

Example Protocol: Lab Retesting Procedure for Concordance

Goal: To systematically assess intra- and inter-plate variability and reproducibility

General Guidelines:

- Try to avoid thawing a new aliquot for retesting. *Do re-testing immediately on next plate.*
- The 'Results' tab in the Excel file will automatically flag samples that require retesting on the subsequent plate because they were equivocal (EqR), need to be diluted/above ULOD (DiLR), or those that were randomly selected (see below).
- *The retesting procedure expressed in this document is a guide only and may need to be adjusted for differences in lab capacity. While regular intra- and inter-plate variability testing with each new plate is encouraged for quality assurance, there may be instances where this is not possible. At the very least an initial retesting procedure should be performed to ensure concordance in assay procedure results before a large-scale rollout of assay processing for the serosurvey.*

Box wise testing strategy:

- For each box there will be 2 plates run:
 - The first plate will include all specimens from the box, plus 4 randomly selected samples to be run in duplicate for intraplate variability (randomly selected by the lab).
 - Leave a blank well between the specimens being test for the first time and the specimens randomly retested on the same plate (see grey cell in plate map below).
 - Note: In plate map 1 below, the random re-test samples are denoted in blue.
 - The second plate will include all samples that require retesting:
 - Specimens above the ULOD on the first plate (dilute and retest – DiLR):
 - In most scenarios, the specimen will only be run once on the second plate, diluted 1:X.
 - Specimen equivocal on the first plate (EqR):
 - Specimen run in duplicate on the second plate
 - Specimens for inter-plate variability: every 20th specimen plus the last specimen from the first plate
 - Example: 20th, 40th, 60th, and last specimen from the first plate
 - Exclude the last four specimens on the first plate that were randomly selected for intraplate retesting
 - If a specimen selected for inter-plate variability was equivocal on first plate, test following equivocal retesting procedures on second plate and results from equivocal testing will be used for inter-plate variability.
 - If a specimen selected for inter-plate variability was above ULOD on first plate, dilute and test on the second plate. Select the preceding specimen (e.g., 19th specimen if 20th specimen was above ULOD) to test on the second plate for inter-plate variability.

Abbreviations: EqR – equivocal test results, ULOD – upper limit of detection, DiLR – dilute and retest

EXAMPLE PLATE 1:

Specimens from Box X run for the first time

- White cells: specimens tested for the first time
- Blue cells: RR1 – random retest specimen #1 [intra-plate variability]
- Green cells: specimens to be run on the second plate for inter-plate variability (20th specimen: 208A02; 40th specimen: 208A02; last specimen: 216B13)
- Orange cells: specimens above ULOD on first plate
- Gold cells: specimens equivocal on the first plate
- Purple cells: Calibrators and controls

	1	2	3	4	5	6	7	8	9	10	11	12
1	Cal1	201A04	201A12	208A01	209A09	209B04	209B12					
2	Cal2	201A05	201A03	208A02	209A10	209B05	209B13					BLANK
3	Cal3	201A06	202B02	208A03	209A11	209B06	214A01					RR1 – 201A12
4	Cal4	201A08	202B11	208A04	209A12	209B07	214A02					RR2- 201A03
5	PC	201A13	201B03	208A05	209A13	209B08	...cont- inued					RR3 – 202B02
6	NC	201B01	201B09	208A06	209B01	209B09					216B10	RR4 – 202B11
7	201A04	201B13	202B01	208A07	209B02	209B10					216B11	IC1
8	201A05	202A02	202B04	208A08	209B03	209B11					216B13	IC2

EXAMPLE PLATE 2:

Retested specimens from Box X

- Orange cells: specimens above ULOD on first plate (run 1:4 dilution)
- Gold cells: specimens equivocal on the first plate (run in duplicate)
- Green cells: specimens run on the second plate for inter-plate variability (20th specimen: 208A02; 40th specimen: 208A02; last specimen: 216B13)
- Purple cells: Calibrators and controls

	1	2	3	4	5	6	7	8	9	10	11	12
1	Cal1	201B09										
2	Cal2	201B09										
3	Cal3	209B06										
4	Cal4	209B06										
5	PC	208A02										
6	NC	209B09										
7	201B09 (1:4 dilution)	216B13										IC1
8	209B13 (1:4 dilution)											IC2

Using the Excel file template to identify all specimens requiring retesting

Once the Results tab has been populated with the OD values and dilution adjustment factor from the plate run, the IU/ml, adjusted result (accounting for dilution), and final qualitative result will be calculated. Equivocal results and results above the upper limit detection will be flagged in the gray columns.

Sr.No.	Participant ID	OD	Category	IU/L (calculated)	Dilution Adjustment Factor (enter 1 except if extra dilution done on retest)	Final Result, IU/L (accounting for dilution)	Final Result, Qualitative	Comments (e.g. Retest for 503A04 or Equivocal, needs retest)	Equivocal	> ULOD
1	201-A-01	1.057	2	849.4	1	849.4	Positive		0	0
2	201-A-02	0.253	3	136.9	1	136.9	Negative		0	0
3	201-A-04	1.928	1	4509.1	1	4509.1	Positive		0	0
4	201-A-05	0.315	3	170.1	1	170.1	Negative		0	0
5	201-A-06	2.5	1	7289.2	1	7289.2	Positive		0	1
6	201-A-07	1.934	1	4538.3	1	4538.3	Positive		0	0
7	201-A-08	1.165	2	958.6	1	958.6	Positive		0	0
8	201-A-09	0.284	3	153.5	1	153.5	Negative		0	0
9	201-A-10	0.573	2	360.2	1	360.2	Positive		0	0
10	201-A-11	0.185	3	100.4	1	100.4	Negative		0	0
11	201-A-13	0.372	3	200.7	1	200.7	Equivocal		1	0

Laboratory analyst should enter '1' in the column 'Interplate random retest' for every 20th specimen and the last specimen tested (prior to the intraplate random retests):

20th specimen:

Sr.No.	Participant ID	OD	Category	IU/L (calculated)	Dilution Adjustment Factor (enter 1 except if extra dilution done on retest)	Final Result, IU/L (accounting for dilution)	Final Result, Qualitative	Comments (e.g. Retest for 503A04 or Equivocal, needs retest)	Equivocal	> ULOD	Interplate random retest (Enter '1' if selected)	Requires retesting
16	201-B-05	0.117	3	63.9	1	63.9	Negative		0	0		0
17	201-B-06	0.345	3	186.2	1	186.2	Negative		0	0		0
18	201-B-08	0.525	2	311.7	1	311.7	Positive		0	0		0
19	201-B-09	1.239	1	1160.4	1	1160.4	Positive		0	0		0
20	201-B-10	0.587	2	374.3	1	374.3	Positive		0	0	1	1

60th specimen:

Sr.No.	Participant ID	OD	Category	IU/L (calculated)	Dilution Adjustment Factor (enter 1 except if extra dilution done on retest)	Final Result, IU/L (accounting for dilution)	Final Result, Qualitative	Comments (e.g. Retest for 503A04 or Equivocal, needs retest)	Equivocal	> ULOD	Interplate random retest (Enter '1' if selected)	Requires retesting
58	203-B-05	0.526	2	312.7	1	312.7	Positive		0	0		0
59	203-B-06	0.198	3	107.4	1	107.4	Negative		0	0		0
60	203-B-08	2.1	1	5345.1	1	5345.1	Positive		0	1	1	1

Last specimen (prior to intraplate random retests):

Sr.No.	Participant ID	OD	Category	IU/L (calculated)	Dilution Adjustment Factor (enter 1 except if extra dilution done on retest)	Final Result, IU/L (accounting for dilution)	Final Result, Qualitative	Comments (e.g. Retest for 503A04 or Equivocal, needs retest)	Equivocal	> ULOD	Interplate random retest (Enter '1' if selected)	Requires retesting
76	204-B-01	0.145	3	79.0	1	79.0	Negative		0	0		0
77	204-B-02	1.283	1	1374.2	1	1374.2	Positive		0	0		0
78	204-B-04	0.211	3	114.3	1	114.3	Negative		0	0	1	1
79	201-A-08	1.064	2	856.5	1	856.5	Positive	Random intraplate retest	0	0		0
80	203-A-05	1.087	2	879.7	1	879.7	Positive	Random intraplate retest	0	0		0
81	203-B-12	0.14	3	76.3	1	76.3	Negative	Random intraplate retest	0	0		0
82	204-A-01	0.9	2	690.7	1	690.7	Positive	Random intraplate retest	0	0		0

The Excel file list can then be filtered to identify all specimens to be retested on the next plate:

Sr.No.	Participant ID	OD	Category	IU/L (calculated)	Dilution Adjustment Factor (enter 1 except if extra dilution done on retest)	Final Result, IU/L (accounting for dilution)	Final Result, Qualitative	Comments (e.g. Retest for 503A04 or Equivocal, needs retest)	Equivocal	> ULOD	Interplate random retest (Enter '1' if selected)	Requires retesting
5	201-A-06	2.5	1	7289.2	1	7289.2	Positive		0	1		1
11	201-A-13	0.372	3	200.7	1	200.7	Equivocal		1	0		1
20	201-B-10	0.587	2	374.3	1	374.3	Positive		0	0	1	1
28	202-A-06	0.372	3	200.7	1	200.7	Equivocal		1	0		1
40	202-B-07	0.162	3	88.1	1	88.1	Negative		0	0	1	1
56	203-B-03	0.44	3	237.1	1	237.1	Equivocal		1	0		1
60	203-B-08	2.1	1	5345.1	1	5345.1	Positive		0	1	1	1
72	204-A-10	0.47	2	256.1	1	256.1	Equivocal		1	0		1
78	204-B-04	0.211	3	114.3	1	114.3	Negative		0	0	1	1

Scan through the list to see if any of the interplate retest specimens were above the ULOD on the first plate (e.g., 203-B-08 below). If so, select the preceding specimen since that specimen will already be retested for another reason.

60th specimen above the ULOD:

Sr.No.	Participant ID	OD	Category	IU/L (calculated)	Dilution Adjustment Factor (enter 1 except if extra dilution done on retest)	Final Result, IU/L (accounting for dilution)	Final Result, Qualitative	Comments (e.g. Retest for 503A04 or Equivocal, needs retest)	Equivocal	> ULOD	Interplate random retest (Enter '1' if selected)	Requires retesting
5	201-A-06	2.5	1	7289.2	1	7289.2	Positive		0	1		1
11	201-A-13	0.372	3	200.7	1	200.7	Equivocal		1	0		1
20	201-B-10	0.587	2	374.3	1	374.3	Positive		0	0	1	1
28	202-A-06	0.372	3	200.7	1	200.7	Equivocal		1	0		1
40	202-B-07	0.162	3	88.1	1	88.1	Negative		0	0	1	1
56	203-B-03	0.44	3	237.1	1	237.1	Equivocal		1	0		1
60	203-B-08	2.9	1	9233.3	1	9233.3	Positive		0	1	1	1
72	204-A-10	0.47	2	256.1	1	256.1	Equivocal		1	0		1
78	204-B-04	0.211	3	114.3	1	114.3	Negative		0	0	1	1

59th specimen selected for interplate retesting, 60th specimen tested for above ULOD:

Sr.No.	Participant ID	OD	Category	IU/L (calculated)	Dilution Adjustment Factor (enter 1 except if extra dilution done on retest)	Final Result, IU/L (accounting for dilution)	Final Result, Qualitative	Comments (e.g. Retest for 503A04 or Equivocal, needs retest)	Equivocal	> ULOD	Interplate random retest (Enter '1' if selected)	Requires retesting
5	201-A-06	2.5	1	7289.2	1	7289.2	Positive		0	1		1
11	201-A-13	0.372	3	200.7	1	200.7	Equivocal		1	0		1
20	201-B-10	0.587	2	374.3	1	374.3	Positive		0	0	1	1
28	202-A-06	0.372	3	200.7	1	200.7	Equivocal		1	0		1
40	202-B-07	0.162	3	88.1	1	88.1	Negative		0	0	1	1
56	203-B-03	0.44	3	237.1	1	237.1	Equivocal		1	0		1
59	203-B-06	0.198	3	107.4	1	107.4	Negative		0	0	1	1
60	203-B-08	2.9	1	9233.3	1	9233.3	Positive		0	1	1	1
72	204-A-10	0.47	2	256.1	1	256.1	Equivocal		1	0		1
78	204-B-04	0.211	3	114.3	1	114.3	Negative		0	0	1	1

Determining final test result for repeated specimens

Scenario	First result	Second result	Third result	Diluted on second run?	Final result – qualitative	Final result – quantitative
Intra or interplate retest	Positive	Positive	N/A	No	Positive	Always use the first result for specimens tested due to random retests
Intra or interplate retest	Negative	Negative	N/A	No	Negative	
Diluted retest	Positive (> ULOD)	Positive	N/A	Yes	Positive	Always use the <u>first</u> diluted result
Equivocal - concordant	Equivocal	Equivocal	Equivocal	No	Equivocal	Select first equivocal result from 2 nd plate
Equivocal - discordant	Equivocal	Positive	Positive	No	Positive*	Select first result from 2 nd plate.
Equivocal - discordant	Equivocal	Negative	Negative	No	Negative*	
Equivocal - discordant	Equivocal	Positive	Equivocal	No	Equivocal*	Select equivocal result from 2 nd plate

*Use the 'best two of three results' for equivocal specimens that are discordant upon retest.