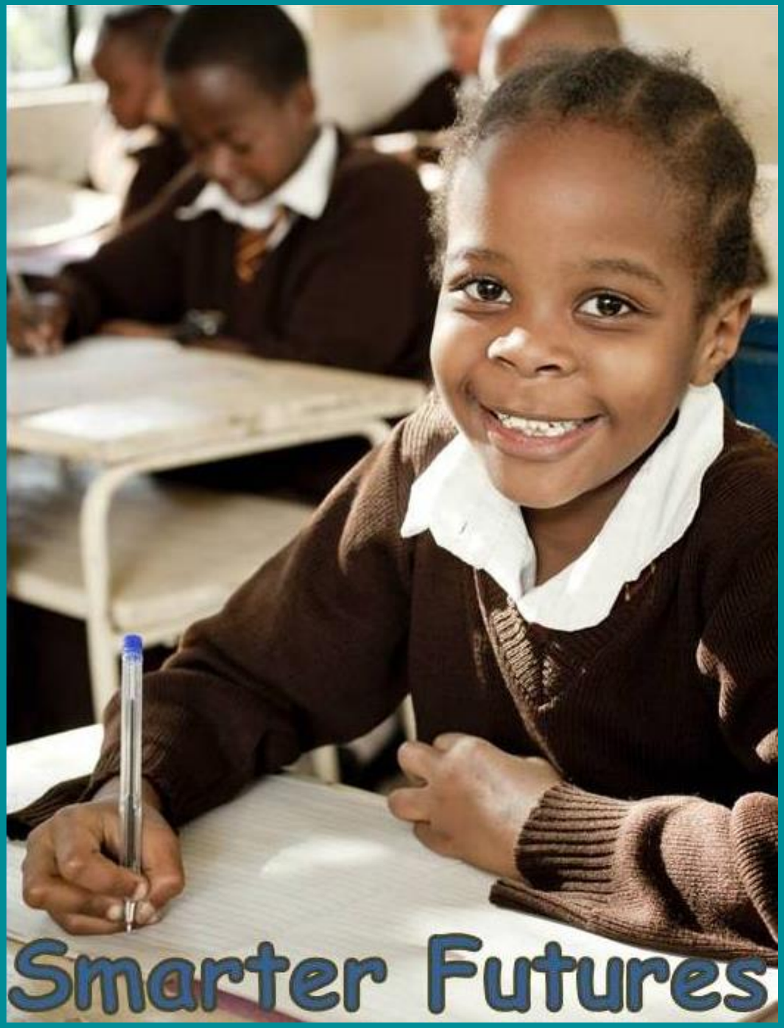
Chemical Tests:
How to understand your measurement method and your result



Kamphala, Uganda| 25.05.2016

Regional Workshop on QA/QC of Flour Fortification

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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. Micro- and rat-bioassay
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



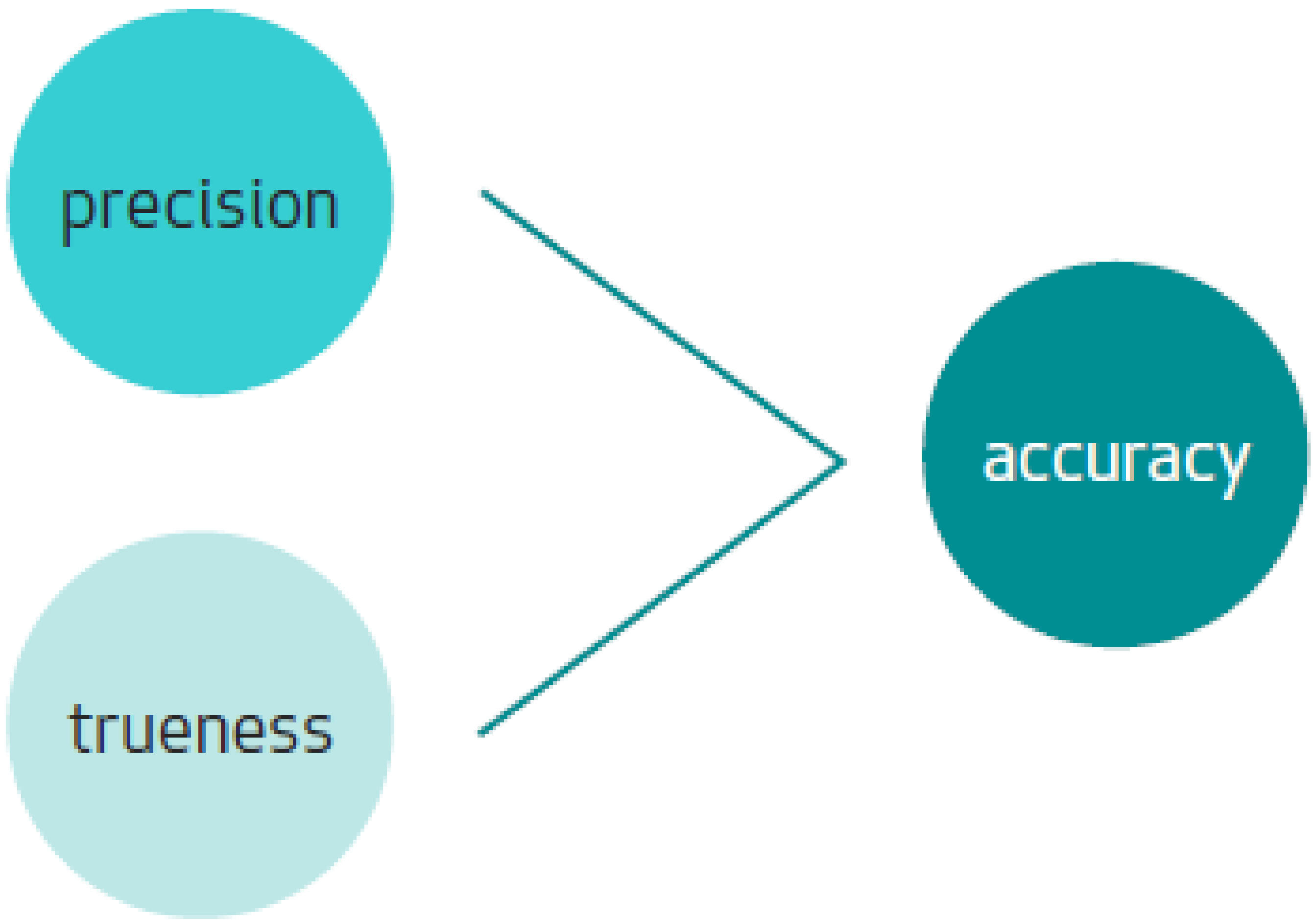
VITAMIN A

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



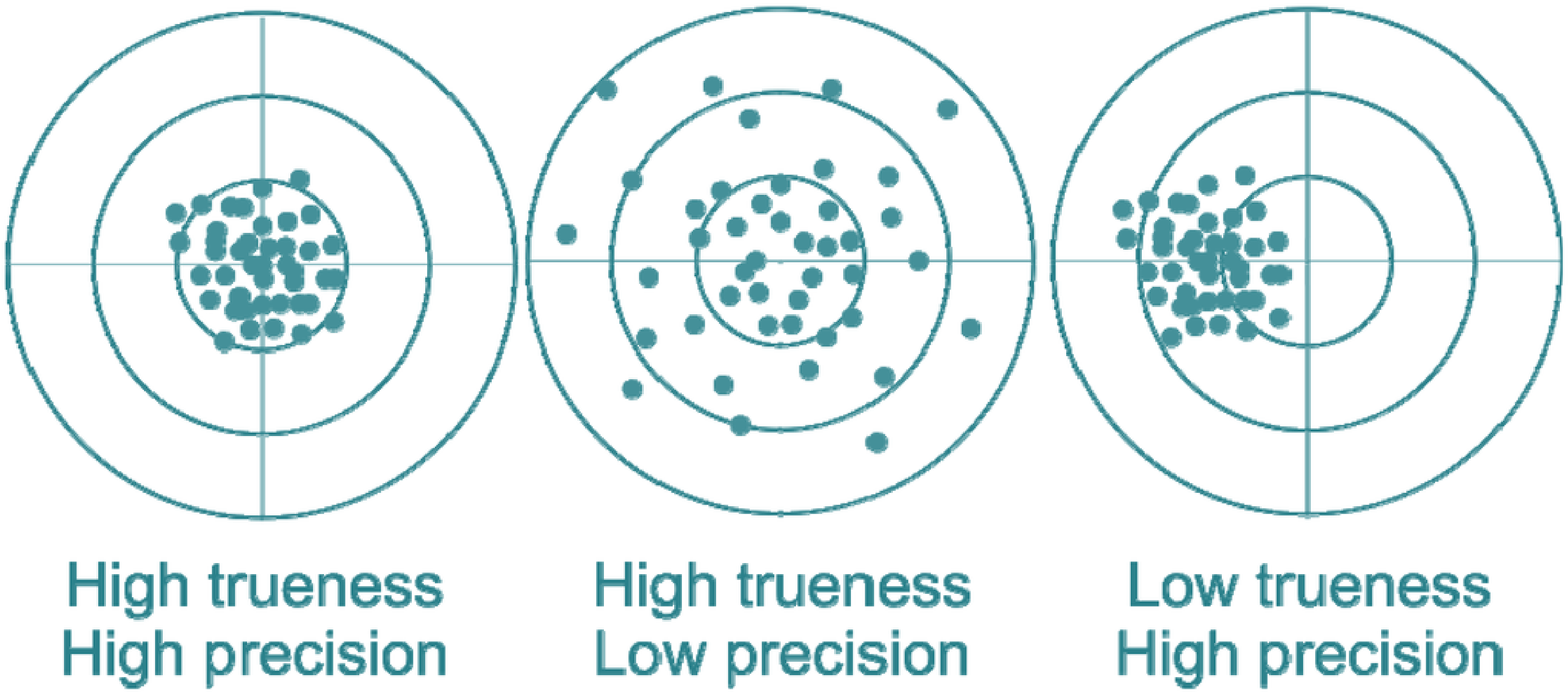


Validation assesses the accuracy of the measurement



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



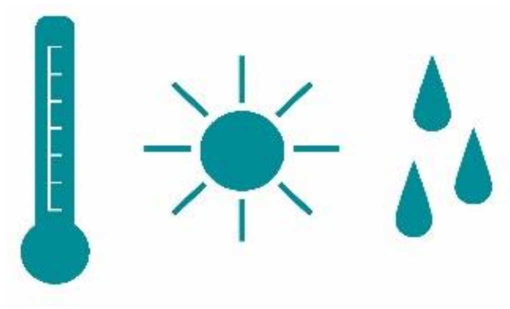
PRECISION

Multiple factors influence the precision of measurement

- the sample itself

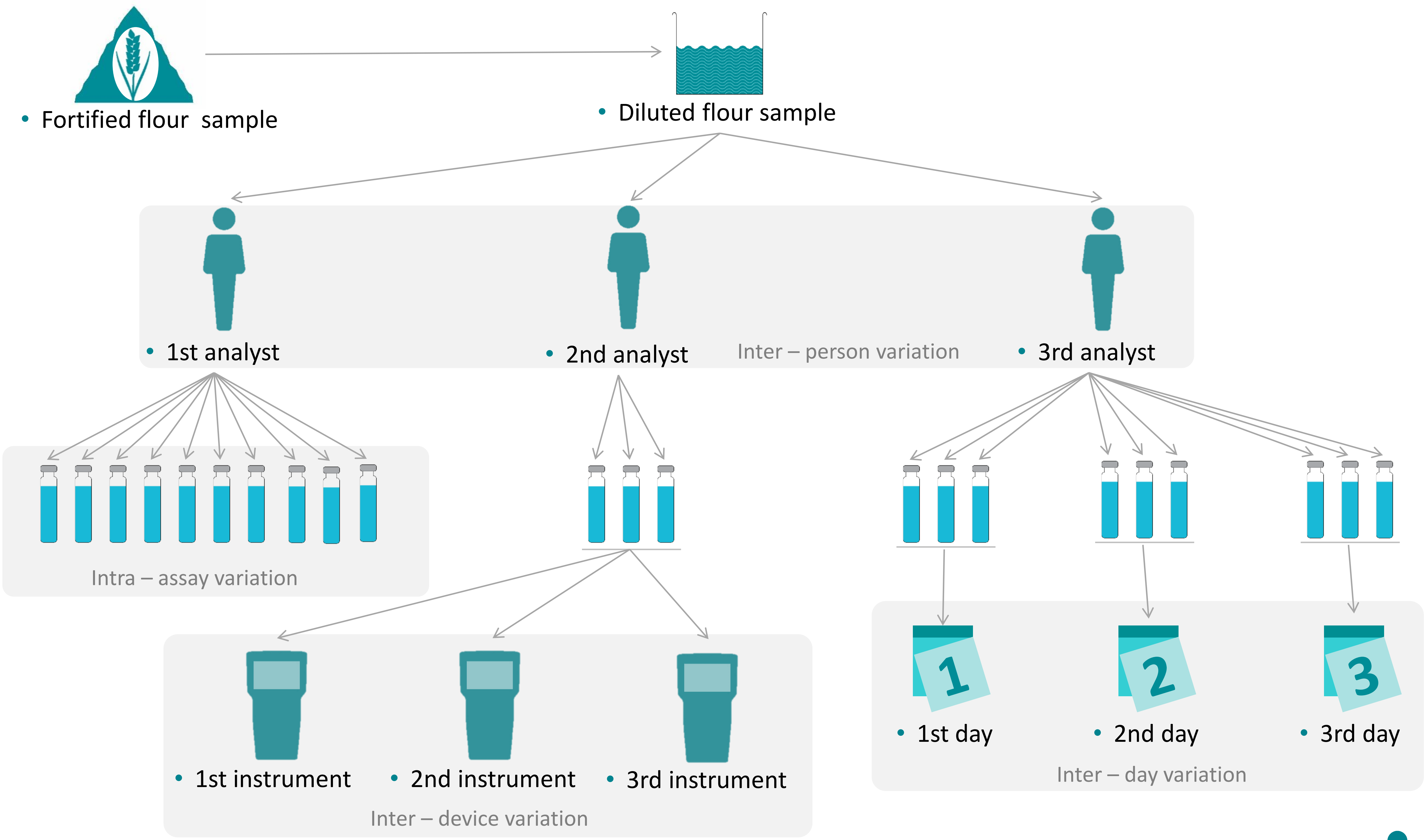
- the analyst

- the instrument



- the environment

Validation protocol example for precision assessment



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%

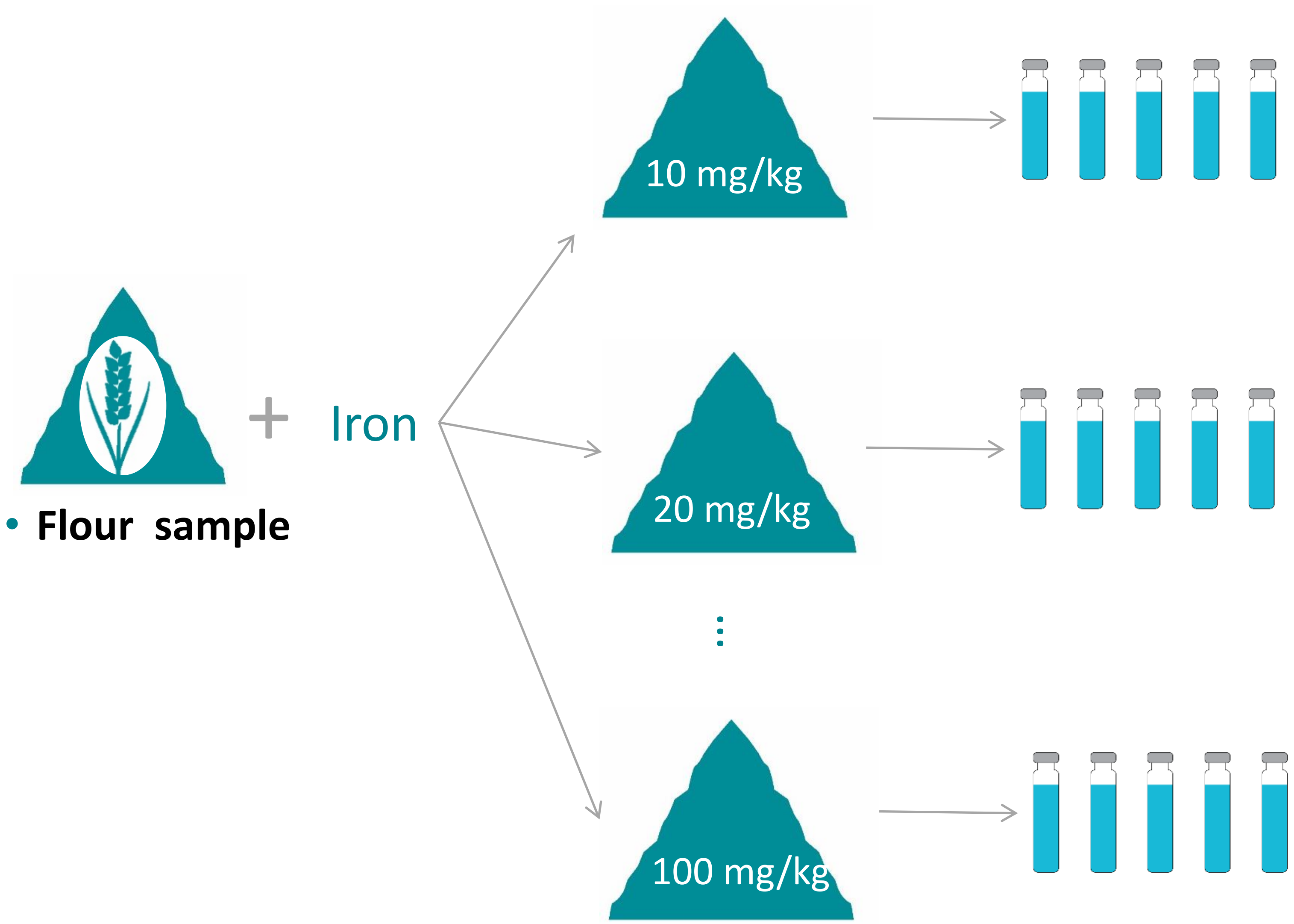
AACC ring trial of 14 USA laboratories with 7 flour samples shows significant variation

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



Calculation of trueness is based on the recovery of added analyte

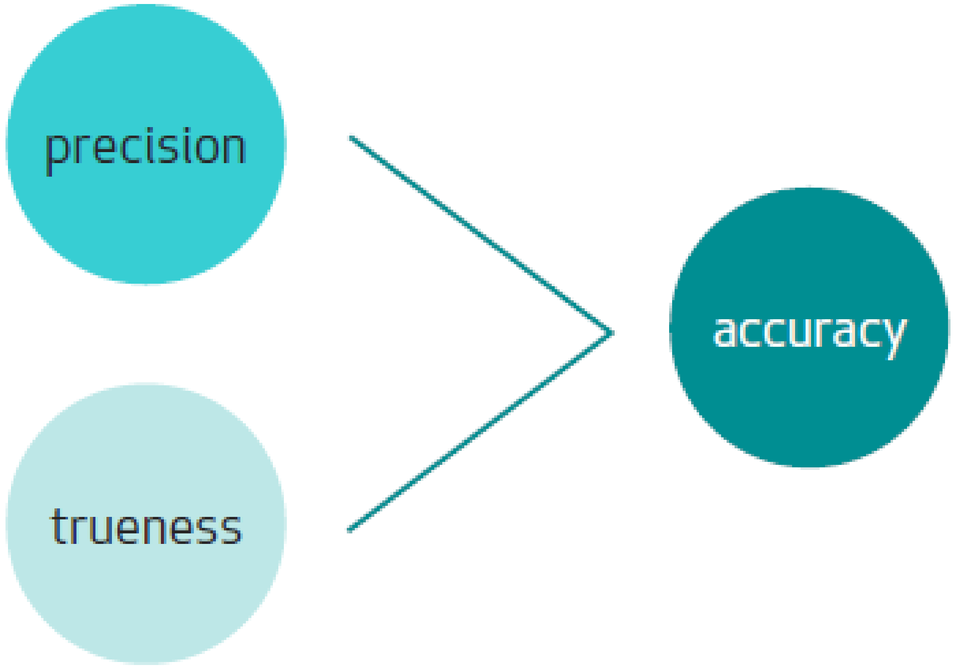
$$\frac{\text{MEASURED AMOUNT}}{\text{ADDED AMOUNT}} \times 100\% = \text{Trueness in \%}$$

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



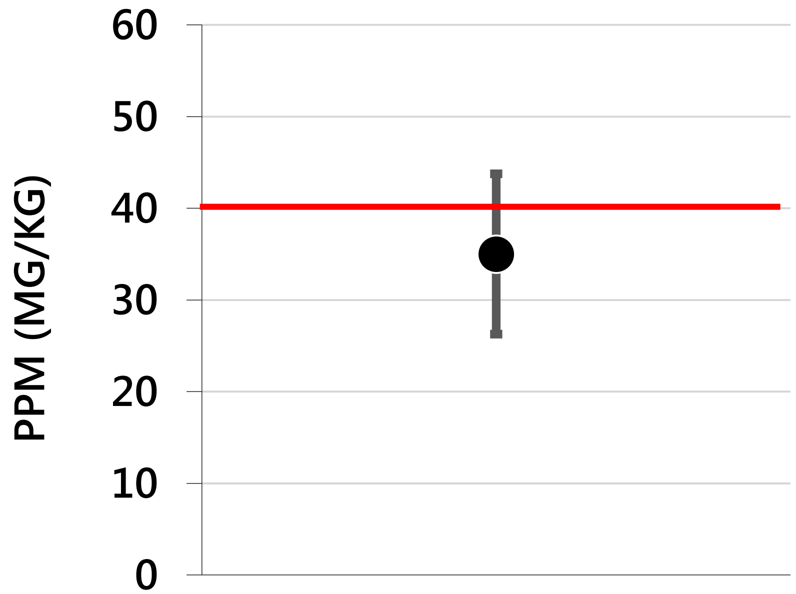
- **Precision** is expressed in terms of coefficient of variation (**CV**) in % at 95% confidence level
- **Trueness** is expressed in terms of **bias**: how far is the result from the true value in %
- **Accuracy** is expressed in terms **Measurement uncertainty,** that combines CV and Bias.

Calculation of measurement uncertainty to express the accuracy

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

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

How to use Measurement Uncertainty to present your analytical result



Example:

- measurement uncertainty was assessed for testing iron in fortified flour using one specific method (i.e. AAS, iCheck Iron, spectrophotometry) and was calculated to be **25%**.
- A sample of fortified flour with unknown concentration was measured and the result is **35 ppm** (mg/kg).
- The standard is set at minimum **40 ppm**.
- Does this sample comply? The answer is „YES“!
- With the measurement uncertainty of 25% the result has a range that is following:
  - 35 ppm ±25%
  - 35 ± 9 ppm
  - **26 – 44 ppm**
- **With measurement uncertainty you are 95% certain that your result is true.**

VERY IMPORTANT

Sampling: Representative sample

1 hour 2 hour 3 hour 4 hour 5 hour 6 hour 7 hour 8 hour 9 hour 10 hour

→ Variation between 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

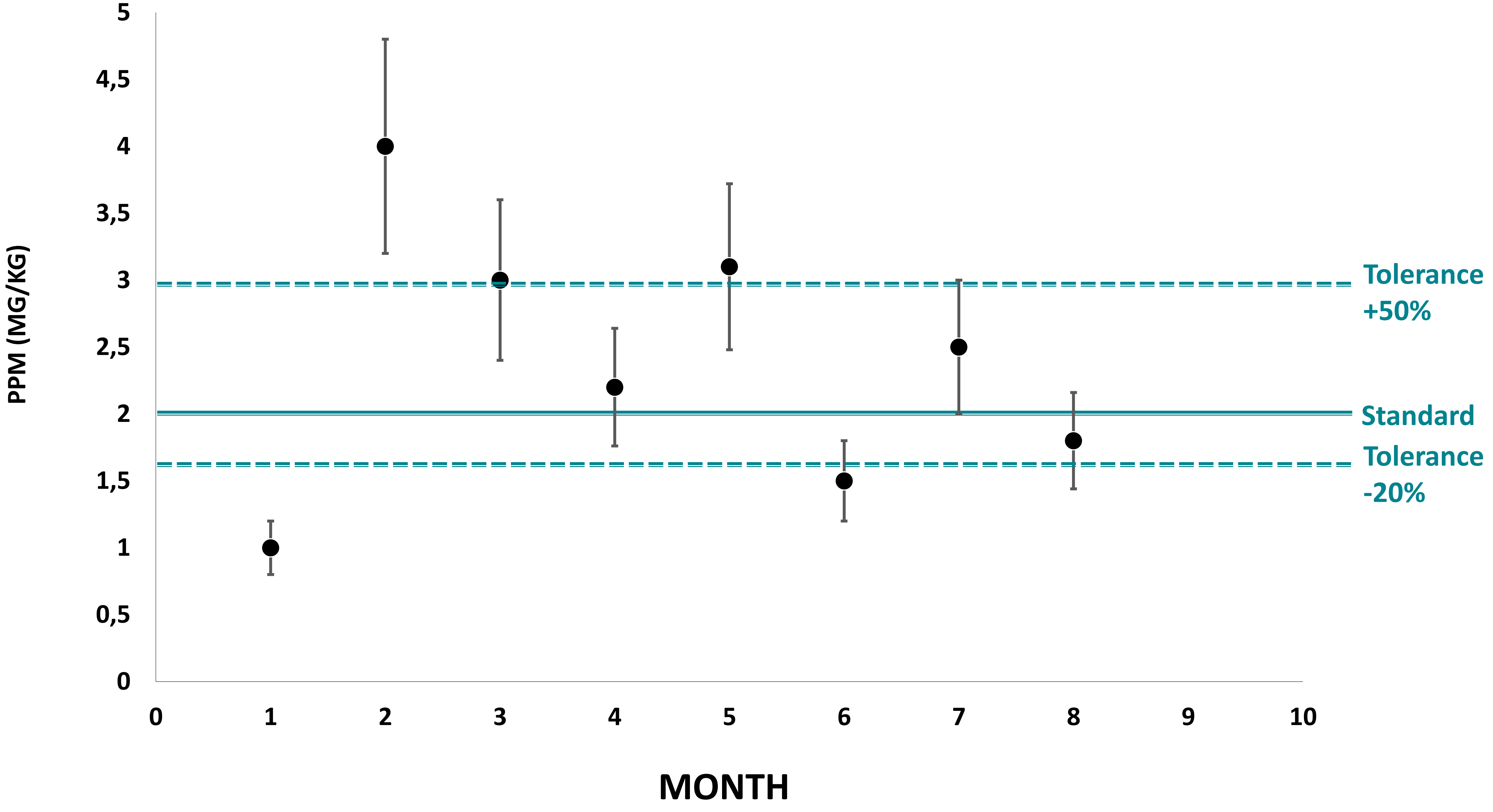
Case Study A: Vitamin A in flour

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

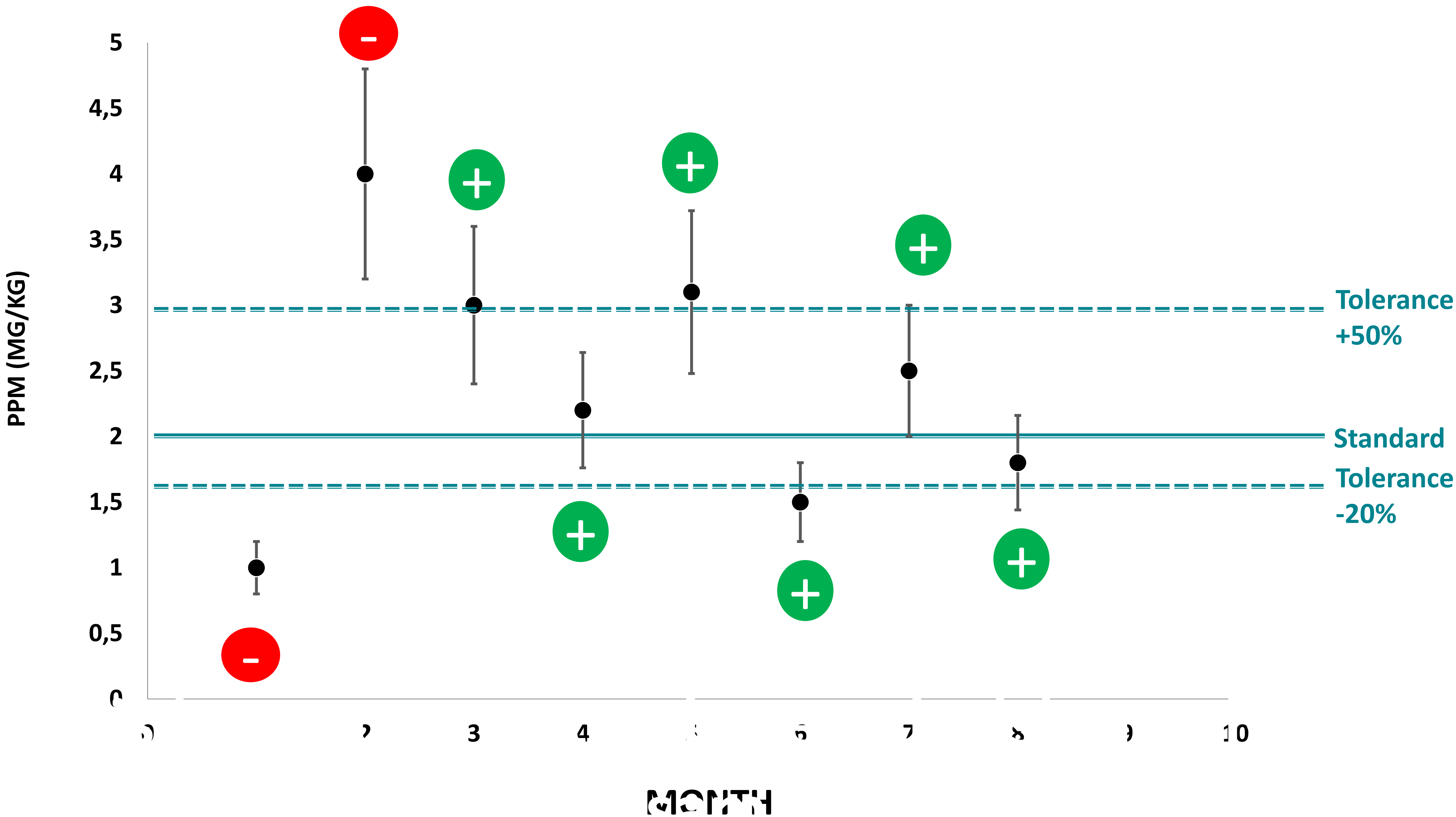
It is important not to base a decision only on **one** result. Collect the samples of the same origin (same brand) during a **period of time** to get an understanding what is **the trend**: is it consistent or is it highly variable?

Permitted tolerance is your definition what is **acceptable difference** of measured result from the target.

Case Study A: Vitamin A in flour



Case Study A: Vitamin A in flour



samples of the same origin during a

Case Study B: Iron in flour

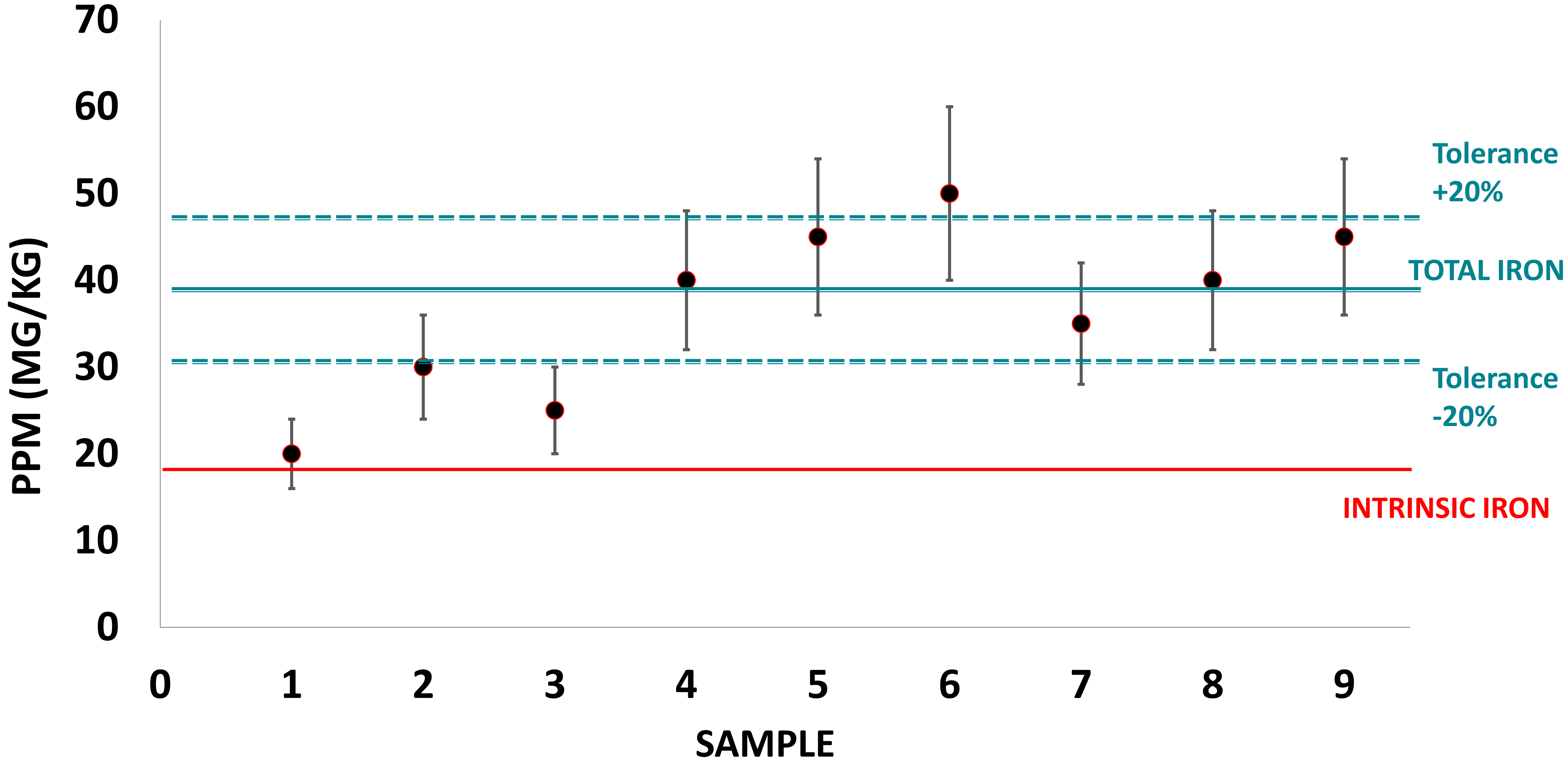
1.	**Measurement Uncertainty**	±25%
2.	**Representative Sample**	✓
3.	**Standard**	20 ppm
4.	**Permitted Tolerance**	±20%

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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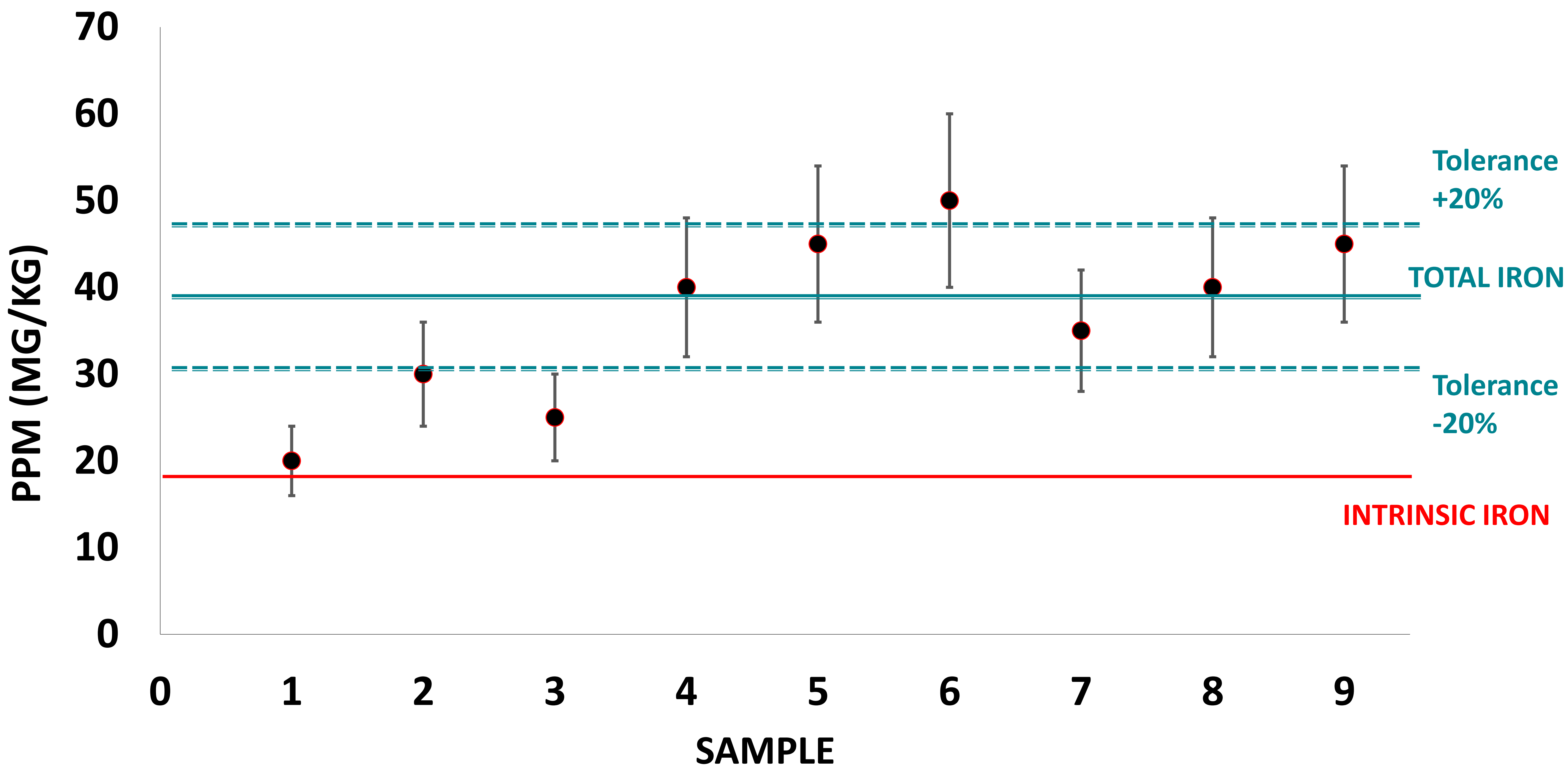

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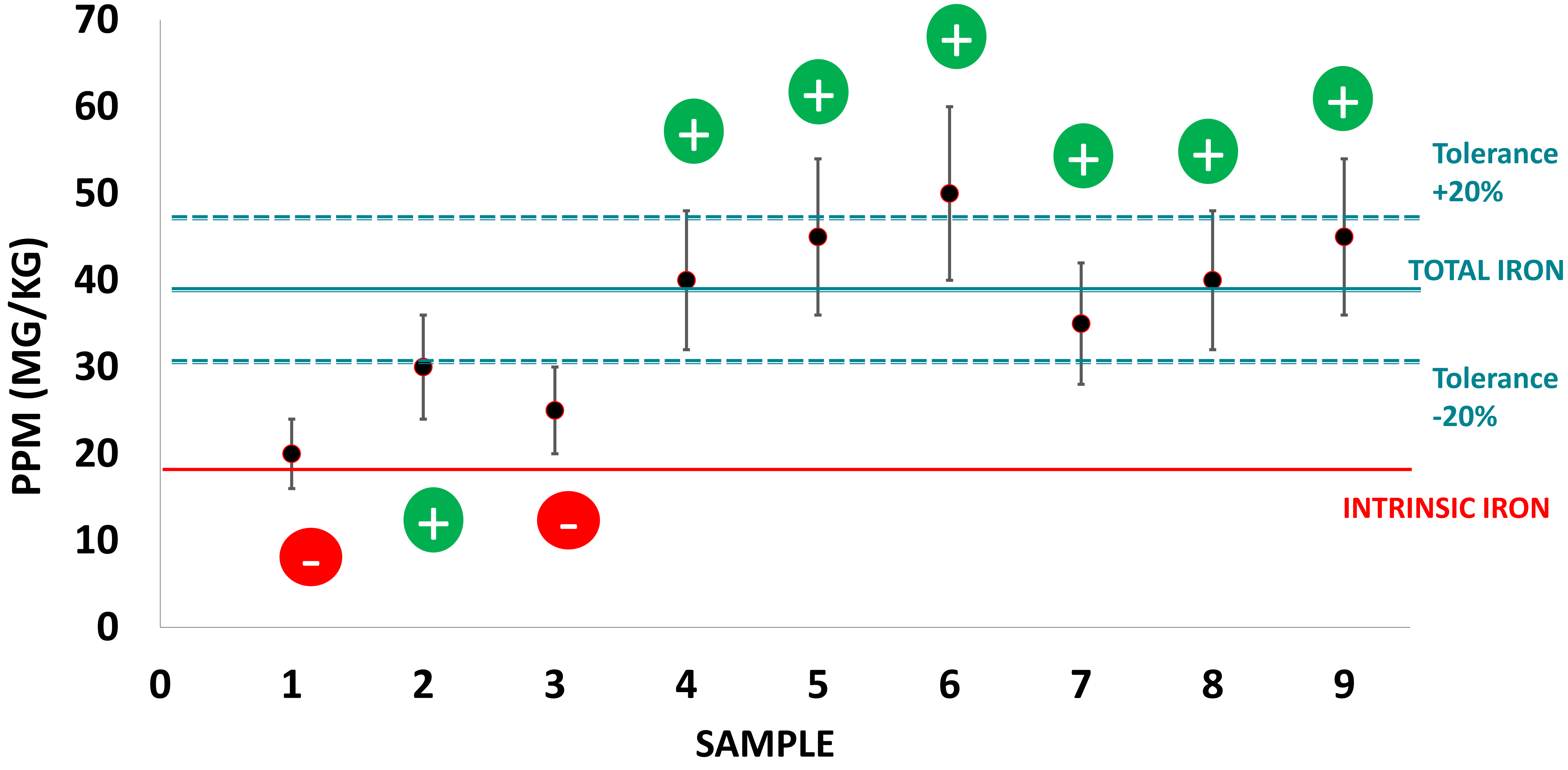
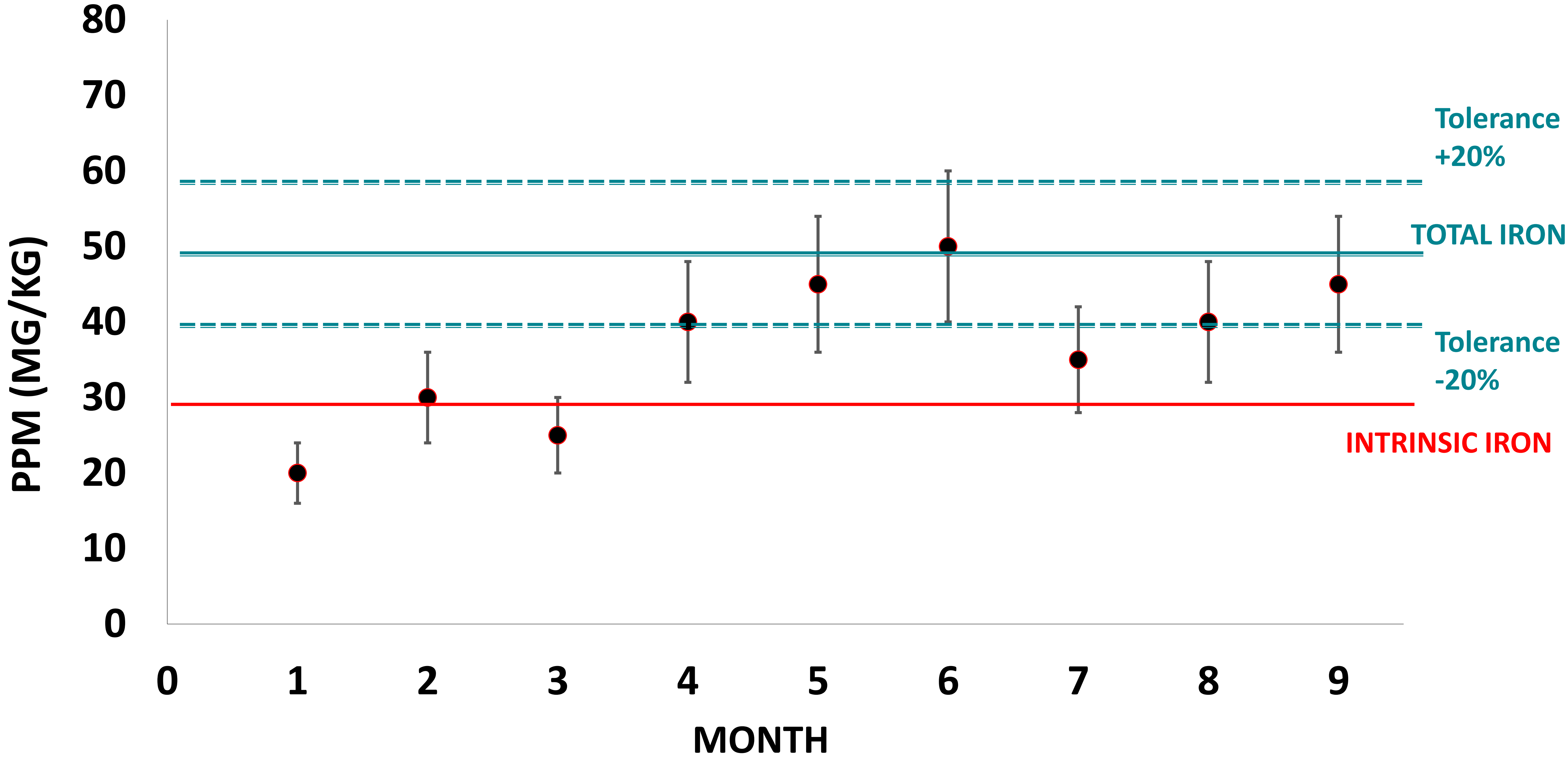
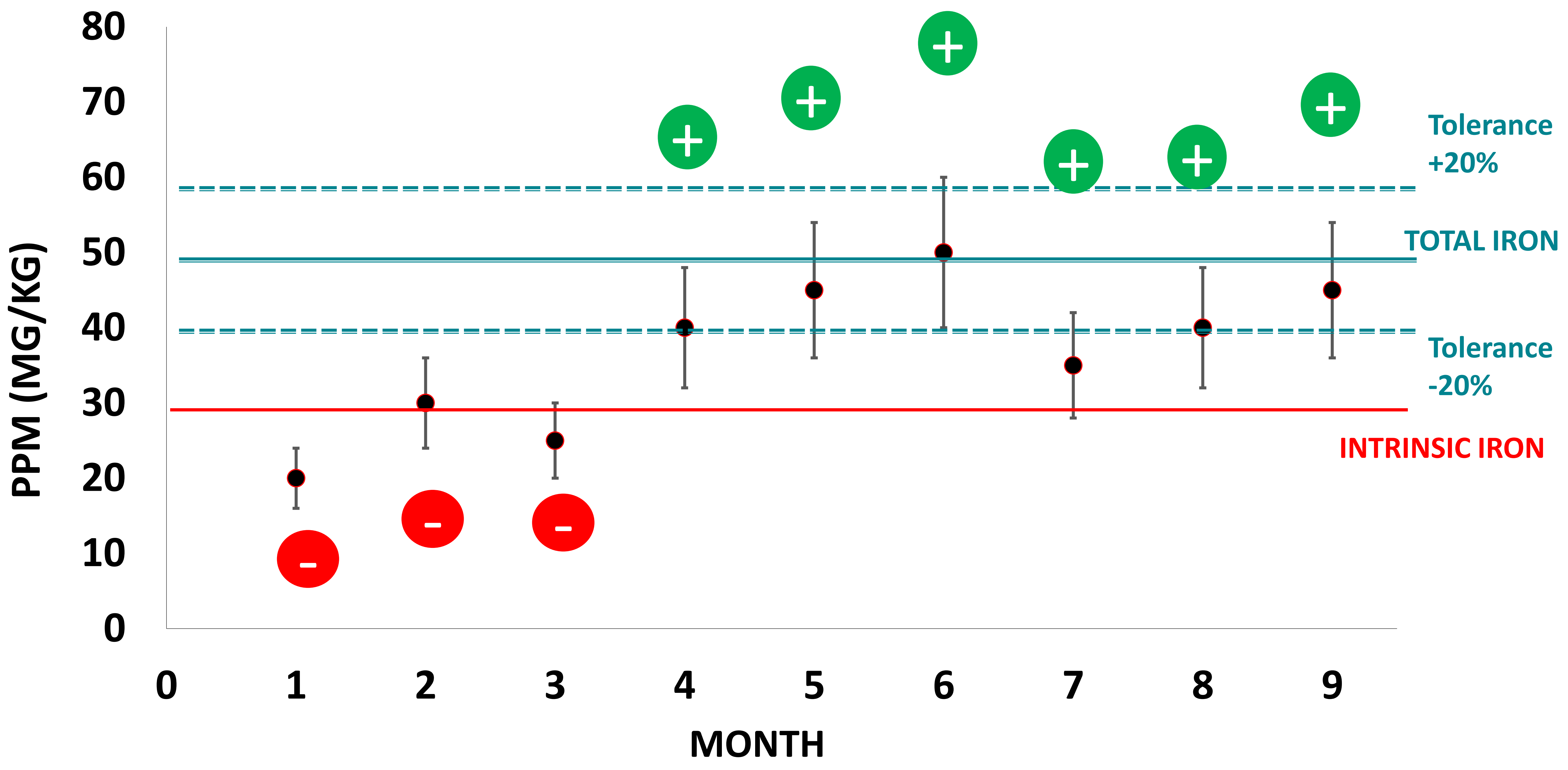


Scenario 2: Bread flour with 30 ppm natural iron; 20 ppm added iron



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

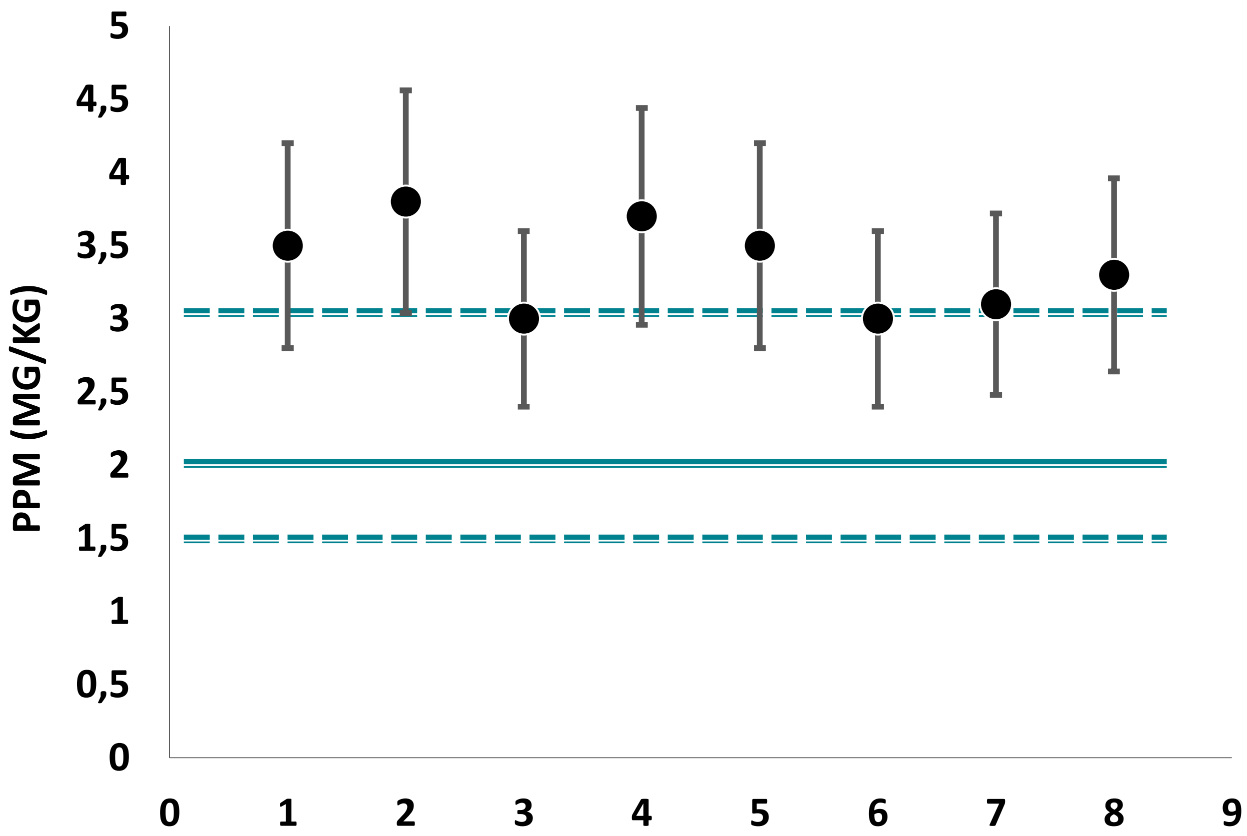


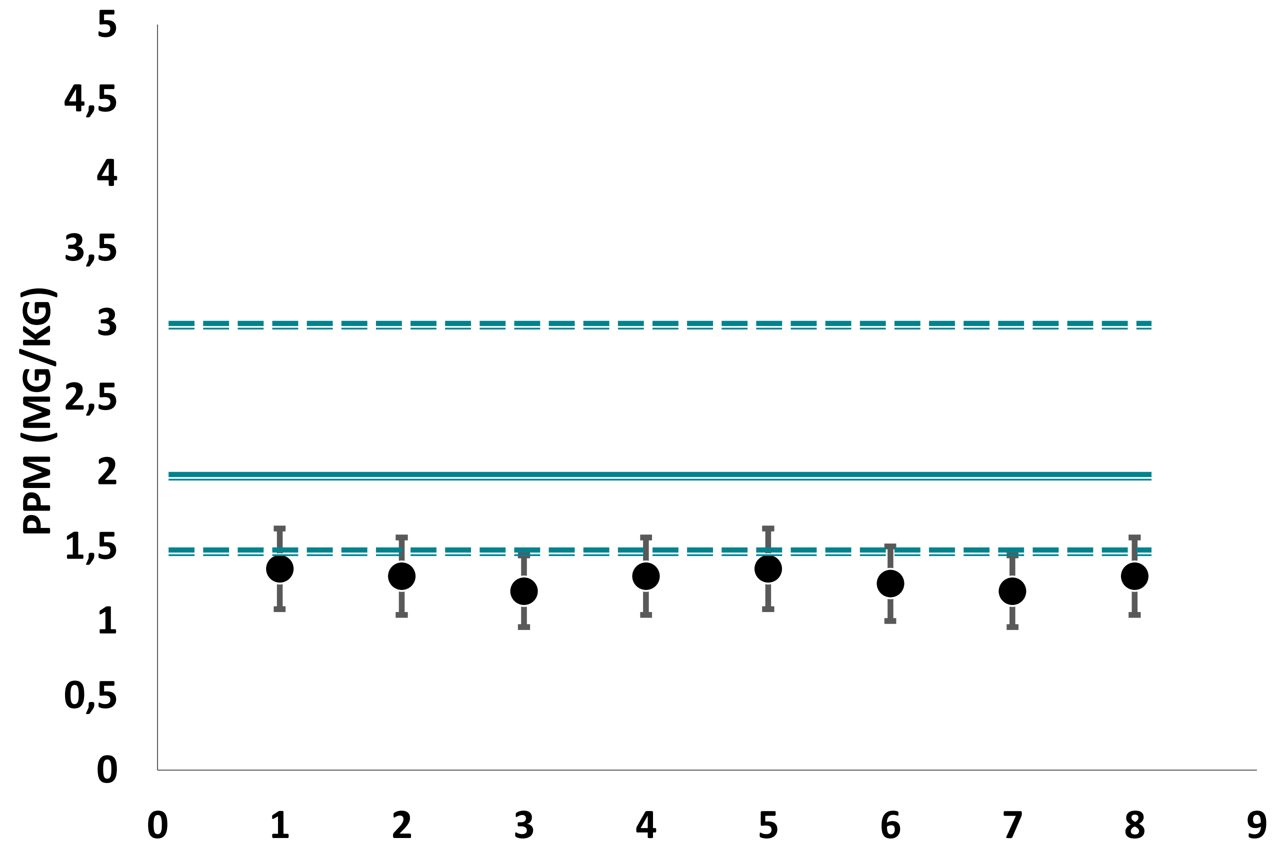
Case Study B: Iron in flour
Additional factor is Instrinsic iron

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!!!!

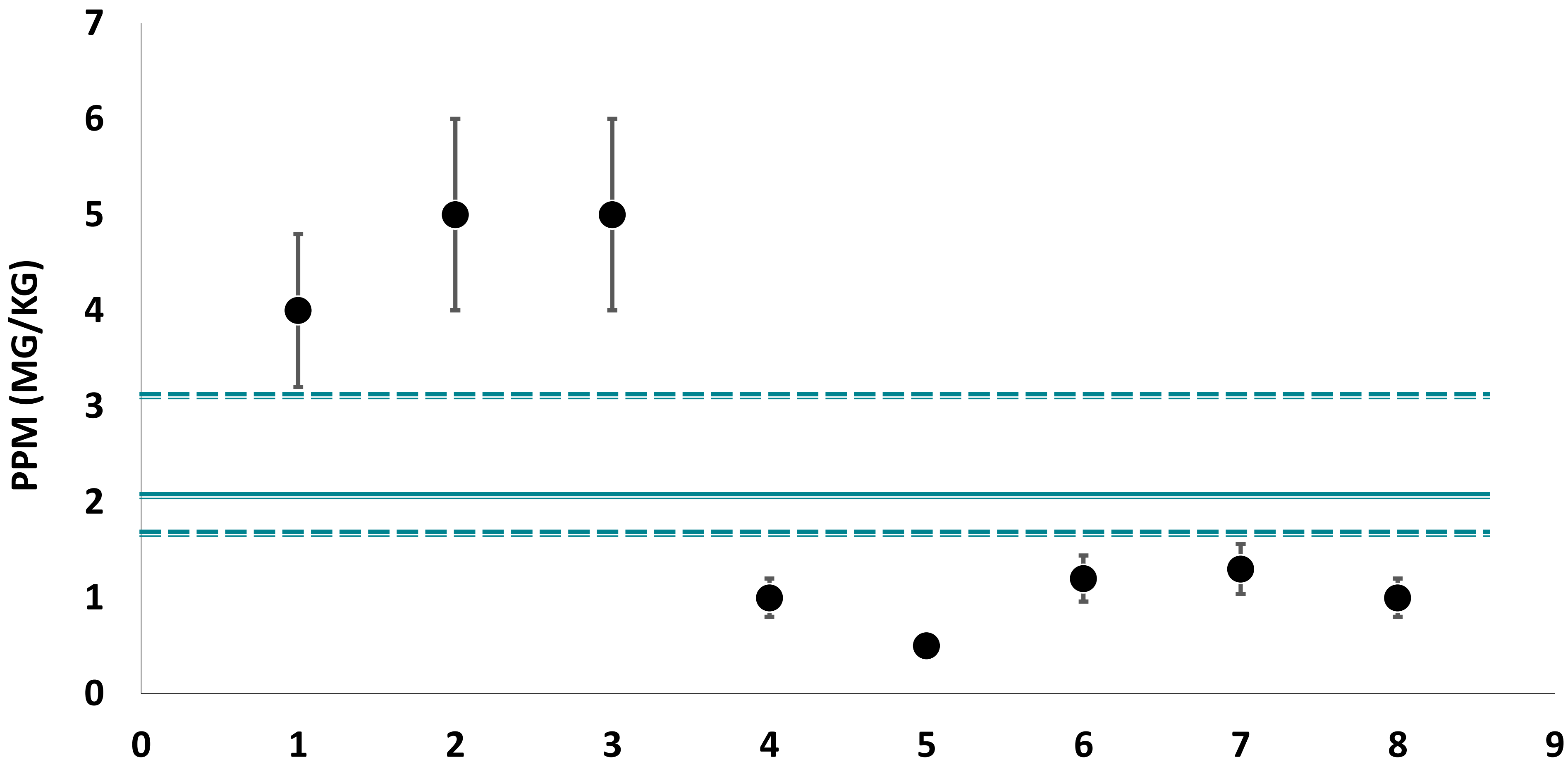
Recommendation 1





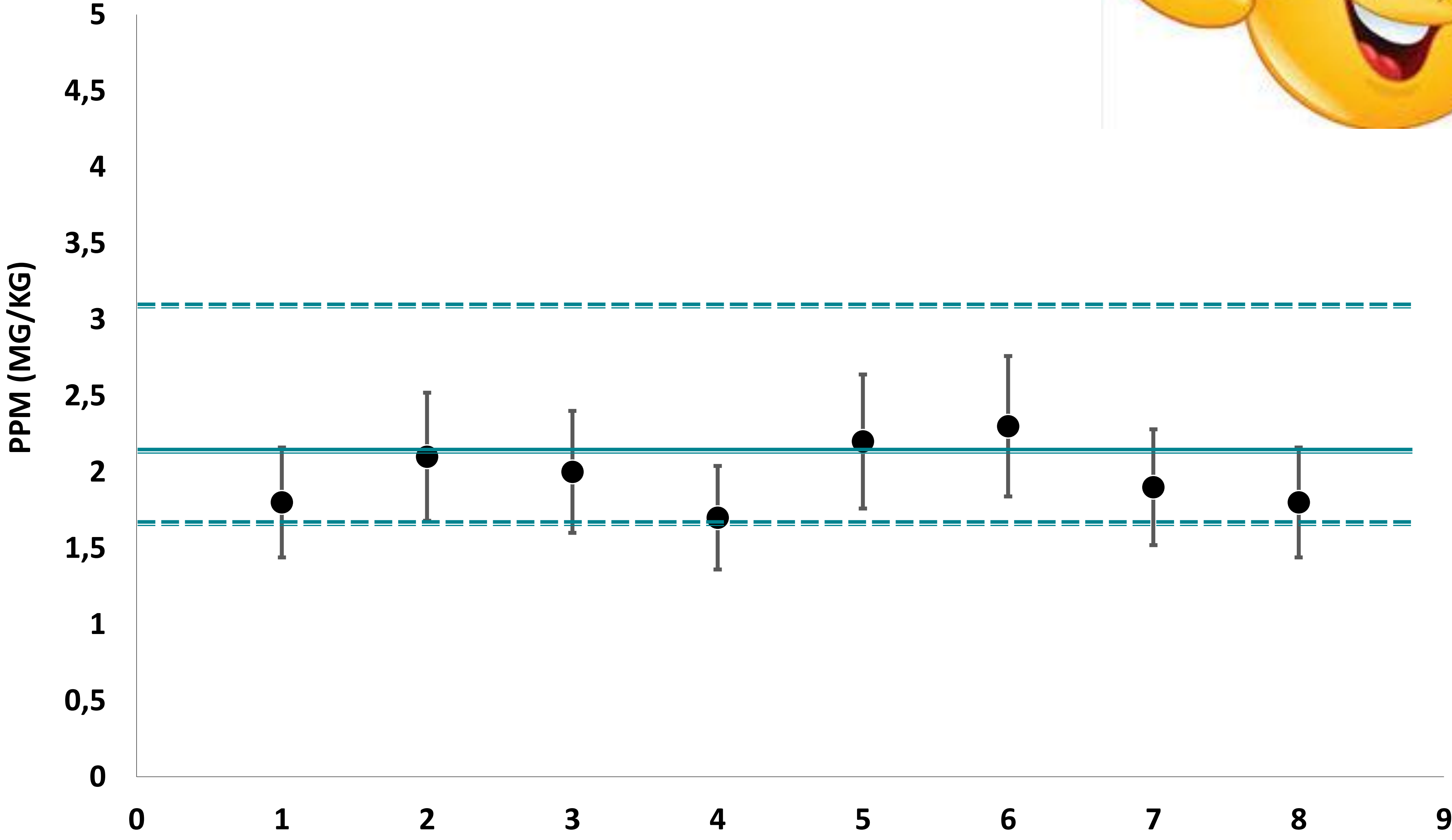
If the analysis results are repeatedly at the upper/lower limits of the permitted tolerance, the food business operator must adopt more effective in-house control and make the necessary changes in the production process or the labelling.

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



THANK YOU!

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