REFERENCE CHANGE VALUE (RCV)

A TOOL FOR INTERPRETING SERIAL LABORATORY RESULTS

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RCV: OBJECTIVES

At the end of this presentation, participants will be able to:

1. Explain the statistical theory that underpins the calculation of the relative (reference) change value (RCV).
2. Identify the inputs needed to calculate the RCV.
3. Propose a research design to generate the inputs needed to calculate the RCV.

RCV: DEFINITION

RCV is the % change in 2 sequential lab results for the same individual that has a specified probability of representing a true change in the state of an individual, as opposed to representing random variation.

$$RCV = \left(\sqrt{2}\right) \times (Z) \times \sqrt{(CVa)^2 + (CVi)^2}$$

CVa = analytical coefficient of variation
Cvi = within-individual coefficient of biological variation
Z = interval of standard deviations (in a Gaussian distribution) between two values that corresponds to a given probability that the difference is due to random variation

RCV: "WHAT PROBABILITY?"

If either an increase or a decrease in a test result is meaningful (i.e. [serum calcium]), and we want the change to have only a 5% chance of being due to random variation, then Z = 1.96.

For only a 1% chance, Z = 2.58

If a change in only one direction is meaningful (i.e. [troponin]), then for a 5% chance of being in one tail of the distribution Z = 1.65

$$RCV = \left(\sqrt{2}\right) \times (Z) \times \sqrt{(CVa)^2 + (CVi)^2}$$

WHY RCV? WHY NOT REFERENCE RANGE?

Many analytes have a low "Index of Individuality" (IoI).

IoI = (CVi / CVg)
CVi is the within-individual biological variation
CVg is the between-individual biological variation

If IoI >1.4, then reference ranges will usually detect biologically significant changes

If IoI <0.6, then reference ranges will usually fail to detect biologically significant changes

WHY RCV? WHY NOT REFERENCE RANGE?

Consider the case of serum creatinine:

A 50% increase in serum [creatinine] is associated with a 50% decrease in GFR

Most individuals in the lower half of the reference range can lose 50% of their renal function and still have a "normal" serum [creatinine].

CVi for serum [creatinine] = 6%
CVa for serum [creatinine] @ 0.74 mg/dL = 3.6%
RCV for serum [creatinine] ~16.3%

RCV: ANALYTICAL VARIANCE

DETERMINATION OF CVa

- ❑ Initial method validation data for imprecision
- ❑ In-house QC data
- ❑ Replicate analysis of specimens in the RCV experiment
- ❑ Within-run vs. total CVa

$$RCV = \left(\sqrt{2}\right) \times (Z) \times \sqrt{(CVa)^2 + (CVi)^2}$$

RCV: ANALYTICAL VARIANCE

DETERMINATION OF CVa

- ❑ Initial method validation data for imprecision
  - (+) should have within-run CVa (repeatability)
  - (-) data may be out of date
- ❑ In-house QC data
  - (+) cumulative s.d. more appropriate for long times between specimens
  - (-) potential matrix effect

RCV: ANALYTICAL VARIANCE

DETERMINATION OF CVa

- ❑ Replicate analysis of specimens in the RCV experiment (within-run only)
  - (+) permits nested ANOVA when all specimens are analyzed in a single run
  - (-) expensive for some reagents
- ❑ Within-run CVa permits statistical modeling to extract both analytical and biological variance from a single run
- ❑ Total CVa better reflects the imprecision inherent in how sequential specimens are actually run

RCV: BIOLOGICAL VARIANCE

DETERMINATION OF CVi

- ❑ Look it up vs. measure it
- ❑ Population to sample
- ❑ Sampling interval
- ❑ # of subjects
- ❑ # of samples per person

$$RCV = \left(\sqrt{2}\right) \times (Z) \times \sqrt{(CVa)^2 + (CVi)^2}$$

RCV: BIOLOGICAL VARIANCE

DETERMINATION OF Cvi

https://www.westgard.com/biodatabase1.htm

Database originally published by Ricos et.al. *Scand J Clin Lab Invest* 1999;59:491-500

Last updated in 2014…now ~370 analytes

$$RCV = \left(\sqrt{2}\right) \times (Z) \times \sqrt{(CVa)^2 + (CVi)^2}$$

RCV: BIOLOGICAL VARIANCE

WHY NOT JUST LOOK IT UP?

Carobene et.al. *Clin Chem Lab Med* 2013; 51:1997-2007

- meta analysis for biological variation in AST, ALT, GGT
- CVi from 3% - 58%
- # subjects from 10–274
- # samples from 2-16
- sampling frequency from daily to monthly

RCV: BIOLOGICAL VARIANCE

WHY NOT JUST LOOK IT UP?

Qi et.al. *Clin Chim Acta* 2015; 450:233-236
vs.
Matyar et.al. *J Clin Lab Anal* 2016; 30:1081-1085

- RCV for neuron-specific enolase 37% vs. 66%
- CVi 13.2% vs. 21.5%
- # subjects 20 vs. 13
- # samples 7 vs. 4
- sampling frequency hourly/daily vs. biweekly
- both used Roche reagents and Cobas analyzers

RCV: BIOLOGICAL VARIANCE

POPULATION TO SAMPLE FOR Cvi
HEALTHY vs. STABLE DISEASE

Braga et.al. *Clin Chim Acta* 2010; 411:1606-1610

- meta analysis for biological variation in HgbA1c
- 4 studies using healthy individuals: CVi all <2%
- 10 studies using diabetics: CVi from 2.4-9.8%

Aakre et.al. *Clin Chem* 2014; 60:838-847

- CVi for cTnI=15.6%, cTnT=8.3% for healthy
- CVi for cTnI=14.3%, cTnT=8.3% for hemodialysis

RCV: BIOLOGICAL VARIANCE

SAMPLING INTERVAL

Wu et.al. *Clin Chem* 2009; 55:52-58
Wu et.al. *Clin Biochem* 2012; 45:714-716

CVi for cTnI

Hourly (4hr)	biweekly (8wks)	9 months (avg)
9.7%	14%	28%

RCV: BIOLOGICAL VARIANCE

SAMPLING INTERVAL

Hilderink et.al. *Clin Chem Lab Med* 2017; 55:1013-1024

- Measured CBC parameters in older adults
- Samples drawn hourly for 24 hours from venous catheter

Fraser et.al. *Am J Clin Pathol* 1989; 92:465-470

- Measured CBC parameters in older adults
- Samples drawn biweekly for 18-20 weeks

CVi similar in both studies except for WBC and platelets

RCV: BIOLOGICAL VARIANCE

of SUBJECTS, # of SAMPLES

Roraas et.al. *Clin Chem* 2012; 58:1306-1313

Used computer simulations (10,000 data sets) and nested ANOVA to study the effect of varying:

- # of subjects (10, 15, 20, 40, 80, 160)
- # of samples per subject (2, 4, 6, 8, 10)
- # of replicates measured per sample (2, 3, 4)
- (CVa/Cvi) (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0)

on the confidence interval for the estimate of CVi, and on the power to estimate CVi

RCV: BIOLOGICAL VARIANCE

of SUBJECTS, # of SAMPLES

Roraas et.al. *Clin Chem* 2012; 58:1306-1313

- The more subjects, the smaller the $CI_{95\%}$ for CVi
- Minimal decrease in $CI_{95\%}$ with more than 8 samples per individual
- If (CVa/CVi) < 1, minimal decrease in $CI_{95\%}$ with more than 2 replicates per sample
- If (CVa/CVi) $\leq$ 1, power to detect CVi > 0 can be optimized with 10 subjects, 8 samples each, analyzed in duplicate
- Fewer samples per subject required if >10 subjects

RCV: EXPERIMENTAL DESIGN

ALL IN ONE RUN vs. REAL TIME ANALYSIS

ALL IN ONE RUN

- Freeze samples as they are collected
- Thaw all samples when study complete
- Analyze all samples in duplicate at the same time
- (+) no between-run or between-day CVa
- (+) data can be analyzed with nested ANOVA

REAL TIME ANALYSIS

- Analyze samples as they are collected
- (+) avoids freeze-thaw effect
- (+) reflects how sequential clinical samples are run

RCV: NESTED ANOVA

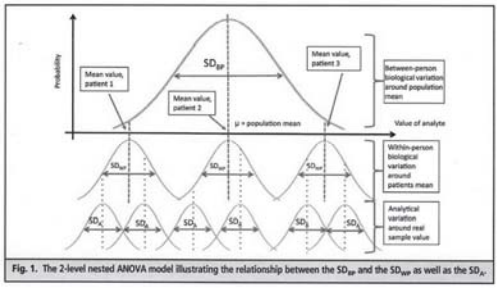


Fig. 1. The 2-level nested ANOVA model illustrating the relationship between the SD_{BP} and the SD_{WP} as well as the SD_A.

Roraas et.al. *Clin Chem* 2012; 58:1306-1313

RCV: ASSUMPTIONS (so far)

- Distributions of measured results are Gaussian
- Differences between measures are random with no autocorrelation between sequential results
- Variances are homogeneous
- No pre-analytical variation

RCV: NON-GAUSSIAN DISTRIBUTION

- Calculate the ln of each data point
- Calculate CVa and CVi using ln-transformed data
- $RCV_{pos} = (\exp[1.96(2)^{1/2}(CVa^2 + CVi^2)^{1/2}]-1)x100\%$
- $RCV_{neg} = (\exp[-1.96(2)^{1/2}(CVa^2 + CVi^2)^{1/2}]-1)x100\%$
- RCV_{pos} may not = RCV_{neg} when underlying distribution is skewed

RCV: CASE STUDY FRUCTOSAMINE vs. FRUCTOSAMINE/ALBUMIN

"Biological Variation of Serum Fructosamine in Healthy Subjects"

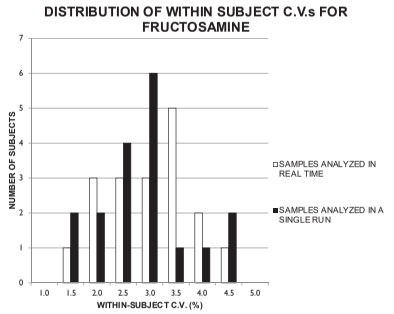
Supported by a Member Grant from the ASCLS E&R Fund and by CTSA #UL1TR000058 from NIH

Does correcting the serum fructosamine result for the serum [albumin] in the same specimen reduce the CVi?

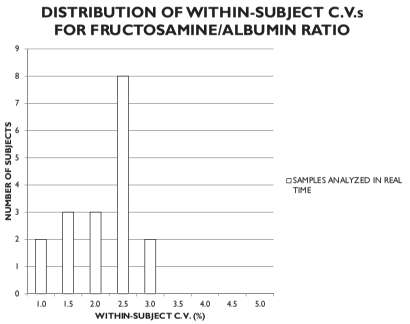
RCV: CASE STUDY FRUCTOSAMINE vs. FRUCTOSAMINE/ALBUMIN

- 20 "apparently healthy" individuals recruited
- Blood drawn biweekly for 20 weeks without regard to fasting, and serum separated within 2 hrs.
- Serum fructosamine, albumin, and glucose measured within 8 hrs of collection (not in duplicate)
- Aliquots of each serum stored at -80°C
- For each individual, all samples thawed and fructosamine remeasured in a single run

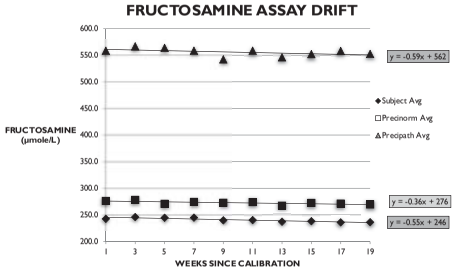
RCV: CASE STUDY FRUCTOSAMINE vs. FRUCTOSAMINE/ALBUMIN



RCV: CASE STUDY FRUCTOSAMINE vs. FRUCTOSAMINE/ALBUMIN



RCV: CASE STUDY FRUCTOSAMINE vs. FRUCTOSAMINE/ALBUMIN



RCV: CASE STUDY FRUCTOSAMINE vs. FRUCTOSAMINE/ALBUMIN

CVi	MEAN/MEDIAN	RANGE
Fructosamine (real time)	3.2% / 3.4%	1.8% - 4.8%
Fructosamine (all in one run)	3.0% / 3.1%	1.6% - 5.0%
Fructosamine/albumin (real time)	2.4% / 2.6%	1.2% - 3.2%

Using total CVa for the low control (1.3%) and Z=1.96:

RCV for fructosamine = 10.1%

RCV: ALTERNATIVE USE

$$RCV = \left(\sqrt{2}\right) \times (Z) \times \sqrt{(CVa)^2 + (CVi)^2}$$

$$Z = \frac{RCV}{\left(\sqrt{2}\right)\sqrt{(CVa)^2 + (CVi)^2}}$$

RCV: SUMMARY

RCV is a more sensitive indicator of a change in the biological / physiological state of an individual than whether or not sequential results fall within or outside the reference range for an analyte, when the within-individual biological CV is less than the between-individual CV.

How the RCV is determined must be customized for each analyte, with respect to:

- the population to which it will be applied
- how often the test is normally ordered
- the achievable CVa and (CVa/CVi)

RCV: QUESTIONS