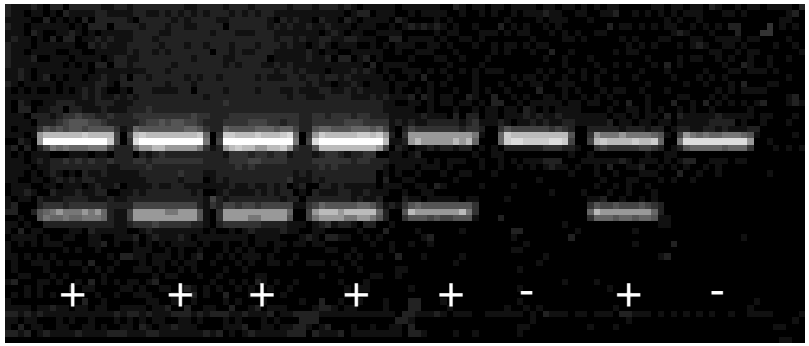
Validation parameters:
An introduction to measures of test accuracy

Types of tests

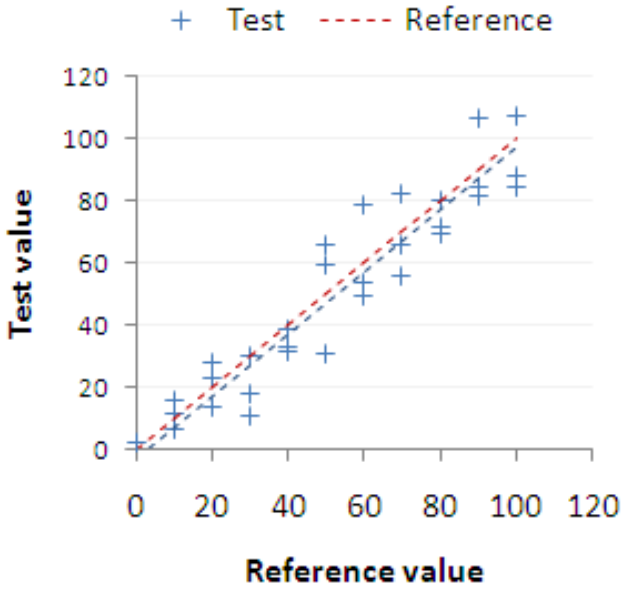
- All tests are fundamentally quantitative
- Sometimes we use the quantitative result directly
- However, it is often necessary to make an inference about the sample based on the quantitative result.



Types of tests

A	**Quantitative** tests. The result can have any value between two limits (including decimals).
B	**Categorical** tests where the quantitative signal is placed into an ordinal series to give the final result.
C	**Categorical** tests where the quantitative signal is placed into one of a limited series of predefined categories to give the final result.
D	**Qualitative** tests where the true quantitative signal can have one of many possible values, but the required result can only have one of two possible values.
E	**Qualitative [binary]** tests where the true quantitative signal can only have one of two possible values

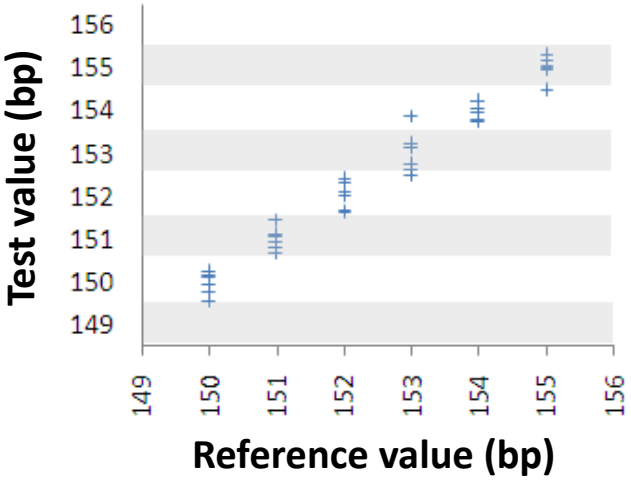
Type A - Quantitative tests

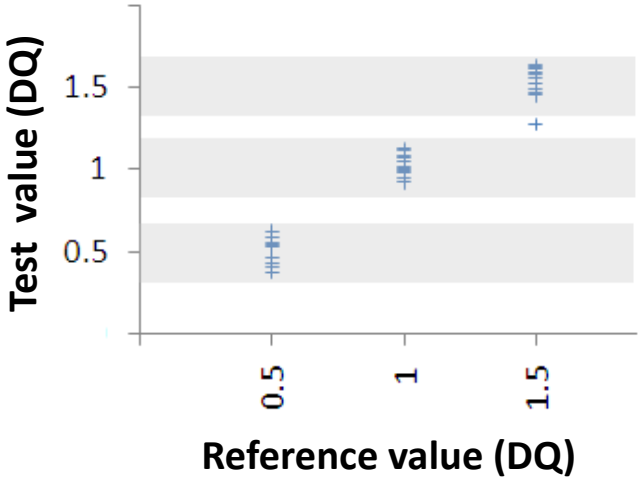


Analysis of methylation load
Analysis of mosaicism
Analysis of heteroplasmy

- Results represent the quantity of the measured analyte
- Reference (true) values can be any number including decimals
- Measurement is referred to as ‘continuous’

Categorical (Semi-quantitative) tests





Type B

Sizing a PCR product
Sizing triplet repeat

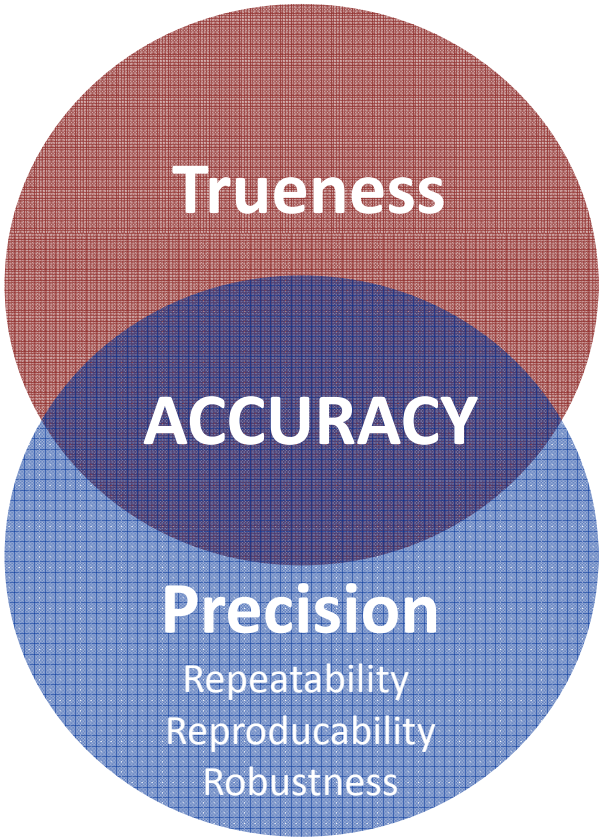
Type C

Allele copy number
Exon deleltion/duplication

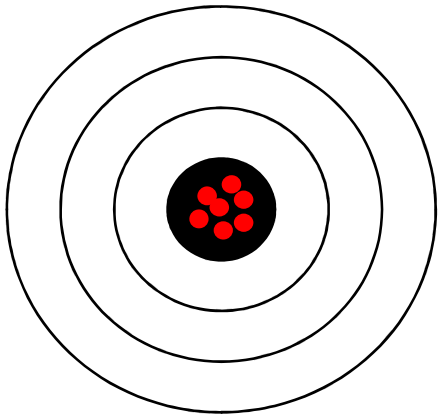
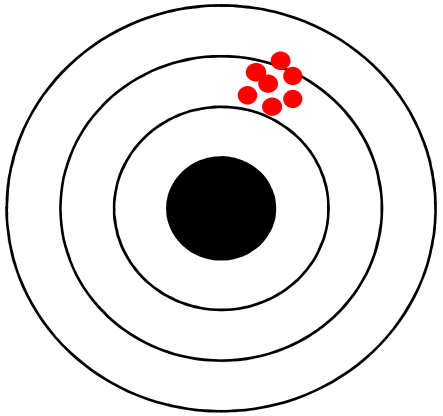
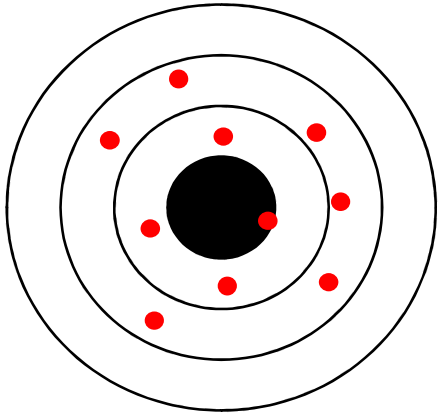
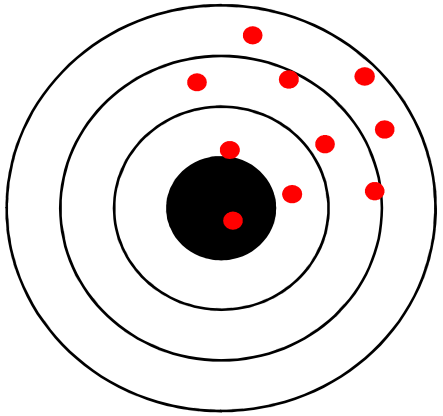
- Test results are grouped into categories using cut-offs
- Measurement is referred to as ‘discontinuous’

Quantitative & Semi-quantitative tests: Components of Accuracy

A key function of validation / verification is to estimate **ACCURACY**



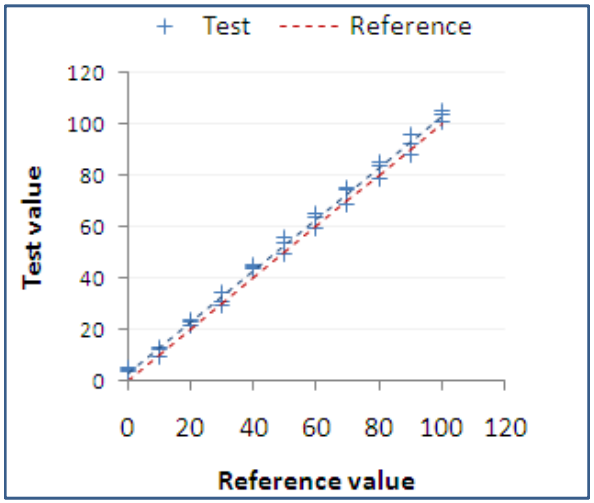
- describes how close the test result is to the true result
- describes how scattered replicate test results are

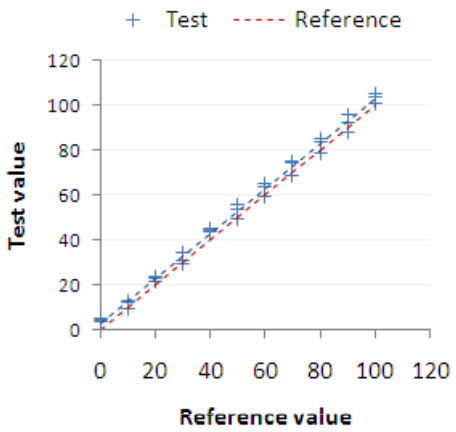
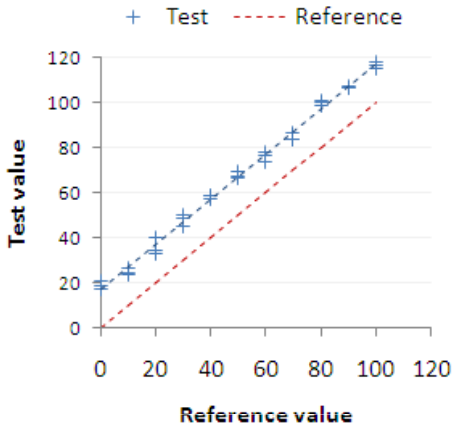
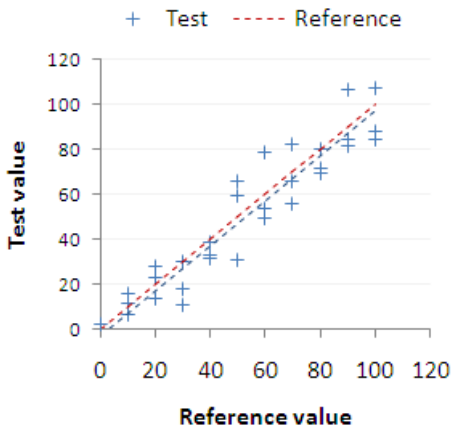
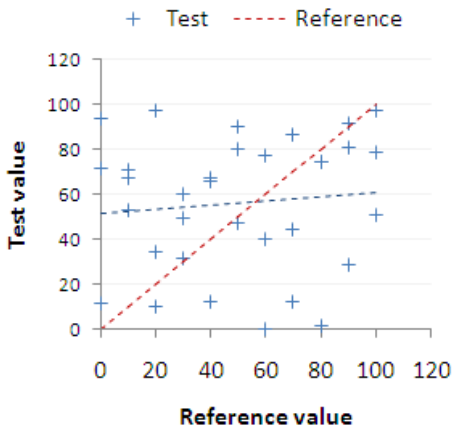
		Trueness	
		True	Not true
Precision	Precise		
	Not precise		

Precision

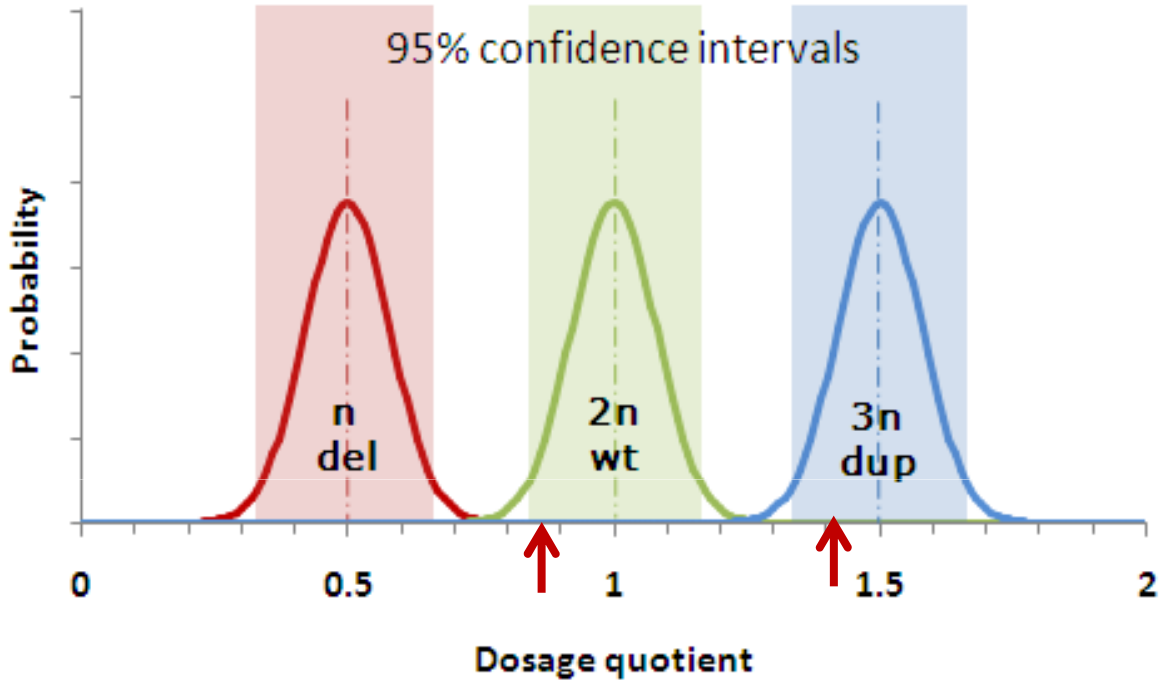
Variation of result under different conditions

- **Repeatability** → same sample same conditions
- **Reproducability** → different samples, operator, PCR machine, lab etc
- **Robustness** → stability of result under specific challenge e.g. Annealing temp, extraction method.



	True	Not true
Precise		
Not precise		

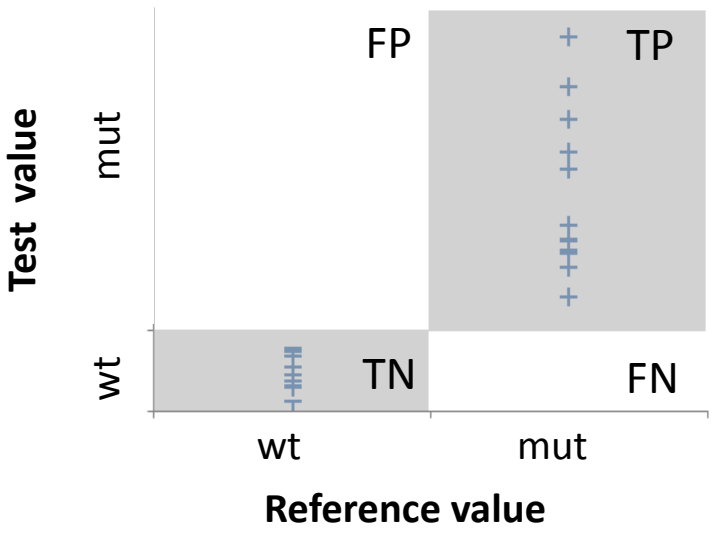
NB: Probability



MLPA assay to detect exon deletion or duplication

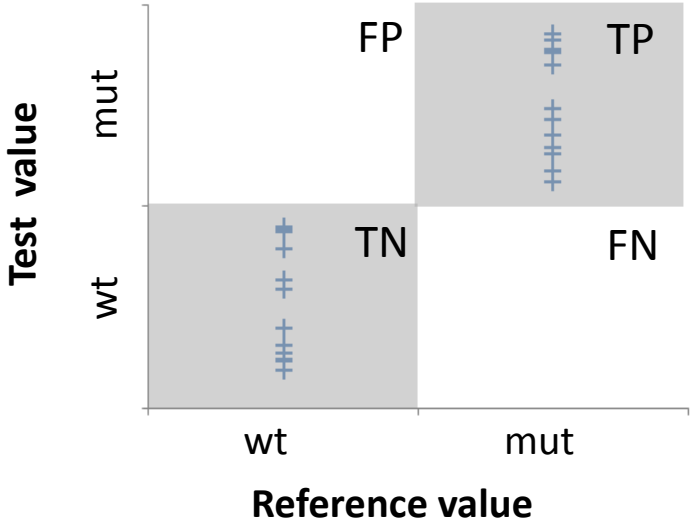
► Probability may be a preferable measure of accuracy for some tests particularly semi-quantitative tests with a low number of pre-defined categories

Qualitative tests



Type D

Mutation scanning

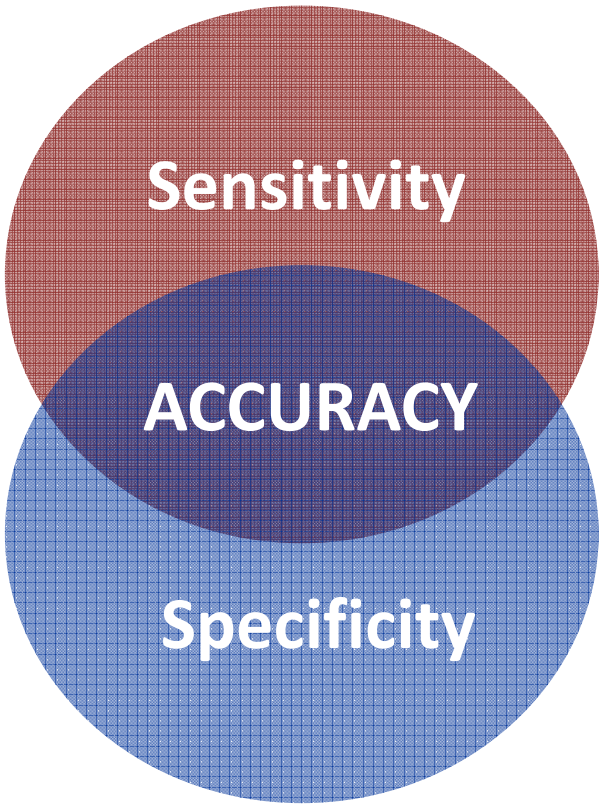


Type E

Genotyping

- Test results are grouped into one of two categories using cut-offs or by manual inspection
- Measurement is referred to as 'Dichotomous'

Qualitative tests:
Components of Accuracy

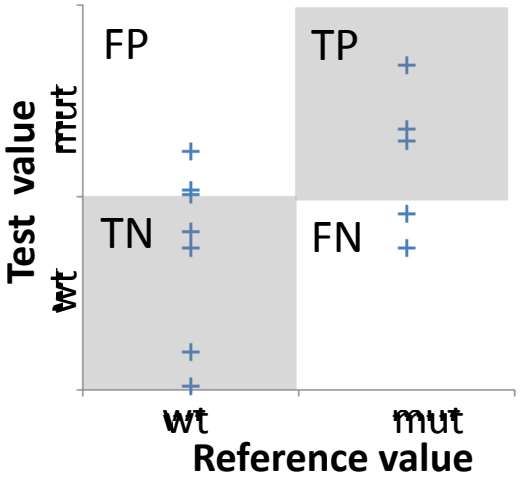


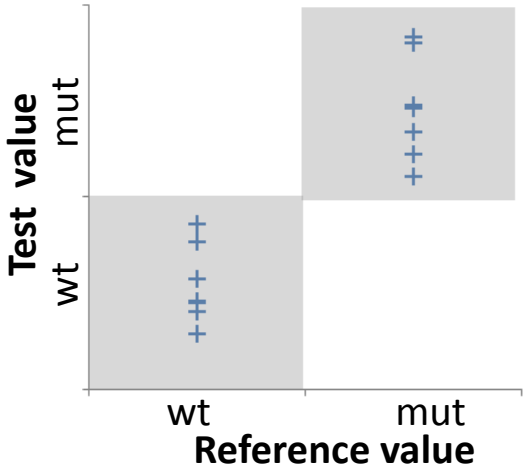
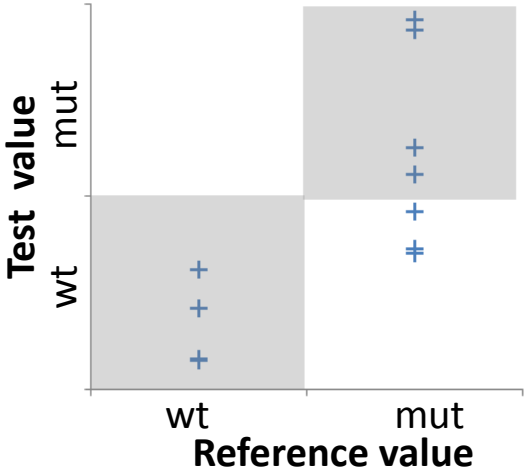
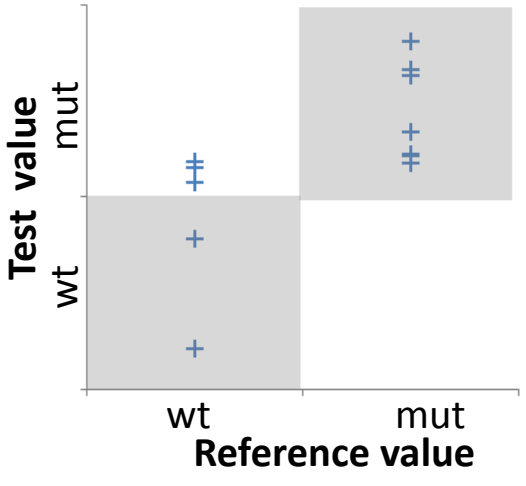
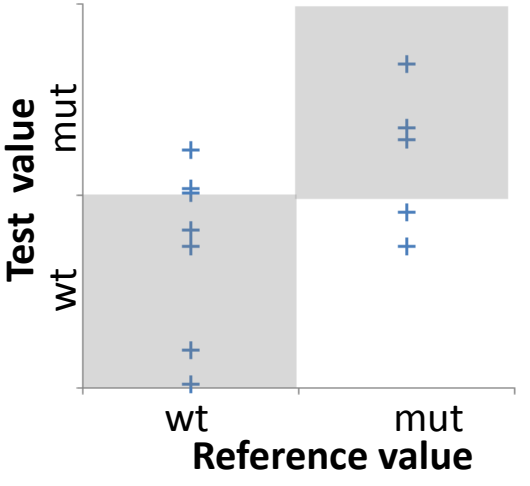
► describes how good the test is at detecting positives (mutants)

► describes how good the test is at detecting negatives (wild types)

NB: the capacity of a test to detect low levels of an analyte in a mixture is sometimes described as sensitivity

THIS SHOULD BE AVOIDED → THE TERM 'LIMIT OF DETECTION' SHOULD BE USED



	Sensitive	Not sensitive
Specific		
Non-specific		

Summary

- A key function of validation / verification is to estimate **ACCURACY**
- For quantitative and semi-quantitative tests **ACCURACY** comprises:
  - **TRUENESS** → measures deviation from truth
  - **PRECISION** → measures deviation from average result
- For some semi-quantitative tests **ACCURACY** may be best described using probability
- For qualitative tests **ACCURACY** comprises:
  - **SENSITIVITY** → measures ability to detect positives
  - **SPECIFICITY** → measures ability to detect negatives

Types of test.

*NB. In addition to the parameters detailed below appropriate **robustness** testing should be carried out for all types of test.*

	Description	Examples	Sensitivity[a]	Specificity[b]	Accuracy[c]	Trueness	Precision[d]	Limits of detection	Probability[e]
A	**Quantitative** tests. The result can have any value between two limits (including decimals).	Determination of methylation load (%); characterization of a mosaic mutation; heteroplasmy of mitochondrial variants.				++	++	++	
B	**Categorical** tests where the quantitative signal is placed into an ordinal series to give the final result.	Sizing a PCR product; determination of triplet repeat size (FRAXA, Huntington disease, etc.)			+	++	++	++	+
C	**Categorical** tests where the quantitative signal is placed into one of a limited series of predefined categories to give the final result.	Determination of copy number using PCR or MLPA.: exon deletion / duplication in *BRCA1*; *PMP22* gene dosage in CMT and HNPP			+	To establish correction factors and/or cut-offs			++
D	**Qualitative** tests where the true quantitative signal can have one of many possible values, but the required result can only have one of two possible values.	Mutation scanning for unknown mutations e.g. by sequencing or high resolution melt.	++	++	+			++	
E	**Qualitative [binary]** tests where the true quantitative signal can only have one of two possible values	Genotyping for a specific mutation e.g. *CFTR* Phe508del in cystic fibrosis or *HFE* Cys282Tyr in hemochromatosis.	++	++	+			++	+

Legend

- Metric used for implementation validation
- Metric used for implementation or ongoing validation
- Metric used for ongoing validation
- ++ Recommended parameter
- \+ Applicable parameter (less used)

Notes

a. Sensitivity = True Positive / (True Positive + False Negative)

b. Specificity = True Negative / (True Negative + False Positive)

c. Accuracy = True Result / (True Result + False Result

d. Precision should be measured in terms of repeatability and intermediate precision (as well as reproducibility for inter-laboratory validations)

e. The term 'probability' is used to describe situations where a probability that the result is correct can be assigned – primarily in ongoing validation (e.g. competitive hypothesis testing)