Quality Control of Fortified Flour

Chemical Test: Understand your measurement method and your result

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Regional Workshop on QA/QC of Flour Fortification

Anna Zhenchuk and Philip Randall

Outline

1. Measurement methods
2. Validation of methods
3. Measurement uncertainty
4. Case study A: vitamin A fortified flour
5. Case study B: iron fortified flour

Measurement methods for micronutrients in food: *CODEX*

Standard 234 – 1999 contain method amendments adopted 2014

1. Fluorometry
2. Colorimetry
3. Spectrophotometry
4. Microbioassay
5. Rat bioassay
6. HPLC (added 2011)

Accessed 2015

Recommended methods for specific applications

IRON

- AAS (Atomic absorption spectroscopy)
- ICP-OES (Inductively coupled plasma atomic emission spectroscopy)
- Colorimetric assay

VITAMIN A

- Colorimetric assay
- Fluorometry
- HPLC (high performance liquid chromatography)

FOLIC ACID

- Microbiological assay
- Immunoassay
- Optical Biosensors

IODINE

- ICP-OES
- Colorimetric assay
- Titration

Each measurement method must be validated for a specific type of food and the type of micronutrient

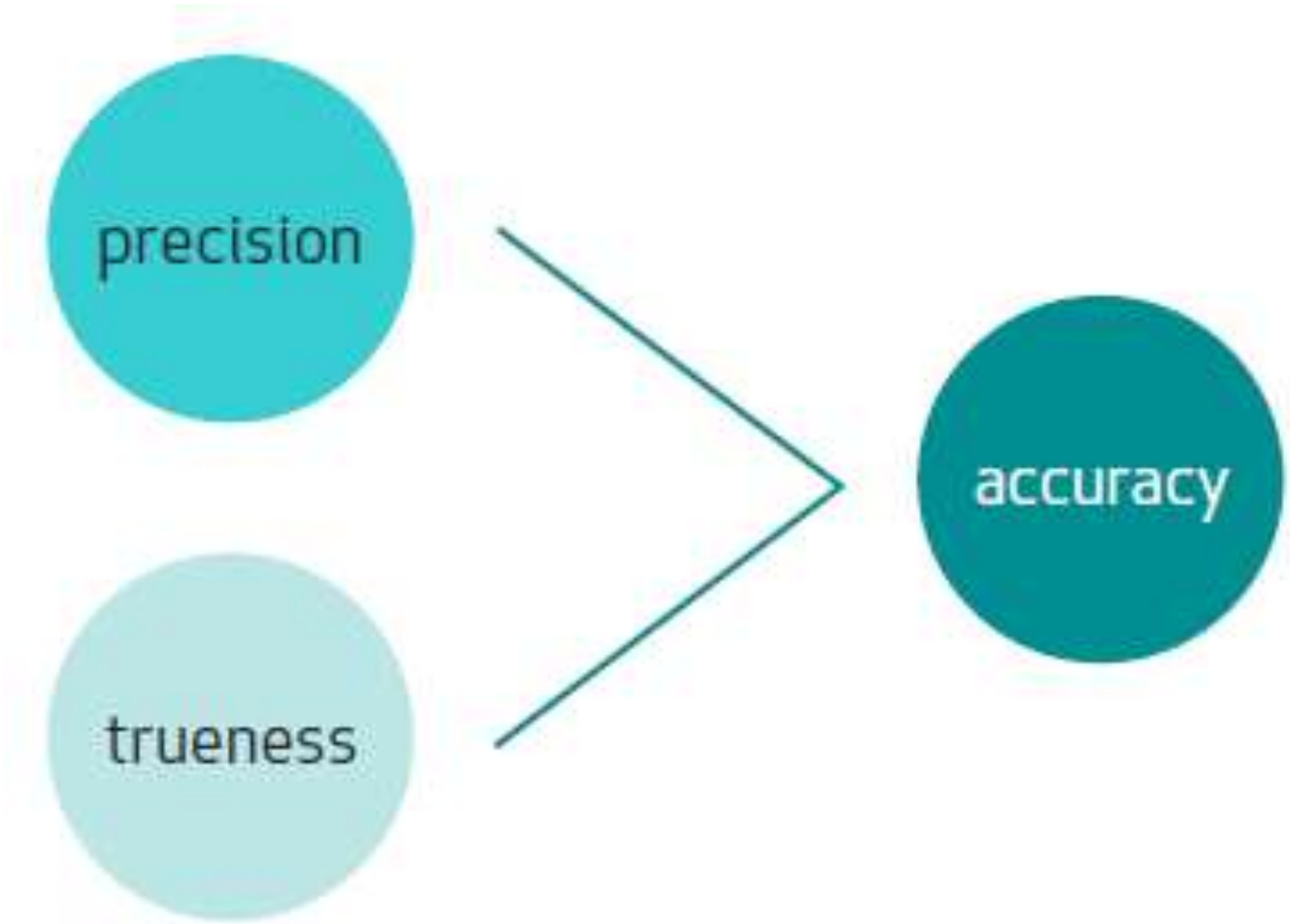
IRON

- NaFeEDTA
- Ferrous fumarate
- Ferrous sulfate
- Electrolytic iron
- Instrinsic iron

VITAMIN A

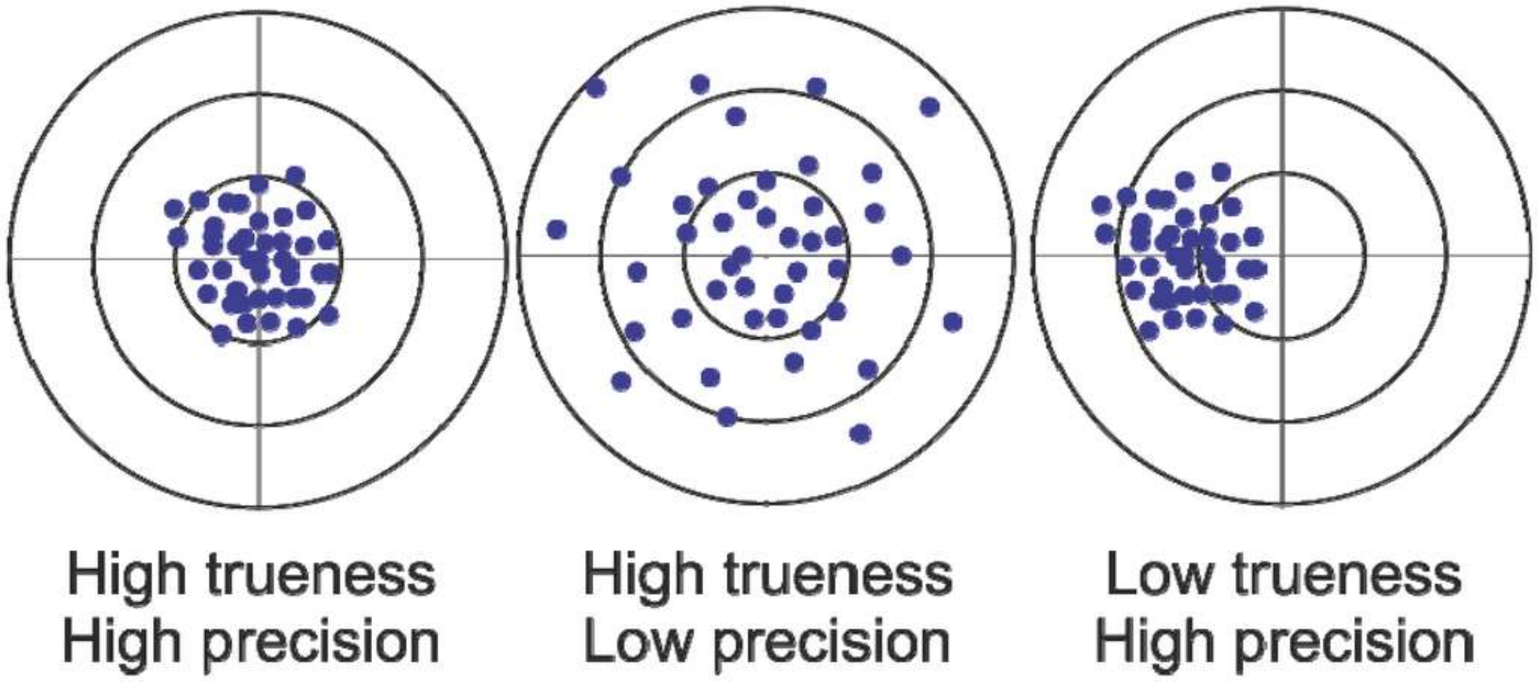
- Vitamin A palmitate
- Vitamin A acetate
- Vitamin A propionate
- Encapsulated vitamin A

Validation assesses the accuracy of the measurement



International Standards Organization definition of accuracy

According to **ISO 5725-1**, **Accuracy** consists of Trueness and Precision
Trueness - proximity of measurement results to the true value
Precision - repeatability or reproducibility of the measurement



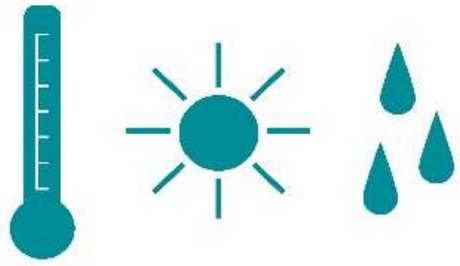
PRECISION

Multiple factors influence the precision of measurement

- the sample itself

- the analyst

- the instrument



- the environment

Validation protocol example for precision assessment

- Fortified flour sample
- Diluted flour sample

- 1st analyst
- 2nd analyst
- 3rd analyst

Inter – person variation

Intra – assay variation

- 1st instrument
- 2nd instrument
- 3rd instrument

Inter – device variation

- 1st day
- 2nd day
- 3rd day

Inter – day variation

Calculation of precision based on results of repeated measurements

$$\frac{\text{STANDARD DEVIATION}}{\text{MEAN}} \times 100\% =$$

= Coefficient of variation (CV) in %

Coefficient of Variation with 95% confidence level = 1.96 x CV

Example: (4 ppm/40 ppm) x 100% = 10%
95% confidence = 1.96 x 10% = 19.6%

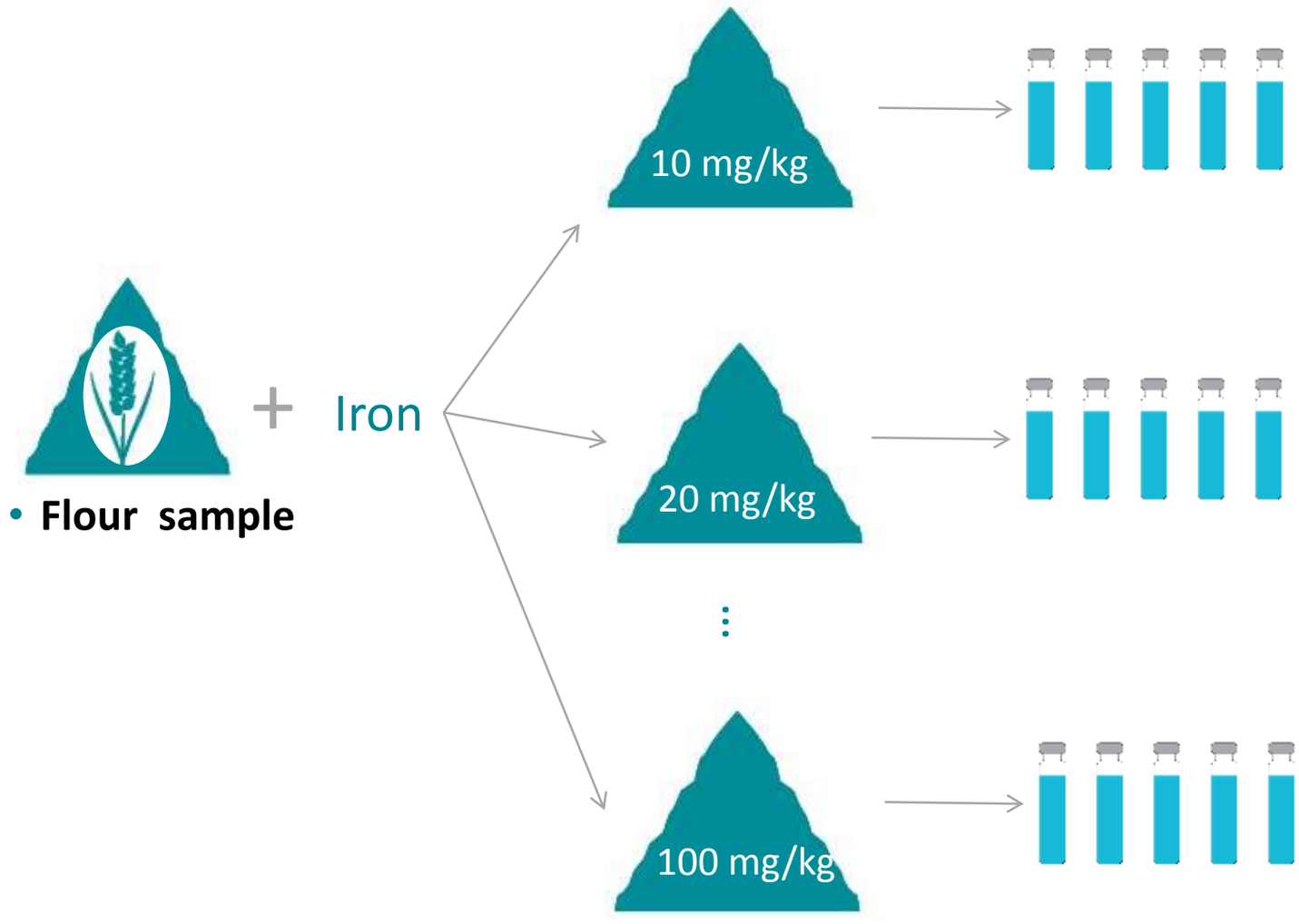
AACC ring trial of 14 USA laboratories with 7 flour samples shows significant variation

Analyte	Minimum CV x 2	Maximum CV x 2	Mean CV x 2
Iron	12%	54%	**22%**
Zinc	9%	16%	**13%**
Vitamin A	63%	370%	**141%**
Folic Acid	30%	82%	**45%**

http://www.aaccnet.org/checksample/

TRUENESS

Validation protocol example for trueness assessment



- **Flour sample**

Calculation of trueness is based on the recovery of added analyte

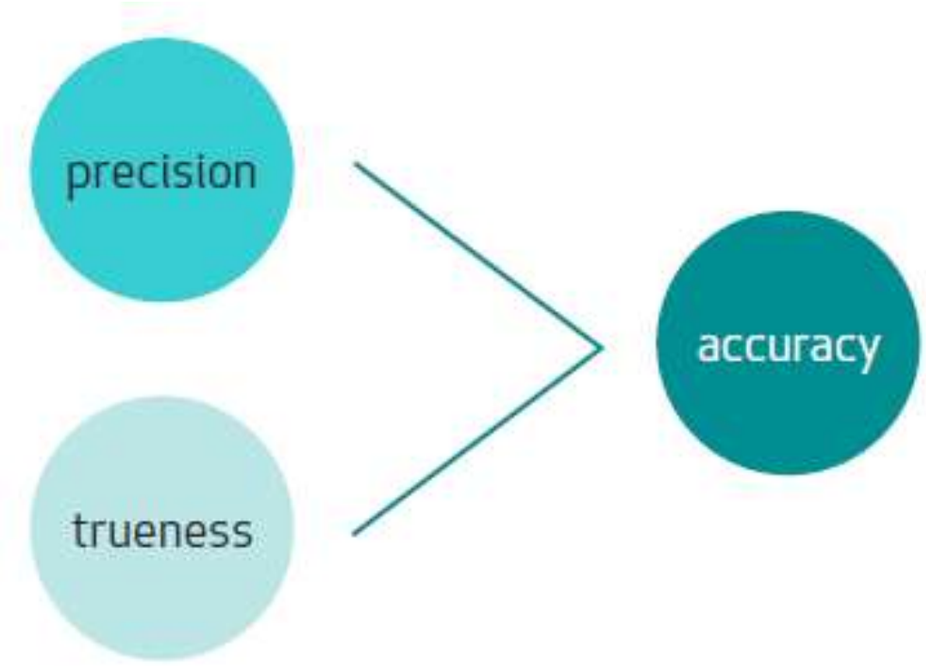
$$\frac{\text{MEASURED AMOUNT}}{\text{ADDED AMOUNT}} \times 100\% = \text{Trueness in \%}$$

Trueness is expressed in terms of bias:
how far is the result from the true value in %?

Example: (38 ppm/40 ppm) x 100% = 95%
Bias: 100% - 95% = 5%

ACCURACY

Accuracy = precision + trueness



- **Precision** is expressed in terms of coefficient of variation (**CV**) in % at 95% confidence level

- **Trueness** is expressed in terms of **bias**: how far is the result from the true value in %

- **Accuracy** is expressed in terms **Measurement uncertainty,** that combines CV and Bias.

Calculation of measurement uncertainty to express the accuracy

BIAS + (1.96 x CV) = Measurement Uncertainty, %

Example: 5% + (1.96 x 10%) = 24.6%

VERY IMPORTANT

Sampling: Representative sample

Variation for single small samples can be as high as ±100%.

Pool/Combine multiple single samples in one composite sample

Control Sample

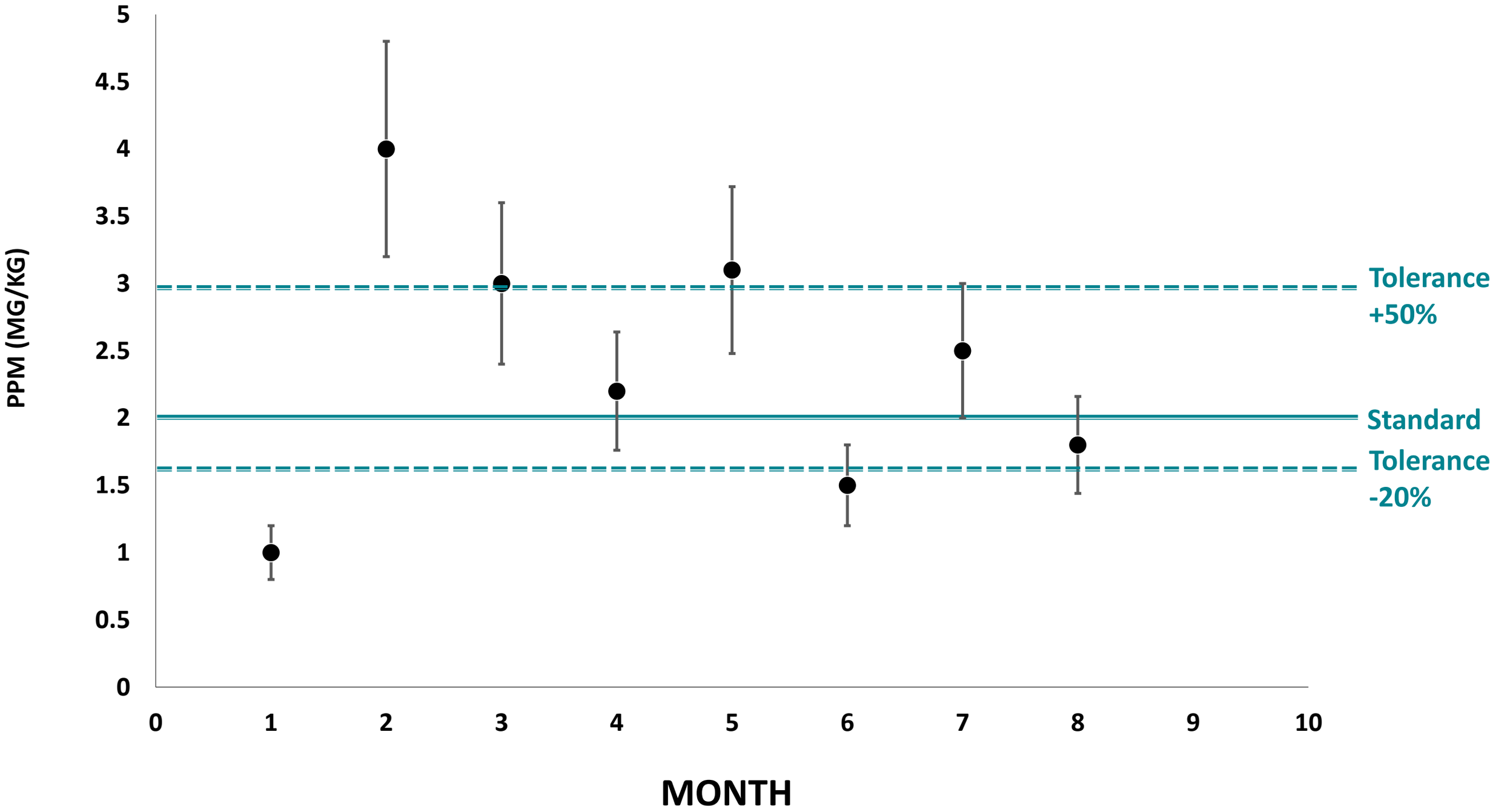
Conditions to be met for proper quality control

1. Measurement Uncertainty is Known
2. Sample is Representative
3. Standard is Set
4. Permitted Tolerance is Set

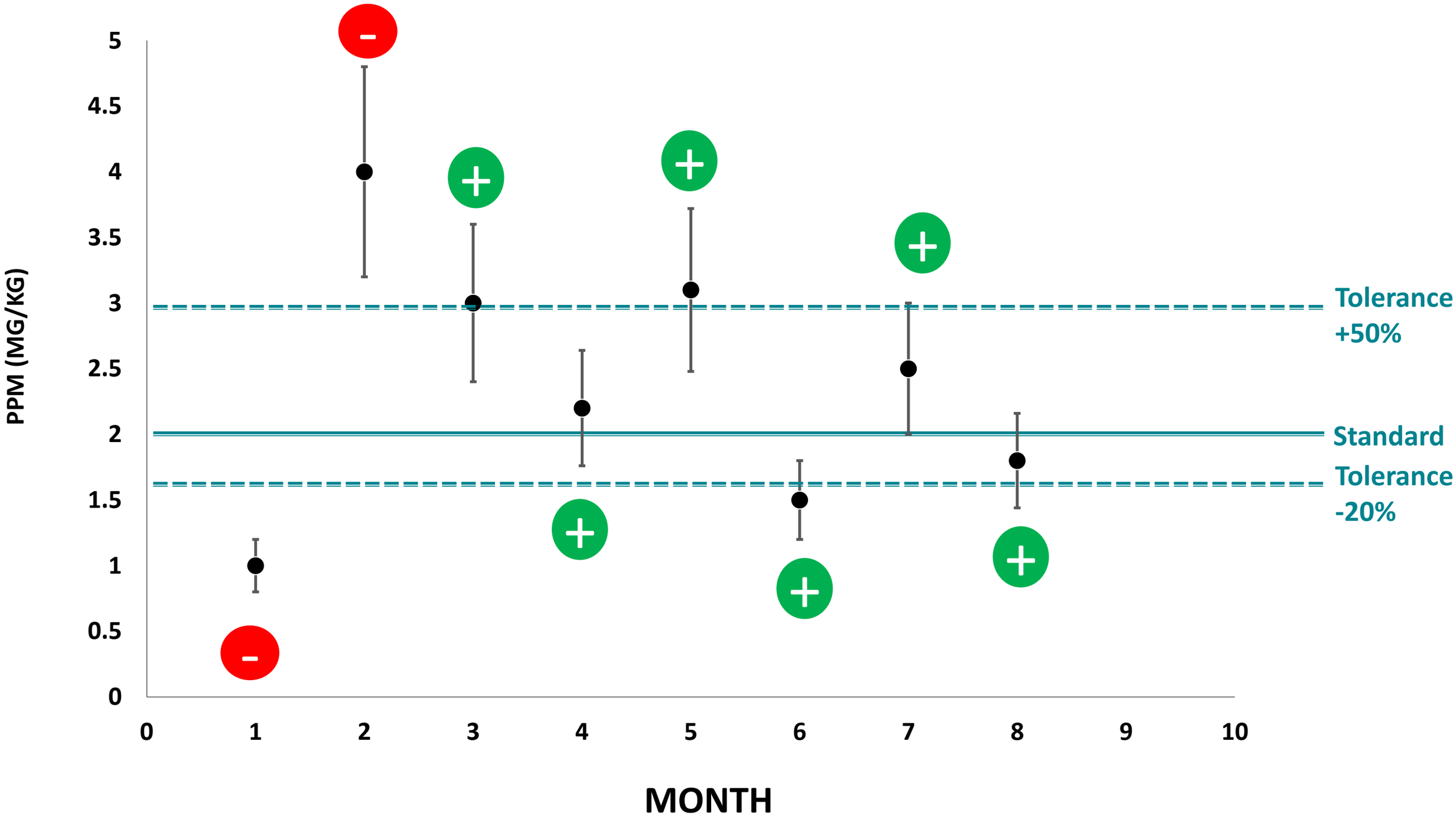
Case Study A: Vitamin A in flour

1. Measurement Uncertainty	±20%
2. Representative Sample	✓
3. Standard	2 ppm
4. Permitted Tolerance	+50% -20%

Case Study A: Vitamin A in flour



Case Study A: Vitamin A in flour



Case Study B: Iron in flour

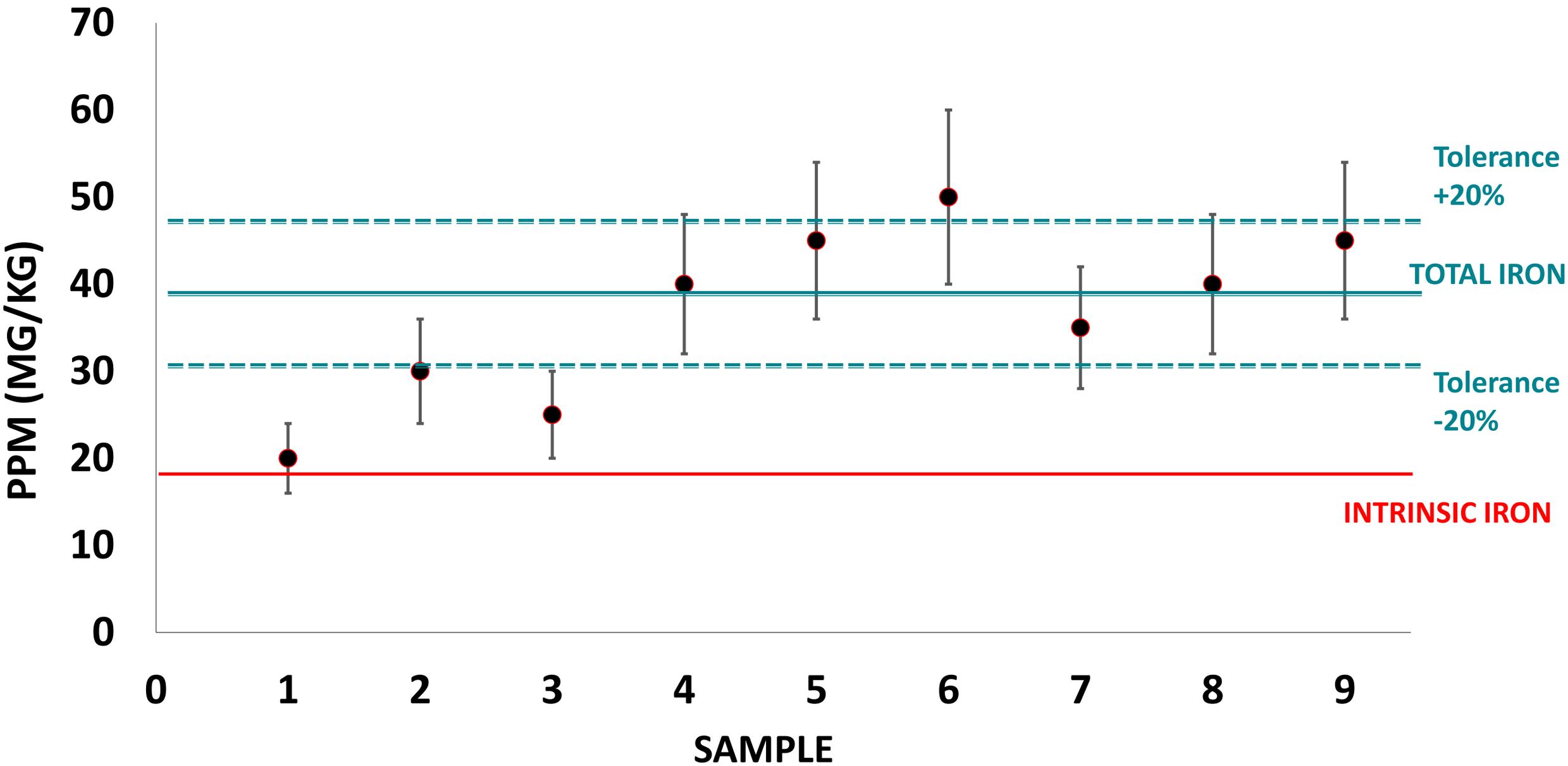
1. Measurement Uncertainty	±25%
2. Representative Sample	✓
3. Standard	20 ppm
4. Permitted Tolerance	±20%

Case Study B: Iron in flour
Additional factor is Instrinsic iron

1. Self-rising white flour: 10-20 ppm
2. Bread flour: 20-40 ppm
3. Brown flour: 40-60 ppm

?

Scenario 1: Self-rising flour with 20 ppm natural iron; 20 ppm added iron



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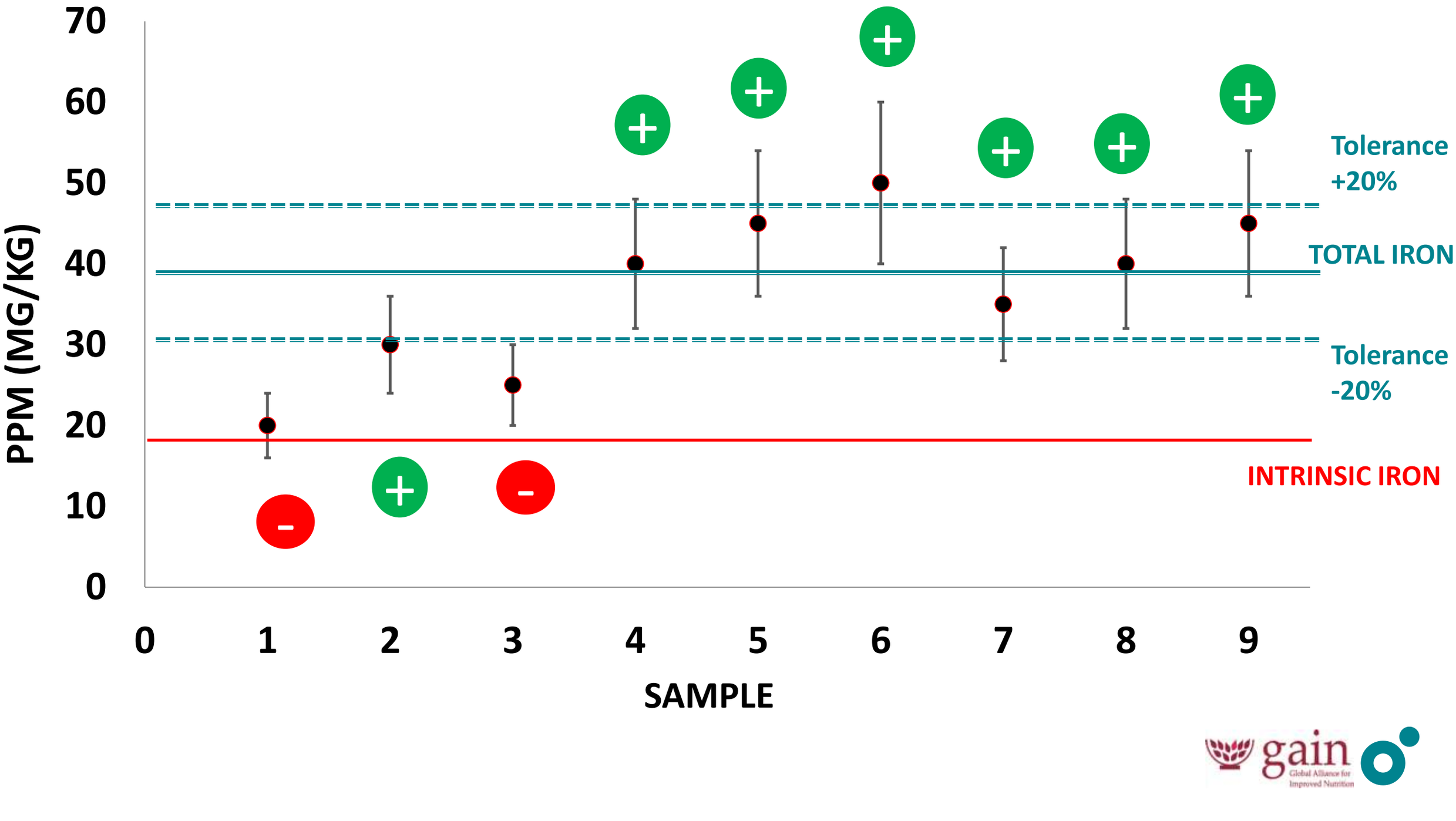
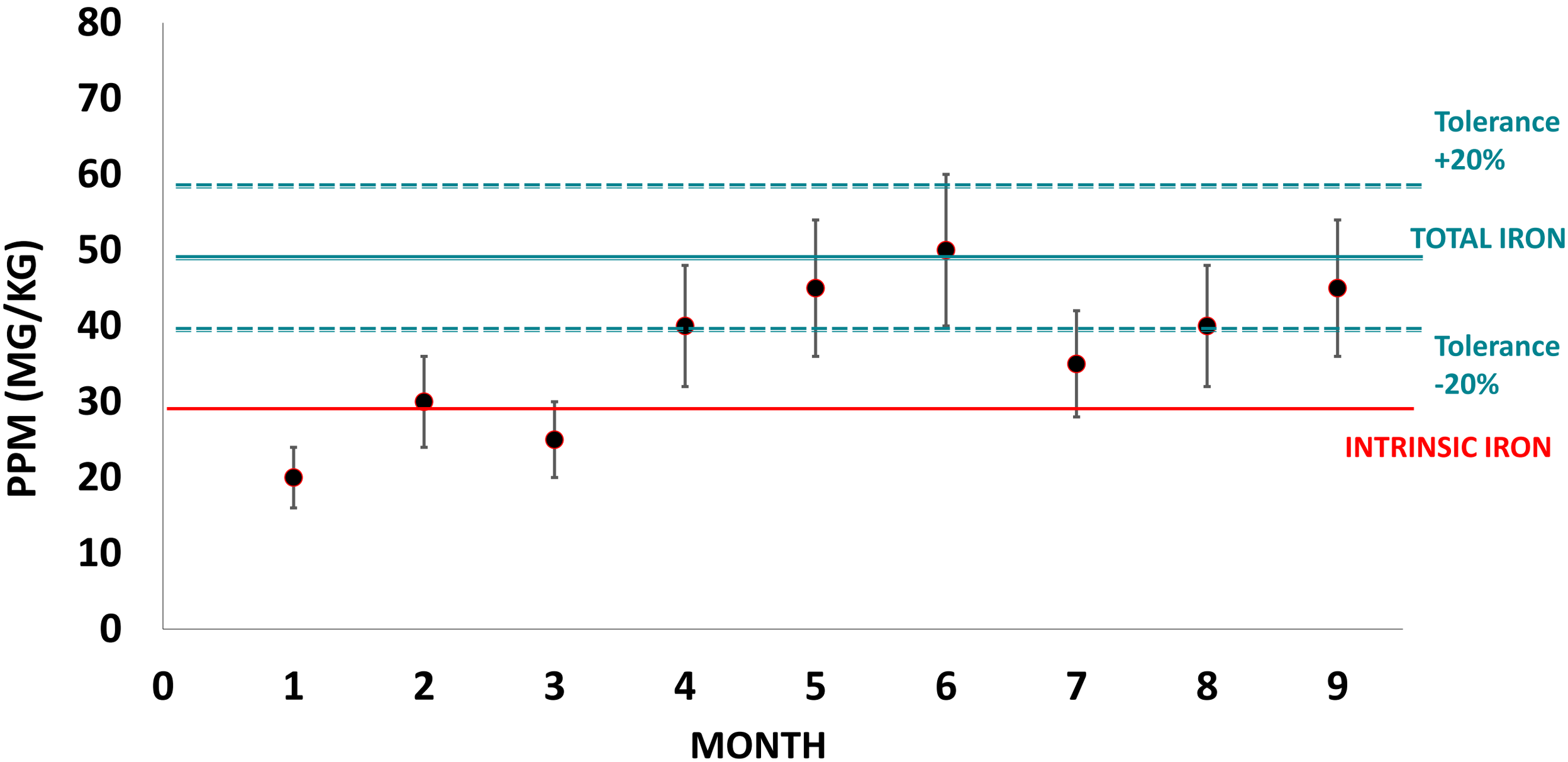


Scenario 2: Bread flour with 30 ppm natural iron; 20 ppm added iron



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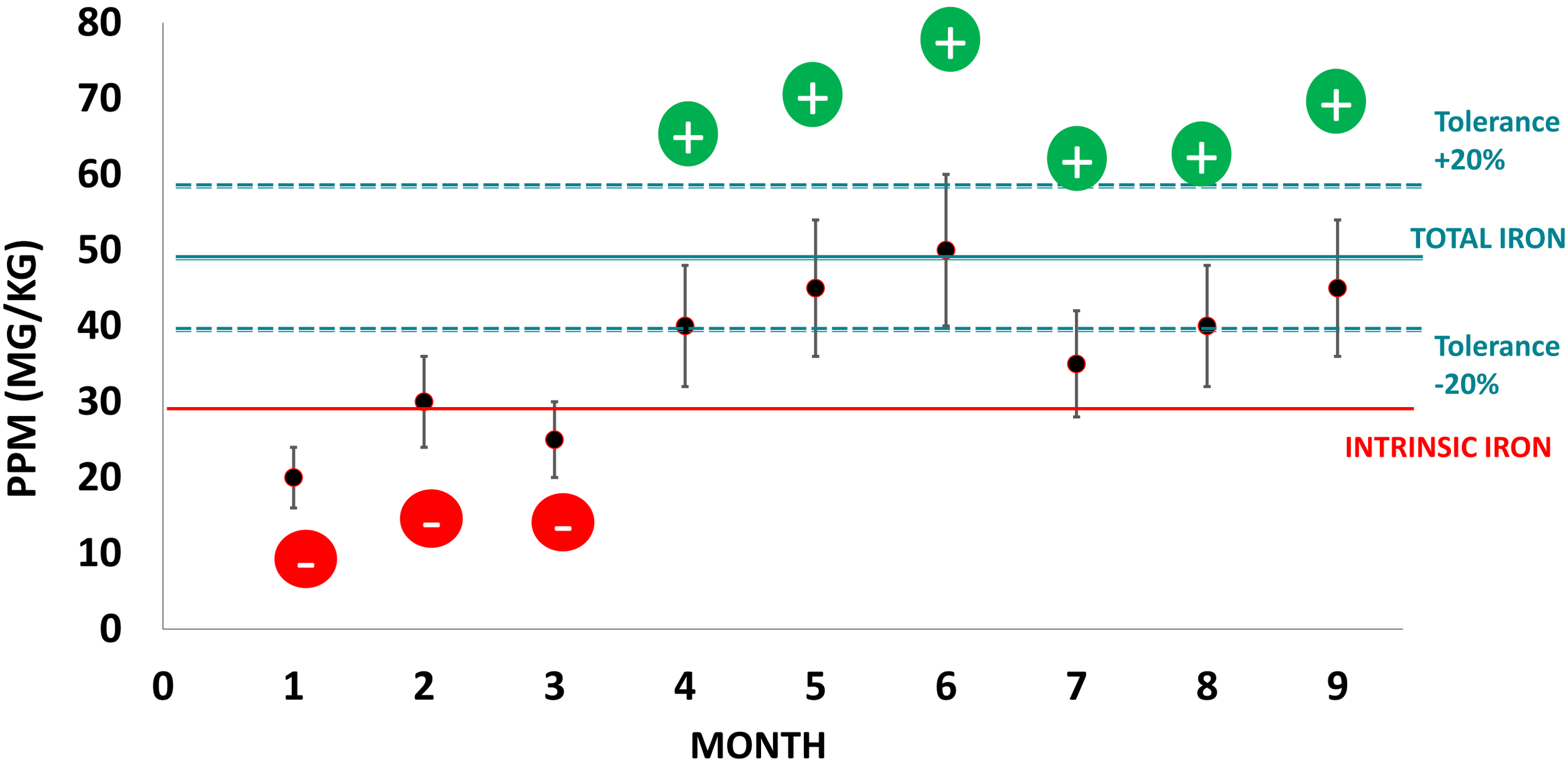
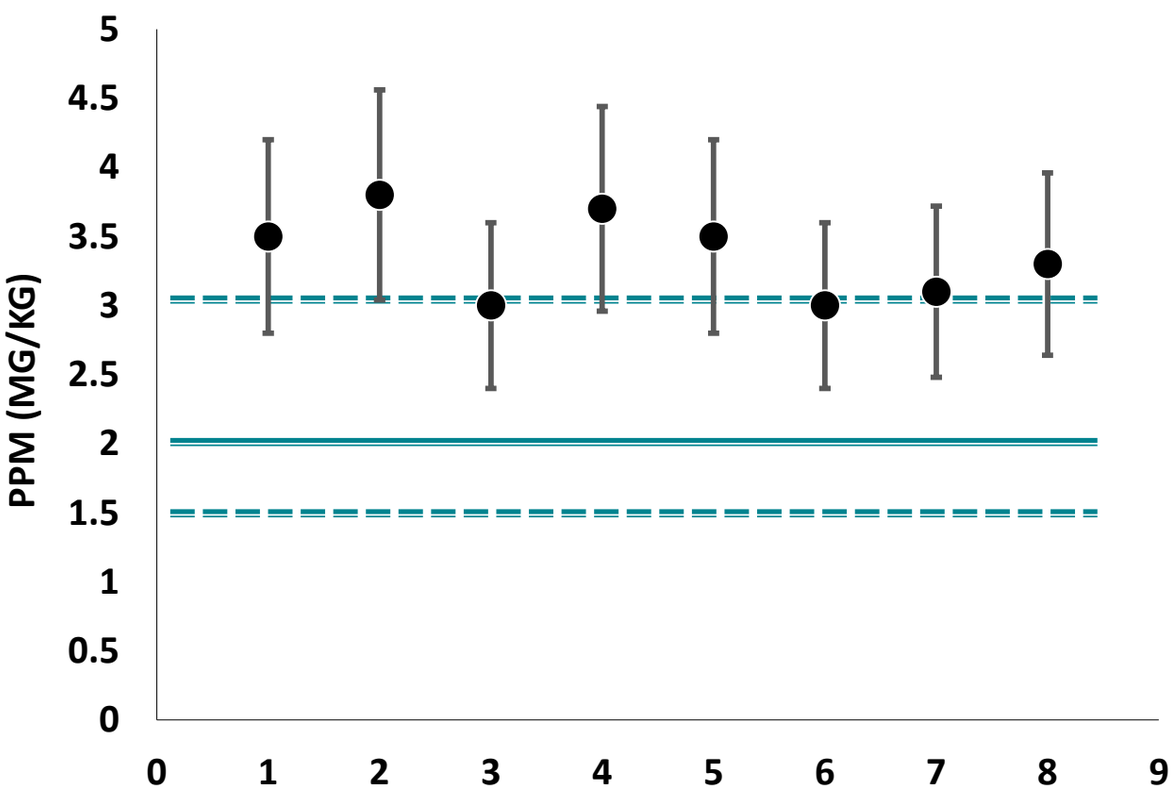


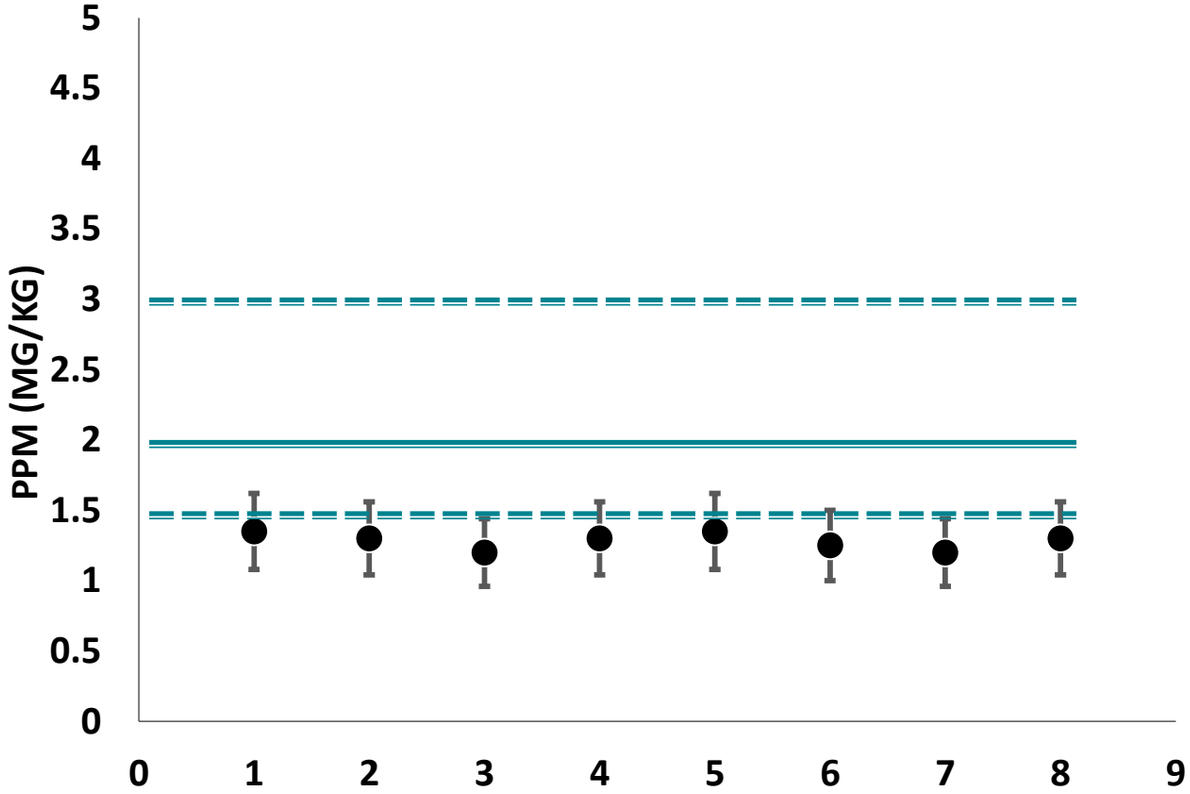
Case Study B: Iron in flour
Additional factor is Instrinsic iron

1. Self-rising white flour: 10-20 ppm
2. White bread flour: 20-40 ppm
3. Brown bread flour: 40-60 ppm

Legal definition of different flour types and implications for total iron standard and tolerance limits!!!!

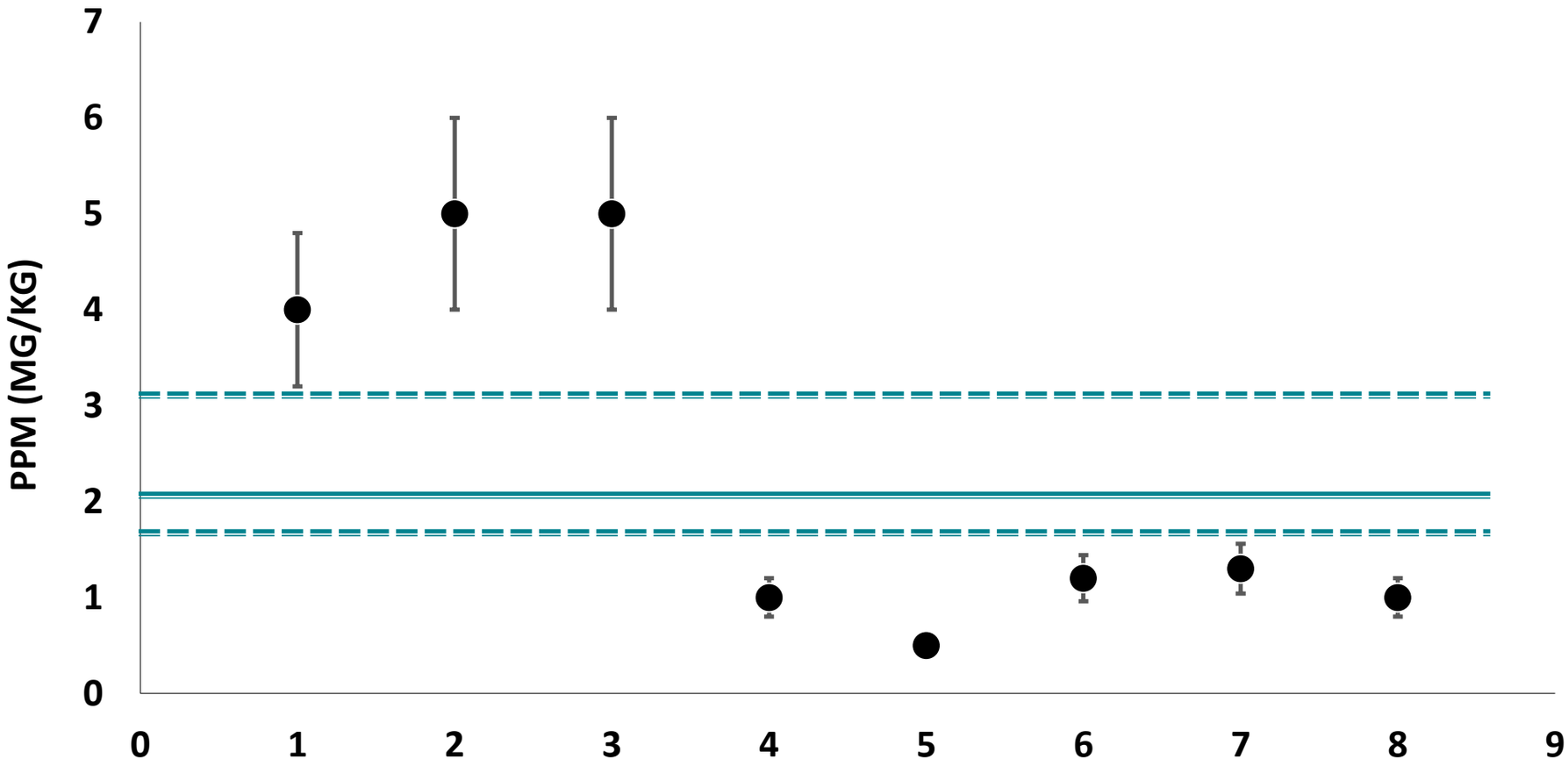
Recommendation 1





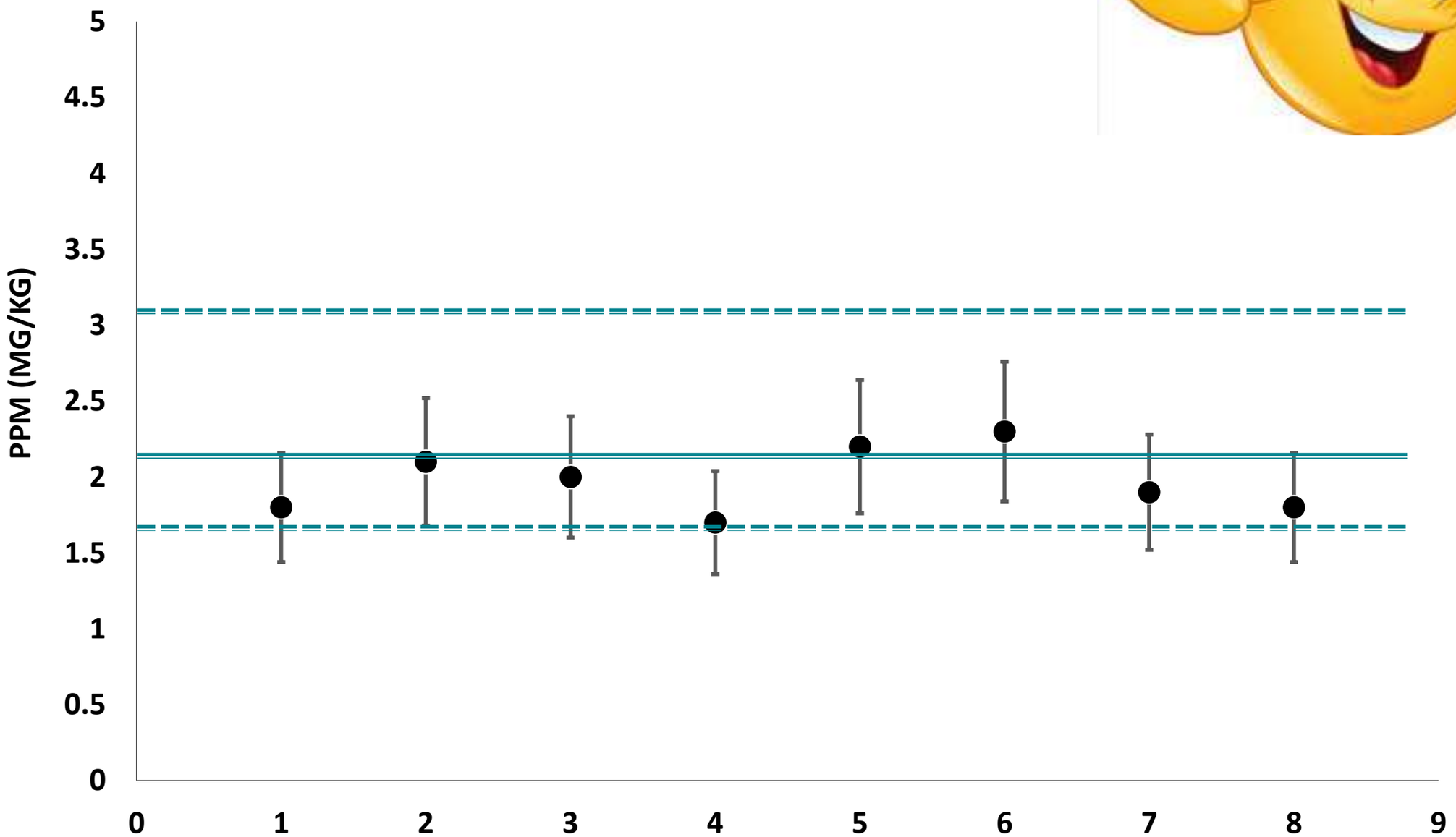
If the analysis results are repeatedly at the upper/lower limits of the permitted tolerance, the food business operator must adopt more effective in-house control and make the necessary changes in the production process or the labelling.

Recommendation 2



If the indicated nutrient contents of the foodstuff deviate repeatedly from the set tolerance limits, taking the measurement uncertainty of the analysis method into account, the foodstuff is not acceptable and may not be kept for sale.

Optimal Situation



Conditions to be met for proper quality control

1. Measurement Uncertainty is Known
2. Sample is Representative
3. Standard is Set
4. Permitted Tolerance is Set

THANK YOU!

Dr. Anna Zhenchuk
Technical Marketing Manager
E-mail: anna.zhenchuk@bioanalyt.com
www.bioanalyt.com

Dr. Philip Randall
Technical Director
E-mail: pcubed@mweb.co.za
www.pcubed.co *(under construction)*