Rapid DON Testing and Method Performance Evaluation at the USDA

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2008 National Fusarium Head Blight Forum
Indianapolis, IN
December 3, 2008

GIPSA–FGIS Facilitates the Marketing of Grain, Oilseeds, and Related Commodities

Use of trade, firm, or corporation names does not constitute an official endorsement or approval by the USDA of any product or service to the exclusion of others that may be suitable.

Official Mycotoxin Testing

- Quantitive or qualitative
  - Based on official samples
  - Appeal process
- Rapid, simple, and low cost
  - Tests done at elevators & loading facilities
  - Non-technical operators
- Need for accurate (unbiased) results
  - Minimize risk to buyer/seller

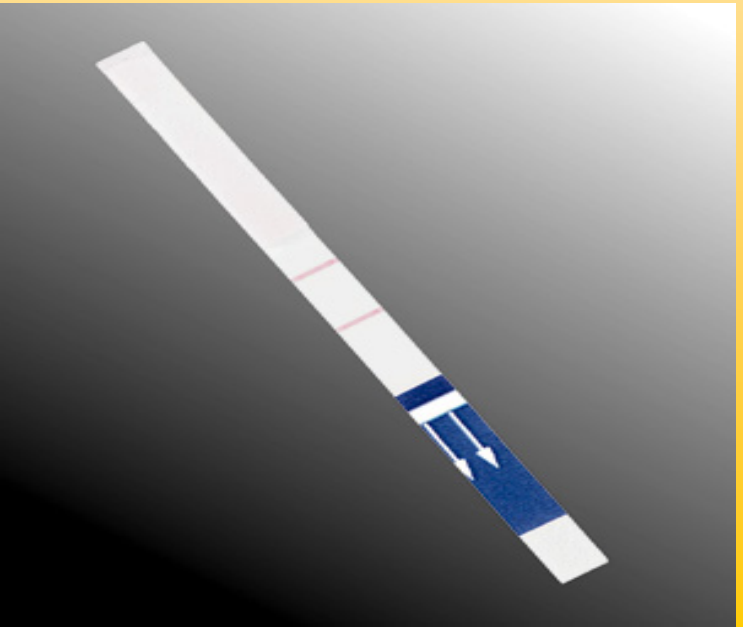
Rapid DON Testing Technology
November – 2008

- Immunoassay formats
  - Lateral flow strip (10)
  - ELISA – microtiter well plate (8)
  - ELISA – antibody coated tubes (2)
  - Homogenous enzyme assay (2)
  - Fluorescence polarization (2)

- Total approved – 24 rapid test kits

Performance Verification Program: DON Tests

Enzyme-Linked Immunosorbent Assay Technology (ELISA)



Lateral Flow Strip Technology (LFS)

Rapid Method Evaluation

- Quantitative
  - Criteria Document
  - Performance Verified
  - GIPSA issues "Certificate of Conformance"
- Qualitative
  - Manufacturer Claims
  - Performance Verified
  - GIPSA issues "Certificate of Performance"

Mycotoxin Test Performance Criteria

- Analysis time
- Commodities
  - Primary grain
  - Other
- Accuracy & precision
  - NC* – primary grain (HPLC)
  - Fortified – other commodities
- Limit of detection
- Equipment sensitivity to electromagnetic fields
- Temperature sensitivity
- Reagent stability
- Avoidance of toxic or hazardous substances
- Performance verification

*NC – naturally contaminated

Quantitative DON Criteria

Accuracy/Precision

Concentration (ppm)	Maximum %RSD	Standard Deviation (ppm)	95% Confidence (ppm)
0.50	25	0.125	0.25 - 0.75
1.00	20	0.20	0.60 - 1.40
2.00	15	0.30	1.40 - 2.60
5.00	10	0.50	4.00 - 6.00

- Naturally-contaminated wheat
- n = 21 at each level

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DON Method Comparison

Method	Quantitative	Time	Training	Cost	LOQ (ppm)	RSD
HPLC	Y	2 hrs	High	$141[a]	0.25	10 - 15%
ELISA	Y	10 - 30 min	Low	$39[a]	0.5	10 - 25%
LFS	Y	10 - 30 min	Low	-	0.5	10 - 25%
FP	N	10 - 30 min	Low	-	1	≤1% Fn[b]

[a] Current GIPSA Fees – 10/2008

[b] False-negatives

Definitions

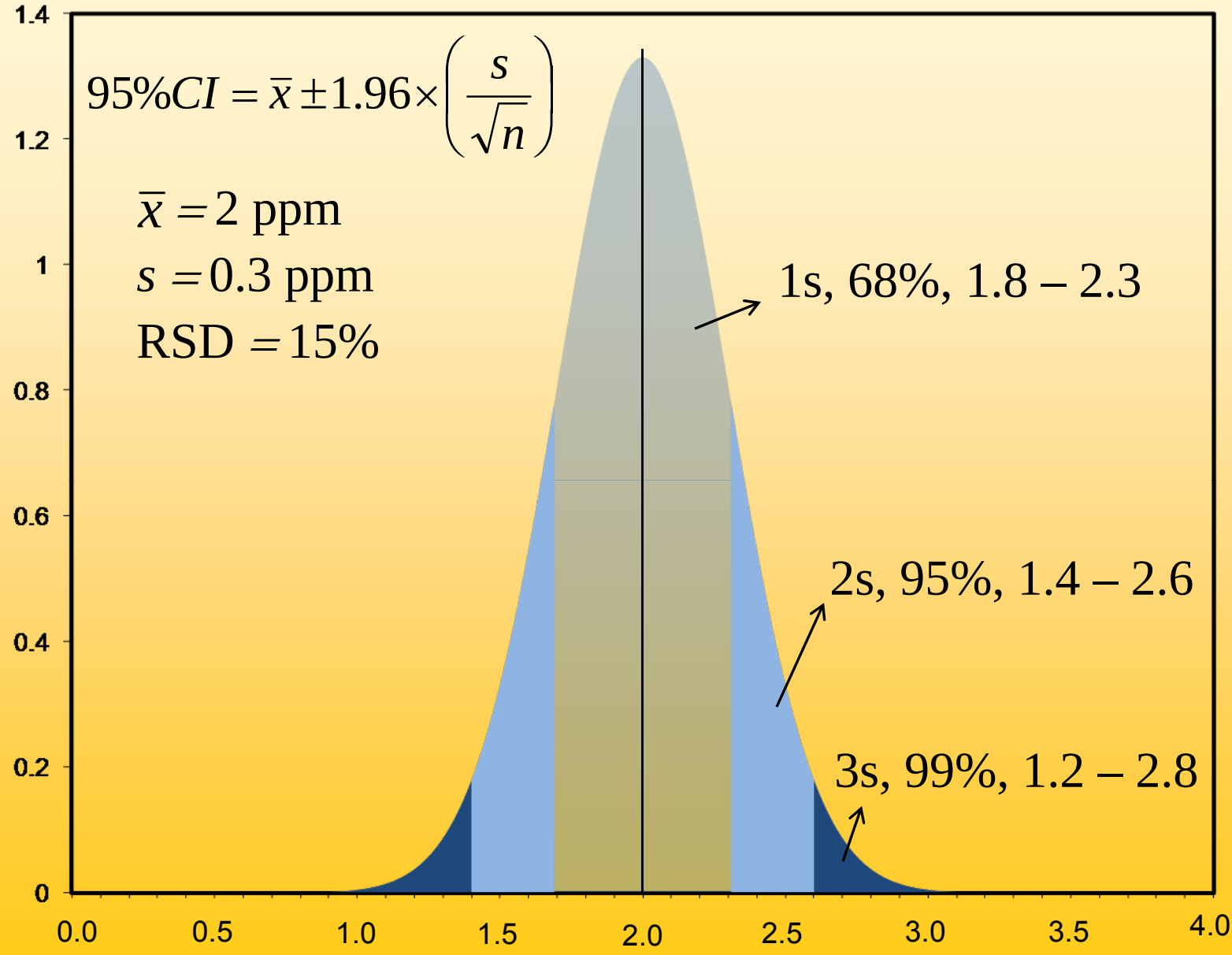
Mean $$\bar{x} = \frac{\sum x}{n}$$

Standard deviation $$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

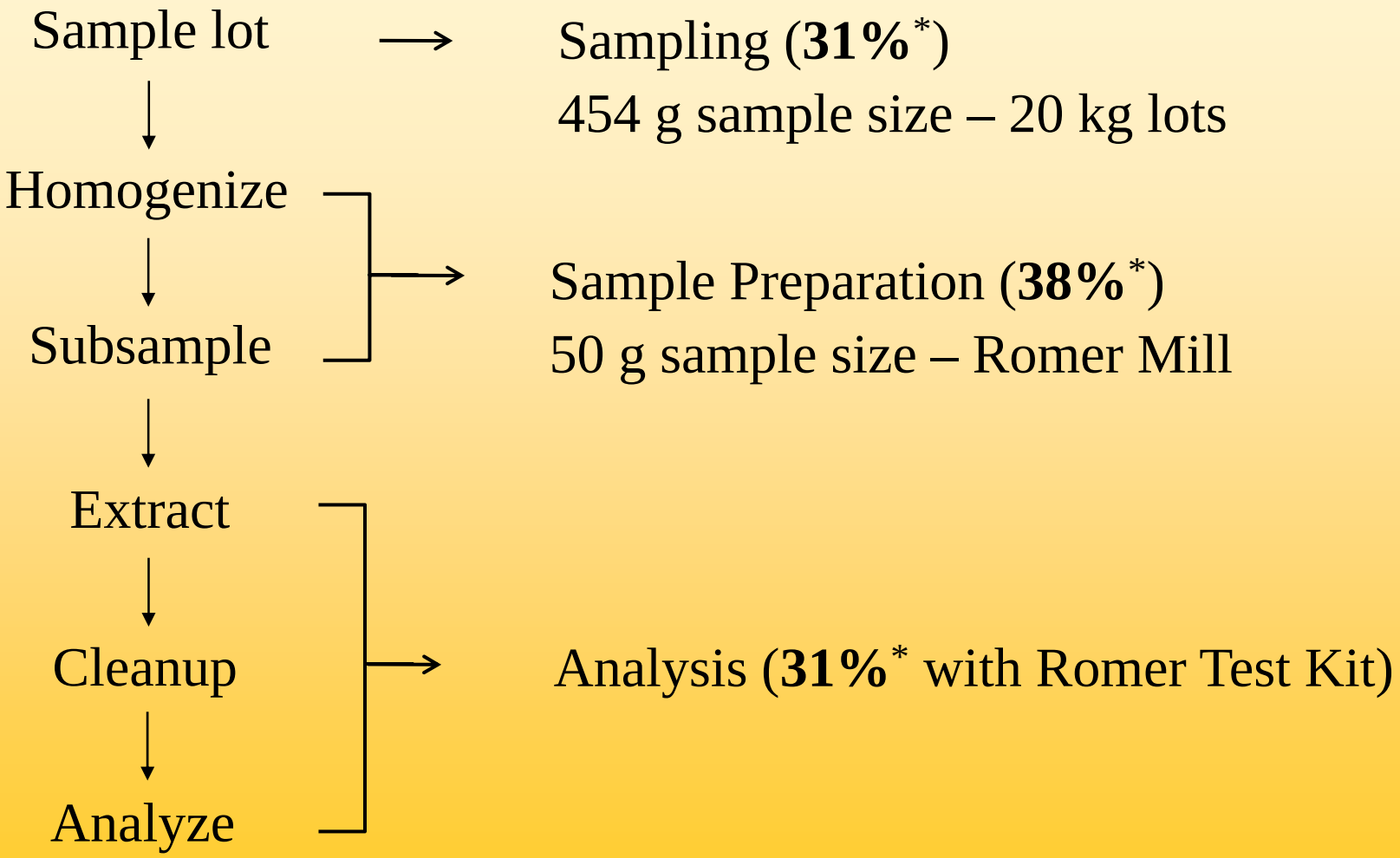
Relative standard deviation $$RSD = \frac{s}{\bar{x}} \times 100$$

95% Confidence Interval $$95\%CI = \bar{x} \pm 1.96 \times \left(\frac{s}{\sqrt{n}} \right)$$

Normal Distribution – Expected Uncertainty

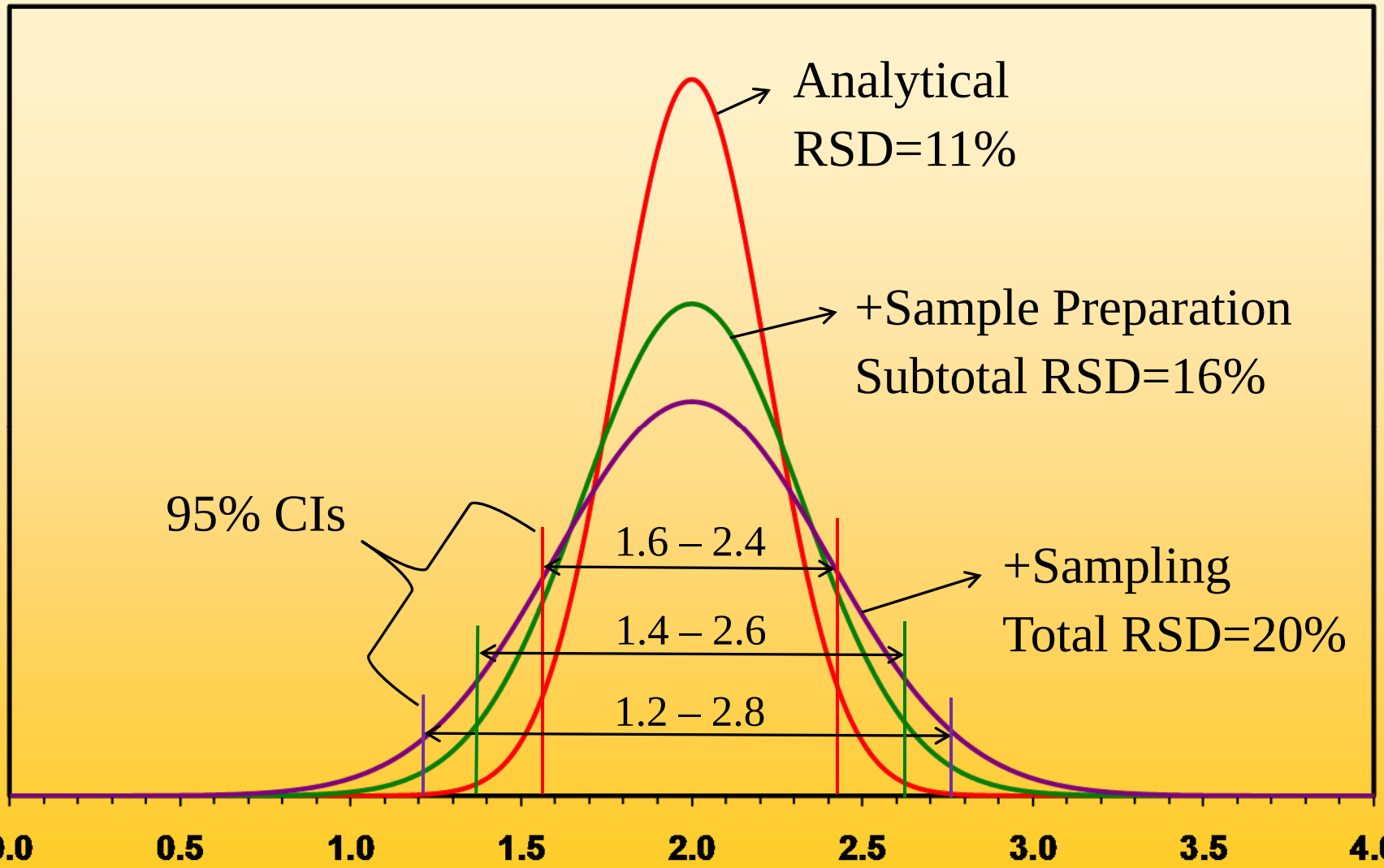


DON Analysis – Sources of Variability



* Percentage of total variance calculated at 2 ppm
Whitaker, T. B. *et al.*, *J. AOAC Int.* **2000**, *83*, 1285.

Components of Variability at 2 ppm*



*Whitaker, T. B. *et al.*, *J. AOAC Int.* **2000**, *83*, 1285.

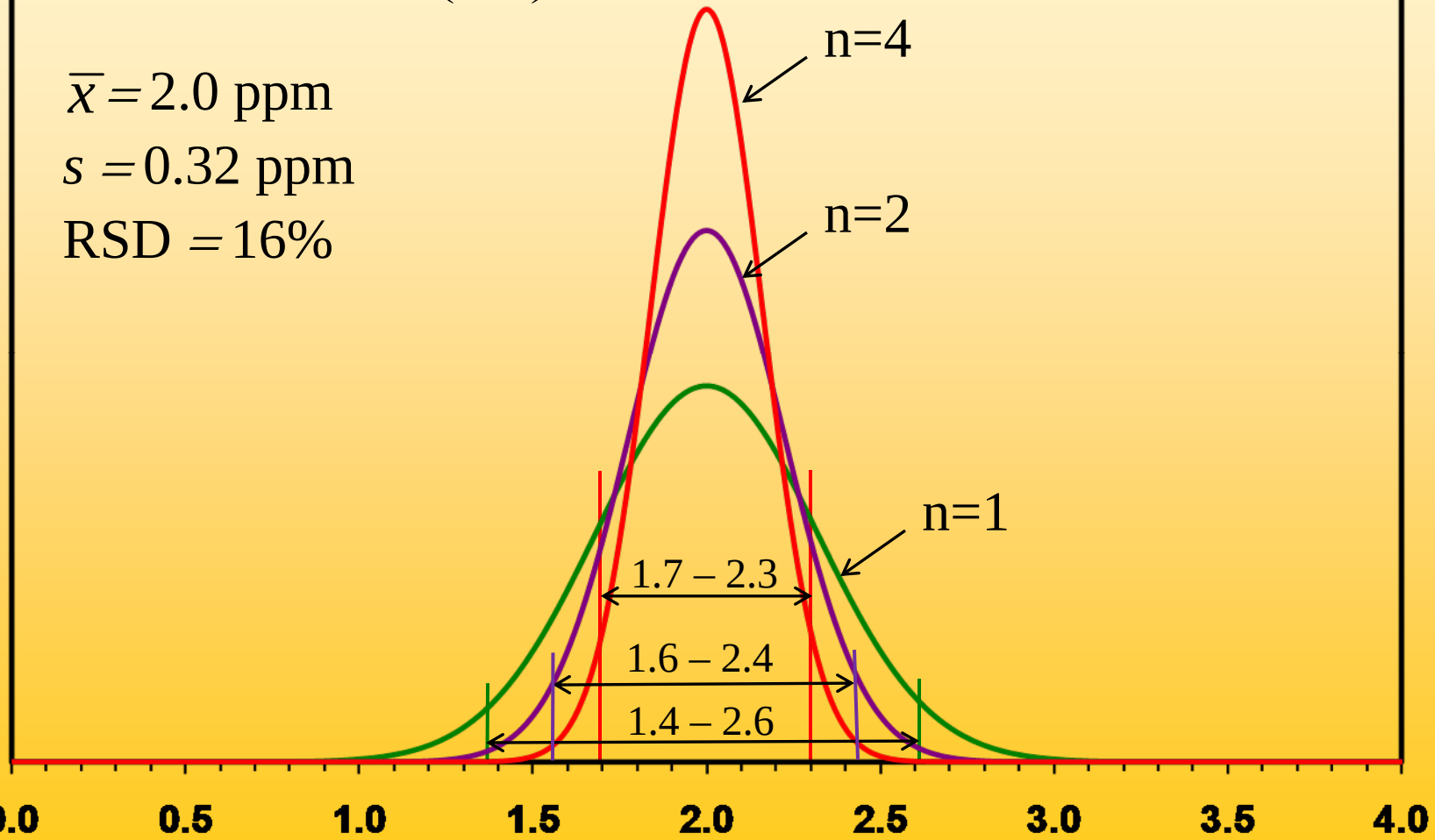
Method Variation – Multiple Samples

$$95\%CI = \bar{x} \pm 1.96 \times \left(\frac{s}{\sqrt{n}} \right)$$

$\bar{x} = 2.0$ ppm

$s = 0.32$ ppm

RSD $= 16\%$



Controlling Bias / Uncertainty

- DON Standard – UV Verification
- Method Spikes (Method Recovery)
- Lab Control Sample – Control Charting
- Certified Reference Standards
  - FAPAS* Proficiency Program (York, UK)
    - www.fapas.com
- Multiple Tests
  - Include sub-sampling and/or sampling

*Food Analysis Performance Assessment Scheme

More Information

- On the Web

  www.usda.gov/gipsa/

  Certified Test Kits

  DON Criteria Document

  DON Handbook

- Contact

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Questions?