

## Technical Aspects of Tumor Marker Studies

### 2<sup>nd</sup> TBCI Correlative Sciences Workshop, Feb 2009

Mitch Dowsett  
Royal Marsden Hospital  
London, UK

Tim (Maughan) and Mitch  
(27/01/09)

Tim: Mitch can I have a word...  
.....d'ya know Mick Helth?

Mitch: Do I know who?

Tim: What?

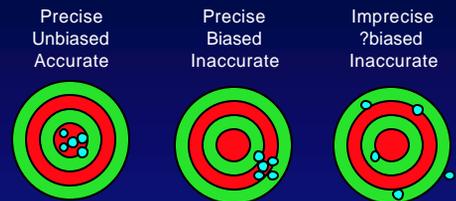
Mitch: You asked if knew Mick somebody

Tim: Pause..... ..Genomic Health?!

Discuss analytical accuracy, reliability, reproducibility, and practicality:  
what does it take to have a "clinical grade" assay?

- performance and control of established assays
- performance and control of development assays

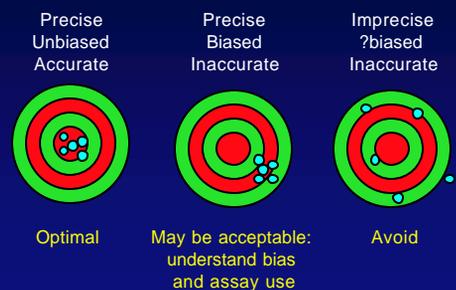
### Basic descriptions of analytical error

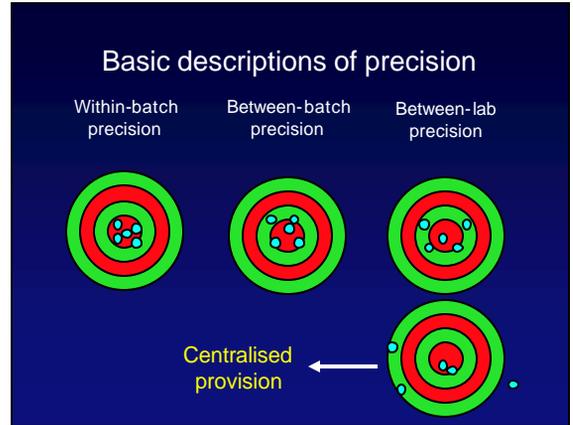
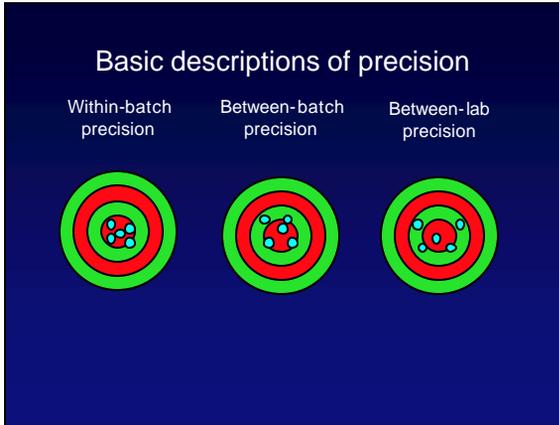


## Error

- Bias
  - non-specificity
  - inappropriate standards
  - incorrect standardisation (NB AFP)
- Variability/imprecision
  - measurement
  - heterogeneous expression
  - physiologic/pathologic

### Basic descriptions of analytical error

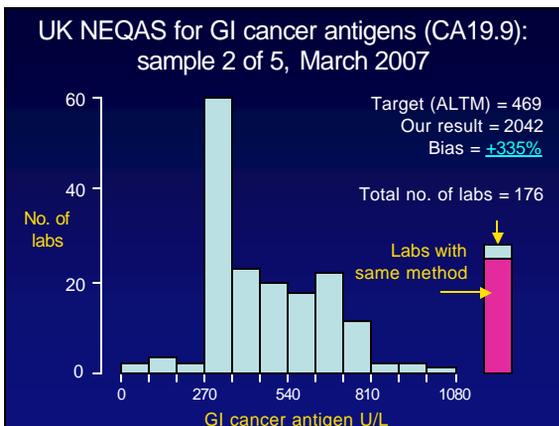
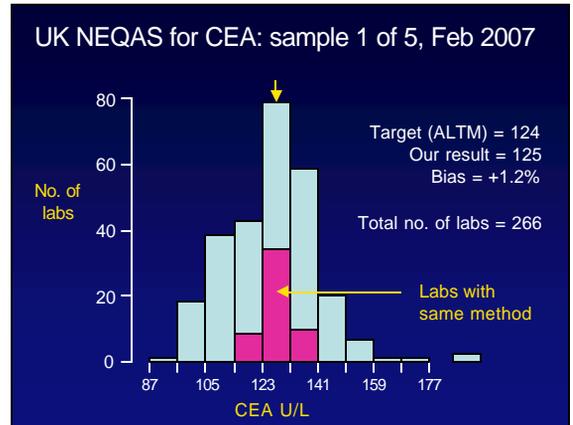




### Established tumour markers in blood

AFP, HCG, CEA, PSA, CA125, CA15.3, CA19.9

- automated immunoassay - high precision
- day-to-day internal controls - decision point
- at least monthly external controls

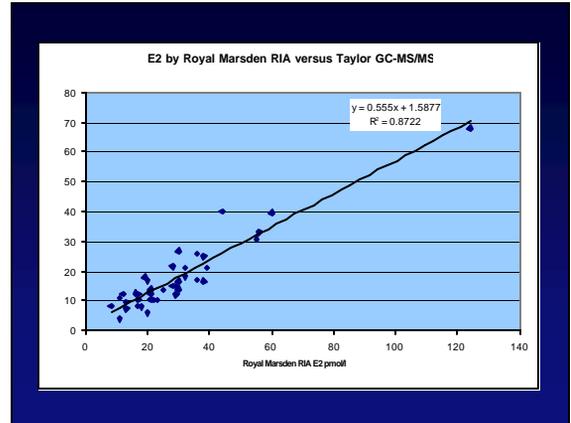
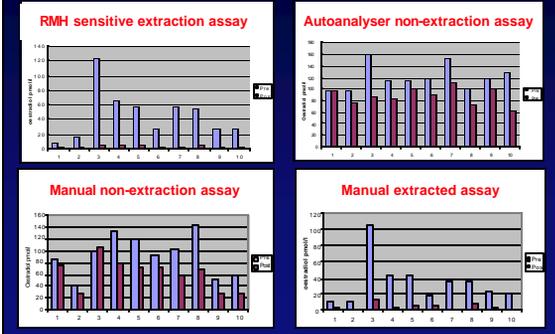


### Plasma estradiol: postmenopausal women

- Used as pharmacodynamic marker for development of aromatase inhibitors
  - specialist sensitive assays
- Sometimes valuable for monitoring compliance and performance of AIs

### Oestradiol assay type seriously affects results related to aromatase inhibitors

(Dowsett and Folked, Breast Cancer Res 7 (2005) 1-4)



### Established tumour marker assays in tissue: breast cancer ER, PgR, HER2 (IHC)

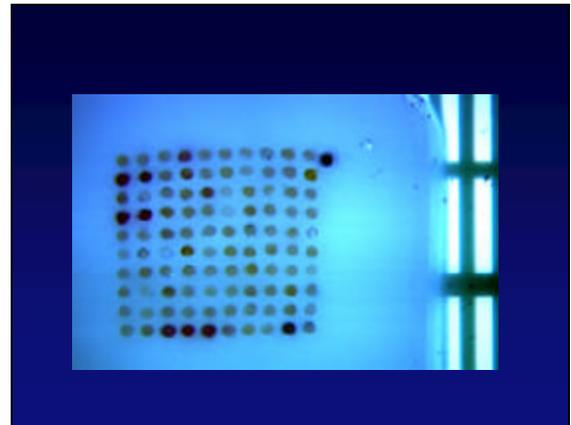
Batch controls:

positive/negative; critical cut-points

Regular audit of performance

NEQAS: 4 assessments per year

- ER/PgR multi-tissue block + own control
- HER2 cell lines + own control
- expert review - confidential report
- on-line images
- detailed analysis of data on methods



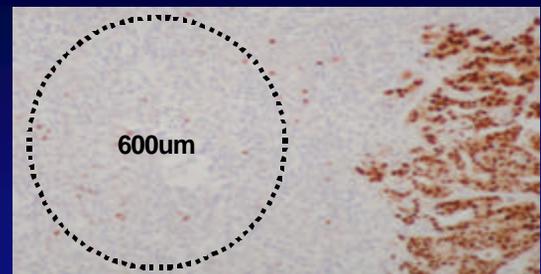
### Concordance between TMA and whole section for PgR

#### Whole section

		+ve	-ve	N/A	Total
TMA	+ve	126	6	1	133
	-ve	29	35	0	64
	N/A	10	3	0	13
	Total	165	44	1	210

+ve >10% cells staining

### Heterogeneity in PgR staining



## Concordance between TMA and whole section for HER2

### Whole section

TMA

	+ve	-ve	N/A	Total
+ve	13	4	0	17
-ve	3	189	1	193
N/A	0	0	0	0
Total	16	193	1	210

+ve IHC 3+ and/or FISH +ve

96.7% concordance

## Concordance between TMA and whole section for HER2

### Whole section

TMA

	+ve	-ve	N/A	Total
+ve	13	4	0	17
-ve	3	189	1	193
N/A	0	0	0	0
Total	16	193	1	210

+ve IHC 3+ and/or FISH +ve

## Ki67: approaching clinical use?

Prognostic marker

Dynamic marker - ? Ki67

Prognostic marker on treatment

good validation data within lab(s)

primary end-point

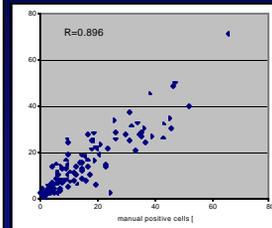
current attempts to agree uniform protocol  
(POETIC/Ellis neoadjuvant triage)

manual or image analysis

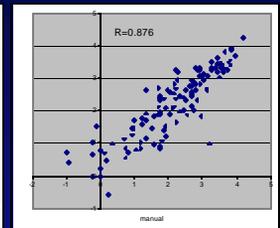
MIB1 or SP6 antibody

## IMPACT Ki67 measurements Manual vs. Ariol

IMPACT %Ki67 pos cells  
61 pairs  
Manual vs. Ariol



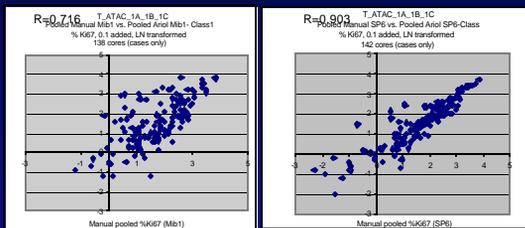
IMPACT %Ki67 pos cells LN transformed  
61 pairs  
Manual vs. Ariol



## T\_ATAC\_1A\_1B\_1C pooled Manual vs. pooled Ariol %Ki67 cases

MIB1

SP6



## Biomarkers in breast cancer studies over last 10 years

In clinical studies	100s
In clinical trials	100s
Worth validation study	10s
Worth (prospective) clinical evaluation	<10
Worth clinical use	<<10

## Biomarkers in breast cancer studies over last 10 years

In clinical studies + analytical validity	100s
In clinical trials + analytical validity	100s
Worth validation study + reproducible method	10s
Worth (prospective) clinical evaluation + rugged, precise method	<10
Worth clinical use + rugged, precise, exportable method (if poss)	<<10

## PACCT

Identify clinical question

Examine potential markers

Select for evaluation based on potential for widespread clinical utility

## New (prospective) tumor marker

- Develop from research assays
- Usually no gold standard
- Full validity often difficult to prove:
  - tests to improve confidence
  - consistency with expectation
- Imagination, no single set of rules
- Understand and *declare* uncertainty

## IHC: approaches to determine (in)validity

1. Western blot of antibody (NB tissue vs cells; fixed vs non-fixed)
2. Immunoabsorption with excess antigen
3. Presence or absence of reaction with known tissue/organelle (*caveolin in caveolae*)
4. Microdissection for RNA studies in cell subpopulations (*aromatase: Miki et al Cancer Res 67,3945*)
5. Anti-phospho-X antibodies: phosphatase, inhibitors
6. Knockouts/knockdowns: siRNA, inhibitors
7. Transfections

## Summary

It is all artifactual  
but know your artifact