

VARIATION IN MEASUREMENT OF HIV RNA VIRAL LOAD

- **SOURCES OF VARIATION (RANDOM VS SYSTEMATIC)**
- **MAGNITUDE OF EACH SOURCE**
- **CONSEQUENCES FOR CONFIDENCE LIMITS AROUND MEASUREMENTS AND CHANGES**
- **DATA FROM ROCHE HIV MONITOR ASSAY**

SOURCES OF VARIATION

- 1) INTRA-ASSAY (AMONG REPLICATES IN A BATCH)**
- 2) INTER-ASSAY (AMONG REPLICATES IN DIFFERENT BATCHES)**
- 3) INTRA-KIT (AMONG KIT LOTS)**
- 4) INTER-KIT (E.G. ROCHE VS OT NASBA)**
- 5) INTER-LAB (SAME KIT)**
- 6) BIOLOGICAL FLUCTUATION (EVEN IF NO SYSTEMATIC CHANGE)**

**ASSUME A PATIENT IS FOLLOWED OVER TIME
WITHIN A LAB
FOCUS ON 1), 2), 6), THEN ON 3)**

MODELING APPROACH FOR ASSAY, BIOLOGICAL VARIATION AND TOTAL

ASSUME THAT DATA ARE NORMALLY DISTRIBUTED AND VARIANCES ARE ADDITIVE:

$$V_{\text{TOT}} = V_{\text{B}} + V_{\text{E}} + V_{\text{A}}$$

WHERE

V_{TOT} = OVERALL VARIANCE

V_{B} = BIOLOGICAL VARIANCE

V_{E} = INTER-ASSAY VARIANCE

V_{A} = INTRA-ASSAY VARIANCE

$$S_{\text{TOT}} = \sqrt{V_{\text{B}} + V_{\text{E}} + V_{\text{A}}}$$

IN BATCHED ASSAYS:

$$S_{\text{TOT}} = \sqrt{V_{\text{B}} + V_{\text{A}}}$$

APPROACH TO ESTIMATION

- **ESTIMATE TOTAL VARIANCE (BIOLOGICAL PLUS ASSAY)**
- **ESTIMATE ASSAY VARIANCE**
- **OBTAIN BIOLOGICAL VARIANCE BY SUBTRACTION**
- **OBTAIN STANDARD DEVIATIONS FROM VARIANCES**

SD_{TOT} FOR BATCH TESTING

STUDY	N	LINEAR MODELS	10 TH -90 TH
			PERCENTILES OF DIFFERENCES
ACTG 076	55	0.27	0.28
ACTG 175, A	95	0.13	0.14
ACTG 175, B	87	0.27	0.16
ACTG 175, C	110	0.27	0.16
ACTG 229	259	0.29	0.21
WITS	57	0.23	0.25
COMBINED	663	0.26	0.18

10%-90% OF DIFFERENCE: 1.8 SD_{TOT}

ASSAY SD'S FROM THE VQA STANDARDS
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	RUNS	STANDARD DEVIATION	
		INTRA-ASSAY	INTER-ASSAY
ACTG 076	48	0.15	0.08
ACTG 175, A	26	0.09	0.05
ACTG 175, B	20	0.09	0.08
ACTG 175, C	37	0.09	0.10
COMBINED	131	0.12	0.08

ASSAY SD'S FROM PROFICIENCY PANELS

**INTRA-ASSAY SD, ALL PARTICIPATING
LABORATORIES**

PANEL	LABS	SOURCE	MEDIAN	RANGE
06R	22	SPIKED SAMPLES	0.15	0.06-0.22
	22	HIV+ PATIENTS	0.16	0.07-0.48
07R	22	SPIKED SAMPLES	0.12	0.07-0.35
	22	HIV+ PATIENTS	0.12	0.07-0.50

**INTRA-ASSAY SD, LABORATORIES WITH
ACCEPTABLE PERFORMANCE**

PANEL	LABS	SOURCE	MEDIAN	RANGE
06R	20	SPIKED SAMPLES	0.11	0.06-0.20
	17	HIV+ PATIENTS	0.12	0.07-0.20
07R	19	SPIKED SAMPLES	0.09	0.07-0.17
	18	HIV+ PATIENTS	0.09	0.07-0.21

INTER-ASSAY SD

SOURCE	ALL LABS	LABS W/C'S
VQA CONTROLS	0.15	0.14
HIV-INFECTED PATIENTS	0.08	0.08

BIOLOGICAL VARIATION

REAL TIME TESTING: $V_{TOT} = V_B + V_E + V_A$

BATCH TESTING: $V_{TOT} = V_B + V_A$

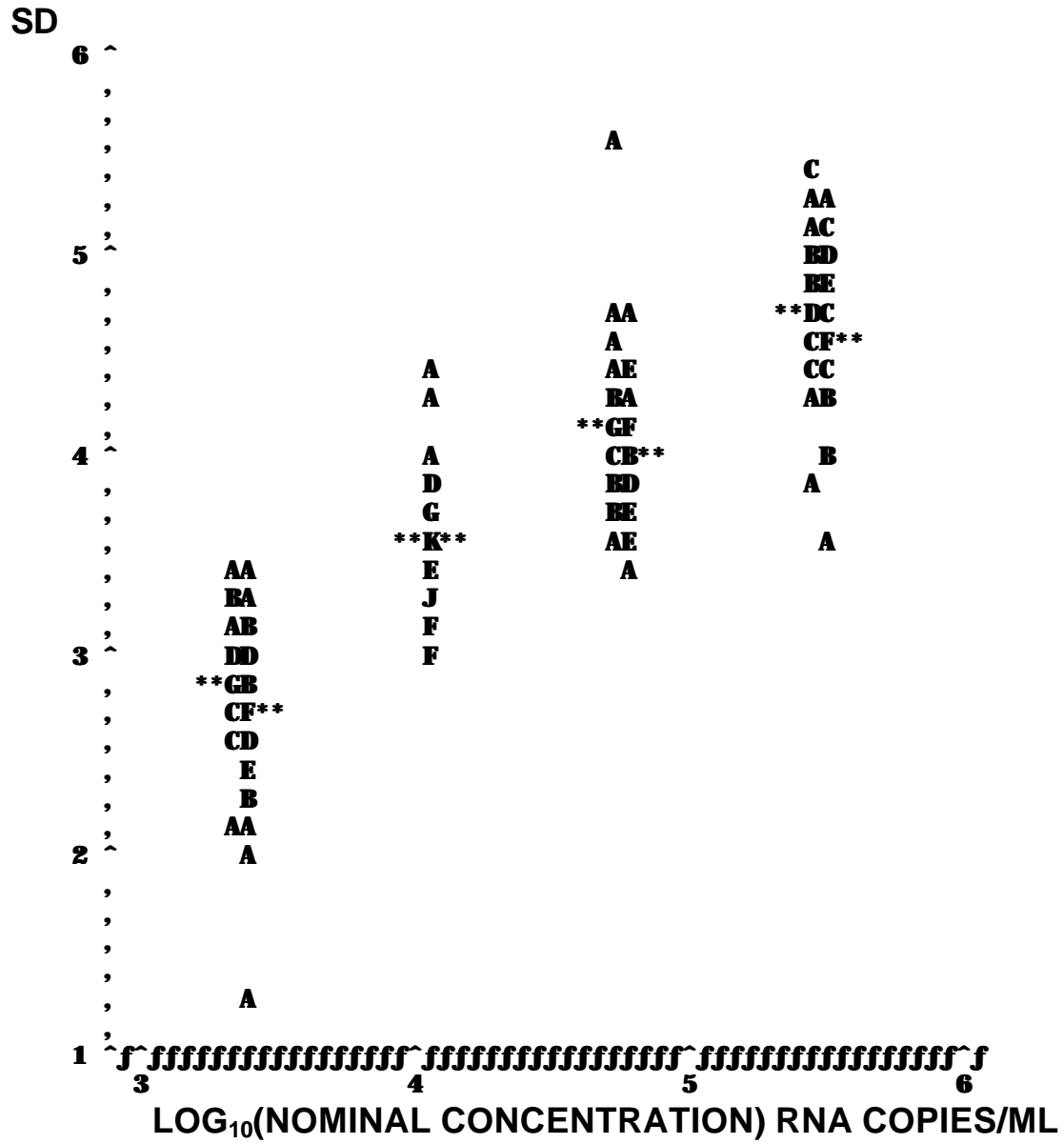
BIOLOGICAL SD: $S_B = (V_{TOT} - V_A)^{1/2}$

% OVERALL VARIATION: $100 * V_B / V_{TOT}$

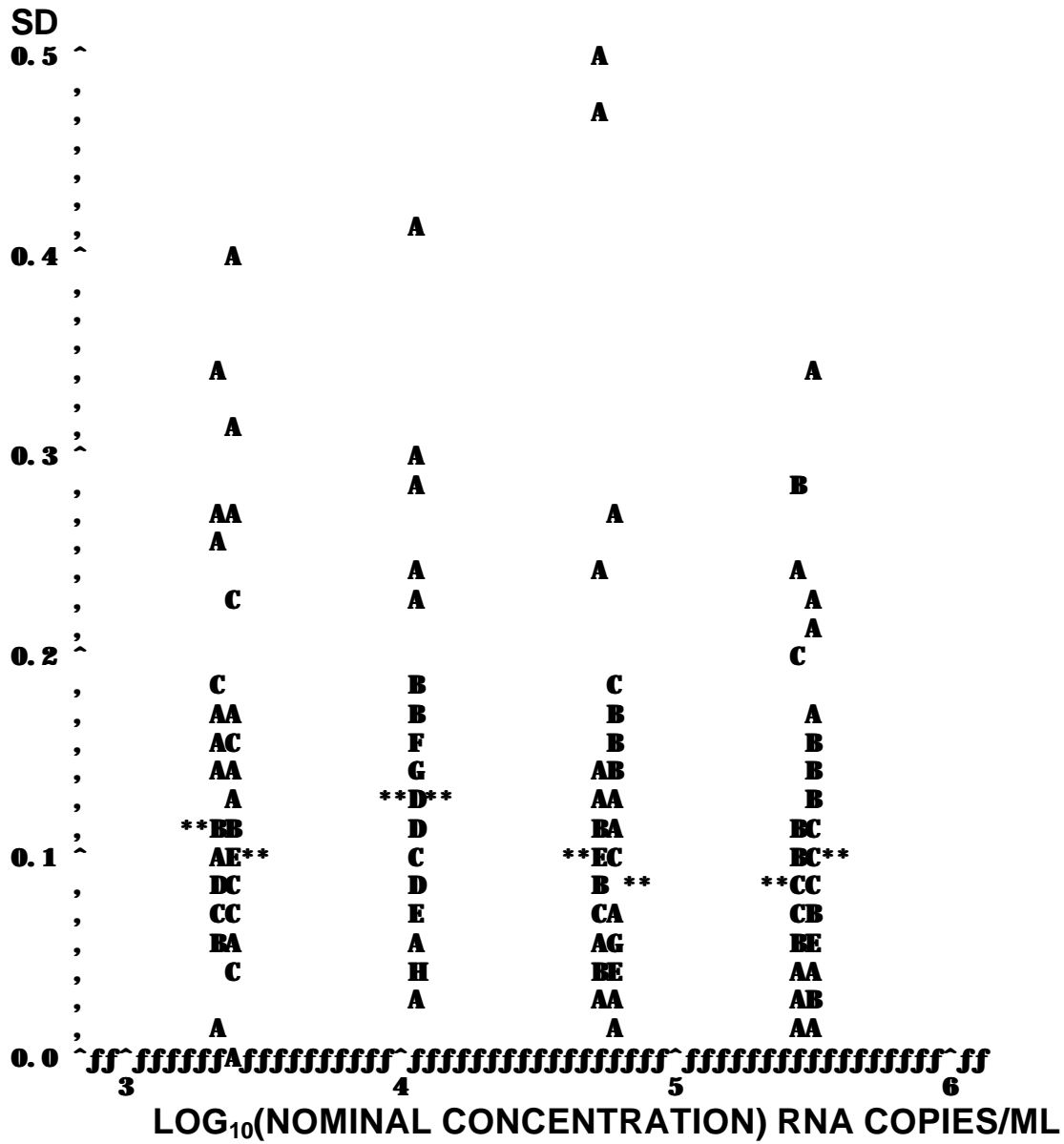
BIOLOGICAL VARIATION

S_{TOT}	S_A	S_B	$100 * V_B / V_{TOT}$
0.26	0.12	0.23	80
0.18	0.12	0.13	56

INTRA-ASSAY SD VS NOMINAL CONCENTRATION, RT-PCR ASSAYS ON PP06R, PP07R



INTRA-ASSAY SD OF LOG₁₀(RNA) VS NOMINAL CONCENTRATION - ON PP06R, PP07R



**95% CONFIDENCE LIMITS (CL) FOR ESTIMATED RNA
COPY NUMBER ASSUMING THE BIOLOGICAL
SD=0.23.**

STANDARD DEVIATION			95% CL	
S_A	S_E	S_{TOT}	LOG₁₀ RNA	@ 10⁴ COPIES/ML
0.12	-	0.26	±0.51	3,100 - 32,000
0.20		0.30	±0.60	2,500 - 40,000
0.25	-	0.34	±0.67	2,100 - 47,000
0.12	0.08	0.27	±0.53	3,000 - 34,000
0.12	0.15	0.30	±0.59	2,500 - 39,000
0.20	0.08	0.32	±0.62	2,400 - 42,000
0.20	0.15	0.34	±0.67	2,100 - 47,000
0.25	0.08	0.35	±0.68	2,100 - 48,000
0.25	0.15	0.37	±0.73	1,900 - 54,000

S_A: INTRA-ASSAY SD
S_E: INTER-ASSAY SD
S_{TOT}: TOTAL SD

STANDARD DEVIATIONS FOR TWO VQA STANDARDS IN FIVE LABORATORIES

LAB	RUNS	SD	
		1,500 COPIES/ML	15,000 COPIES/ML
A	191	0.27	0.25
B	102	0.20	0.21
C	65	0.23	0.18
D	75	0.19	0.16
E	58	0.28	0.21

**MEDIAN LOG10 RECOVERY FOR EACH QS LOT IN EACH LABORATORY.
DASHES: <8 TOTAL ASSAYS PER QS LOT IN THAT LABORATORY. P: P-
VALUE FOR DIFFERENCES AMONG QS LOTS WITHIN EACH
LABORATORY.**

LAB	QS INPUT COPIES									P
	72	78	79	81	82	87 ₁	87 ₂	88	91	
A		0.32	-0.03	-0.18		0.09		0.24	0.11	<0.001
B	-0.12		-0.11	-0.15	-0.06	0.21			0.05	<0.001
C	-0.06				-0.10	0.09			0.004	0.0047
D		0.12					0.013	0.12		<0.001
E		0.19					0.10	0.17		0.081
ALL	-0.09	0.18	-0.06	-0.17	-0.07	0.12	0.04	0.19	0.06	

EFFECTS OF QS VARIATION ON A HYPOTHETICAL STUDY

ASSUMPTIONS

- LONGITUDINAL STUDY WITH ASSAYS IN REAL TIME
- 2 QS LOTS USED IN SUCCESSION
- LOT CHANGE OCCURS BETWEEN TWO PATIENT VISITS

95% CL FOR THE DIFFERENCE IN RNA BETWEEN VISITS

$$0.20 \pm 1.96S_{TOT} \sqrt{2}$$
$$= 0.20 \pm 0.72, \text{ IF } S_{TOT} = 0.26$$

IF NEW LOT READS 0.20 LOG10 HIGHER THAN OLD,

- P(APPARENT 3-FOLD INCREASE) = 0.33
- P(APPARENT INCREASE IS CONFIRMED) = 0.10

IF NEW LOT READS 0.30 LOG10 HIGHER THAN OLD,

- P(APPARENT 5-FOLD INCREASE) = 0.14
- P(APPARENT INCREASE IS CONFIRMED) = 0.02

MEDIAN LOG10 RECOVERY FOR EACH QS LOT IN EACH LABORATORY FOR THE 150-COPY STANDARD ON THE ULTRANSENSITIVE MONITOR ASSAY.

LAB	QS INPUT COPIES									P
	72	74	76	78	82	82.5	87	88	91	
A	-0.03				-0.22					0.038
B	-0.39	0.05	0.003	-0.02	-0.42	-0.02	-0.03	0.08	0.03	<0.001
C		0.16	-0.08		0.07	0.03	0.004		0.004	0.24
D	-0.29				-0.28					0.80
E	-0.32			0.28	-0.14			0.17		<0.001

QS LOTS IN CHRONOLOGICAL ORDER

LAB	QS INPUT COPIES									P
	72	82	78	88	87	76	82.5	91	74	
A	-0.03	-0.22								0.038
B	-0.39	-0.42	-0.02	0.08	-0.03	0.003	-0.02	0.03	0.05	<0.001
C		0.07			0.004	-0.08	0.03	0.004	0.16	0.24
D	-0.29	-0.28								0.80
E	-0.32	-0.14	0.28	0.17						<0.001

SUMMARY

- **TOTAL STANDARD DEVIATION: 0.18 – 0.26**
- **INTRA-ASSAY SD: 0.12 IN PROFICIENT LABORATORIES**
- **INTRA- + INTER-ASSAY SD: 0.14-0.17**
- **BIOLOGICAL SD: 0.13-0.23**
- **BIOLOGICAL VARIANCE (BATCH): 56-80% OF TOTAL**
- **ASSAY VARIANCE (BATCH): 20-44% OF TOTAL**
- **LOT-TO-LOT VARIATION: SYSTEMATIC COMPONENT**
 - **USUALLY SMALL**
 - **SHOULD BE VERIFIED FOR NEW LOTS**