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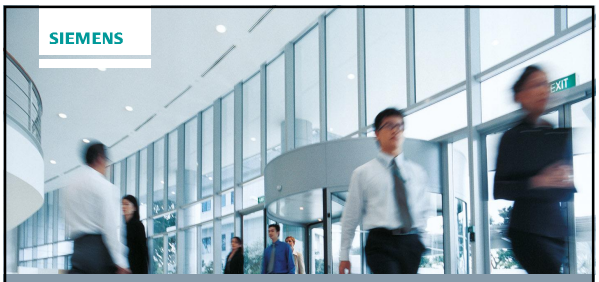

Verifying New Reagent Lot Performance

Julianne Addison
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Answers for life.

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Objectives

- Describe issues relating to changing reagent lots
- Discuss the reasons QC material cannot be relied on for evaluating new reagent lots
- Describe the new CLSI guideline EP26
- Outline steps to verify performance of a new reagent lot

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Lot-to-Lot Variation Is a Fact

- In spite of quality efforts by manufacturers, new reagent lots can occasionally exhibit different performance
- Regulatory and accreditation organizations mandate verification of new lot performance
- To date, there has not been a standard protocol to verify new reagent lot performance

Letters

Letters to the Editor

Validating New Reagents:
Roadmaps Through the Wilderness

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Abstract

[1]Martindale, et al., Validating New Reagents: Roadmaps through the Wilderness, LabMed, 37, 2006, 347-351

"We QC each new lot, isn't that enough?"

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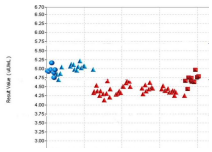
QC material does not interact with reagents exactly the same way as fresh patient samples

- Often QC material shows change in performance with a new reagent lot when no change is noted with patient samples
- Sometimes QC material shows no change, but patient samples do

Why?



What's a "matrix" ?

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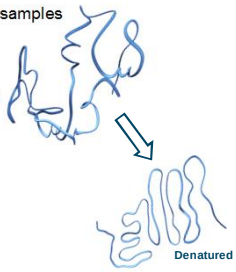
Matrix: everything in the sample except what we are measuring

- QC sample matrix different from patient samples
  - Matrix proteins in particular
  - Matrix modified in manufacture
- Proficiency Testing (PT), EQA, Linearity and other manufactured samples also have an altered matrix



Matrix Modified

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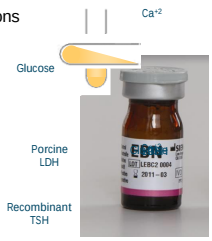
Pool serum

Remove selected analytes

Add analytes back at target concentrations

Stabilize for storage



Matrix effects on methods

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Methods sensitive to sample matrix

Examples:
Immunoassays
Enzyme activity assays

Measured value impacted by altered sample matrix

Matrix effects may change in new reagent lot

Reagents contain multiple biologic components

One or more components may be different in a new reagent lot

Impact on QC results varies by

- Method
- Lot
- QC material

Matrix Interactions Vary Between QC Materials

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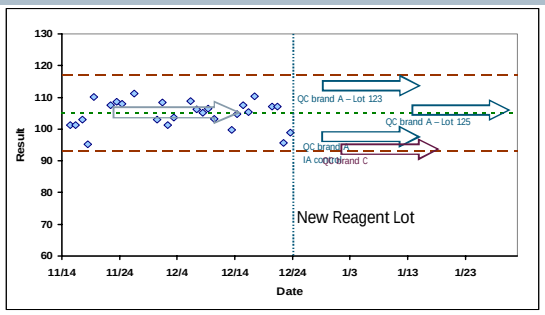

Abbreviations: IA, immunoassay; QC, quality control.

Managing Lot to Lot – verifying result quality

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Demonstrate lack of impact using patient samples

CAP requires validation before using new lot:

CHM.12900: Are new reagent lots and/or shipments validated before or concurrent with use for patient testing ?

NOTE: Good clinical laboratory science includes patient based comparisons when possible

Test patient samples with old and new reagent lots

- Does not require large numbers of samples
- Can evaluate based on clinical judgment
- Decide if lot to lot difference is significant

What is an acceptable lot to lot difference for patient results ?

What is significant lot to lot difference?

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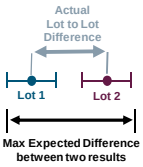
A single criteria like “Less that 10%” will not work

Typically, comparison done by testing patient samples once with each lot

- Must take into account method imprecision
- Since imprecision varies by method, no single criteria will work for all

Should be based on clinical significance



Verifying Lot to Lot result quality

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The challenges

- Starting a new reagent lot is frequent event
- Can happen at any time on any day
- Often there is very little of the “current” lot left
- Must be done quickly
- Little time to find necessary patient samples
- Limited resources available

Protocol used needs to

- **Be simple to perform when needed**
- **Require few resources or samples**
- **Provide a quick screen to detect clinically significant differences**

New CLSI Guideline

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*EP26-A User Evaluation of Between-Reagent Lot Variation

- Intended to provide laboratories with a practical tool to ***screen*** new reagent lots for difference in performance
- Is useful within typical constraints of the clinical lab
- Designed to use minimum number of fresh patient samples
  - Required number of samples determined by…
    - Size of the critical lot to lot difference
    - Imprecision of the measurement procedure
    - Goal for probability of detecting the critical difference
  - May use as few as three patient samples

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EP26 – two part protocol

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Set up – needs time and thought

- Decisions and calculations ***made in advance***
- Determines the number of samples to be tested
- Generally, done one time for each analyte
- Create summary checklist or spreadsheet for routine use

Actual evaluation of a new lot – simple & quick

- Readily performed when needed
- Simple protocol, simple arithmetic
- Clear criterion for acceptance

EP26 – Part 1 - Set up

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Need to determine

- **Critical Difference (CD)**

- Maximum acceptable difference between lots
- Based on clinical use of analyte*
  - Total Allowable Error cab be basis
- CD will be unique for each analyte
- Smaller CD requires more samples

*1999 Stockholm Conference:
Kenny D, Fraser CG, Hyltoft Petersen P, Kallner A. Strategies to set global analytical quality specifications in laboratory medicine. Consensus agreement. *Scand J Clin Lab Invest*. 1999;59:585

Determining Critical Difference

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Total Allowable Error

RE **CD**

- Random Error (RE) = imprecision – e.g. 2 * SD
- Critical Difference (CD)
  - CD/SD determines number of samples that need to be tested
  - CD/SD ~ 3 may need 3 samples
  - CD/SD ~1 may need 22 samples

EP26 – Part 1 - Set up

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Need to determine

- Critical Difference (CD)
- **Medical decision concentration(s)**

- May not be practical to obtain fresh patient samples that span measuring interval
- Focus on concentrations that are medically important
- Typically use concentration interval near decision point
- Typically look at 1 – 3 concentration intervals

EP26 – Part 1 - Set up

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Need to determine

- Critical Difference (CD)
- Medical decision concentration(s)
- **Historical precision performance**

- Necessary to account for contribution of imprecision to verification of new lot
- Three suggested sources
  - Manufacturers documentation
  - Historical data from routine QC testing
  - Studies performed for this purpose
- At each concentration need
  - S_R – Repeatability (aka within run)
  - S_{WRL} – Within lot total precision
- Poorer precision requires more samples

EP26 – Part 1: Setup (cont'd)

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Need to determine

- Critical Difference (CD)
- Medical decision concentration(s)
- Historical precision performance
- **Desired statistical power**

Statistical power determines probability of detecting CD

Example: Power = 0.9
- Implies 90% probability of detection

Goal for power depends on clinical use of results
- Critical analytes may warrant 0.95
- For other analytes, 0.80 may be acceptable

Higher power requires more samples

For example, with all other factors constant…
- To achieve power = 0.80 may require 2 samples
- To achieve power = 0.95 may require 13 samples

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Example: Setup Data Summary

Analyte (units)	Target Concentration (range)	CD	S_{WRL}	S_R	Desired Power	CD/S_{WRL}	S_R/S_{WRL}
Glucose (mg/dL)	50 (40–60)	4	1	0.6	0.9	4.0	0.60
	150 (150–200)	11	2	1.5	0.9	5.5	0.75
	300 (300–400)	9	4.5	3.5	0.9	4.7	0.78
AST (IU/L)	40 (30–50)	4	1.3	0.8	0.8	3.2	0.62
	200 (200–400)	26	4.1	1.3	0.8	6.3	0.32
Na (mmol/L)	140 (130–150)	2.4	0.8	0.4	0.8	3.0	0.5
TSH (mIU/L)	0.35 (0.3–0.6)	0.04	0.017	0.017	0.8	2.1	1.0
	5.4 (3–6)	0.68	0.2	0.16	0.8	3.4	0.8

Abbreviations: CD, critical difference; AST, aspartame transaminase; Na, sodium; S_R, repeatability (within-run imprecision); S_{WRL}, within-reagent lot imprecision; TSH, thyroid-stimulating hormone.

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EP26 – Part 1 - Set up

Need to determine

- Critical Difference (CD)
- Medical decision concentration(s)
- Historical precision performance
- Desired statistical power
- **Rejection limit**
- **Number of samples (at each concentration)**

- Use EP26 Tables in Appendix A to obtain Rejection limit and number of samples
- Choice of table depends on how many different concentration intervals will be evaluated

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Using the Tables

Example: Evaluating two medical decision concentrations

$CD/S_{WRL} = 4.5$ $S_R/S_{WRL} = 0.85$

Desired statistical power = 0.9

Table A2. Continued)

		Number of Samples to Compute Mean Difference (False Rejection Rate, Power if True Difference = CD) Rejection Limit for Mean Difference				
CD/S_{WRL}	S_R/S_{WRL}	0.90•CD	0.80•CD	0.70•CD	0.60•CD	0.55•CD
4.5	0.95	1 (0.004, 0.625)	1 (0.011, 0.738)	2 (0.003, 0.901)	2 (0.010, 0.957)	2 (0.018, 0.973)
4.5	0.90	1 (0.004, 0.625)	1 (0.011, 0.738)	2 (0.004, 0.892)	2 (0.013, 0.951)	2 (0.023, 0.968)
4.5	0.85				2 (0.017, 0.944)	3 (0.015, 0.977)
4.5	0.80	1 (0.004, 0.625)	1 (0.011, 0.738)	2 (0.007, 0.876)		971)
4.5	0.75	1 (0.004, 0.6				970)
4.5	0.70	1 (0.004, 0.6		0.864)	020, 0.940)	023, 0.967)
4.5	0.60	1 (0.004, 0.625)				934)
4.5	0.50	1 (0.004, 0.625)	1 (0.011, 0.738)	2 (0.01		
4.5	0.40	1 (0.004, 0.625)	1 (0.011, 0.738)	2 (0.02		
4.5	0.30	1 (0.004, 0.625)	1 (0.011, 0.738)	2 (0.023, 0.836)	—	—

$2(0.017, 0.944)$

Number of samples at each concentration

false positive rate

Statistical power

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Set up complete

Summarize the information

Analyte (units)	Target Concentration (range)	CD	S_{WRL}	S_R	Desired Power	CD/S_{WRL}	S_R/S_{WRL}	Rejection Limit	Number of samples	Power Achieved
Glucose (mg/dL)	50 (40–60)	4	1	0.6	0.9	4.0	0.60	0.7*CD	9	0.848
	150 (150–200)	11	2	1.5	0.9	5.5	0.75	0.6*CD	2	0.967
	300 (300–400)	9	4.5	3.5	0.9	4.7	0.78	0.6*CD	3	0.946
AST (IU/L)	40 (30–50)	4	1.3	0.8	0.8	3.2	0.62	0.9*CD	5	0.599
	200 (200–400)	26	4.1	1.3	0.8	6.3	0.32	0.7*CD	1	0.898
Na (mmol/L)	140 (130–150)	2.4	0.8	0.4	0.8	3.0	0.5	0.9*CD	2	0.590
TSH (mIU/L)	0.35 (0.3–0.6)	0.04	0.017	0.017	0.8	2.1	1.0	0.6*CD	7	0.933
	5.4 (3–6)	0.68	0.2	0.16	0.8	3.4	0.8	0.6*CD	9	0.934

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Set up complete

Summarize the information

Create guide for routine use

This is what will be used each time a new lot needs to be evaluated

Analyte	Concentration Interval	# Samples	Rejection Limit
Glucose	40-60 mg/dl	9	2.8
	150-200 mg/dl	2	6.6
	300-400 mg/dl	3	12.6
AST	30-50 IU/L	5	4
	200-400 IU/L	1	18
Na	130-150 mmol/L	2	2
TSH	0.3– 0.6 mIU/L	7	0.021
	3-6 mIU/L	9	0.41

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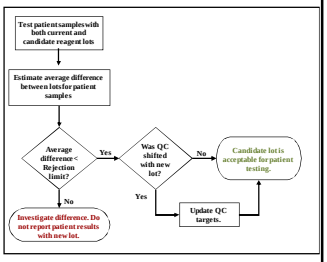
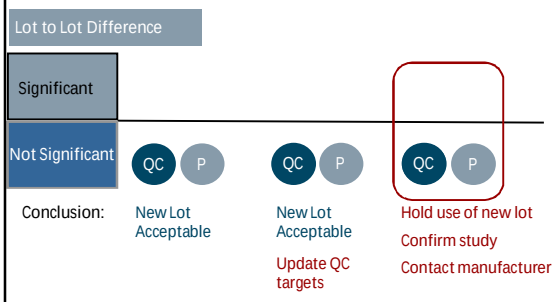
EP26 – Part 2 - Actual new lot evaluation

For each new reagent lot:

1. Locate the specified number of fresh patient samples within the concentration interval(s)
2. Measure the samples using both reagent lots
3. Calculate the average difference between lots for each concentration
4. Compare absolute value of average difference to rejection limit



Evaluating Lot to Lot Difference



EP26 also contains

- Guidance for situations where fresh patient samples are not practical
- Guidance for situations where desired goals cannot be practically achieved
- Suggestions for follow up if a significant difference is detected
- Discussion of multi-system and multiple location laboratory environments

By design, EP26 is …

- **Not** intended to characterize difference between lots
- **Not** for monitoring long term trends across multiple lots
- **Not** for investigating root cause of lot to lot differences
- **Not** the ultimate tool to address all issues concerning lot to lot performance

EP26 is …
an effective, practical tool to screen new reagent lots for clinically significant changes in performance with patient samples

Summary

- Verifying new reagent lot performance is an important part of laboratory quality assurance
- Most common change that occurs with a new reagent lot is seen in QC material only and is matrix related
- Effective screening of new reagent lots is best done using fresh patient samples
- CLSI EP26 provides a standardized protocol for verifying new lot performance
- EP26 protocol is practical for typical routine laboratory environment
- There is more to be done in monitoring lot to lot performance, especially long term changes