Controlling Variability to Increase Accuracy in the BET Assay

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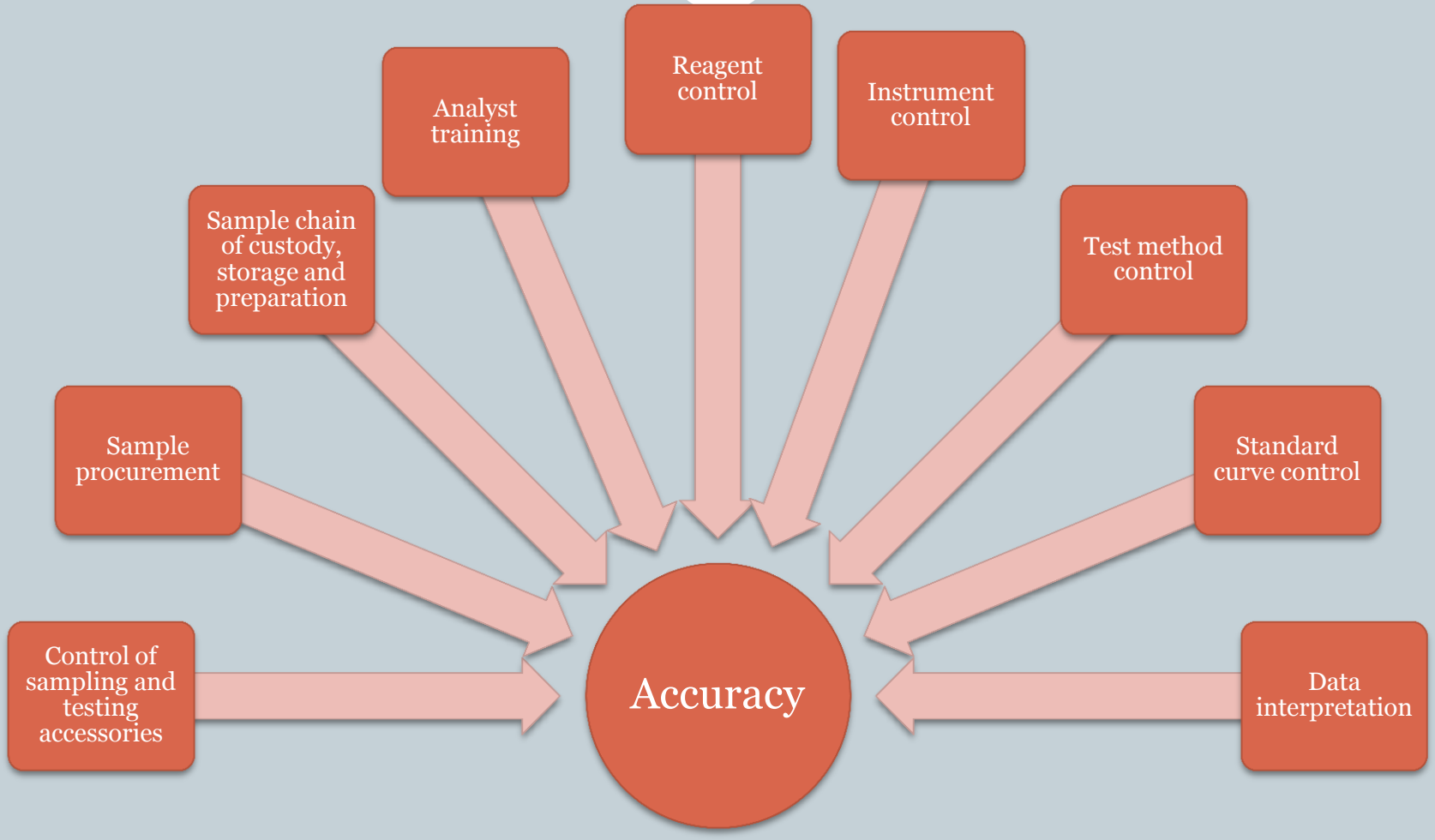
Definition of Accuracy

The degree to which the result of a measurement, calculation, or specification conforms to the correct value or a standard.

VARIABILITY in the BET assay affects accuracy

What Affects Accuracy in a BET Assay?



Control of Sampling and Testing Accessories

- Depyrogenated sampling equipment
  - Dry heat for glass bottles/metal scoops
  - Demonstration of lack of interference for plastics
- Testing accessories must be shown to be free of interference (plastics) – Reference USP <85>
  - Detectable endotoxin
  - Leachables
  - Assign a low limit and test according to AAMI ST72 or USP <161> (medical devices)
  - Polystyrene is usually OK. Polypropylene can pose problems
  - No requirement for spike/recovery in USP <85>, <161>, or AAMI ST72
  - Manufacturer's CoA should be confirmed

Sample Procurement

- Sample vessels are non-interfering
- Sample (water, in process product stream, raw materials) procured aseptically as not to introduce contamination
  - Clear training and supervision for samplers

Sample Chain of Custody, Storage, Preparation

- For non-steriles, transport in a manner that will not support microbial proliferation
  - Validate time/temperature of transport/hold time
- Validate storage conditions
- Always mix/vortex any sample prior to testing and between dilutions to assure that endotoxin is distributed
  - Especially important when working with CSE “spikes” because of the natural aggregation of the purified LPS
  - Important as well for natural endotoxin in samples, even though the surrounding cell wall components help to keep the endotoxin in solution

Analyst Training

- Assay is hugely analyst/technique dependent
- Need training in
  - Aseptic handling of samples during preparation
  - Preparing serial dilutions/special sample preparation
  - Pipetting
    - Serological and mechanical pipettors - technique
  - Analysis of data – when are tests invalid and when are they OOS?
- Best to have a combination of
  - “Book” training (theory and practical matters)
  - SOP training
  - Demonstration of competency

Reagent Control

- Confirm sensitivity of reagents
- Be attentive to manufacturer's expiration dates – don't re-invent reagent stability
- Storage of diluted standards should be validated
  - Storage containers
  - Storage temperature
  - Length of storage

Instrument Control

- Instruments must be properly qualified
  - Water baths/heat blocks mapped for heat distribution
  - Incubating plate/tube readers checked for temperature distribution and control, optics, data collection/transfer, data management, calculations
    - Some manufacturers calculate averages off of raw onset times, others off of test results
  - Pipettors must be calibrated. For example, for a 10µL spike, if the pipettor is off by 20%, there could be a significant impact on reported spike recovery

Test Method Control

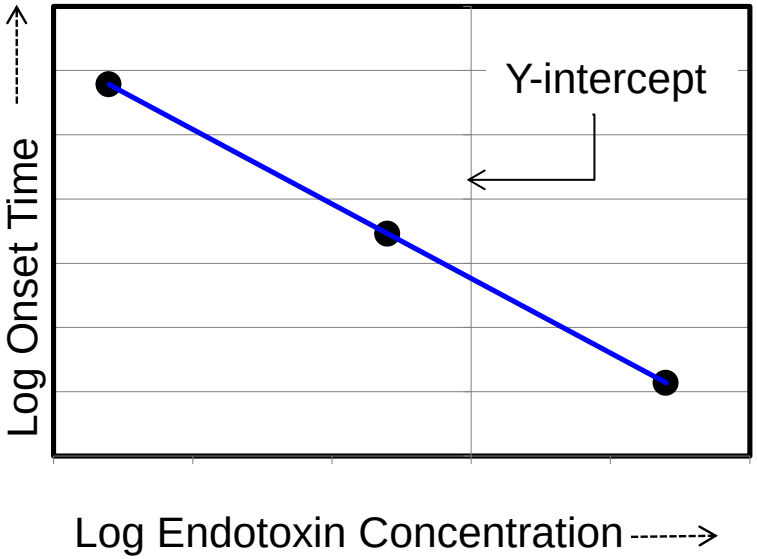
- Suitability testing (inhibition/enhancement)
  - Per compendia (BET chapter is harmonized)
  - Understand your product – many conditions can affect the aggregation of endotoxin in solution
    - Lack of spike recovery may require a change in lysate vendor and/or test method
    - Lack of spike recovery may require treatment of the sample (heat, ultrafiltration, etc)
  - Keep abreast of dosing or administration changes as reflected in the package insert that could affect the endotoxin limit
    - If the endotoxin limit and MVD change as the result of the PI change, the method may need re-qualification

Standard Curve Control

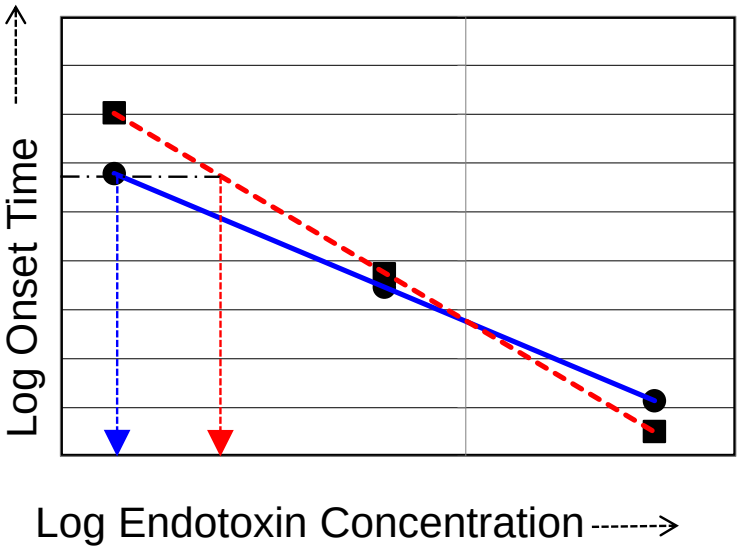
- Attributes that affect accuracy
  - Slope
  - Y-intercept
  - Linearity
  - % cv

Standard Curve Control

"Perfect" curve

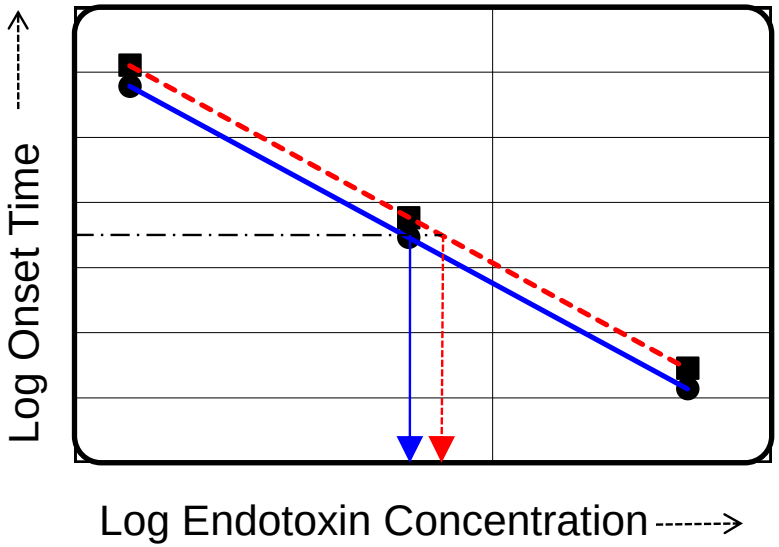


Change in slope



Standard Curve Control

1% change in y-intercept



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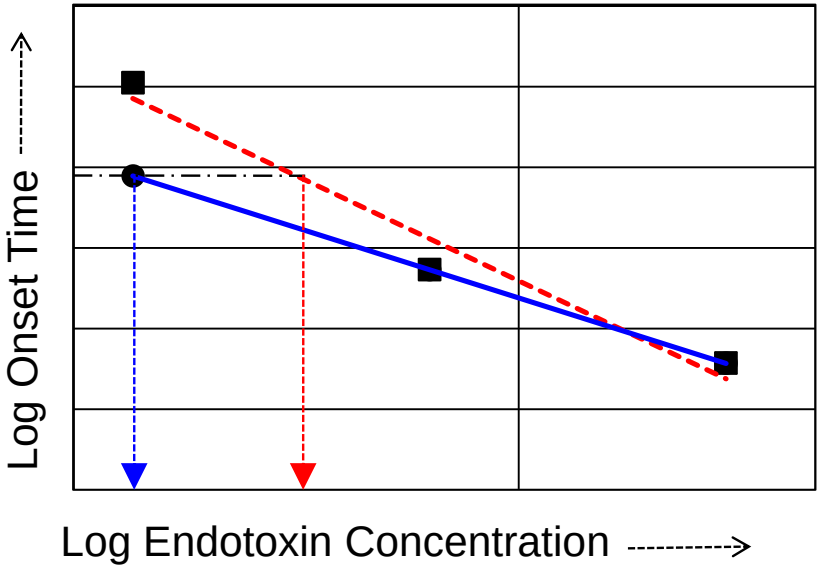

Data Interpretation

- What is an invalid test and what is OOS?
  - What happens on a quantitative test where one replicate is over the limit and one is under the limit and the average is OK?
- Trend invalid tests to identify issues in the laboratory CAPA
  - By product
  - By invalidity
  - By analyst
- Trend test results to identify adverse trends in manufacturing

Acme Pharmaceuticals 2012 BET Excursions and Invalid Tests

Date	Problem	Lysate Lot	Analyst	Product	correlation
1/1/12	invalid spike	XYZ123	BA	WFI port 2	0.991
1/15/12	OOS	XYZ123	KS	holding tank	0.998
2/12/12	void curve	XYZ123	BA	n/a	0.979
2/14/12	hot well	XYZ123	CC	NSA	0.999
2/22/12	invalid spike	XYZ123	BA	lipid emulsion	0.995
3/17/12	OOS	XYZ123	CC	ampicillin	0.999
4/1/12	invalid spike	XYZ123	BA	NSA	0.986
4/5/12	negative cont	XYZ123	MG	NaCl	0.996
5/1/12	hot well	XYZ123	CC	WFI port 2	0.999
5/9/12	OOS	XYZ123	BA	holding tank	0.997
5/29/12	invalid spike	ABC234	BA	Ringer's	0.993
6/8/12	invalid spike	ABC234	MG	lipid emulsion	0.999
6/14/12	hot well	ABC234	CC	recrystallized	0.986
7/5/12	OOS	ABC234	MG	holding tank	0.999
8/30/12	missed well	ABC234	KS	ampicillin	0.994
9/3/12	void curve	ABC234	BA	Ringer's	0.976
9/13/12	negative cont	ABC234	KS	WFI port 2	0.998
10/7/12	hot well	ABC234	CC	NaCl	0.999
10/31/12	invalid spike	ABC234	BA	holding tank	0.991
11/5/12	invalid spike	ABC234	CC	lipid emulsion	1.000
11/25/12	OOS	ABC234	KS	holding tank	0.998
12/15/12	negative cont	ABC234	CC	NSA	0.997
12/31/12	void curve	ABC234	BA	NaCl	0.975

Analysis: Acme Pharmaceuticals 2012 BET Excursions and Invalid Tests. Sorted by problem and analyst

Date	Problem	Lysate Lot	Analyst	Product	Correlation
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Thank You!!!!!

QUESTIONS?