CHEMICAL ASSAYS
– Tests; Theory
(Vitamin A, Folic acid, Iron)

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Chemical Analysis

- Is only as good as the sample itself
- Requires skilled analysts
- For fortification - requires relatively expensive to very expensive equipment and consumables
- Is time consuming and
- Most importantly it's expensive
Validity of Analysis

• Inspectors frequently take a grab sample - as they are overworked as well – so the sample is not representative but it is considered legal

• Mills are not pharmaceutical level processors and fortified foods are not like vitamin tablets (every single one the same) – we can get mills to that level of homogeneity but not economically
• The general public isn’t like an astronaut taking pills and pastes they eat bulk quantities of a food vehicle i.e. Bread which has undergone a further mixing process

• The analyst takes 0.5g of sample and tries to find the micronutrients – the consumer eats 200g of sample and lets the body find the micronutrients
A Case in Point

• 2 internationally accredited (for vitamin and mineral analysis) laboratories plus 5 pre-mix supplier laboratories participate in a ring trail to assess how much reliance can the RSA Department of Health place on an external analysis for prosecution purposes.

• The 2 accredited laboratories had already been verified against the Canadian accredited reference laboratory for such analysis.
Method

• Laboratories are provided with freshly prepared pre-mixes which are then adulterated to be below the legal limit.
• Each lab receives 2 original, but different, pre-mix formulations, 2 adulterated by 10% and 2 adulterated by 20%
• Each of the above is provided to the laboratory on 2 or 3 different occasions i.e. Blind duplicate or triplicate samples
Results

• Each laboratory is requested to analyse the pre-mixes for Vitamin A, Riboflavin, Thiamine, Niacin, Pyridoxin, Folic acid, Iron and Zinc

• Each laboratory correctly identifies the 100%, 90% and 80% samples.

• The coefficient of variation (CV) within anyone laboratory was <5%

• The CV between laboratories was typically 10-12% depending on micronutrient
Conclusion

• If you fool around with fortification pre-mix any reasonably competent laboratory will catch you out.
On Fortified Product??

- Same experimental design using pre-mixes designed to be used at 200g/MT i.e. 1:5000
- Samples prepared in laboratory using the same food vehicle (wheat flour) but the 2 different pre-mixes (avoids variability in intrinsic value issues) and made thoroughly homogenous.
Results

• Each laboratory is requested to analyse the pre-mixes for Vitamin A, Riboflavin, Thiamine, Niacin, Pyridoxin, Folic acid, Iron and Zinc
• Individual laboratory CV’s >10% so even within a laboratory compliance verification questionable.
• Between laboratory CV’s >40%
Conclusions

• Group could definitely not distinguish even at 20% adulteration level so disputes are inevitable.

• Compliancy or not would depend on luck
So we scrap chemical assays?

- No – vital role to play in fortification programme.
- Ensure pre-mix is “fit for purpose” – note this is different to “conforms to specification” (concrete life jacket)
- Recognise the limitations of wet chemistry and use it not abuse it.
Rapid Tests

- Iron
- Vitamin A
- iCheck
- Semi quantitative i.e. BASF; Akzo Nobel
- Spot Test
Iron
Vitamin A

±250 IU/100g  ±300 IU/100g  ±500 IU/100g  ±700 IU/100g
Mix with Water

- Transfer 10 g of fortified flour into a 50-mL test tube
- Add 30 mL water and mix thoroughly to suspend all flour
- Shake resulting suspension vigorously for 1 minute
Centrifuge at 4000 rpm for e.g. 10 minutes until all flour has been precipitated at the bottom of the tube.

Then take out 15 ml of the supernatant liquor and transfer this to a 50 ml tube.
Add Reagents

1. 5.0 ml of vitamin C solution (1 g/L)
2. 5.0 ml of phenanthroline solution (1 g/L)
3. Fill up to 50 mL and mix
4. After **10 minutes** the color reaction has completed
Transfer to Cuvette
and Compare Visually
and Measure with Spectrophotometer

Measure absorbance at 510 nm against sample from unfortified flour
Assume in flour:

10 ppm Fe as Ferrazone

→ cuvette: 1 mg/L Fe

Absorbance: ~ 0.196
Three different Methods to assess Vitamin A in fortified Oil

### HPLC, BASF test kit and iCheck

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<td><img src="image1" alt="HPLC image" /></td>
<td><img src="image2" alt="BASF Test kit image" /></td>
<td><img src="image3" alt="iCheck test kit image" /></td>
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| - Lab equipment needed  
- Technically highly trained staff  
- High-Priced  
- Quantitative  
- Golden Standard | - Applicable in the field  
- Technically trained staff  
- Low-Priced  
- Semi-quantitative  
- Screening tool | - Applicable in the field  
- Low trained staff  
- Middle-Priced  
- Quantitative  
- Precise easy-to-use test kit |
Vitamin A
Field Test Kits Towards Cost-Effectiveness in Quality Control/Monitoring

Company Level

Producers: In-house Quality Check

FDA/Bureau of Standards: Screening

External QC

Laboratories: Control

Screening with Test Kit

HPLC (High performance liquid chromatography)

Number of Samples

$0.02 - 0.05

$8-15

$50-100