The Art of Quality Control, Selected Topics

Laboratory Education Expo, April 2019

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Objectives

- How to reliably derive QC statistics
- Use resources to determine correct data configuration
- Explain selected Westgard Rules and their behavior
- Consider different ways to capture blood bank, microbiology and infectious disease QC

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Most FAQ…Why Does QC Matter?

Point-of-care testing for HIV: HIV counselling and testing

BL Johnston, MD FRCPC[1] and JM Conly, MD CCFP FRCPC FACP[2]

HIV Testing After a First Positive Rapid Diagnostic Test: A Role for Nucleic Acid Testing?

Anne M Neilan ✉, Jennifer E Cohn, Jean-Francois Lemaire, Emma Sacks, Rebecca Alban, Kenneth A Freedberg, Rochelle P Walensky, Andrea L Ciaranello

Open Forum Infectious Diseases, Volume 5, Issue 8, August 2018, ofy170, https://doi.org/10.1093/ofid/ofy170

Published: 29 August 2018 **Article history ▾**

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Arriving at Decision Limits - New Control Testing

•Account for Variability
•Detect and Eliminate Outliers
•Calculate a +/- 2 SD Range (~ 95% CI*)

*CI = Confidence Interval

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Initial Control Value Testing

Philosophy: Have as much variety in test conditions as seen in daily testing

- Personnel
- Testing Parameters
  - **Reagents**
  - **Calibrations**
  - **Environment (Temperature & Humidity)**
  - **Electrical Supply**

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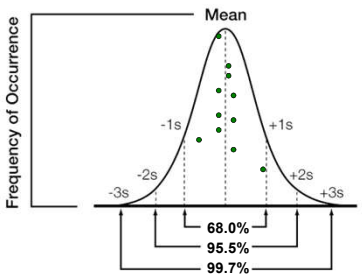
Gathering Data Points



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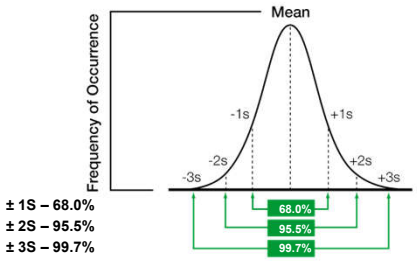
Gaussian Distribution – the Key



± 1S – 68.0%
± 2S – 95.5%
± 3S – 99.7%

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Coefficient of Variation [CV], Uses

Compare methods, reagents and instruments under similar conditions

Analyte **Sample** **Level of Analyte**

Use to Compare:

- Proficiency Survey Results
- Manufacturer's Claims
- Monthly QC Peer Group Reports
- Your Own Historical Performance

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Effect of Mean Value on CV

HDL (mg/dL)			
n	80	80	80
Mean	20.6	51.4	128.7
Within run SD	1.1*	SD Similar	1.4*
Within run CV (%)	5.5	2.6	1.1
Total SD	1.4	1.4	2.8
Total CV (%)	6.9	2.8	2.2

* SDs similar

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Effect of Mean Value on CV

HDL (mg/dL)			
n	80	80	80
Mean	20.6	51.4	128.7
Within run SD	1.1	1.3	1.4
Within run CV (%)	5.5*	CV 5x ↑	1.1*
Total SD	1.4	1.4	2.8
Total CV (%)	6.9	2.8	2.2

* CVs 5X different due to different means

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Coefficient of Variation Ratio [CVR]*

Calculation

$$\frac{\text{Lab's Monthly CV}}{\text{Peer Monthly CV}}$$

Ideally, CVR ≤ 1.0, since your values are from a single lab, while the peer CV is from several.

If CVR = 1.5 to 2.0, the lab is 50-100% less precise than its peer group, usually requiring investigation.

*Also known as CVI, Coefficient of Variation Index

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Coefficient of Variation Ratio [CVR]

Calculation

$$CVR = \frac{5.0}{5.0} = 1.0$$

Your imprecision is same as peers

$$CVR = \frac{10.0}{5.0} = 2.0$$

- What is the probability? 1 out of ? Labs?
- Can the CVR be too 'small'? Answer later

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Standard Deviation Index, SDI

•Calculation:

•SDI = $\frac{\text{[Lab Mean – Peer Group Mean]}}{\text{Peer Group's 1 standard deviation}}$

•Use to assess bias compared to peer group

TARGET SDI = 0.0, so lab's mean value is the same as the peer's ➡ NO bias

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Standard Deviation Index, SDI

CALCULATION

SDI = $\frac{100 - 100}{1.0} = 0.0$

Your mean has no bias against peers

SDI = $\frac{120 - 100}{10.0} = 2.0$

What is the probability? 1 out of ? Labs?

Can the SDI be too small? Answer later.

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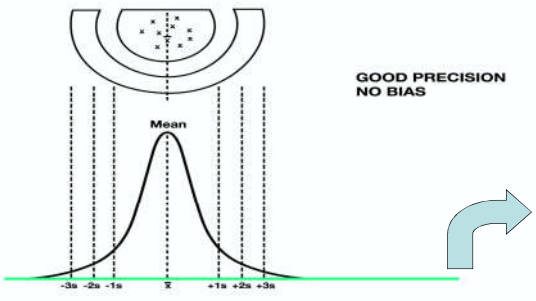
Textbook Example #1 – Imprecision versus Bias



GOOD PRECISION
NO BIAS

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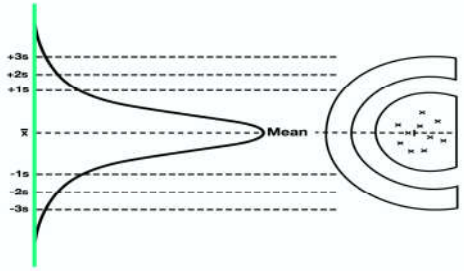
Levey-Jennings



Levey-Jennings Chart

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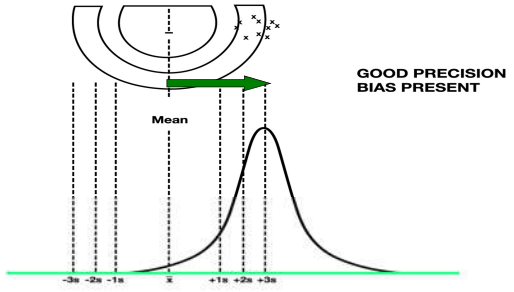
Textbook Example #2, Bias present



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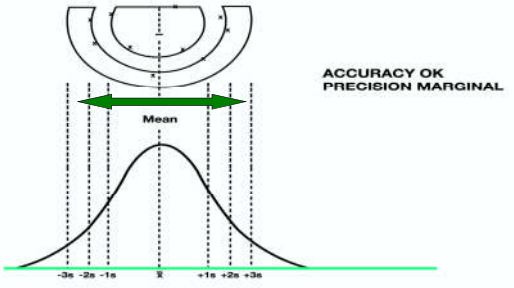
Textbook Example #3, Imprecision present



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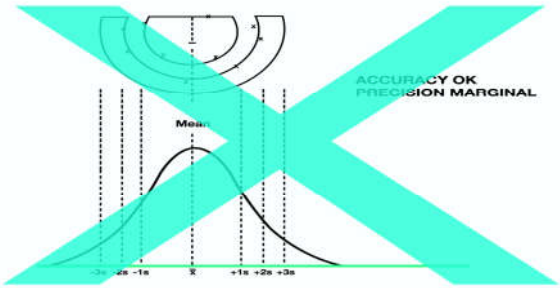
Imprecision Present – Bias Present?



For troubleshooting, verify precision first

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Traditional Calculation of +/- 2SD Range

Calculation of A 95% CI

Estimation of a 2s Range for a small sample by calculation a 95% confidence interval.

Data Set: Level II — Fibrinogen

408	397	400	400
402	393	391	389
401	403	393	385
401	404	393	389
394	404	389	387
402	418	385	392
392	400	411	
392	388	398	

n = 30
$\bar{x}$ = 396.7
s = 7.94
2s range = 380.8 – 412.6

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Traditional Calculation of +/- 2SD Range

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Estimation of a 2s Range for a small sample by calculation a 95% confidence interval.

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402	393	391	389
401	403	393	385
401	404	393	389
394	404	389	387
402	418	385	392
392	400	411	
392	388	398	

CV = 7.94/396.7 x 100% = 2.0%

n = 30
$\bar{x}$ = 396.7
s = 7.94
2s range = 380.8 – 412.6

IF +/- 2 SD, total SD = 4. Divide by 4 to find 1 SD.
(1 SD/mean)/100% = CV%. Compare with Insert Range

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In-Lab Resources

- Manufacturer Kit / Reagent Insert
- Reports, Often Online
- Your Own Historical Data

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Reading an Insert - Imprecision

INSTRUCTIONS FOR USE
Performance Characteristics

GLU
Glucose

Instrument Models

Sample:
- **Serum**
- **Urine**
- **CSF**

Different Conc.

Serum

	Conventional Units (mg/dL)			SI Units (mmol/L)					
	Mean Conc.	Within Day SD*	Within Lab SD**	Mean Conc.	Within Day SD*	Within Lab SD**	Within Lab CV%**	No. Observ.	No. Days
250	86	0.5	1.5	4.8	0.03	0.08	1.7	77	20
	286	1.4	4.1	15.9	0.08	0.23	1.4	78	20
5,1 FS†	83	0.4	1.2	4.6	0.02	0.07	1.5	85	21
	292	1.1	3.5	16.2	0.06	0.20	1.2	88	22
5600††	75	0.6	1.0	4.2	0.03	0.06	1.3	88	22
	284	1.2	3.7	15.8	0.07	0.21	1.3	88	22

Urine

	Conventional Units (mg/dL)			SI Units (mmol/L)					
	Mean Conc.	Within Day SD*	Within Lab SD**	Mean Conc.	Within Day SD*	Within Lab SD**	Within Lab CV%**	No. Observ.	No. Days
250	44	0.3	0.4	2.5	0.02	0.02	0.9	88	22
	77	1.1	1.5	4.3	0.06	0.08	1.9	84	21
	232	2.9	4.7	12.9	0.16	0.26	2.0	88	22
	278	2.0	3.8	15.4	0.11	0.21	1.4	88	22
5,1 FS†	26	0.2	0.3	1.5	0.01	0.02	1.2	88	22
	291	2.1	3.9	16.1	0.11	0.22	1.3	80	22
5600††	28	0.3	0.5	1.6	0.02	0.03	1.8	84	21
	294	2.8	4.0	16.3	0.16	0.22	1.4	84	21

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Look for Imprecision Claims

Within Day versus Within Lab

Within Day = fewer variables = best precision

Within Lab = more time & variables

Serum

	Conventional Units (mg/dL)		
	Mean Conc.	Within Day SD*	Within Lab SD**
250	86	0.5	1.5
	286	1.4	4.1
5,1 FS†	83	0.4	1.2
	292	1.1	3.5
5600††	75	0.6	1.0
	284	1.2	3.7

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In-Lab Resources, Manuf Controls

- Manufacturer Controls, Bio-Rad Mfr Reports

Roche/Hitachi **cobas c** 502 analyzer

Value sheet

Short name / component	Methods	ACN	Value	Range	1s	Unit
NH3L Ammonia	enzymatic	8478	227 397	182 - 272 309 - 465	15 26	µmol/L µg/dL

CV = (15/227) x 100% = 6.6%

Unity

Manufacturer Report for Roche

Ethanol/Ammonia • Lot 54270 • Exp 31-Oct-2020

CV ~ 3-5% at comparable concentration

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Look for Imprecision Claims

Precision Roche NH3L, 'c' models

Precision was determined using human samples and controls in an internal protocol with repeatability* (n = 21) and intermediate precision** (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

*Repeatability**	*Mean* µmol/L (µg/dL)	*SD* µmol/L (µg/dL)	*CV* %
AEC Control N	60.7 (103)	1.4 (2)	2.3
AEC Control A	202 (344)	2 (3)	0.8
Human plasma 1	28.6 (48.7)	2.5 (4.3)	8.8
Human plasma 2	585 (996)	1 (2)	0.2
*Intermediate precision***	*Mean* µmol/L (µg/dL)	*SD* µmol/L (µg/dL)	*CV* %
AEC Control N	56.9 (97.1)	2.8 (4.8)	4.9
AEC Control A	203 (346)	4 (7)	1.8
AEC Control N 1:2 dil.	28.1 (47.7)	2.6 (4.4)	9.4
AEC Calibrator	318 (542)	5 (9)	1.5

* repeatability = within-run precision

** intermediate precision = total precision / between run precision / between day precision

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Group Values by Method

Unity **Worldwide Report**

Unassayed Chemistry • Lot 31880 • Exp 31-May-2020

Glucose Hexokinase mg/dL

Beckman Coulter AU Series

	Level 1 Mean	Level 1 Mon	Level 1 Cum	Level 2 Mean	Level 2 Mon	Level 2 Cum
Mean	85.03	85.08		283.8	283.8	
SD	1.82	1.85		5.75	6.37	
CV	2.1	2.2		2.0	2.2	
# Points	5333	25491		5327	25800	
# Labs	137	154		138	155	

Beckman AU — 85 / 284 mg/dL

Roche cobas 6000/8000/c 311

	Level 1 Mean	Level 1 Mon	Level 1 Cum	Level 2 Mean	Level 2 Mon	Level 2 Cum
Mean	84.63	84.78		282.4	282.9	
SD	1.61	1.70		5.08	5.30	
CV	1.9	2.0		1.8	1.9	
# Points	16945	84266		16667	83786	
# Labs	336	389		334	387	

Roche cobas — 85 / 283 mg/dL

Method Group • Hexokinase

	Level 1 Mean	Level 1 Mon	Level 1 Cum	Level 2 Mean	Level 2 Mon	Level 2 Cum
Mean	84.49	84.71		281.6	282.4	
SD	2.20	2.18		6.13	6.23	
CV	2.6	2.6		2.2	2.2	
# Points	39294	209K		38941	209K	
# Labs	913	1044		913	1042	

Hexokinase — 85 / 282 mg/dL

Group Values by Method

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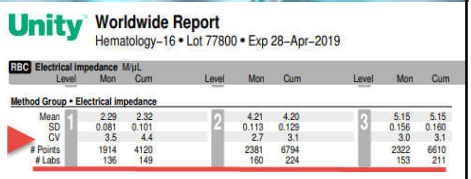
Group Values by Method, Hematology

Models from Abbott to Sysmex



Hundreds of labs, different manufacturers/models, and **%CV < 5%**

ALL using method ELECTRICAL IMPEDANCE

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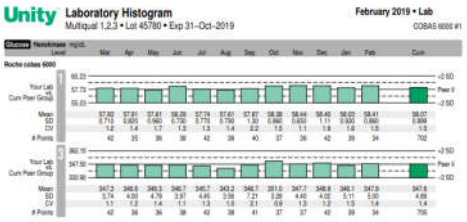
Your historical performance



Your historical performance to use for reference

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Use historical CV to set new ranges

Coefficient of Variation, CV

Measure of variability (random error or imprecision)

Expressed as a % value

CALCULATION: $\frac{\text{1 standard deviation, (s)}}{\text{Mean Value}} \times 100\%$

Example: Fibrinogen $\frac{\textbf{28} \text{ mg/dL}}{280 \text{ mg/dL}} \times 100\% = 10.0\%$ CV

CV REMAINS the same; SD CHANGES to match new mean; so, now 28 mg/dL is a reasonable 1 SD value

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Good initial control testing – WHY?

Establishing Data Parameters

Determination of valid mean and standard deviation values are crucial to successful data acceptance and data rejection by error detection schemes

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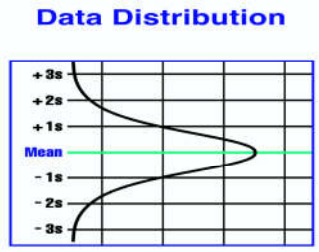
Range Defined After Initial Testing



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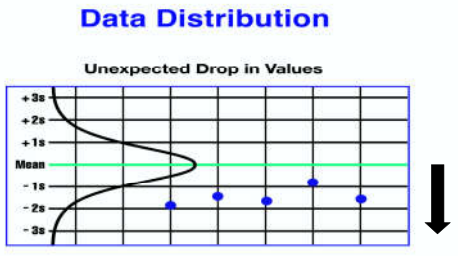
Shift (down) in Values – Systematic Error



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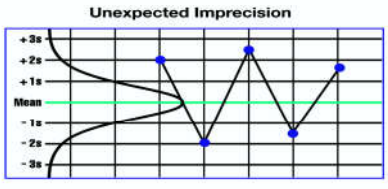
Erratic Values – Random Error

Data Distribution



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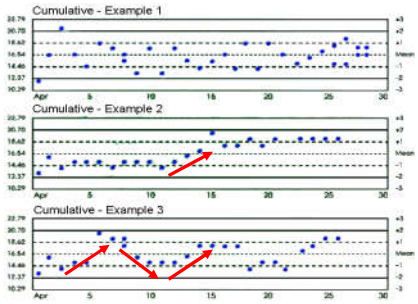
Same Mean, 1 SD and CV: Different Stories

'normal'

increase ~ 11th

cyclical



In a month of results, do YOU see any values > 2SD?

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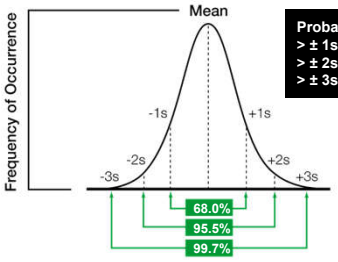
Gaussian Distribution

± 1S – 68.0%
± 2S – 95.5%
± 3S – 99.7%

1. Probability
2. Size of Error
3. Important?



Probability
> ± 1s: 1/3
> ± 2s: 1/20
> ± 3s: 1/333

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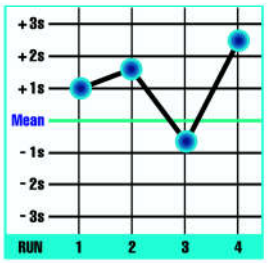
1_{2s} RULE

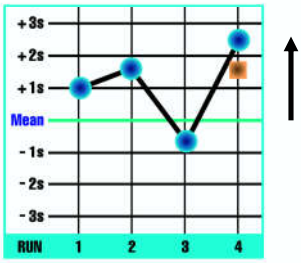
- Warning rule only: look for errors in the current run and in previous runs
- Any control value greater than two standard deviations from the mean
- Does not require run rejection



1_{2s} RULE

- Warning rule only: look for errors in the **current** run and in previous runs
- Any control value greater than two standard deviations from the mean
- Does not require run rejection

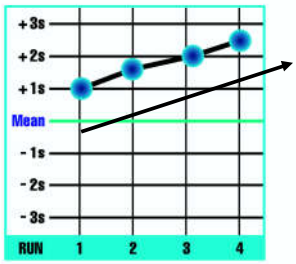
Level 1 Level 2



- **Level 1 & 2 LJ's superimposed**

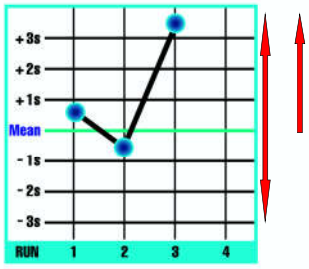
1_{2s} RULE

- Warning rule only: look for errors in the current run and in **previous** runs
- Any control value greater than two standard deviations from the mean
- Does not require run rejection



1_{3s} RULE

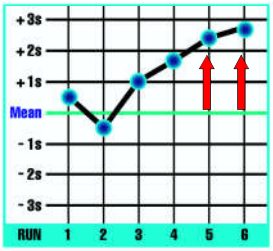
- Any control value greater than three standard deviations on either side of the mean
- Detects principally random error
- May indicate the beginnings of large systematic error
- Applied in a single run
- May be a cause for run rejection



- **1 of 2 Westgard rules for imprecision**

2_{2s} RULE (Across Run)

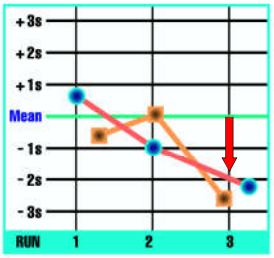
- Two Consecutive Control values each from separate runs between two and three standard deviations from the mean both on the same side of the mean.
- Detects systematic error which is potentially affecting a specific portion of the method curve.
- May be a cause for run rejection



2_{2s} RULE (Within Run)

- Two control values between 2 and 3 standard deviations from the mean, both on the same side of the mean.
- Detects systematic error which is potentially affecting the entire method curve.
- May be a cause for run rejection

Level 1 Level 2

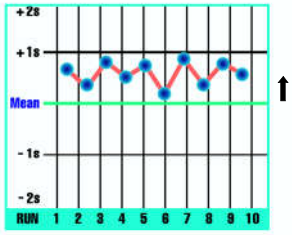


- **Level 1 & 2 LJ's superimposed**

Any labs using this Westgard Rule?

10$\bar{x}$ RULE (Within Control Materials)

- Ten consecutive values for the same level of control on the same side of the mean.
- Indicates a bias in the test system potentially affecting a specific portion of the method curve.
- Usually is not a clinically significant bias.
- Run rejection is not required.



Frequently Asked Questions

My control values do not fall within your package insert range. What should I do?

- **Obtain peer information online**
- **Any changes to instrument, reagents/calibrators, software? Any manufacturer notifications?**
- **Call your QC program**
- **Confirm change affects patients / QC? Values?**

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Bad Habits of Quality Control

- Repeat the Control
  - If **out** 1 out of 20, then **in** 19 out of 20, right?
  - If rules chosen properly, less need to repeat
  - See next section
- Open a New Bottle of Control
  - Proper preparation & storage – training
  - Expensive
- Recalibrate
  - Introduces bias, may mask other problems

From "QC - The Out of Control Problem", Elsa Quam, Westgard.com

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A New View of 1-2s Rule Usage

•Repeat the Control

– If **out** 1 out of 20, then **in** 19 out of 20, right?


Clinical Chemistry 58:5 925-929 (2012)

Should I Repeat My 1:2s QC Rejection?

AACC:

Clinical Chemistry

58:5; pg 925-9

2012

http://clinchem.aaccjnls.org/content/clinchem/58/5/925.full.pdf

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Functional Performance of 1-2s Repeat

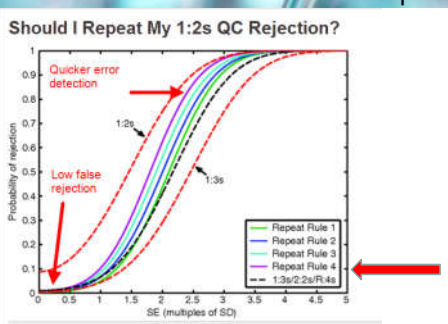

Fig. 1. Power function curves for the 4 repeat sampling strategies described in Table 1 compared to a 1:2s QC rule, a 1:3s QC rule, and a 1:3s/2:2s/R:4s multirule when 2 concentration levels of control material are examined.

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Qualitative Responses Selected Considerations

- **Urinalysis**
  - **Added by Analyte/Instrument/Strip**
    - **In the past, Reagent = Dedicated**
    - **Now, designate dipstick 'name'**
      - **10SG, 9SG = same responses**
      - **May differ when manual vs instrument**
      - **If so, need to create new combo**
      - **May differ geographically**
      - **Would like to compare, if possible**

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Qualitative Responses Selected Considerations

- Microbiology
  - Antimicrobial Susceptibility Testing (AST)
    - Analyte currently ANTIBIOTIC (µg)
    - Method currently agar
      - Considerations for additives, atmosphere, time, and temperature
    - Future to build MIC (Min Inhib Conc), same considerations, but liquid media
    - Etest MIC, various incubations, reporting in ranges of concentrations
    - Specs may differ by source/site of specimen

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Qualitative Responses Selected Considerations

- Microbiology, continued.
  - AST testing scheme, 'Lot' & AMIRT
    - LOT, selected as ATCC (American Type Culture Collection)
    - Analyte, the antibiotic (AST or MIC)
    - Method, the growth conditions
    - Instrument, manual or automated, if significant
    - Reagent, disk or broth manufacturer
    - Temperature, growth condition
    - Unit, quantitative (mm)

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Qualitative Responses Selected Considerations

- ImmunoHematology (IH = Blood Bank) QC
- Covers 3 common tasks: ABO-Rh; Ab Screening/Ab Identification
- Even within ABO-Rh, there are multiple combinations of Ab-Ag testing, especially the Rh subgroups; QC testing of BB materials is done daily
- Initially, the QC will be a documentation of daily QC testing
- Eventually, hope to have applications for troubleshooting marginal reagents and evaluating changes in titer for Ab and Ag

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Qualitative Responses Selected Considerations

- **Infectious Disease Testing**
- **These tests are normally reported qualitatively as Neg/Nonreactive, Pos/Reactive; sometimes Indeterminate.**
- **Reporting standards – per manufacturer – are usually based on Sample/Cutoff ratios, which are measured quantitatively**
  - **'Reactive' may be based on S/CO of > 1.00**
  - **'Reactive' may be based on S/CO of < 1.00**

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Infectious Disease Testing

- How to monitor QC of testing that is reported as Reactive or Non-Reactive?
- Look at the insert:
- First you have RLU's and S/CO:

RESULTS

Calculations

- The ARCHITECT *i* System calculates the cutoff RLU from the mean RLU of the three Calibrator 1 replicates and stores the result. The cutoff RLU is determined by multiplying the Calibrator 1 Mean RLU by 0.40.

  Cutoff RLU = Calibrator 1 Mean RLU x 0.40
- The ARCHITECT *i* System calculates a result based on the ratio of sample RLU to the cutoff RLU (S/CO) for each specimen and control.

  S/CO = Sample RLU/Cutoff RLU

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Infectious Disease Testing

- How to monitor QC of testing that is reported as Reactive or Non-Reactive?
- Look at the insert:
- Consider the interpretation

Interpretation of Results

ARCHITECT HIV Ag/Ab Combo Initial Result		
Initial Result (S/CO)	**Instrument Interpretation**	**Retest Procedure**
< 1.00	NONREACTIVE (NR)	No retest required.
≥ 1.00	REACTIVE (R)	Retest in duplicate.

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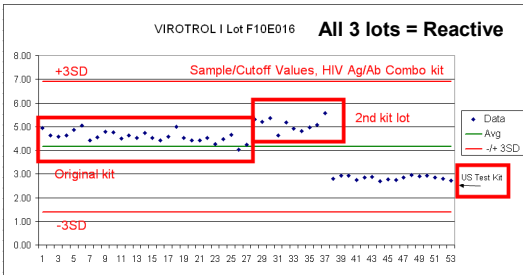
Infectious Disease Testing

- Look at the data. Example LJ:



Mean for Original kit = 4.61; SD = 0.2; CV = 4.29%

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The Art of QC, Selected Topics

Questions?

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