Validation of mutliplex ligation-dependent probe amplification: A competitive hypothesis testing approach

Validation options

- MLPA results categorised into a limited set of pre-defined categories – typically
  - Normal (*2n* alleles present)
  - Exon deletion (*n* alleles present)
  - Exon duplication (*3n* alleles present)

- Could be treated as a Categorical (type C) test:
  - Test *p* positive and *n* negative samples
  - Count 'correct' and 'incorrect' results
  - ACCURACY= correct / (correct + incorrect)

- Could be treated as a Qualitative (type D) test:
  - Test *x* deletions, y duplications and *n* negative samples
  - Construct a contingency table for results
  - SENSITIVITY = TP/(TP+FN)
  - SPECIFICITY = TN/(TN+FP)

	+	-
+	TP	FP
-	FN	TN

- **Disadvantages:**
  - Requires good numbers of different deletions and duplications – these may not be available
  - Performed once before implementation – good control measures / ongoing validation are required to ensure conditions are maintained and the accuracy measure is a true reflection of current usage

Probability as a measure of accuracy

- Based on a number of control reactions the probability that a given result should be placed in a particular category can be calculated

- Probability may be a preferable measure of accuracy for some tests particularly semi-quantitative tests (like MLPA) with a low number of pre-defined categories.

- **Advantages:**
  - Probability is test run specific
  - Full validation does not require large numberrs of positive controls

Dosage quotient (DQ)

⇒DQ=relative height of the test peak expressed as a proportion of the relative height of a reference peak[s]).

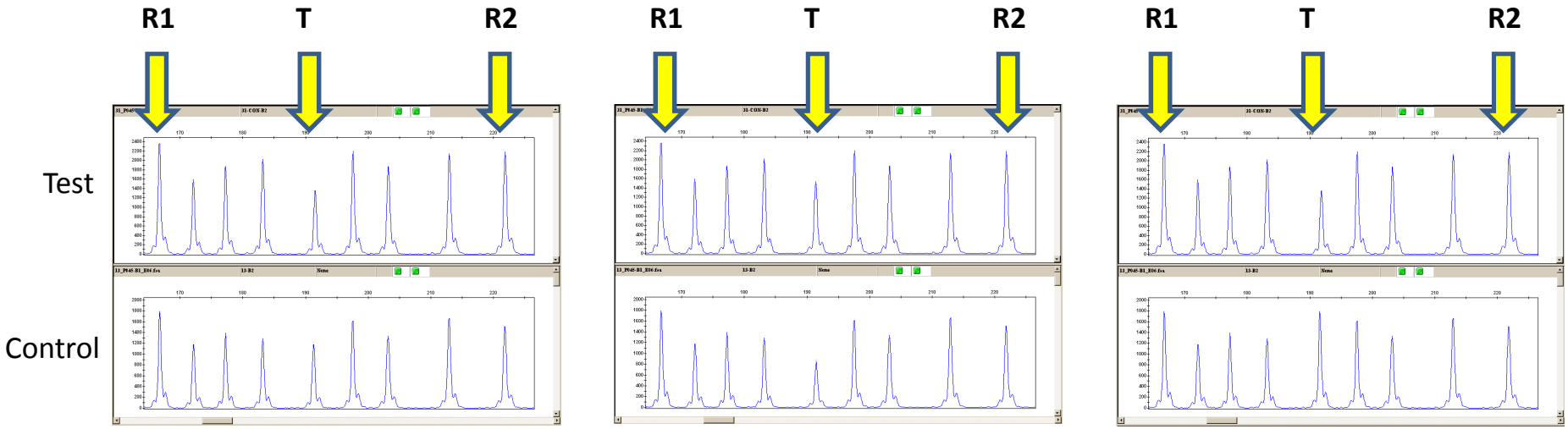
i.e.

$$\frac{\left(\frac{peak\ of\ interest\ [control]}{average\ of\ ref\ peaks\ [control]}\right)}{\left(\frac{peak\ of\ interest\ [test]}{average\ of\ ref\ peaks\ [test]}\right)}$$

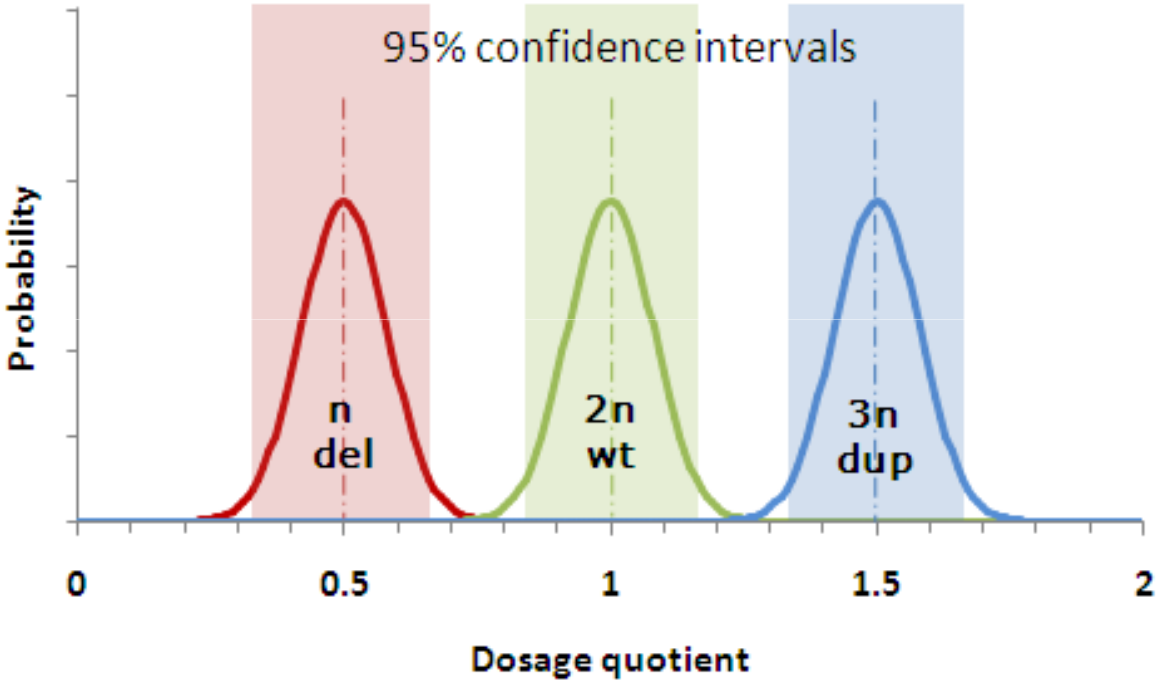
Normal *(2n)*:
DQ = 1.0

Exon deletion (n):
DQ = 1.0

Exon duplication (3n):
DQ = 1.0



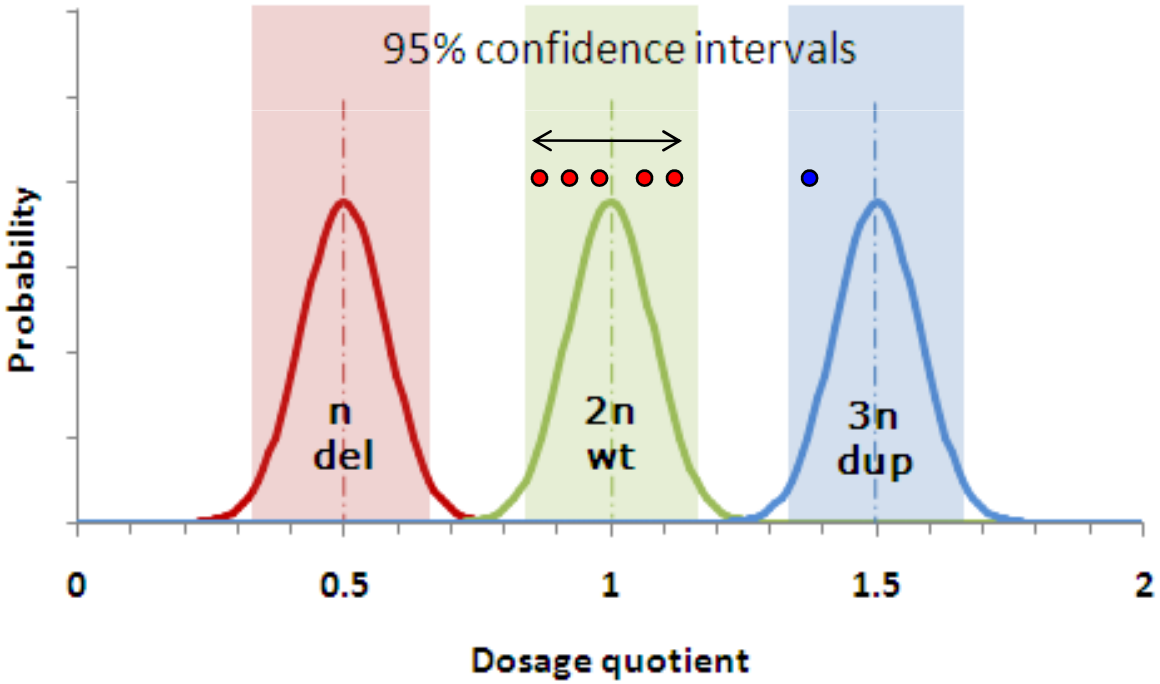
Validation of MLPA on an ongoing basis



MLPA assay to detect exon deletion or duplication

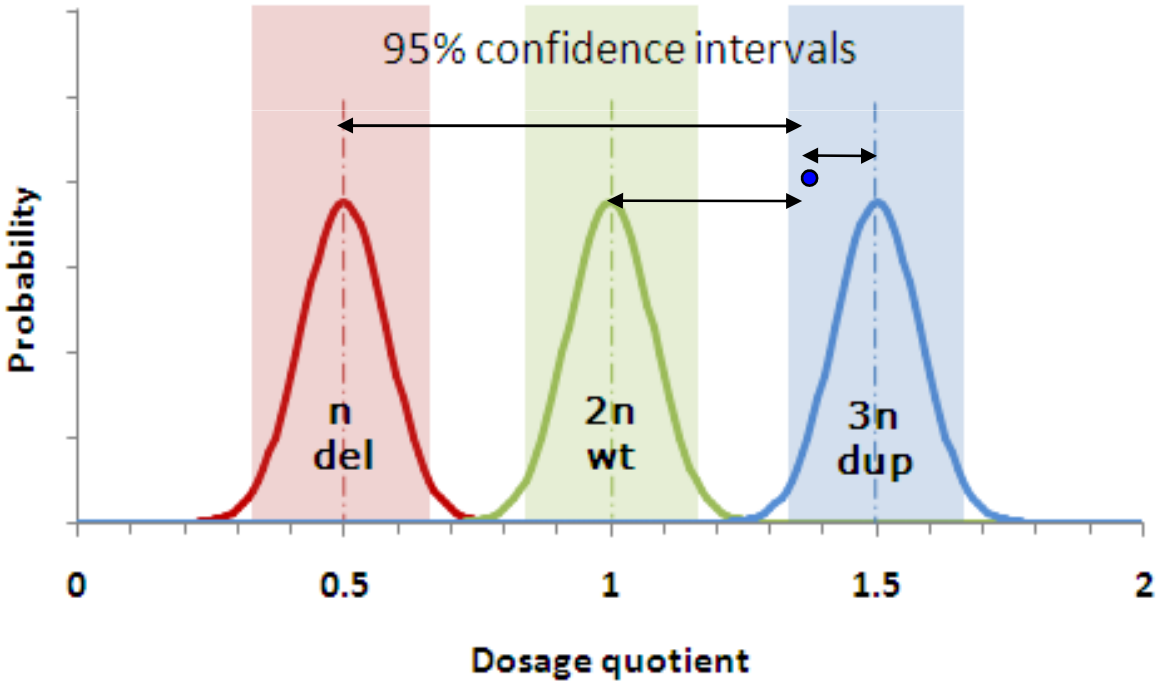
Validation of MLPA on an ongoing basis

⇒5x wild type controls run with each batch
⇒Distribution of wt results calculated (mean & SD)
⇒Assumptions:
2 x deletion result should follow wt distribution
2/3 x duplication result should follow wt distribution



Validation of MLPA on an ongoing basis

⇒Calculate each result as number of SD from mean for each hypothesis
⇒Use t-statistic to determine probability for each hypothesis
⇒Compare hypotheses by odds ratios:
⇒p(wt):p(del) and p(wt):p(dup)



[MLPA spreadsheet analysis instructions]

http://www.ngrl.org.uk/Manchester/Downloads