ISO Standards - Ensuring Accuracy for Your Liquid Handlers and Assay Results

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Accuracy

Closeness of agreement between a **measured** quantity value and a **true** quantity value of a measurand

Source: ISO Guide 99:2007

$$Y = f\,(X_1, X_2, \ldots, X_N)$$

The measurement result Y is itself dependent on other measurements, such as temperature, mass, volume, chemical amount, absorbance, fluorescence, etc. These input quantities (X_i) must be measured with sufficient accuracy to achieve the desired accuracy in *Y*.

Source: ISO Guide 98-1:2009

Metrological Confirmation

set of operations required to ensure that **measuring equipment** conforms to the **requirements** for its intended use

Source: ISO 9000:2015

Intended use or "fit-for-purpose" is ***not*** based on manufacturers specifications or product requirements. Requirements for intended use depend on the desired accuracy of the assay result, and the sensitivity of the assay result to errors in the input quantity.

Accuracy requires sufficiently small errors. Errors can be systematic or random.

systematic error

component of **measurement error** that in replicate **measurements** remains constant or varies in a predictable manner

random error

component of **measurement error** that in replicate **measurements** varies in an unpredictable manner

Source: ISO Guide 99:2007

Liquid handling devices always (almost always) have published specifications for random error.

Some liquid handling devices also have specifications for systematic error.

Manufacturer perspective: Often interested in the per channel perspective. Is the device broken? Is repair, maintenance or adjustment required?

Laboratory perspective: What is happening in the microplate?

References: ISO 8655 and ISO IWA 15

Example:

We specify an acceptance test where a liquid handling system will fill three 96 well plates and the random error, expressed as CV (RSD) must be less than 2%.

For 100 µL, plus or minus 2 SD → 96 µL to 104 µL

Source: ISO IWA 15:2015, Example 2

Example from ISO IWA 15 – Performance of automated liquid handling systems

Example 2
8 channel device, $L = 8$
three replicate runs, $M = 3$
12 replicates per channel, $N = 12$
measured in three 96 well plates

Run 1, $m = 1$

	1	2	3	4	5	6	7	8	9	10	11	12
A	101,72	101,17	100,87	102,38	101,44	100,92	101,17	102,21	100,79	103,27	101,44	102,19
B	97,03	96,15	98,14	97,67	98,51	98,14	97,41	99,15	99,01	96,82	97,95	97,36
C	99,55	101,91	97,83	100,25	99,72	99,60	100,04	97,47	98,78	99,35	99,91	101,99
D	99,44	100,09	100,54	100,23	100,01	100,02	101,32	100,99	100,10	99,45	101,89	98,76
E	103,21	102,69	105,47	102,15	102,57	103,80	103,99	103,90	105,39	105,27	103,35	104,53
F	102,33	103,78	101,74	102,14	102,83	101,94	103,73	103,37	100,97	103,74	102,09	102,37
G	103,61	101,71	101,20	102,11	102,39	102,31	101,00	102,13	104,14	102,92	101,29	102,54
H	100,78	100,78	100,53	100,99	100,58	99,10	99,27	98,20	100,73	99,84	100,67	99,58

Channel Statistics, Run 1		
Form. 1	Form. 3	Form. 7
$V(l,m)$	$e_S(l,m)$	$CV(l,m)$
101,63	1,63%	0,74%
97,78	-2,22%	0,91%
99,70	-0,30%	1,36%
100,24	0,24%	0,86%
103,86	3,86%	1,09%
102,59	2,59%	0,88%
102,28	2,28%	0,93%
100,09	0,09%	0,88%
max $e_S(l,m)$	3,86%	
min $e_S(l,m)$	-2,22%	
max $CV(l,m)$	1,36%	

Run 2, m = 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	102,75	101,86	100,11	102,28	100,49	101,37	102,17	101,09	102,54	101,81	101,16	102,37
B	95,86	96,51	99,81	99,12	97,14	97,94	99,26	98,25	96,94	98,89	96,30	95,05
C	101,22	99,44	99,59	99,29	99,59	97,86	98,92	98,19	98,01	99,91	99,79	99,53
D	99,22	101,46	99,09	100,01	100,33	99,22	100,39	101,03	100,30	99,63	99,18	99,35
E	103,49	102,06	101,60	103,17	104,01	103,54	104,55	104,50	105,74	103,99	103,25	101,88
F	102,86	105,07	102,15	102,51	100,86	101,60	104,07	104,36	102,79	103,89	102,55	102,54
G	102,09	101,11	100,88	101,52	103,30	101,82	100,84	101,15	101,63	103,00	103,13	102,76
H	100,27	100,29	101,33	99,52	96,28	98,66	100,27	101,69	99,92	100,46	99,21	101,61

Channel Statistics, Run 2		
Form. 1	Form. 3	Form. 7
$V(l,m)$	$e_S(l,m)$	$CV(l,m)$
101,67	1,67%	0,82%
97,59	-2,41%	1,55%
99,28	-0,72%	0,94%
99,93	-0,07%	0,78%
103,48	3,48%	1,17%
102,94	2,94%	1,17%
101,94	1,94%	0,89%
99,96	-0,04%	1,48%
max $e_S(l,m)$	3,48%	
min $e_S(l,m)$	-2,41%	
max CV(l,m)	1,55%	

Run 3, m = 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	101,22	101,74	101,06	101,37	102,62	103,10	102,31	101,18	99,65	101,10	103,78	103,25
B	96,63	96,76	98,15	98,49	98,36	96,94	97,28	95,90	98,91	98,47	98,95	98,89
C	100,39	98,10	101,67	100,32	101,14	100,10	99,90	100,17	100,29	99,50	99,23	99,47
D	101,05	101,51	98,41	98,30	98,73	98,46	101,06	100,79	99,60	100,05	99,48	100,65
E	103,87	104,61	103,98	101,97	104,47	102,07	105,93	105,82	104,02	104,63	103,71	104,33
F	102,46	102,94	102,55	103,27	102,93	101,97	103,37	102,49	103,56	103,46	102,71	103,16
G	103,79	100,68	102,23	103,08	103,34	101,81	102,76	101,45	101,89	103,34	102,01	101,70
H	102,32	99,52	100,39	100,32	98,37	99,12	99,48	99,56	100,52	100,31	100,40	100,12

Channel Statistics, Run 3		
Form. 1	Form. 3	Form. 7
$V(l,m)$	$e_S(l,m)$	$CV(l,m)$
101,87	1,87%	1,15%
97,81	-2,19%	1,07%
100,02	0,02%	0,91%
99,84	-0,16%	1,17%
104,12	4,12%	1,15%
102,91	2,91%	0,47%
102,34	2,34%	0,90%
100,04	0,04%	0,97%
max $e_S(l,m)$	4,12%	
min $e_S(l,m)$	-2,19%	
max $CV(l,m)$	1,17%	

Run Order Statistics, all three runs combined

	$n \rightarrow$	1	2	3	4	5	6	7	8	9	10	11	12
Form. 9	$V(n)$	101,13	100,91	100,80	100,94	100,83	100,48	101,27	101,04	101,09	101,38	100,98	101,08
Form. 3	$e_S(n)$	1,13%	0,91%	0,80%	0,94%	0,83%	0,48%	1,27%	1,04%	1,09%	1,38%	0,98%	1,08%
Form. 11	$CV(n)$	2,23%	2,30%	1,81%	1,62%	2,20%	1,95%	2,21%	2,39%	2,25%	2,25%	1,95%	2,23%

Overall Statistics	
Grand Avg	Form. 12
V_{GA}	101,00

← calculated as average of all 288 wells

Overall CV	Form. 13
CV_{OA}	2,10%

← calculated as CV of all 288 wells

Channel Statistics, All Runs		
Form. 4	Form. 3	Form. 8
$V(l)$	$e_S(l)$	$CV(l)$
101,72	1,72%	0,92%
97,73	-2,27%	1,21%
99,67	-0,33%	1,09%
100,00	0,00%	0,95%
103,82	3,82%	1,14%
102,81	2,81%	0,89%
102,19	2,19%	0,91%
100,03	0,03%	1,14%
max $eS(l)$	3,82%	
min $eS(l)$	-2,27%	
max $CV(l)$	1,21%	

Form. 14
CV_{C2C}
1,97%

Take away:
Test specification must include how the data will be analyzed and interpreted.

Summary

- Specify accuracy goals for the assay, and for each input quantity.
- Use input quantity accuracy goals as requirements for the metrological confirmation program.
- Communicate requirements using standardized terminology.
- Exploit standardized test methods and calculation formulae to remove ambiguity about verification and validation processes.

References

- ISO Guide 99 – International vocabulary of metrology (VIM)
- ISO 9000 – QMS fundamentals and vocabulary

- ISO Guide 98 – Uncertainty of measurement (GUM)

- ISO 9001 – QMS requirements
- ISO 17025 – Requirements for competence of testing and calibration laboratories

- ISO 8655 – Piston operated volumetric apparatus
- ISO IWA 15 – Performance of automated liquid handling systems

- *Coming soon* ISO 23783 – Automated liquid handling systems

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