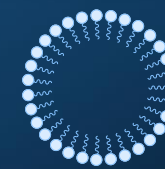
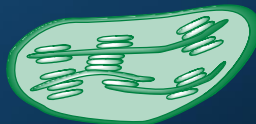
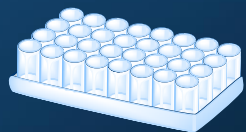
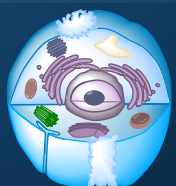
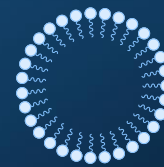
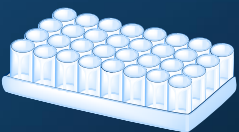
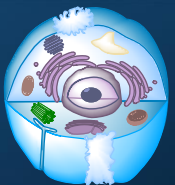


Development of Novel Assay for Protein Quantitation

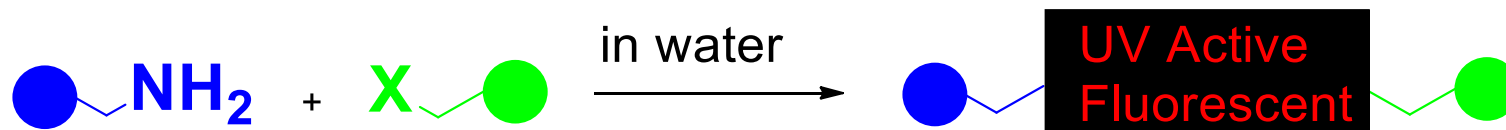
www.x-lyz.com



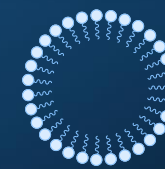
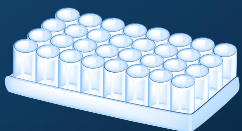
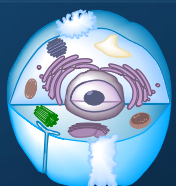
- **Chemistry basis**
- **About X-Lyz Assay**
- **Market analysis**
- **Assay performance**
- **Milk or Melamine**
- **Marketing Preparation**



Conjugation with Amine

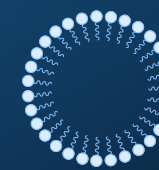
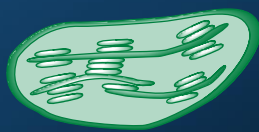
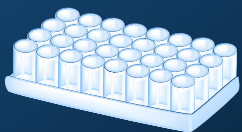
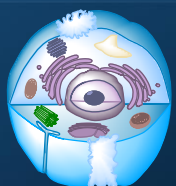


- Water compatible
- Irreversible
- UV and fluorescent



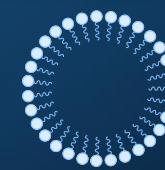
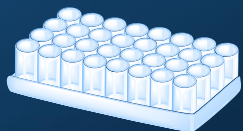
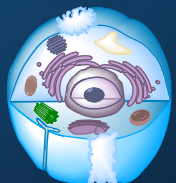
Potential Products

- Amino acid derivatization
- Total protein quantitation
- Amine detection
- Staining for gel electrophoresis
- Crosslinking
- Molecular probes (NIR dyes)



Content in X-Lyz™ Kit

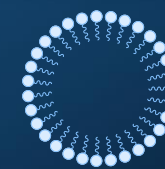
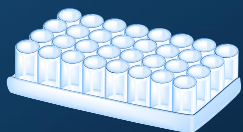
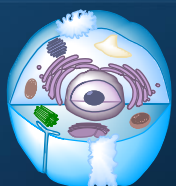
- Reagent A
- Reagent B
- BSA standard
- TCA Precipitation Solution
For interferent removal



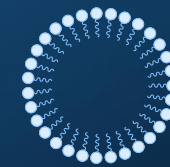
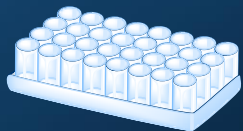
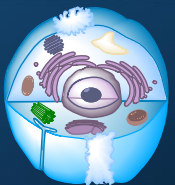
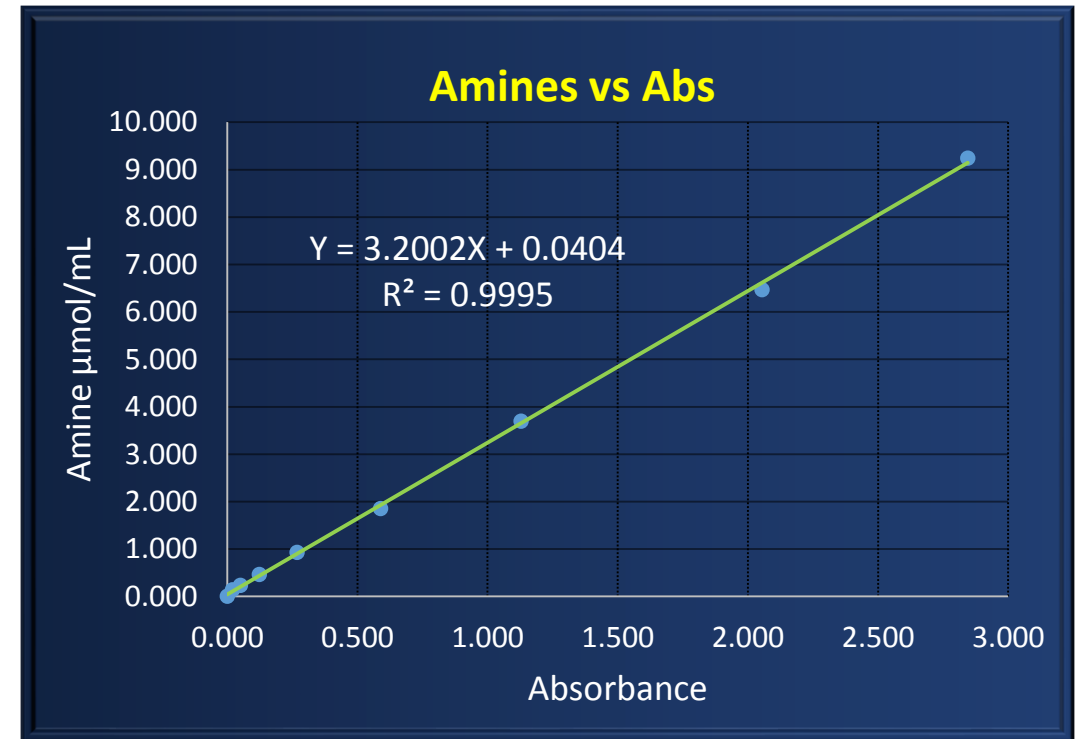
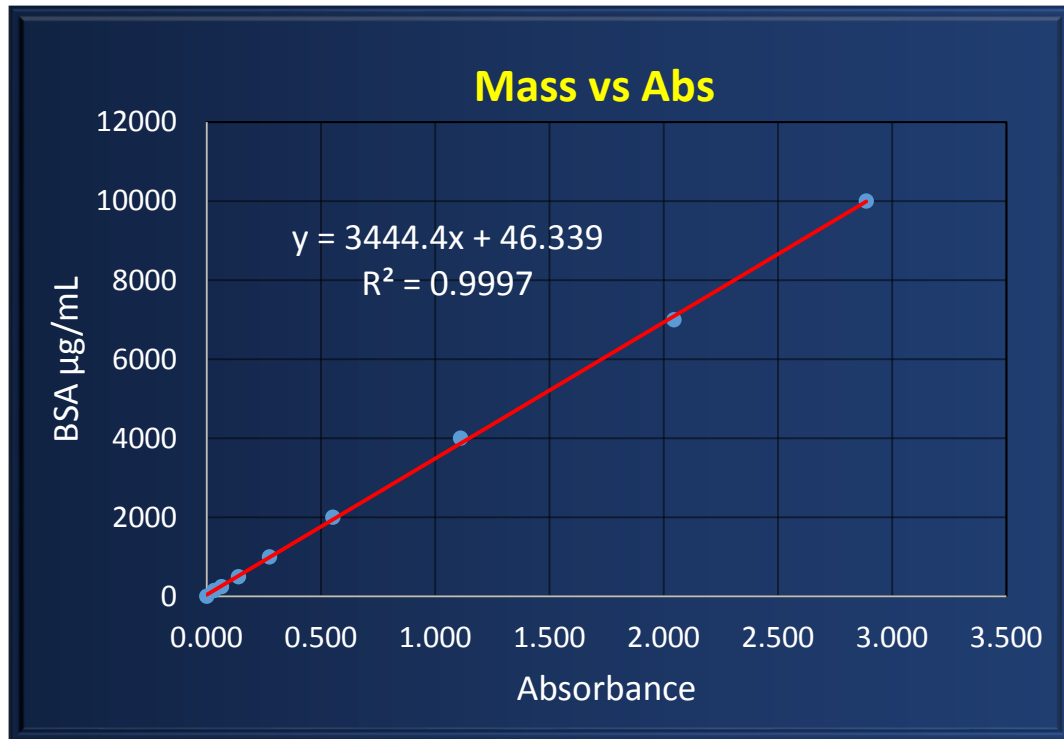
Assay Procedure

Mechanism: Reaction with amines and form chromophore

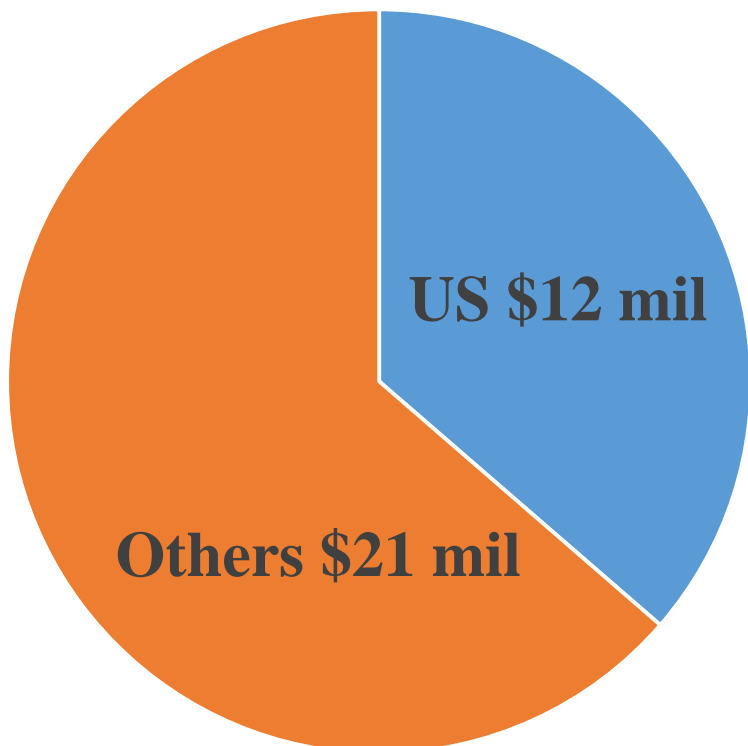
- **Prepare Working Reagent by combining Reagent A and B**
- **Mix samples with Working Reagent**
- **Incubate for 15min at temperature between 23-37°C**
- **Read absorbance at wavelength between 350-405nm**



Calculation Approaches

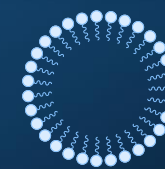
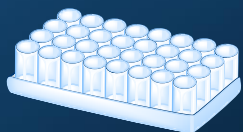
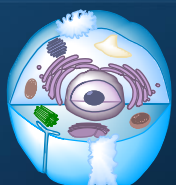


Protein Quantitation Market



Targets

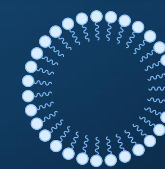
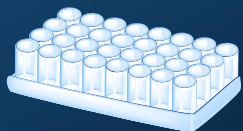
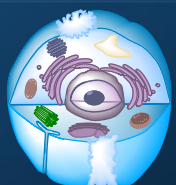
- Bio & Pharm
- Environment
- Food
- Pet feed
- Membrane separation



Existing Quantitation Methods

Assay	UV	Mechanism	Advantages	Disadvantages
280nm	280 nm	Tyrosine tryptophan	Rapid Low cost	Incompatibility High variability
BCA	562 nm	Cu ¹⁺	Low variability	Low compatibility with reducing agents
Bradford	470 nm	Dye	Compatible with reducing agents	Incompatibility with detergents
Lowry	750 nm	Cu ¹⁺	High sensitivity	Long procedure

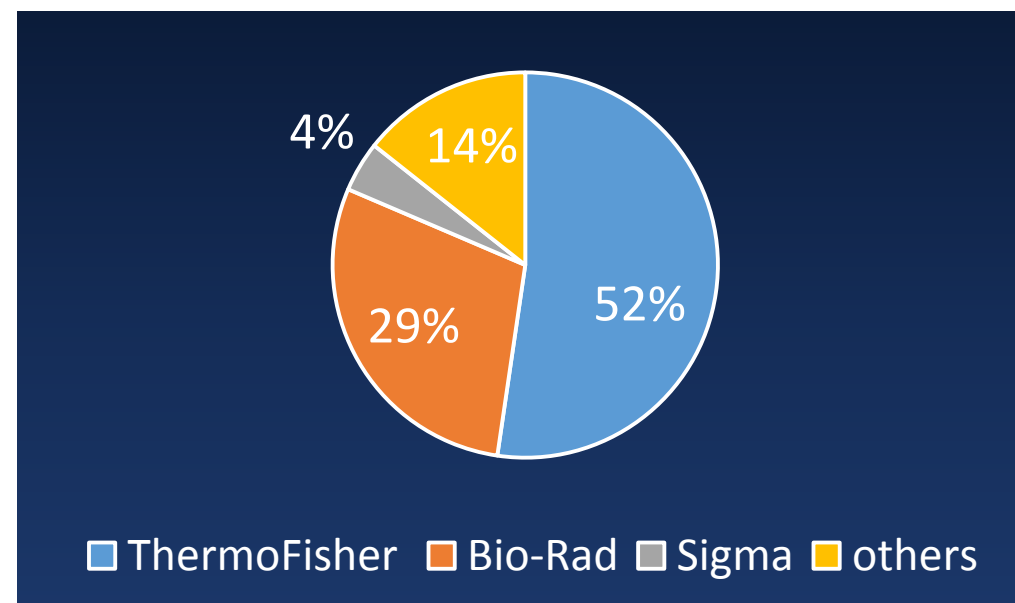
Mary Johnson at Labome 2017 DOI//dx.doi.org/10.13070/mm.en.2.115



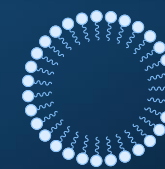
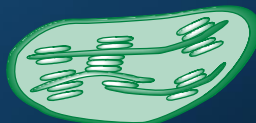
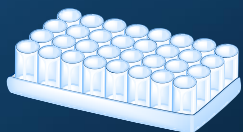
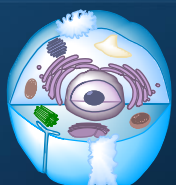
Popularity by Methods

Methods	# Citing /250 Papers	Popularity
BCA	140	56%
Bradford	78	31%
Lowry	21	8%
280 nm	12	5%

Popularity by Vendors

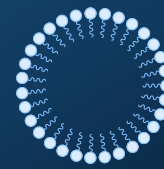
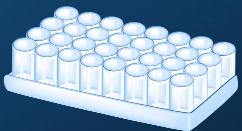
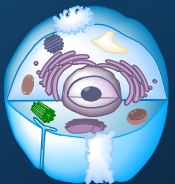


Mary Johnson at Labome **2017** DOI//dx.doi.org/10.13070/mm.en.2.115



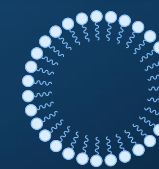
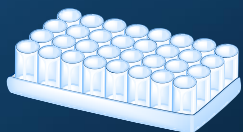
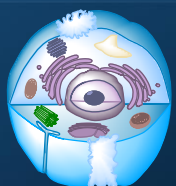
Evaluation of a Method

- Ease of operation
- Protein variation
- Linear range
- Accuracy
- Calibration frequency

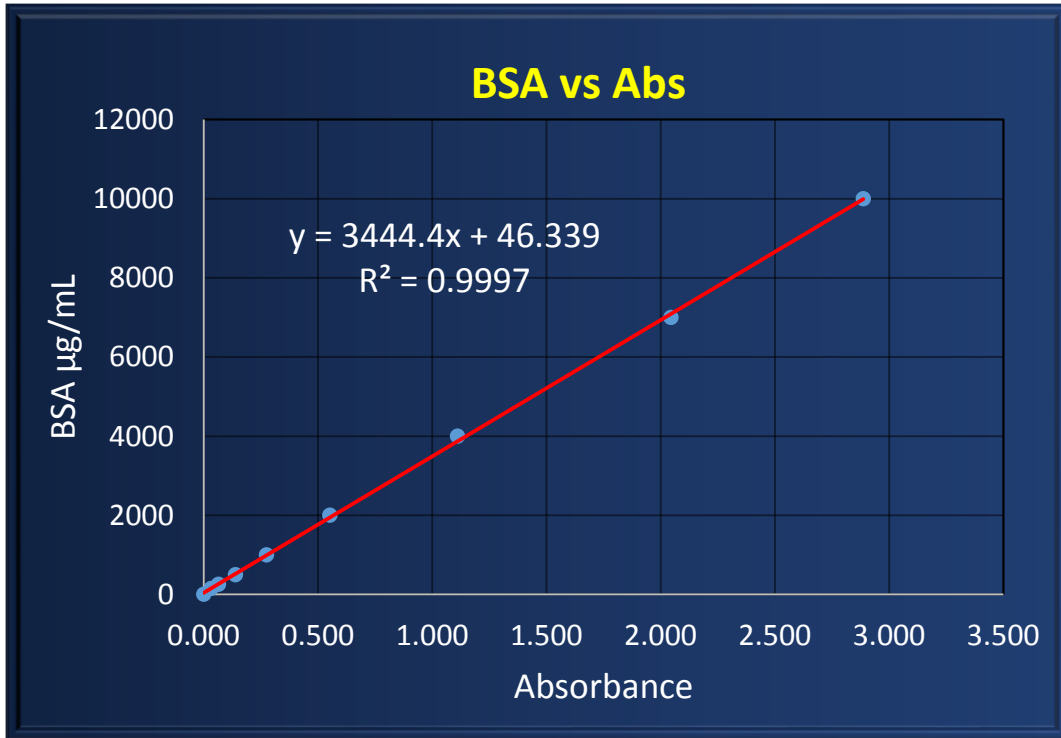


Protein Variations

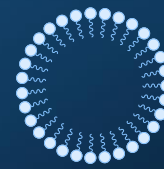
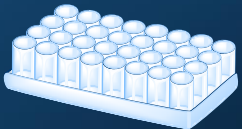
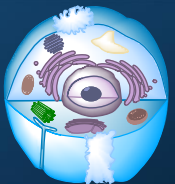
Method	X-Lyz (mass)	X-Lyz (amine)	BCA	Pierce 660	Bradford
Coefficient of Variation	21%	8.7%	15%	37%	44%



Linear Ranges



Methods	Ranges
BCA	20-2000 $\mu\text{g/mL}$
Bradford	1-1500 $\mu\text{g/mL}$
Pierce 660	25-2000 $\mu\text{g/mL}$
X-Lyz	10-10,000$\mu\text{g/mL}$



Accuracy/Recovery

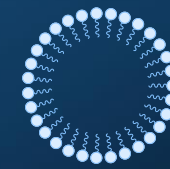
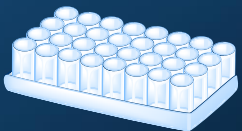
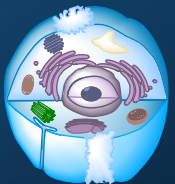
True Values ($\mu\text{g/mL}$)	Average Absorbance	Estimates ($\mu\text{g/mL}$)	Deviations
200	0.049	215.1	7.50%
300	0.074	301.2	0.40%
600	0.160	597.4	-0.40%
900	0.260	941.9	4.70%
1900	0.562	1982.1	4.30%
5000	1.406	4889.2	-2.20%
9000	2.600	9001.8	0.00%

True Values: The true concentrations of BSA samples in $\mu\text{g/mL}$.

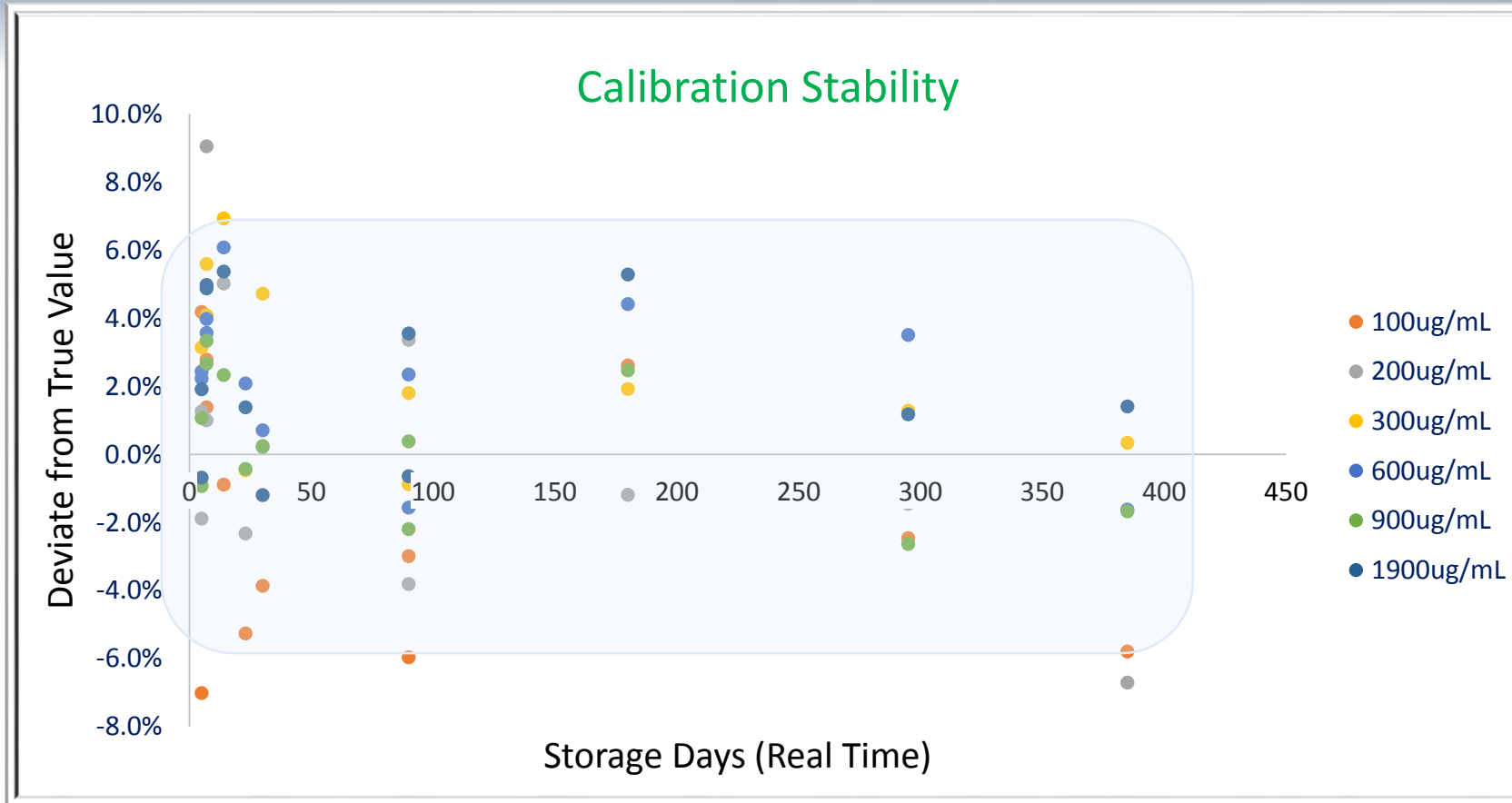
Average Absorbance: Average absorbance in five replicates.

Estimates: Estimates of sample concentrations.

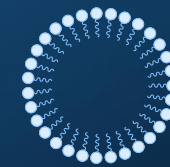
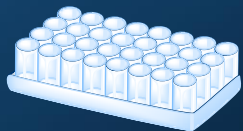
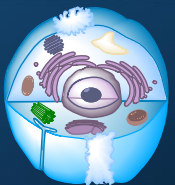
Deviations: Percentage deviations of the estimates over the true values.



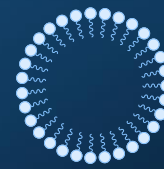
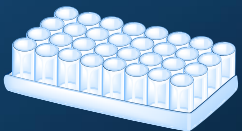
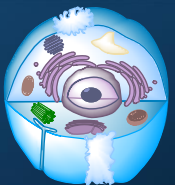
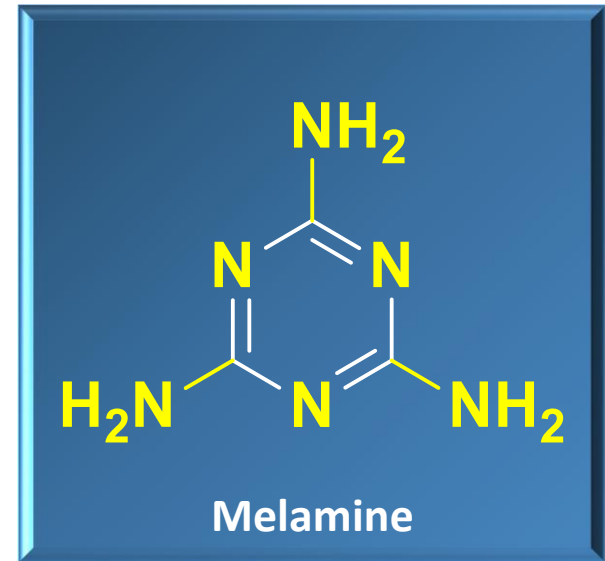
Re-Use of Standard Curve



The same standard curve was used for twelve assays over a year.

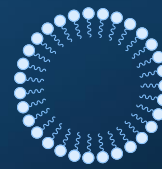
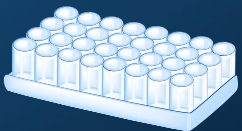
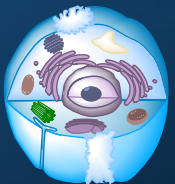


Milk or Melamine (三聚氰胺)



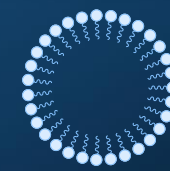
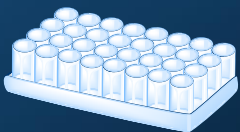
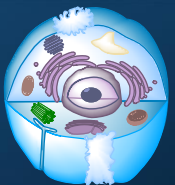
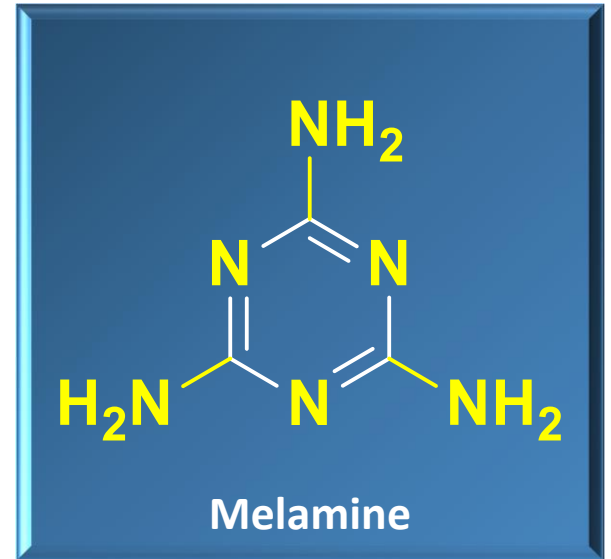
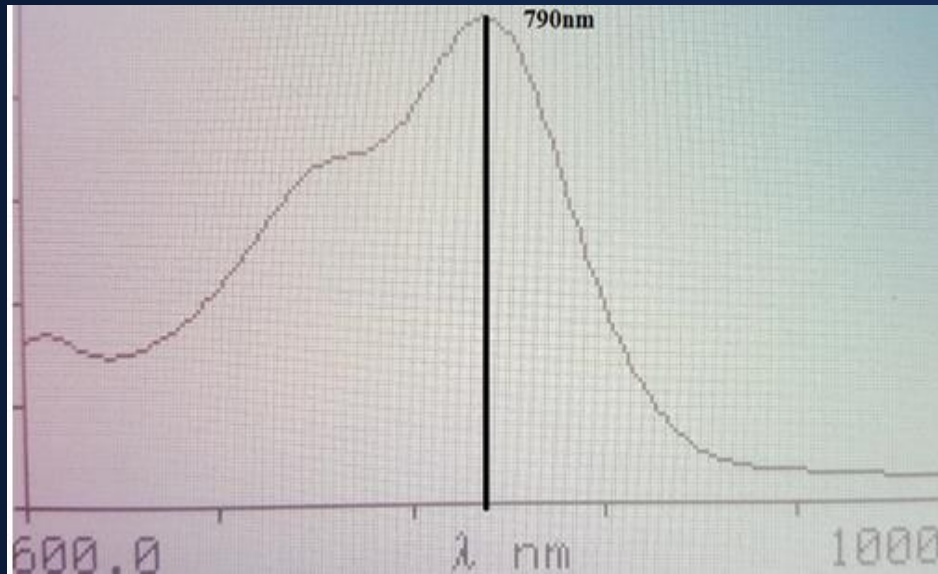
Milk Protein Concentration

	Reported by Horizon	Milk Only	Melamine Only	Mix
Content (mg/mL)	42	46	-0.4	24
Deviation	NR	9.1%	NR	13.5%



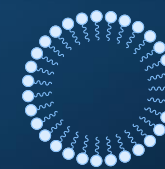
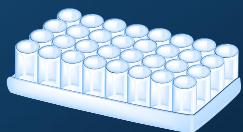
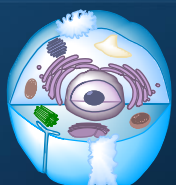
Melamine Quantitation?

UV Trace in Presence of Melamine



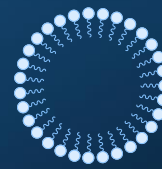
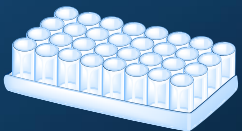
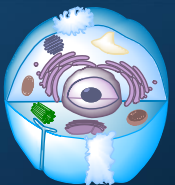
Other Applications

- Lysine determination
- Proteolysis monitoring
- Cross-linking estimation
- Polyamine measurement



Marketing Preparation

- Pricing
- Instruction
- Package
- X-lyz.com
- [Intro video](#)



Thank You!

www.x-lyz.com

