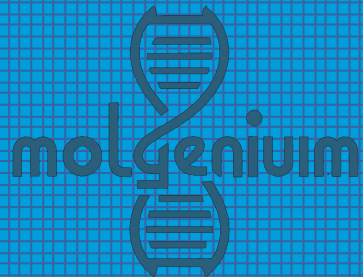


Harnessing Platform Methods for mRNA Quality Control: Challenges, Solutions, and Future Perspectives.

USP mRNA Virtual Summit

Joining Forces to Advance the Quality of mRNA Therapeutics

March 11-12, 2025



Mohamad Toutounji, Ph. D
- Molgenium -

Why mRNA?

- Industry growth: \$50B+ market by 2030 (roots in COVID-19 vaccines).
- Therapeutic expansion: Oncology, gene therapy, personalized medicine.
- QC as the Bottleneck: Scalability demands platform approaches.
- Roadmap: Challenges Platform Solutions Future-ready QC.

Key Challenges in mRNA QC

1. Raw Material Variability

Lipid sources: Lipid batches from different vendors altered LNP size.

Impact: Failed specs, costly delays.

2. Analytical Gaps

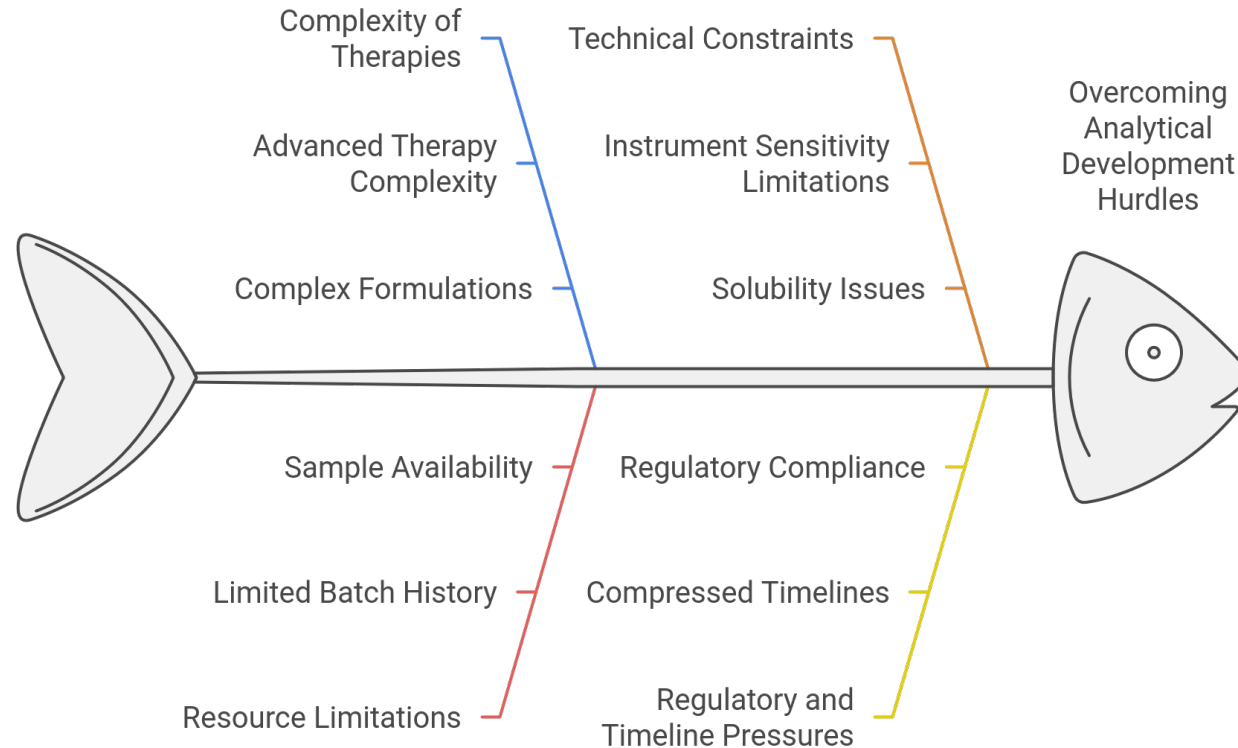
Integrity: Ribogreen/qPCR lacks resolution for fragmented mRNA.

dsRNA: ELISA false positives/positives due to antibody cross-reactivity and assay range.

Poly(A): NGS bias in tail length quantification.

Key Challenges in mRNA QC: Analytical Methods

Challenges in Developing Analytical Platform Methods

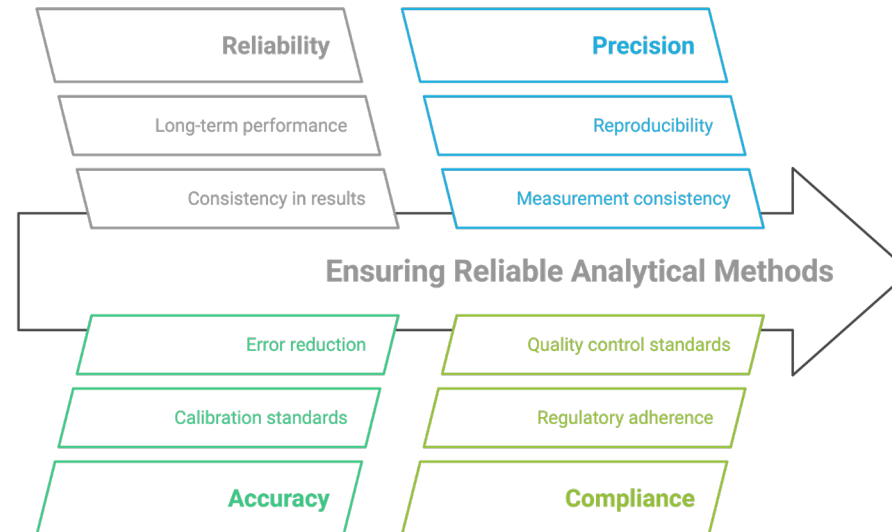


Method Performance Expectations

Defining Method Performance Expectations

Purpose

- To validate that analytical methods consistently produce data that are reliable and conform to the predefined performance.



Method Performance Expectations

Performance Metrics in Development



Fit-to-Platform Assessment

What is Fit-to-Platform Assessment?

Definition

Evaluating the suitability of a new analytical method or process to a pre-established platform technology.

Ensures the method aligns with platform standards for performance, compatibility, and reproducibility.

Key Goals

Determine if the method meets platform expectations (e.g., sensitivity, accuracy).

Optimize the platform's capacity to address product-specific challenges.

Significance

Reduces development time by leveraging prior platform knowledge.

Streamlines validation by focusing on critical gaps or enhancements.

Fit-to-Platform Assessment

Fit-to-Platform Assessment – Key Components

Alignment with Platform Capabilities

- Is the new method compatible with the platform's standard design space?
- Does it meet the required performance metrics, including sensitivity, specificity, and robustness?

Gap Analysis

- Identification of areas where the new method deviates from platform standards.
- Example: Adaptation required for novel analytes or detection targets.

Fit-to-Platform Assessment

Fit-to-Platform Assessment – Key Components

Risk Assessment

- Quantifying the potential impact of method-platform misalignment on overall product quality.
- Focus on critical quality attributes (CQAs) that could be compromised.

Optimization

- Adjustments to enhance specificity, linearity, and robustness to meet platform requirements.

Fit-to-Platform Assessment

Case Study – Optimizing Specificity in dsRNA Detection

Background

- dsRNA Detection is a critical step for ensuring mRNA DS safety, as dsRNA is an impurity linked to immune responses.
- ELISA-based assay.

Challenge

- Standard kits showed cross-reactivity with unrelated nucleic acids, compromising specificity.
- Achieving high specificity while maintaining sensitivity was critical for assessing product safety.

Fit-to-Platform Assessment

Case Study – Optimizing Specificity in dsRNA Detection

Approach to Optimization

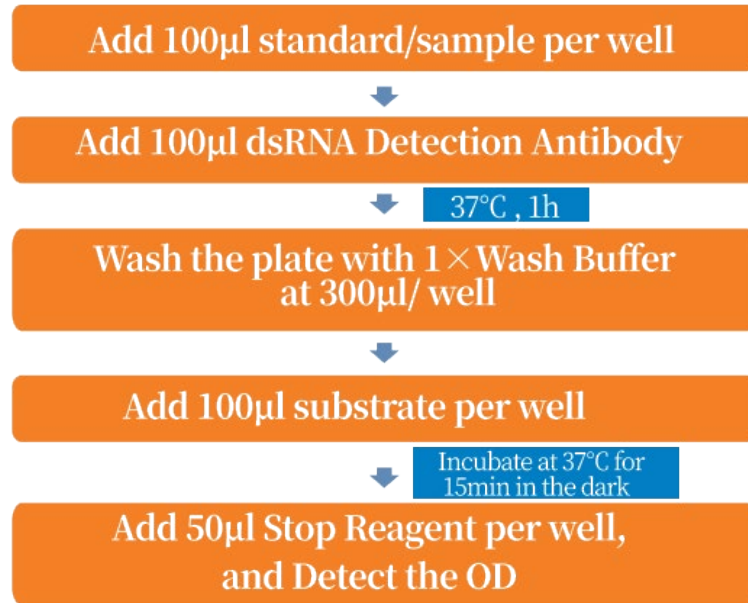
1. dsRNA quantification using ELISA and Fit-to-Platform Evaluation

- Evaluated the compatibility of the dsRNA detection kit with the existing analytical platform.
- Identified specificity as the primary limitation through gap analysis.

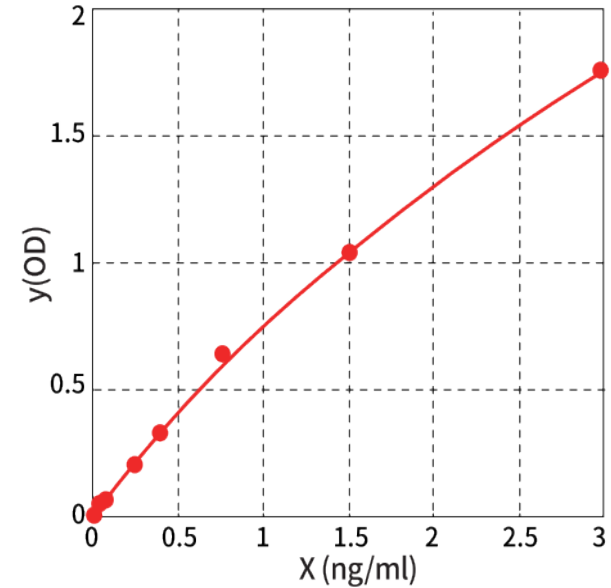
2. Optimizing Specificity

- Adjusting Kit Design: Modified capture and detection antibodies to reduce cross-reactivity.
- Validation with Controls: Introduced a broader range of negative controls (e.g., single-stranded RNA, DNA) to confirm absence of non-specific binding.

dsRNA Impurities Control & Detection

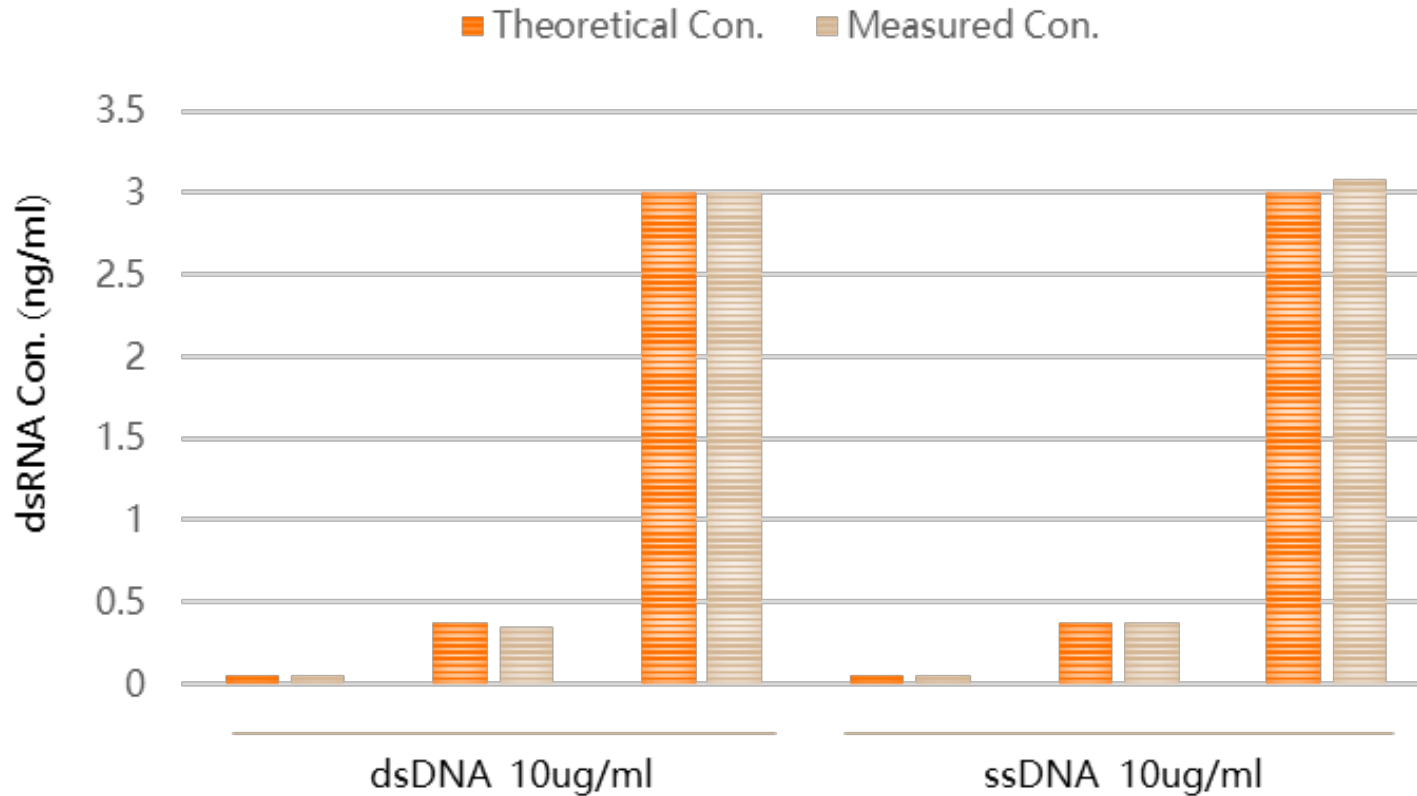


NovoFast dsRNA ELISA Kit, Cat. No.: RD017



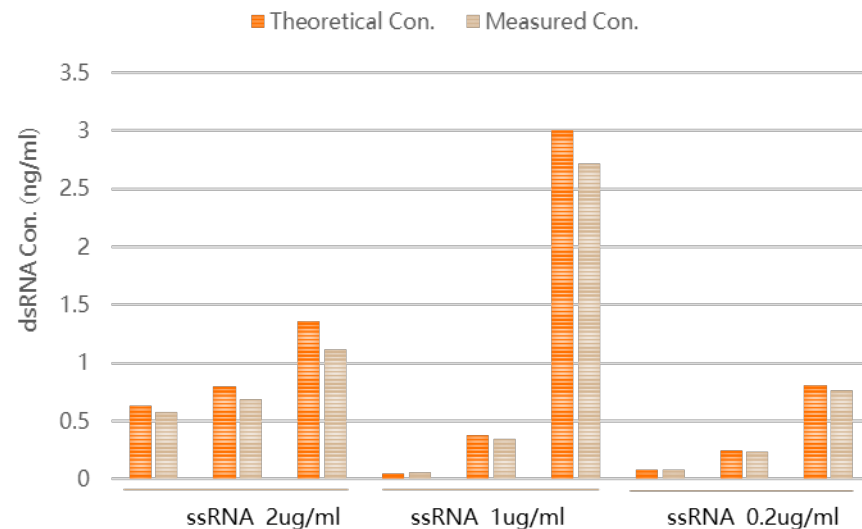
- One-step detection takes only 1.5 hours
- Sensitivity: 0.047ng/ml
- Detection range: 0.047-3ng/ml

dsRNA Impurities Control & Detection – assessing the Specificity



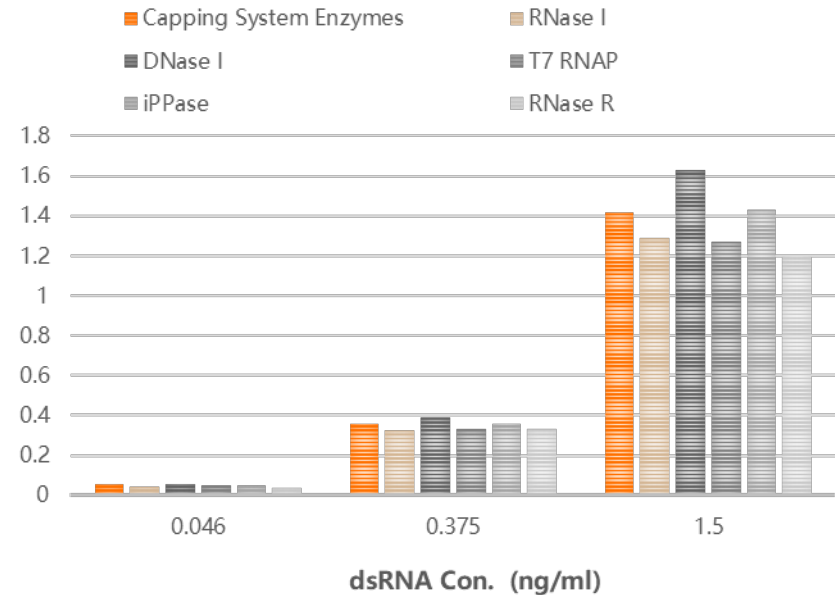
dsRNA Impurities Control & Detection – assessing the Specificity

ssRNA µg/ml	Background dsRNA Con. (ng/ml)	dsRNA Con. (ng/ml)	Theoretical dsRNA Con. (ng/ml)	Measured dsRNA Con. (ng/ml)	Recovery%
mRNA1 1019nt 0.2 µg/ml	0.061669	0.000	0.062	0.062	
		0.023	0.085	0.079	6%
		0.188	0.249	0.239	4%
		0.750	0.812	0.764	6%
mRNA 2 2156 nt 1.0 µg/ml	Below detection limit	0.000	Below detection limit	----	
		0.046	0.046	0.057	24%
		0.375	0.375	0.349	7%
		3.000	3.000	2.715	9%
mRNA3 1019nt 2.0 µg/ml	0.609262	0.000	0.609	0.609	
		0.023	0.632	0.579	8%
		0.188	0.797	0.689	14%
		0.750	1.359	1.112	18%



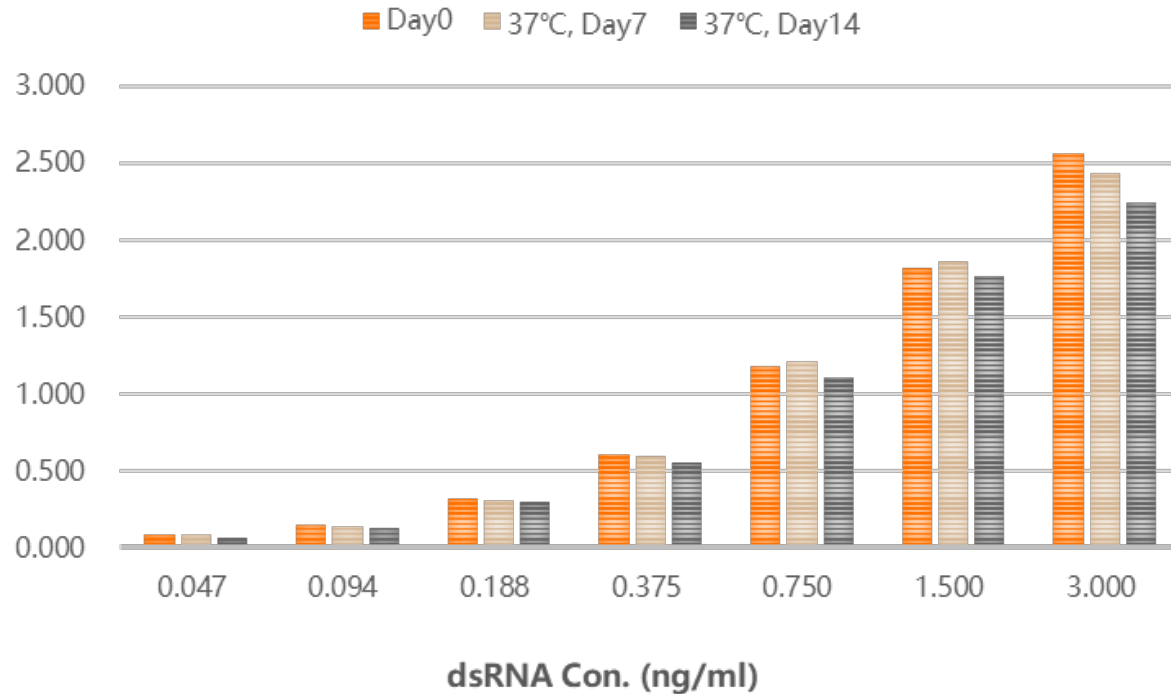
dsRNA Impurities Control & Detection – assessing the Specificity

Enzymes (10µg/ml)	Background dsRNA Con. (ng/ml)	dsRNA Con. (ng/ml)	Theoretical dsRNA Con. (ng/ml)	Measured dsRNA Con. (ng/ml)	Recovery%
Capping System Enzymes	Below the detection limit	0.046	0.046	0.055	19%
		0.375	0.375	0.359	4%
		1.500	1.500	1.415	6%
RNase inhibitor	Below the detection limit	0.046	0.046	0.041	11%
		0.375	0.375	0.326	13%
		1.500	1.500	1.286	14%
DNase I	Below the detection limit	0.046	0.046	0.053	16%
		0.375	0.375	0.392	4%
		1.500	1.500	1.628	9%
T7 RNAP	Below the detection limit	0.046	0.046	0.049	7%
		0.375	0.375	0.333	11%
		1.500	1.500	1.268	15%
iPPase	Below the detection limit	0.046	0.046	0.051	11%
		0.375	0.375	0.360	4%
		1.500	1.500	1.428	5%
RNase R	Below the detection limit	0.046	0.046	0.038	18%
		0.375	0.375	0.331	12%
		1.500	1.500	1.200	20%



dsRNA Impurities Control & Detection – assessing the Specificity

Accelerated Stability of RD017



Design Space and Robustness

What is Design Space?

- Defined by ICH Q8 as the multidimensional range of input variables and process parameters that ensure method performance.

For dsRNA detection:

- Includes variables such as antibody concentration, reaction time, buffer composition, and temperature.

What is Robustness?

- The method's capacity to remain unaffected by small variations in operating conditions.
- Ensures reliable performance under real-world conditions.

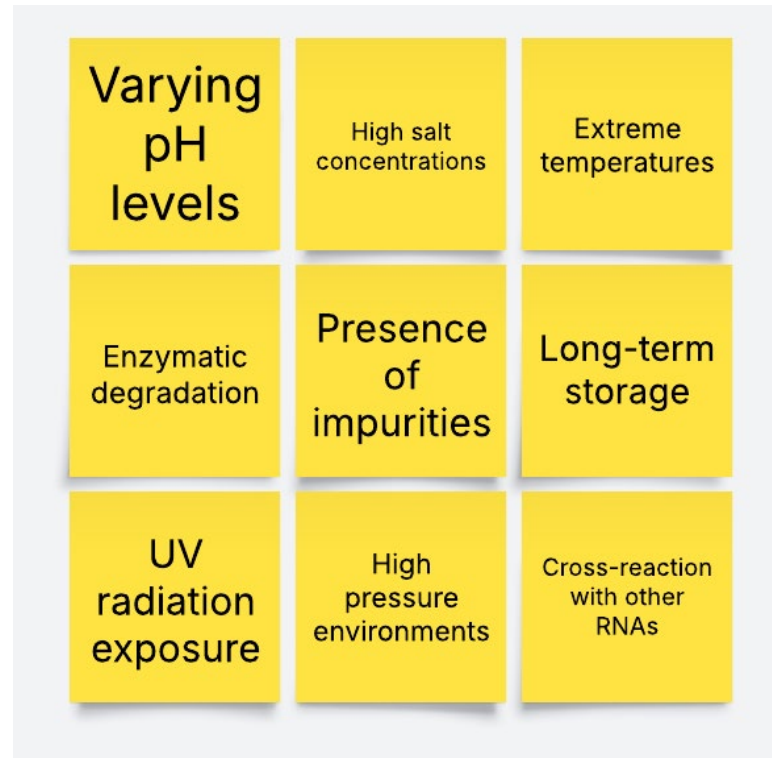
Design Space and Robustness

Defining the Design Space for dsRNA Detection - Key Variables Explored



Design Space and Robustness

Robustness Testing for dsRNA Detection - Key Stress Conditions Evaluated



Total Analytical Error (TAE)?

Definition

- Total Analytical Error (TAE): A statistical measure that combines systematic error (bias) and random error (imprecision) to assess the overall performance of an analytical method.

Significance

- Provides a holistic view of method reliability.
- Ensures the method delivers results within acceptable accuracy and precision limits.

Total Analytical Error (TAE)?

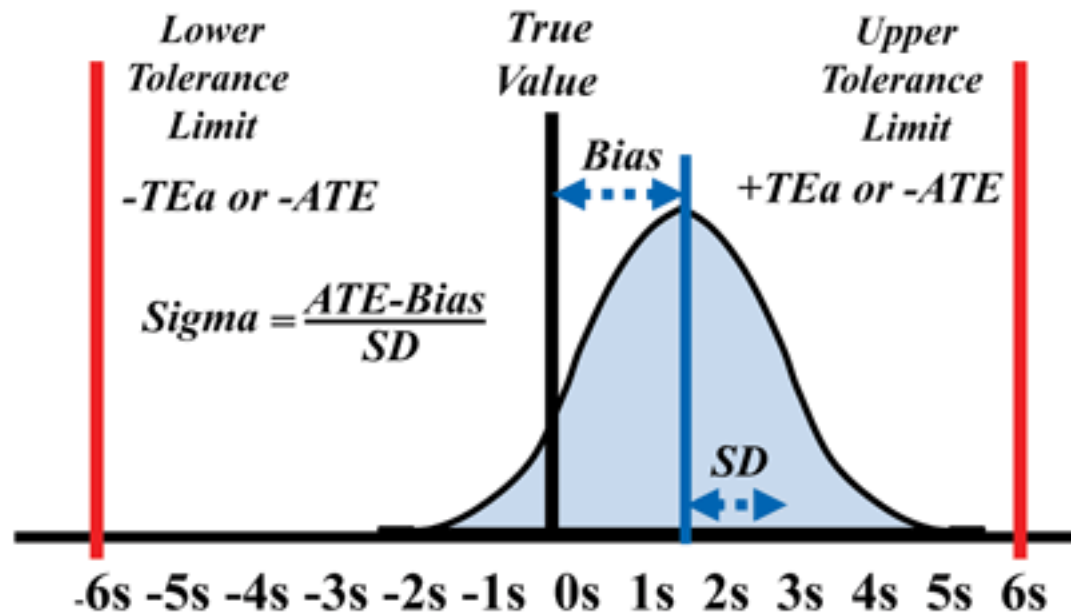
Components

1. Systematic Error (Bias)

Deviation of the measured value from the true value.

2. Random Error (Imprecision)

Variability observed between repeated measurements.



Total Analytical Error (TAE)?

Calculating Total Analytical Error

$$\text{TAE} = |\text{Bias}| + z \times \text{Imprecision (SD)}$$

Where:

Bias = Mean of measured values - True value.

z = Standard score for a chosen confidence level (e.g., z = 1.96 for 95%).

SD = Standard deviation of measurements.

Interpreting TAE

- Compare TAE to predefined Total Error Allowance (TEA).
- A method is acceptable if:

$$\text{TAE} \leq \text{TEA}$$

Total Analytical Error (TAE)?

Calculating Total Analytical Error for the dsRNA detection method

True Value (spike): 1 ng/mL dsRNA.

Measured Mean: 0.97 ng/mL (Bias = -0.3 ng/mL).

Standard Deviation (SD): 0.25 ng/mL.

TAE Calculation

$$\text{TAE} = |-0.3| + (1.96 \times 0.25) = 0.3 + 0.49 = 0.79 \text{ ng/mL.}$$

TEA Benchmark: Defined TEA: ± 1 pg/mL.

Conclusion: Since TAE(0.79) < TEA(1), the method is acceptable.

Total Analytical Error (TAE)?

Challenges and Best Practices in TAE Evaluation

Challenges

- Selecting appropriate TEA limits for complex matrices.
- Balancing sensitivity with precision in low-concentration analytes.
- Addressing variability introduced by operator or equipment changes.

Best Practices

- Define Clear TEA Limits: Align with product-specific and regulatory requirements.
- Perform Rigorous Testing: Use multiple replicates and conditions to ensure reliability.
- Iterative Optimization: Adjust method parameters to minimize bias and imprecision.

Leveraging Prior Knowledge for Platform Validation Across Development Stages

Role of Prior Knowledge

Preclinical/IND Stage

Utilizing prior knowledge to minimize experimental burdens in early stages



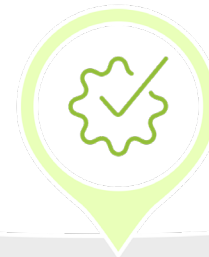
Phase 1 and Phase 2

Using historical data to optimize validation protocols



Phase 3/Commercial

Ensuring method reliability through thorough validation or verification



Regulatory Considerations for Analytical Platforms

Expectations for Analytical Platforms



Suitability

Assess the appropriateness for specific products.



Key Parameters

Focus on accuracy, precision, and specificity.



Lifecycle Management

Monitor continuously after product approval.



Justification

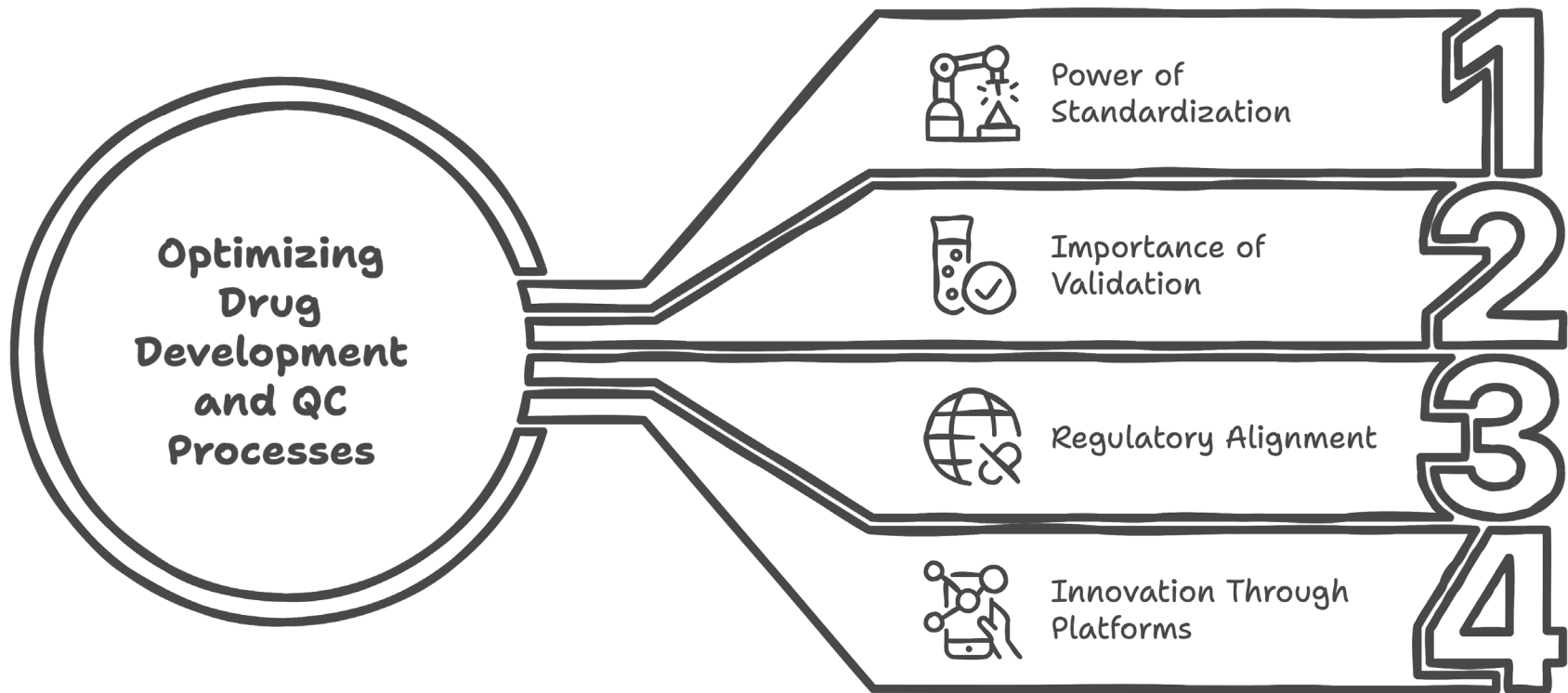
Provide data to support any changes.



Consistency

Ensure reproducibility across various drug products.

Take Home Message



Thank You for Your Attention!



Transforming Quality into Excellence

"Together, we pave the way for reliable and innovative drug solutions"