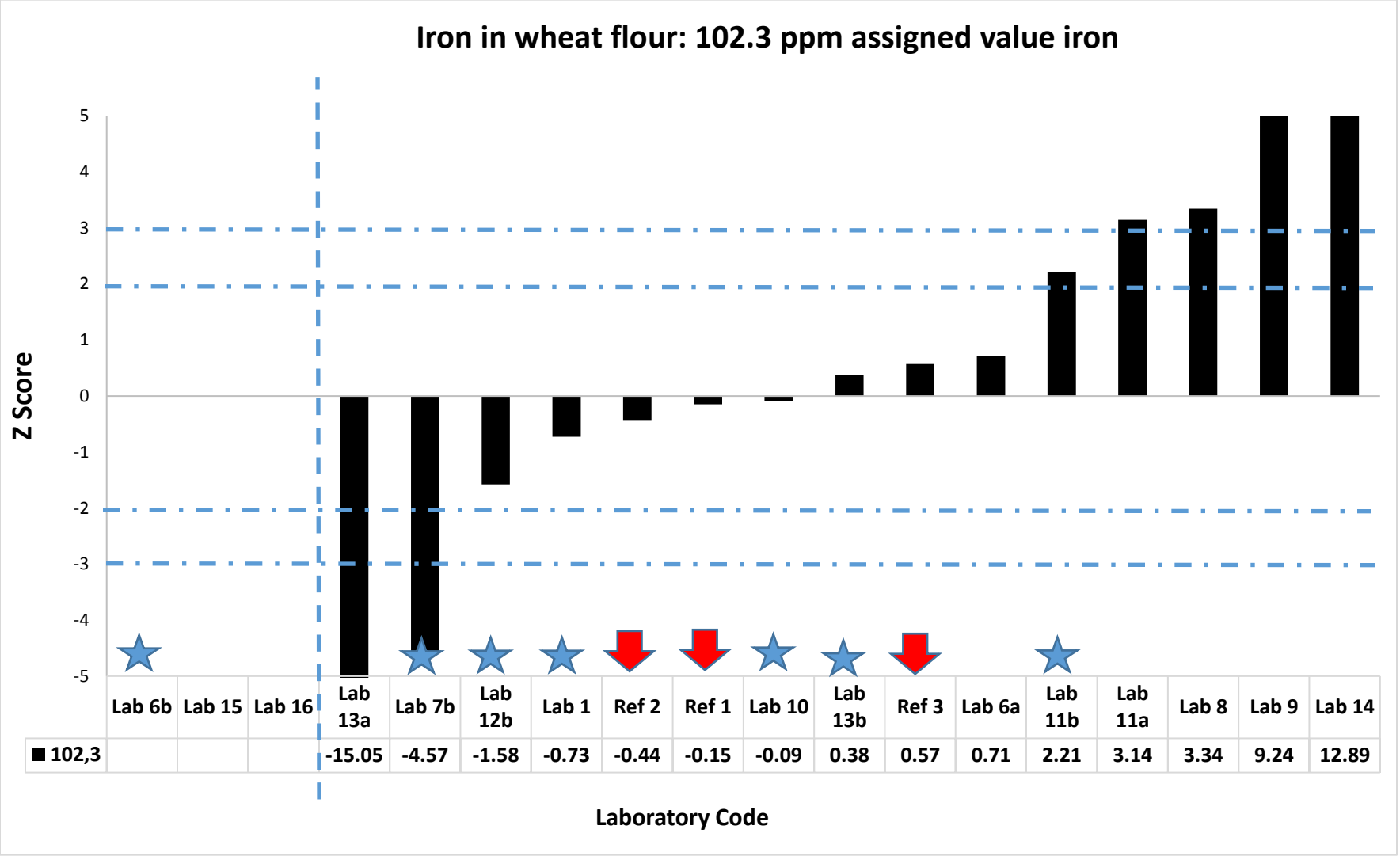
Graph 22 – Z Score Iron in Wheat flour assigned value 29.8 ppm



Graph 23 – Z Score Iron in Wheat flour assigned value 58.4 ppm



Graph 24 – Z Score Iron in Wheat flour assigned value 102.3 ppm



Discussion

As mentioned above BioAnalyt prepared samples at five different concentrations. For rounds 1 and 2 the samples were high, middle and low. For round 3 the range of samples was tightened with the intention of looking at the ability of laboratories to distinguish between samples with smaller differences in concentration of micronutrient. Whilst laboratories did analyse these samples it was noted, after completion of the study and the decision to not include BioAnalyt in the baseline group, that there was not enough data in the baseline group to carry out adequate baseline statistical analysis on these intermediate samples for comparison with the participating laboratories. The support group [David Morgan, Gerhard Rimkus, Phillip Makhumula) therefore, recommended that the analytical data for these samples be dropped from the database for this report.

Table 1 deals with the homogeneity of the samples and it can be seen that low concentration samples have a higher CV (95%) than the other two concentrations. Homogeneity at low concentrations is always difficult to achieve and part of the reason for the high CV could be the low number of samples used to check homogeneity combined with the samples being prepared by BioAnalyt as 3 different batches for the three rounds. Another influencing factor would be the iCheck reproducibility. Mitigating the impact of apparent lack of homogeneity would be the aliquot process and random selection from those sub samples to the different laboratories. The laboratories themselves, baseline and participating, did not show the same pattern with most laboratories having the higher CV's at target and high concentrations.

The impact of a low number of samples can be clearly seen in the high concentration iodine in salt (level 90 mg/kg). When the Z scores were calculated using the baseline data from Table 7 the resultant scores were very high for most laboratories. This was believed to be due to the very low U value which in turn results from the very low robust standard deviation. To mitigate this the U value was assumed to be 13 for Table 13b and the robust standard deviation recalculated as 6.77. This changed the Z-score picture significantly with the number of participating laboratories scoring >±3 dropping from 11 to 4 and the number of participating laboratories <±2 increasing from 1 to 6

It should be noted that the robust standard deviation and median were used in calculating the Z-score for all participating laboratories and the only deviation from this

policy was for the baseline vitamin A in oil as the data was clearly bi-modal and normally distributed as required by robust statistics.

Table 15 below summarises the level of participation throughout the study and the Z-Scores of the participating laboratories. Data in ( ) indicates iCheck values. IChecks represented 45.8% of the participants, 67.8% of the participants with a Z Score ≤ ± 2 and 71.9% of those with a Z Score >±2 ≤ ± 3

Table 15 – Summary of Results

Matrix	Nutrient	Spike Value	Assigned value	Number participants	Not Delivered	Z Score ≤ ± 2	Z Score >±2 ≤ ± 3	Z Score > ± 3
Salt	Iodine	15	15.7	17 (8)	5 (2)	7 (5)	0 (0)	5 (1)
		50	52.2	17 (8)	3 (1)	8 (4)	2 (1)	4 (2)
		90	102.0	17 (8)	5 (2)	6 (1)	2 (2)	4 (3)
Oil	Vitamin A	5	4.9	19 (8)	7 (3)	4 (2)	2 (2)	6 (1)
		15	13.0	19 (8)	4 (0)	10 (5)	3 (3)	2 (0)
		30	25.6	19 (8)	6 (2)	10 (6)	1 (0)	2 (0)
Maize	Vitamin A	1	1.2	15 (7)	7 (1)	6 (5)	1 (1)	1 (0)
		3,5	3.9	15 (7)	7 (1)	3 (3)	1 (1)	4 (2)
		6	7.6	15 (7)	7 (1)	1 (1)	1 (1)	6 (4)
Wheat	Vitamin A	1	1.2	15 (7)	7 (1)	3 (3)	1 (1)	4 (2)
		3,5	4.2	15 (7)	7 (1)	6 (6)	0	2 (0)
		6	8.1	15 (7)	7 (1)	5 (4)	2 (2)	1 (1)
Maize	Iron	15	23.1	15 (7)	7 (1)	4 (3)	1 (1)	7 (2)
		45	51.6	15 (7)	7 (1)	4 (3)	2 (2)	5 (1)
		90	97.0	15 (7)	7 (1)	1 (1)	1 (0)	10 (5)
Wheat	Iron	15	29.8	15 (7)	7 (1)	3 (2)	1 (1)	8 (3)
		45	58.4	15 (7)	7 (1)	4 (3)	2 (1)	5 (2)
		90	102.3	15 (7)	7 (1)	5 (4)	1 (1)	6 (1)

From table 15 in comparing the spike value with the assigned value the latter is higher in every case except for the vitamin A in oil. For iodine in salt there is no clear explanation whereas for vitamin A in oil Reference laboratory 1 was clearly closer to the spiked value that Reference laboratory 2. The apparent over recovery of nutrients in the grains is probably due to overages in the premix for vitamin A and for iron the overages plus the intrinsic content. The Certificate of Analysis for that premix was used in the calculation

of addition but this study has already shown differences between laboratories, techniques and methods.

With vitamin A in oil the U value is very low at the low assigned value. The CV's are comparable but the mean and median are markedly different and so reflect the use of robust statistics. The baseline clearly shows average recoveries of < 90% though clear differences between the two HPLC methods with Laboratory 1 closer to theoretical than Laboratory 2. This indicates that the choice of reference method within a technique could be critical plus it is of importance to note that few industries use HPLC – the instrument of choice amongst regulators – but use iCheck and/or UV/Vis spectrophotometers

With vitamin A in maize meal – which has higher CV and U at low assigned values than wheat flour – this cannot be the explanation as the mean and median are identical. This very high U (and CV) may be due to the apparent lack of homogeneity identified from table 1 but this was not repeated in the vitamin A in wheat nor was it repeated in the iron in either grain even though the CV in Table 1 is significantly higher at the low spike value.

Applicable to all methodologies is that total iron is measured and discussion with the laboratories at the ECSA meeting indicated that some laboratories are confusing added iron, intrinsic iron and total iron. As many countries have regulations that specify maximum values this would lead to conflict situations as the intrinsic iron content is known to vary significantly, and uncontrollably, according to grain environmental factors as well as grain product quality. The question of iron recovery is discussed below.

In terms of analytical methods used the iCheck provided slightly less of half the data (46%) but provided over two thirds (68%) of the Z-Scores < ±2 and 72% of those < ±3. In terms of missing data iCheck accounted for 19% of that total and official reference methodology the rest which is very concerning as the laboratories knew well in advance this study was being planned. That 40% of the data was not reported is also a clear message that laboratory capacity needs to be investigated in depth and laboratories be honest with themselves over the constraints they are obviously facing.

The lack of reference method analysis of vitamin A in cereal products from the regulatory authority laboratories is of particular concern.

Iron Recovery

All of the laboratories measured TOTAL iron as the intrinsic level was not provided – nor would it be likely to be available in a regulatory situation.

The intrinsic iron content of maize meal and wheat flour was measured by one of the reference laboratories on two separate occasions. Iron concentrations in maize meal of 11.7 mg/Kg with a standard deviation of 0.6 and U of 12.5% and in wheat flour 19.5 mg/Kg with a standard deviation of 1.1 and U of 11.3% were found.

Table 16 below indicates that reportable value for iron ranges from 10% to 100% greater than the addition level with the low iron addition indicating close to 100% over recovery and the high iron addition circa 10% over recovery. It would be on the basis of these values that the regulatory authorities would determine if fortification had been complied with or, as is the concern of many regulators, fortification had been over dosed.

Using the assigned value (the median iron content determined by the baseline laboratories) and subtracting the intrinsic iron content determined by one of those laboratories a significantly different picture emerges. By deducting the intrinsic value the baseline laboratories indicate that iron was actually under-recovered with the data indicating recoveries of between 70% and 95% with the low iron addition having a 76% and 70% recovery and the high iron addition 95% and 92% recovery.

Table 16 – Recovery data for iron in maize meal and wheat flour

	Added iron	Recovery	Assigned value	Intrinsic	Assigned - Intrinsic	Recovery
Iron in Maize meal mg/Kg	15	152.1	23.1	11.7	11.4	76.0
	45	113.9	51.5		39.8	88.4
	90	108.0	97.0		85.3	94.8
Iron in Wheat flour mg/Kg	15	199.9	30.0	19.5	10.5	70.0
	45	130.4	58.4		38.9	86.4
	90	114.0	102.3		82.8	92.0

This data starts to indicate that having a fixed correction value for intrinsic iron can not only be questioned on the basis of the grain and the level of processing it has undergone (factors which are recognised but vary from country to country) but that the correction value could, and this would need further investigation, be dependent on the addition

level of the iron. As the addition rates for iron are typically in the 15 mg/Kg to 30 mg/Kg range this could lead to a conclusion of non-compliance.

Conclusions

Several laboratories indicated willingness to participate using both regional reference methodologies (ECSA) and rapid test kits –iCheck. Either through lack of management support, consumables or a combination of both many could only provide data by one technology. At GAIN's request BioAnalyt had checked with the laboratories to ensure laboratories had vials and that those vials were not past their use by date.

In discussions with laboratory staff at the ECSA meeting in Mozambique it became clear that as iCheck is a single source supplier procurement by Government entities (that require multiple quotes) this is a significant constraint. This issue has been raised with BioAnalyt who have advised this is not an issue as a similar situation arises with WFP. If laboratories properly plan their requirements then the issue can be managed – the procurement process for many laboratories, especially in the regulatory area, is overly complicated and time consuming.

Most of the laboratories have reason to investigate at least part of their systems and a couple are in need of extensive technical support. Factors affecting the quality of the laboratory analysis included:

1. Failure to read manuals and strictly follow method protocol,
2. Use of differing calibration techniques,
3. Apparent failure to use control samples[9],
4. Equipment instability – especially reference spectrophotometers
5. Incorrect and/or different correction factors – especially with iCheck dilution factor calculations and correction for background and/or intrinsic content.

Of particular concern is lack of reference method for the analysis of vitamin A in cereal products from, especially, the regulatory authority laboratories.

[9] In order to assure that a test run is valid and results are reliable, Quality Control Samples should be used in the performance of each assay. http://www.who.int/diagnostics_laboratory/quality/control/en/

Bullet 5 above applied particularly to the use of iCheck technology where some discussions with laboratories would use a correction factor for vitamin A in grains to correct for background and others would not.

The iCheck methodology gave minimal indications of problem. Where issues were noted it appeared that the relevant laboratories had analysts that were not the original ones trained or the laboratories themselves had received minimal training. One laboratory indicated they did not require training. Laboratories that are known to have undergone in-depth training on iCheck performed significantly better.

Recommendations

A deep root cause analysis is clearly indicated that should result in a clear set of Corrective and Preventive Actions (CAPA) for each analysis, by matrix if necessary, and by methodology.

The derivation of such an exercise above should be organised by - at least within the ECSA Region – bringing in support from the ECSA Laboratory Working Group. Further that support be given to the working group especially as the question of validation of the ECSA methodologies themselves is being openly questioned by some and vehemently rejected by others (who feel it unnecessary). On balance the ECSA methodologies did give more reason for concern.

If such a proficiency scheme is launched it is strongly recommended that the participant base be widened to include all regulatory authorities, 3rd party laboratories and industry. Expanding the exercise to include all countries with mandatory fortification should be considered as a gold standard BUT it is recommended such an initiative set itself a goal to be self-funding in a short period of time for sustainability issues (previous attempts have failed due to slow delivery by laboratories). Fee for service maybe an option to explore but whether a subscription system would speed up laboratory performance is open to debate.

Prior to the implementation of the above it is strongly recommended that:

1. Training and consultancy be offered to laboratories in all countries with fortification programmes
2. 'Reference material' (see below) be supplied to laboratories as support material and to assist laboratories in identifying potential problems in their analyses

3. In view of the apparent issues, as indicated in the Z scores, with ECSA methodology it is recommended that validation of the methods be implemented. At this stage it is unclear if the problems are with the methods themselves or with in-house variations of the methods [at least one laboratory stated they used ECSA methods and performed well]. A separate study will be required.

'Reference material' needs to be explained and defined. According to various ISO Guides, such as ISO Guide 33 through 35, it is important to distinguish between a Certified Reference Material (CRM) and a Reference Material (RM):

1. Certified Reference Material
    a. Material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability
2. Reference Material
    a. Material sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

CRMs are expensive and probably not available for fortified food vehicles but RMs can be prepared for specific food vehicles on a regular basis to assist laboratories in ensuring their systems are functioning correctly. Due to the quantities required specific mixing equipment would be required as scaling up from preparing a few hundred grams to over a kilogram is difficult with normal laboratory equipment (as this exercise demonstrated). Scaling up to produce circa 10 Kg of sample would be several orders of magnitude more difficult. The production of the sugar samples was problematic for just that reason – it was not feasible to produce spiked samples using a 50:1 pre-blend that was sufficiently homogeneous.

CRM's and Reference materials are not commercially available, or at least readily available. Some laboratories do create reference materials for their clients and entities such as FSANZ/MMI, AACCI, AOAC and FAPAS have proficiency schemes but do not target fortified food vehicles and this is a major weakness in technical support available to countries taking on fortification programmes.

In this exercise a commercial premix was used for wheat flour and maize meal vitamin A and iron. That the premix itself was not homogeneous cannot be discounted so for future studies the use of pure chemicals is strongly recommended.

As many countries use NaFeEDTA as the source of the added iron it is recommended that as NaFeEDTA is water soluble be exploited and resources be found to assess the viability of solubilising the iron from the flour and testing the supernatant rather than wet or dry ashing (or direct injection) of the whole sample.

Annex 1 - Outlier Analysis

The study of outliers or wild observations has been of interest to statisticians and researchers for over half a century. Two of the most quoted articles in this regard are:

Kruskal, W.H. 1960 "Some remarks on wild observations" Technometrics Vol 2 (1) pp 1-3

Grubbs[10], F.E. 1969 "Procedures for detecting outlying observations in samples" Technometrics Vol 2 (1) pp 1-21

Paraphrasing from Kruskal

1. Whether you use or do not use apparent wild observations it is important that a statement is provided noting which data and why it was/was not excluded.
2. Dangerous to oversimply apparently wild observations and classify them simply as include or exclude. An apparently wild observation is a signal that says: "Here is something from which we may learn a lesson, perhaps of a kind not anticipated beforehand, and perhaps more important than the main object of the study." A frequently used example of such a situation is Fleming's recognition of the virtue of penicillium. This often refers to methods of sampling, measurement, and data reduction, instead of to the underlying physical phenomenon.
3. If we 'know' the observation is anomalous do we include it or not?
    a. If we are determining the content of chemical A and one observation is anomalous and we find an equipment calibration error that affected that observation or set of observations we can correct for it if the magnitude of the error is known.
    b. What if the magnitude is not known? If the objective is only estimating the quantity of chemical we can exclude the observation. If the objective is mainly, or even partly, investigating the method of measurement of the quantity of chemical A (in setting up a routine procedure to be based on a single observation), then it may be important to keep the observation in.
    c. In the latter case the observation is telling us something about the frequency and magnitude of serious errors in the method.
4. It is often useful to classify degrees of knowledge about the apparently wild observation:
    a. Known before – sensitive instrument was jarred during measurement

[10] This is a more reader friendly and expansive work than previous pure mathematical publications circa 1950

b. Known after – check the laboratory notebook and see there was a procedural error (the danger here is to bias one's approach to the observation)
c. No evidence – perhaps the most difficult and one that gives rise to various rule of thumb approaches.

5. For 4c the classical approach is to create a test statistic, chosen so as to be sensitive to the kind of wildness envisaged, to generate its distribution under some sort of hypothesis of non-wildness, and then to 'reject' (or treat differently) an observation if the test statistic for it comes out improbably large under the hypothesis of no wildness.

6. Kruskal concluded by observing that his own approach was to carry out an analysis both with and without the suspect observations. If the broad conclusions of the two analyses are quite different, I should view any conclusions from the experiment with very great caution.

Return to text

Paraphrasing Grubbs

1. An outlying observation maybe an extreme manifestation of random variability inherent in the data and so should be retained
2. An outlying observation maybe a result of deviation from experimental procedure or calculation.
    a. Maybe necessary to investigate the reason for the value and even reject it, though not necessarily so
3. "When a skilled experimenter is clearly aware that a gross deviation from prescribed experimental procedure has taken place, the resultant observations should be discarded, whether or not it agrees with the rest of the data and without recourse to statistical tests for outliers." Return to text
4. Many times the evidence of a deviation is the value itself. In such cases a cautious attitude is recommended. Use of one of the criteria below may permit a clear cut judgement but in doubtful cases the experimenter's judgement will have "considerable influence" and the rationale behind that judgement should be recorded along with the extent to which it has been used
5. Screening for outlying samples is as follows:
    a. Physical reason known
        i. Reject
        ii. Correct physically
        iii. Reject and possibly take additional observations

   b. Physical reason unknown
      i. Reject
      ii. Correct statistically
      iii. Reject and possibly take additional observations
      iv. Employ truncated sample theory for censored observations
6. Statistical test may be used to support judgement that a physical reason exists or as a basis to initiate action to find a physical cause
7. Significance levels > 5% should not be routinely used
8. Most test assume data is normally distributed
9. Since the estimate of within-laboratory variation is independent of any differences between laboratories then repeated outlier testing can be used
10. When dealing with multiple outliers a situation can develop in which some results mask others also anomalous. In such situations, if the data is available, ANOVA can be used to detect differences between laboratories creating outliers (i.e. using a non-standard technique) by testing the laboratory averages using statistical tests such as David's T Criteria etc.

Return to text

Annex 2 - Analytical Protocols for Reference Methods

Samples

All samples are homogeneous as possible. As per good laboratory practise thoroughly mix or shake the sample as received before commencing analysis (to circumvent the possibility of some segregation during transportation).

Each sample is marked with a once off code number. Some samples may be marked indicating if they are for the iCheck or the reference method (where applicable), others may be marked BLANK for methods such as UV/VIS vitamin A in oil.

Please confirm by e-mail receipt of arrival and if the samples where received in good condition.

Important for Sugar

We have been advised that each sugar sample aliquot has been made up individually and it is strongly recommended that the sample aliquot NOT be sub-divided but extracted in its entirety.

Repeats

Enough sample has been provided to perform triplicate analyses [**except for sugar**]. Please perform **triplicate sample preparations (i.e. extractions)**. In the methods below you will be asked to provide specific data you generated to calculate the final result. This information will be used to calculate other factors such as recovery.

> [Should you wish you can additionally carry out triplicate analyses on the same extract but that is an optional extra and will depend on your laboratory capacity. Not all of the analysis methods will have enough sample to complete this option. If you do this option please clearly indicate the results are triplicate analyses on the same extract].

Data

All data (sample result, methodology and additionally requested information such as sample weight) is treated confidentially. Each participating laboratory will be allocated a unique code which identifies neither the laboratory nor the country involved – your specific code will be forwarded to you separately.

Each laboratory is requested to report the results within **14 days** of receipt. Please show all calculations to assist in identifying potential systematic bias factors.

Reporting

Please send all data in the Excel table attached to p3away@mweb.co.za .

In the subject line of the email identify your laboratory.

This trial is not a 'proficiency test' in the normal use of the term. This trial is primarily aimed at establishing a baseline for laboratory capacity and as a result your data from this trial will only be reported at the conclusion of the exercise when all of the laboratories have submitted their results. All of the data will then be subject to statistical analysis and the results of that exercise will be reported to you separately.

From this baseline decisions can be made regarding the establishment of a proficiency scheme (in which results are reported after each round) and possible laboratory training exercises and capacity building.

Units

In all calculations please clearly indicate the units for concentration, volume and weight being used.

Techniques and Methodologies

Please advise any modifications (other than those on sample size requested below) that the laboratory has made to the analytical method as prescribed by AOAC and/or ECSA Laboratory Manuals.

Please also advise if you are using methodology/techniques different to those mentioned below. The quantity of sample is limited.

Iodine in Salt (added as iodate)

AOAC 925.56 and ECSA Laboratory Manual Part I Section B1 instruct to take 50 g of salt and make up to 250 ml then take 50 ml of this solution and perform the titration.

During this ring-trial, due to limited sample weights, for the titration method take circa 10 g weighed accurately and make up to 50 ml then proceed as normal. Please report the actual sample weight used, the actual weight of sodium thiosulphate used to make up the standard solution and the titration start and finish values on each sample.

For the WYD Checker follow the normal method. Please report the actual sample weight or scoop size used.

For all methods please report the date of receipt of the samples, date of actual analysis and the analyst's name (in case additional or missing information may be requested later).

Vitamin A (added as Retinyl palmitate)

HPLC

AOAC 2001.13, ECSA Laboratory Manual Part II Section C4 and Part III Section C2 all use <5 g on low fat samples and <2 g on high fat samples.

Please show the calculations for RFa (give values for low, medium and high standard), the sample weights and full calculations.

UV/VIS Spectrophotometer

ECSA Laboratory Manual Part II Section B3 B notes that this adaption uses 100 g of sugar

As the homogeneity has been mitigated for steps F (a) 1-3 of the ECSA protocol use 60 g [this is the content weight of 1 pouch] and make up to 150 ml then proceed as normal. Please state the method used to make up to 150 ml (i.e. volumetric flask or measuring cylinder).

ECSA Laboratory Manual Part II Section C 3 requires 2 g oil. Please advise if the solvent used was Dichloromethane or Hexane.

Please report sample weight to at least 3 decimals (ECSA advises 4 for oil) and show all calculations.

For UV/VIS methodology a sample identified as BLANK has been provided. All of the samples have been made from the same oil or sugar as the blank.

QUERY - If you did not have a blank (i.e. Regulator at market level how do you compensate for background?)

For all methods please report the date of receipt of the samples, date of actual analysis and the analyst's name (in case additional or missing information may be requested later).

Iron (added as NaFeEDTA)

UV/VIS Spectrophotometer

ECSA Laboratory Manual Part III Section II. As the homogeneity has been mitigated step 2 H a 3 can be omitted. For step 2 H a 4 use triplicate samples then proceed as normal.

Please report sample weights to 3 decimals and show all calculations.

For all methods please report the date of receipt of the samples, date of actual analysis and the analyst's name (in case additional or missing information may be requested).

Return to text

Annex 3 Analytical Protocol for iCheck

Iodine in salt (added as iodate)

For the iCheck IODINE take circa 5 g of salt, weighed accurately and add water to 50 ml. Proceed as per the manual. Please report the actual sample weight used and show all calculations.

Vitamin A (added as retinyl palmitate) – Wheat and maize flour

IMPORTANT

The same sample flour dilution is used for both vitamin A and iron analysis. For vitamin A analyse immediately after dilution. Ensure that you inject a well-mixed slurry. Use the wide tip needle.

For iCheck FLUORO take circa 5 g of flour, weighed accurately and add water to 50 ml. Proceed as per the manual. Please report the actual sample weights used and show all calculations.

Vitamin A (added as retinyl palmitate) - Oil

For the iCheck CHROMA or CHROMA 3 proceed as per the manual. Indicate if you used a CHROMA or CHROMA 3. The oil type has been selected to work on both CHROMA and CHROMA 3.

Vitamin A (added as retinyl palmitate) - Sugar

IMPORTANT

The production of homogeneous sugar samples is problematic. You have been provided with coded 50g sample aliquots, which have been individually produced. DO NOT split up the aliquot – each sample must be used in its entirety (all 50g).

For the iCheck FLUORO take the 50g sample aliquot and record its actual weight accurately. Add water to 500ml and proceed as per the manual. Please report the actual sample weights used and show all calculations. All sugar must be solubilised and the solution analysed quickly - DO NOT let vitamin A

form a layer on top of the solution – mix well just before taking the solution up into the syringe.

Iron (added as NaFeEDTA) – Wheat and maize flour – TOTAL IRON

IMPORTANT

The same flour dilution is used for both vitamin A and iron. If you do both vitamin A and iron analyse vitamin A first. For iron ensure you use new additive (vial with green top) and that you inject the well-mixed slurry. Incubate the sample in the reagent vial for 1 hour. Use the wide tip needle.

For the iCheck IRON take circa 5 g of flour, weighed accurately and add water to 50 ml. Proceed as per the manual. Please report the actual sample weights used and show all calculations.

NOTE: With the new additive all incubations are now 1 hour

Return to text

Appendices – Individual Laboratory Data

Laboratory 1

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 1	17.67	47.44	87.55
	17.74	48.44	82.81
	17.17	47.76	79.57
	18.62	48.23	
		46.19	
Median	17.71	47.76	82.81
Z-Score	1.82	-1.29	-2.86
Standard Deviation	0.60	0.89	4.86
U	4.73	2.89	11.5

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 1	4.70	14.17	28.63
	4.20	15.25	27.08
	4.20	12.96	27.27
	4.37	14.28	
		13.91	
Median	4.29	14.17	27.27
Z-Score	-2.05	0.59	0.32
Standard Deviation	0.24	0.82	0.85
U	5.83	5.39	2.05

	Vitamin A in Maize meal Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4**	**4.6**
Laboratory 1	0.98	3.77	6.59

	Vitamin A in Maize meal Concentration mg RE/Kg		
	1.22	4.02	7.77
	1.47	4.01	6.49
	1.58	3.31	
		4.72	
Median	1.35	4.01	6.59
Z-Score	0.29	0.02	3.98
Standard Deviation	0.27	0.51	0.21
U	39.35	17.60	4.46

	Vitamin A in Wheat flour		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4.1**	**8.1**
Laboratory 1	1.52	3.07	6.01
	1.64	3.73	9.05
	1.27	3.22	
		4.86	
		4.48	
Median	1.52	3.73	7.53
Z-Score	1.60	-0.46	-0.57
Standard Deviation	0.19	0.78	2.15
U	23.21	52.02	59.35

	Iron in Maize meal		
	Concentration mg /Kg		
Assigned Value from Baseline	**23.1**	**51.5**	**97**
Laboratory 1	18.72	50.19	100.10
	20.59	47.06	94.91
	16.98	46.27	96.87
	17.20	45.00	
		42.28	
Median	17.96	46.27	96.87
Z-Score	-3.21	-2.01	-0.05

Standard Deviation	1.67	2.89	2.62
U	14.24	8.07	5.95

	Iron in Wheat flour		
	Concentration mg /Kg		
Assigned Value from Baseline	**30**	**58.4**	**102.3**
Laboratory 1	21.25	53.61	98.88
	19.86	48.64	107.42
	21.09	47.63	96.93
		47.73	
		58.82	
Median	21.09	48.64	98.88
Z-Score	-5.94	-2.87	-0.73
Standard Deviation	0.76	4.88	5.58
U	2.23	6.10	5.80

Laboratory 2

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 2	15.28	43.72	4.16
	15.44	44.07	
	15.13	41.08	56.31
		42.14	67.09
Median	15.28	42.93	56.31
Z-Score	-0.38	-2.96	-6.82
Standard Deviation	0.16	1.40	33.66
U	2.89	6.61	56.28

Laboratory 3

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 3	14.65	38.50	65.22

	15.75	42.48	62.43
	15.70	41.24	69.68
		41.83	
Median	15.70	41.54	65.22
Z-Score	0.00	-3.44	-5.49
Standard Deviation	0.62	1.75	3.66
U	0.94	4.39	12.58

Laboratory 4a

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 4a	3.40	10.32	19.69
	3.09	10.11	87.58
	14.40	36.17	
	13.82	38.82	
		16.89	
		16.79	
Median	8.61	16.84	53.64
Z-Score	12.37	1.92	5.29
Standard Deviation	6.28	12.75	48.01
U	183.20	115.66	186.07

Laboratory 4b

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 4b	<3	2.90	15.54
	<3	11.18	
Median		7.04	15.54
Z-Score		-2.98	-1.90
Standard Deviation		5.85	
U		172.89	

Laboratory 5a

	Vitamin A in Oil Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 5a	4.98	15.07	29.58
	5.11	15.23	26.61
	4.88	15.18	27.51
	4.85	13.83	
		13.91	
Median	4.93	15.07	27.51
Z-Score	0.14	0.80	0.44
Standard Deviation	0.12	0.71	1.52
U	0.10	1.04	0.36

Laboratory 5b

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 5b		15.32	
		14.40	
Median		14.86	
Z-Score		0.93	
Standard Deviation		0.65	
U		9.10	

Laboratory 6a

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 6a		84.50	
		87.04	
Median		85.77	

Z-Score		11.82	
Standard Deviation		1.80	
U		4.35	

	Iron in Maize meal		
	Concentration mg /Kg		
Assigned Value from Baseline	**23.1**	**51.5**	**97**
Laboratory 6a	25.25	45.89	67.12
	32.84		89.69
	22.15	53.33	99.24
			89.73
Median	25.25	49.61	89.71
Z-Score	1.09	-0.77	-3.04
Standard Deviation	5.50	5.26	13.64
U	36.10	22.05	15.65

	Iron in Wheat flour		
	Concentration mg /Kg		
Assigned Value from Baseline	**30**	**58.4**	**102.3**
Laboratory 6a	28.95	50.47	91.68
	30.58	52.55	
	29.26	55.03	105.64
		55.24	109.97
Median	29.26	53.79	105.64
Z-Score	-0.49	-1.36	0.71
Standard Deviation	0.87	2.26	9.56
U	3.11	7.35	12.05

Laboratory 6b

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 6b		10.18	
		11.41	

Median		10.80	
Z-Score		-1.10	
Standard Deviation		0.87	
U		16.85	

Laboratory 7a

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 7a	21.20	59.36	108.12
	23.32	55.65	100.35
	25.97	53.00	100.17
		53.53	
Median	23.32	54.59	100.35
Z-Score	6.93	1.07	-0.25
Standard Deviation	2.39	2.89	4.54
U	26.73	7.14	0.53

Laboratory 7b

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 7b		40.35	
		40.22	
Median		40.29	
Z-Score		-3.87	
Standard Deviation		0.09	
U		0.47	

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**

Laboratory 7b	3.16	12.77	26.64
		7.31	6.31
		3.91	
		7.28	
		5.23	
		8.74	
Median	3.16	7.30	16.48
Z-Score	-5.80	-2.85	-1.72
Standard Deviation		3.08	14.38
U		70.73	181.40

	Vitamin A in Maize meal		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4**	**4.6**
Laboratory 7b	1.09	2.65	5.54
	1.48	3.34	11.00
	1.61	3.47	7.23
	1.00	2.45	
		2.98	
Median	1.29	2.98	7.23
Z-Score	0.17	-2.55	5.26
Standard Deviation	0.30	0.44	2.80
U	54.91	35.52	68.72

	Vitamin A in Wheat flour		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4.1**	**8.1**
Laboratory 7b	3.67	5.69	4.97
	4.51	4.84	7.20
	1.43	3.82	7.18
	1.63	3.93	
		4.41	
Median	2.65	4.41	7.18
Z-Score	7.25	0.39	-0.92

Standard Deviation	1.52	0.76	1.28
U	124.26	32.00	0.82

	Iron in Maize meal		
	Concentration mg /Kg		
Assigned Value from Baseline	**23.1**	**51.5**	**97**
Laboratory 7b	22.23	47.43	82.93
	22.67	41.53	83.43
	17.23	42.80	79.70
	23.03	46.50	
		44.50	
Median	22.45	44.50	82.93
Z-Score	-0.41	-2.69	-5.86
Standard Deviation	2.73	2.46	2.02
U	5.24	13.21	1.77

	Iron in Wheat flour		
	Concentration mg /Kg		
Assigned Value from Baseline	**30**	**58.4**	**102.3**
Laboratory 7b	28.60	52.60	90.13
	27.30	55.50	80.80
	27.07	49.00	80.37
	25.47	59.73	
		56.30	
Median	27.19	55.50	80.80
Z-Score	-1.88	-0.85	-4.57
Standard Deviation	1.28	4.04	5.52
U	8.27	15.36	1.56

Laboratory 8

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**

Laboratory 8	22.01	49.89	96.25
	20.40	52.90	110.86
	13.76	50.45	97.50
	19.05	62.49	
		51.48	
Median	19.73	51.48	97.50
Z-Score	3.66	-0.01	-0.67
Standard Deviation	3.57	5.19	8.10
U	22.06	8.11	3.77

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 8	11.11	22.20	49.92
	6.24	24.75	46.62
	10.27	23.59	42.76
		28.07	
		33.89	
Median	10.27	24.75	46.62
Z-Score	17.90	5.88	3.97
Standard Deviation	2.60	4.67	3.58
U	24.05	30.29	20.81

	Vitamin A in Maize meal		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4**	**4.6**
Laboratory 8	1.30	1.82	1.32
	1.70	0.80	1.87
	2.04	1.67	3.76
	1.40	1.05	2.16
Median	1.55	1.36	2.02
Z-Score	0.70	-6.60	-5.17
Standard Deviation	0.33	0.49	1.05
U	47.42	83.23	61.28

	Wheat vitamin A		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4.1**	**8.1**
Laboratory 8	13.06	26.23	17.99
	20.38	24.43	0.14
	0.49	0.32	
	7.26	4.06	
		17.32	
Median	10.16	17.32	9.07
Z-Score	44.80	16.53	0.97
Standard Deviation	8.46	11.77	12.62
U	181.87	151.24	289.46

	Iron in Maize meal		
	Concentration mg /Kg		
Assigned Value from Baseline	**23.1**	**51.5**	**97**
Laboratory 8	248.87	66.29	53.26
	23.66	26.51	112.05
	39.71	37.91	125.46
	117.43	57.38	3109.46
Median	78.57	47.65	118.76
Z-Score	34.67	-1.48	9.06
Standard Deviation	102.81	18.10	1506.59
U	175.44	87.56	89.37

	Iron in Wheat flour		
	Concentration mg /Kg		
Assigned Value from Baseline	**30**	**58.4**	**102.3**
Laboratory 8	64.31	89.04	6.00
	58.29	39.52	230.02
	2247.75	2330.00	
	13.30	37.00	

		64.88	
Median	61.30	64.88	118.01
Z-Score	20.87	1.91	3.34
Standard Deviation	1101.46	1016.46	158.41
U	122.32	114.92	279.05

Laboratory 9

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 9	24.38	59.87	122.26
	35.24	60.45	104.47
	27.10	57.73	106.46
		55.19	
		45.39	
Median	27.10	57.73	106.46
Z-Score	10.36	2.15	0.67
Standard Deviation	5.65	6.14	9.75
U	29.51	12.94	5.50

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 9	6.98	16.07	24.78
	9.59	13.27	23.37
	7.20	10.59	
	14.27	17.32	
		13.45	
Median	8.40	13.45	24.08
Z-Score	11.65	0.23	-0.29
Standard Deviation	3.39	2.63	1.00
U	45.70	59.89	8.61

	Iron in Maize meal		
	Concentration mg /Kg		
Assigned Value from Baseline	**23.1**	**51.5**	**97**
Laboratory 9	34.31	59.55	111.50
	36.28	106.82	102.64
	30.86	64.86	139.69
	35.30	62.38	
		67.27	
Median	34.81	64.86	111.50
Z-Score	7.32	5.14	6.04
Standard Deviation	2.36	19.58	19.35
U	8.32	11.24	23.36

	Iron in Wheat flour		
	Concentration mg /Kg		
Assigned Value from Baseline	**30**	**58.4**	**102.3**
Laboratory 9	12.92	65.91	121.97
	45.56	65.62	
	47.02	104.71	171.00
	60.63	71.87	145.72
		68.56	
Median	46.29	68.56	145.72
Z-Score	10.86	2.99	9.24
Standard Deviation	20.25	16.61	24.52
U	47.86	12.61	47.92

Laboratory 10

	Vitamin A in Maize meal		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4**	**4.6**
Laboratory 10	1.31	2.04	5.52
	1.13	1.96	3.99
	0.84	2.12	4.35
	1.99		4.17

Median	1.22	2.04	4.26
Z-Score	0.04	-4.90	-0.68
Standard Deviation	0.49	0.08	0.69
U	56.63	11.53	12.42

	Vitamin A in Wheat flour		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4.1**	**8.1**
Laboratory 10	1.31	3.44	5.68
	1.47	3.22	5.79
	2.01	3.11	5.63
	1.27	3.52	
		3.73	
Median	1.39	3.44	5.68
Z-Score	0.95	-0.83	-2.42
Standard Deviation	0.34	0.25	0.08
U	21.15	18.80	2.59

	Iron in Maize meal		
	Concentration mg /Kg		
Assigned Value from Baseline	**23.1**	**51.5**	**97**
Laboratory 10	31.80	51.97	84.30
	24.97	50.97	82.17
	23.27		86.15
	17.23	51.53	99.47
Median	24.12	51.53	85.23
Z-Score	0.64	0.01	-4.91
Standard Deviation	5.99	0.50	7.80
U	47.17	2.51	6.86

	Iron in Wheat flour		
	Concentration mg /Kg		
Assigned Value from Baseline	**30**	**58.4**	**102.3**

Laboratory 10	45.20	62.57	96.50
	40.67	62.23	101.90
	31.90	76.93	110.80
	29.57	69.43	
		60.03	
Median	36.29	62.57	101.90
Z-Score	4.19	1.23	-0.09
Standard Deviation	7.34	6.94	7.22
U	44.97	11.93	15.58

Laboratory 11a

	Iron in Maize meal		
	Concentration mg /Kg		
Assigned Value from Baseline	**23.1**	**51.5**	**97**
Laboratory 11a	31.67	62.97	102.52
		59.60	
		61.28	
		14.40	
		14.10	
Median	31.67	59.60	102.52
Z-Score	5.36	3.12	2.30
Standard Deviation		25.79	
U		16.62	

	Iron in Wheat flour		
	Concentration mg /Kg		
Assigned Value from Baseline	**30**	**58.4**	**102.3**
Laboratory 11a	40.68	68.91	123.39
	36.02	18.44	110.76
Median	38.35	43.68	117.08
Z-Score	5.57	-4.33	3.14
Standard Deviation	3.30	35.69	8.93
U	17.86	169.87	15.86

Laboratory 11b

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 11b	18.45	46.55	85.65
	17.25	47.35	50.00
	8.50	26.50	49.90
		26.75	
		46.80	
		56.90	
Median	17.25	46.68	50.00
Z-Score	1.41	-1.66	-7.76
Standard Deviation	5.43	12.39	20.61
U	20.45	34.33	0.59

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 11b	5.75	16.10	27.72
	5.17	17.93	24.31
		16.07	36.52
		17.49	
		23.34	
Median	5.46	17.49	27.72
Z-Score	1.87	2.25	0.40
Standard Deviation	0.41	3.00	6.30
U	15.62	23.37	36.17

	Vitamin A in Maize meal		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4**	**4.6**
Laboratory 11b	1.63	3.30	6.45
	0.10	4.04	5.59
	1.13	2.49	7.08

		3.21	7.41
		3.65	
Median	1.13	3.30	6.77
Z-Score	-0.14	-1.75	4.33
Standard Deviation	0.78	0.58	0.80
U	130.09	31.18	20.86

	Vitamin A in Wheat flour		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4.1**	**8.1**
Laboratory 11b	1.63	3.71	5.59
	0.77	3.60	6.78
	2.29	3.27	3.59
		3.63	5.40
		2.53	
Median	1.63	3.60	5.50
Z-Score	2.15	-0.62	-2.61
Standard Deviation	0.76	0.49	1.32
U	119.04	8.98	36.92

	Iron in Maize meal		
	Concentration mg /Kg		
Assigned Value from Baseline	**23.1**	**51.5**	**97**
Laboratory 11b	21.70	62.97	102.52
		59.60	66.20
		61.28	73.55
		52.35	
		36.75	
		35.65	
Median	21.70	55.98	73.55
Z-Score	-0.88	1.72	-9.77
Standard Deviation		12.35	19.20
U		32.30	29.38

	Iron in Wheat flour		
	Concentration mg /Kg		
Assigned Value from Baseline	**30**	**58.4**	**102.3**
Laboratory 11b	40.68	68.91	123.39
	36.02	79.20	110.76
	32.40	40.50	114.65
	30.30	50.60	27.40
Median	34.21	59.76	112.71
Z-Score	2.81	0.40	2.21
Standard Deviation	4.55	17.48	44.75
U	24.58	69.90	16.47

Laboratory 12a

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 12a	15.17	47.27	85.87
	15.90	47.63	94.70
	16.97	48.07	
	16.27	50.50	
		48.43	
		50.53	
Median	16.09	48.25	90.29
Z-Score	0.35	-1.12	-1.75
Standard Deviation	0.75	1.43	6.24
U	9.78	4.87	14.38

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 12a	5.38	12.89	22.95

	6.59	15.31	27.00
	5.07	16.98	27.55
		13.76	31.14
		17.20	
Median	5.38	15.31	27.28
Z-Score	1.60	1.16	0.32
Standard Deviation	0.80	1.91	3.35
U	16.94	32.07	22.31

Laboratory 12b

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 12b	16.00	45.37	86.07
	15.83	47.20	82.17
	17.17	47.47	82.70
		46.93	
		48.63	
		49.83	
Median	16.00	47.34	82.70
Z-Score	0.27	-1.44	-2.88
Standard Deviation	0.73	1.52	2.12
U	3.12	5.28	1.88

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 12b	4.38	12.86	22.45
	4.45	8.42	25.31
	2.99	9.35	20.84
		11.17	17.43
		14.22	
Median	4.38	11.17	21.65
Z-Score	-1.73	-0.92	-0.75
Standard Deviation	0.82	2.40	3.29

U	4.70	47.90	30.36

	Vitamin A in Maize meal		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4**	**4.6**
Laboratory 12b	0.62	3.74	6.81
	1.46	2.65	5.41
		3.85	5.75
		2.94	
		2.87	
		3.62	
		3.67	
Median	1.04	3.62	5.75
Z-Score	-0.32	-0.95	2.30
Standard Deviation	0.59	0.49	0.73
U	118.73	18.68	17.38

	Vitamin A in Wheat flour		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4.1**	**8.1**
Laboratory 12b	1.33	3.54	8.05
	1.00	3.59	7.64
	1.70	3.14	7.26
	1.69	3.54	6.12
Median	1.51	3.54	7.45
Z-Score	1.55	-0.70	-0.65
Standard Deviation	0.33	0.21	0.83
U	36.02	2.08	15.59

	Iron in Maize meal		
	Concentration mg /Kg		
Assigned Value from Baseline	**23.1**	**51.5**	**97**
Laboratory 12b	16.67	42.90	86.80
	21.32	45.07	91.96
		50.13	89.63
		46.56	
		44.71	
		47.53	
		48.00	
Median	19.00	46.56	89.63
Z-Score	-2.57	-1.90	-3.07
Standard Deviation	3.29	2.40	2.58
U	35.99	9.41	7.64

	Iron in Wheat flour		
	Concentration mg /Kg		
Assigned Value from Baseline	**30**	**58.4**	**102.3**
Laboratory 12b	25.33	55.10	100.23
	31.53	54.33	93.33
	27.75	34.15	90.97
	28.98	38.81	96.46
Median	28.37	46.57	94.90
Z-Score	-1.09	-3.48	-1.58
Standard Deviation	2.58	10.70	4.01
U	18.92	51.42	8.50

Laboratory 13a

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 13a	14.43	48.05	94.41
	12.95	46.73	94.97
	15.07	49.29	91.88
	15.95	47.67	93.68

Median	14.75	47.86	94.05
Z-Score	-0.86	-1.26	-1.19
Standard Deviation	1.26	1.06	1.34
U	15.15	4.05	2.02

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 13a	12.09	12.00	22.37
	9.06	14.58	
	7.09	18.01	
	4.05	15.74	
		6.37	
		2.70	
Median	8.08	13.29	22.37
Z-Score	10.58	0.15	-0.61
Standard Deviation	3.38	5.90	
U	91.02	79.31	

	Iron in Maize meal		
	Concentration mg /Kg		
Assigned Value from Baseline	**23.1**	**51.5**	**97**
Laboratory 13a	13.52	22.29	36.26
	11.83	23.00	36.05
	16.35	25.55	30.32
	15.35	23.91	28.96
	28.53		
	19.04		
	18.30		
Median	16.35	23.46	33.19
Z-Score	-4.22	-10.79	-26.59
Standard Deviation	5.46	1.41	3.80
U	48.37	10.15	26.31

	Iron in Wheat flour		
	Concentration mg /Kg		
Assigned Value from Baseline	**30**	**58.4**	**102.3**
Laboratory 13a	17.35	20.97	32.56
	20.92	25.65	33.01
	14.30	28.08	33.33
	17.98		30.61
	24.08		26.54
	23.33		22.26
Median	19.45	25.65	31.59
Z-Score	-7.03	-9.63	-15.05
Standard Deviation	3.78	3.61	4.44
U	45.20	27.85	14.75

Laboratory 13b

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 13b	20.57	53.26	91.32
	19.28	51.46	89.39
		50.81	102.03
		62.86	
Median	19.93	52.36	91.32
Z-Score	3.84	0.30	-1.59
Standard Deviation	0.91	5.60	6.81
U	9.52	6.88	6.21

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 13b	3.75	15.00	28.50
	3.75	15.25	31.00
	4.00	14.75	30.00
	6.50	14.50	
	6.25	15.00	

Median	4.00	15.00	30.00
Z-Score	-3.00	1.00	0.83
Standard Deviation	1.40	0.29	1.26
U	18.38	4.90	9.80

	Vitamin A in Maize meal		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4**	**4.6**
Laboratory 13b	2.22	5.43	9.44
	2.08	5.91	8.79
	2.60	2.49	10.95
			10.53
			11.08
Median	2.22	5.43	10.53
Z-Score	2.04	3.58	11.86
Standard Deviation	0.27	1.85	1.00
U	18.54	25.99	15.36

	Vitamin A in Wheat flour		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4.1**	**8.1**
Laboratory 13b	2.61	7.30	12.22
	2.79	6.83	6.81
	1.85	3.70	6.27
	1.76	2.79	
		3.05	
Median	2.23	3.70	6.81
Z-Score	5.15	-0.50	-1.29
Standard Deviation	0.52	2.16	3.29
U	56.03	72.31	23.31

	Iron in Maize meal		
	Concentration mg /Kg		
Assigned Value from Baseline	**23.1**	**51.5**	**97**

Laboratory 13b	28.35	90.81	184.73
	27.60	128.10	210.22
	58.36	100.69	208.45
	20.01	70.47	232.25
		71.37	95.78
			93.89
Median	27.98	90.81	196.59
Z-Score	3.05	15.12	41.50
Standard Deviation	16.94	23.82	60.80
U	43.82	62.94	36.86

	Iron in Wheat flour		
	Concentration mg /Kg		
Assigned Value from Baseline	**30**	**58.4**	**102.3**
Laboratory 13b	57.11	122.22	210.92
	52.78	121.12	76.21
	22.53	76.39	74.58
	26.14	93.15	104.08
	73.96	83.07	107.46
Median	52.78	93.15	104.08
Z-Score	15.19	10.22	0.38
Standard Deviation	21.77	21.37	55.93
U	117.98	52.90	78.73

Laboratory 14

	Iodine in Salt		
	Concentration mg/Kg		
Assigned Value from Baseline	**15.7**	**51.5**	**102**
Laboratory 14	8.33	16.64	21.60
	10.67	19.87	26.63
	5.07	14.65	21.04
			24.29
			30.75
Median	8.33	16.64	24.29

Z-Score	-6.70	-12.02	-11.60
Standard Deviation	2.81	2.63	3.98
U	82.59	35.16	32.56

	Vitamin A in Oil		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**4.9**	**13**	**25.6**
Laboratory 14	25.15	98.79	36.88
	19.55	4.59	44.12
	9.23	9.28	4.67
	-1.65	1.13	
Median	14.39	6.94	36.88
Z-Score	31.63	-3.03	2.13
Standard Deviation	11.82	47.01	21.00
U	162.63	172.75	57.72

	Vitamin A in Maize meal		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4**	**4.6**
Laboratory 14	13.02	10.59	19.60
	13.68	41.51	1.02
	1.02	0.44	
		0.47	
Median	13.02	5.53	10.31
Z-Score	23.64	3.83	11.42
Standard Deviation	7.13	19.43	13.14
U	14.90	269.81	264.91

	Vitamin A in Wheat flour		
	Concentration mg RE/Kg		
Assigned Value from Baseline	**1.2**	**4.1**	**8.1**
Laboratory 14	36.91	54.03	66.20
		0.49	37.72

		0.68	37.13
Median	36.91	0.68	37.72
Z-Score	178.55	-4.28	29.62
Standard Deviation		30.86	16.62
U		82.15	4.60

	Iron in Maize meal		
	Concentration mg /Kg		
Assigned Value from Baseline	**23.1**	**51.5**	**97**
Laboratory 14	119.33	354.13	192.50
	47.72	180.03	
		36.64	
		28.58	
Median	83.53	108.34	192.50
Z-Score	37.77	21.86	39.79
Standard Deviation	50.64	152.93	
U	126.03	205.50	

	Iron in Wheat flour		
	Concentration mg /Kg		
Assigned Value from Baseline	**30**	**58.4**	**102.3**
Laboratory 14	281.37	192.09	165.17
		19.63	46.69
		33.83	162.86
Median	281.37	33.83	162.86
Z-Score	167.58	-7.23	12.89
Standard Deviation		95.73	67.75
U		123.41	4.17