Demonstrating control over multiplexed biomarker methods – can the context of use save the day?

Michael Wright

EBF Autumn Focus Workshop 2019

Disclaimers

- The opinions expressed in this presentation are those of the author
- They do not purport to reflect the views of LGC or the industry as a whole
- This presentation will look at ways of demonstrating control over multiplexed biomarker assays but is not exhaustive

other flavours of control are available…

Multi-Com-Plex Assays

Additional considerations

- Ranges & Expected changes
- Freeze Thaw limitations
- Different Stabilities
- Difference in kit performance between biomarkers
- Kit lot variations
- Differential sample requirements for additional assays

Drivers

- Cost
- Time
- Sample volume
- Single measurement Index analysis (IVDMIA)
- Precision (Personalised) medicine

http://www.e-b-f.eu/wp-content/uploads/2018/12/bcn2018-47.-James-Beecroft-LGC.pdf

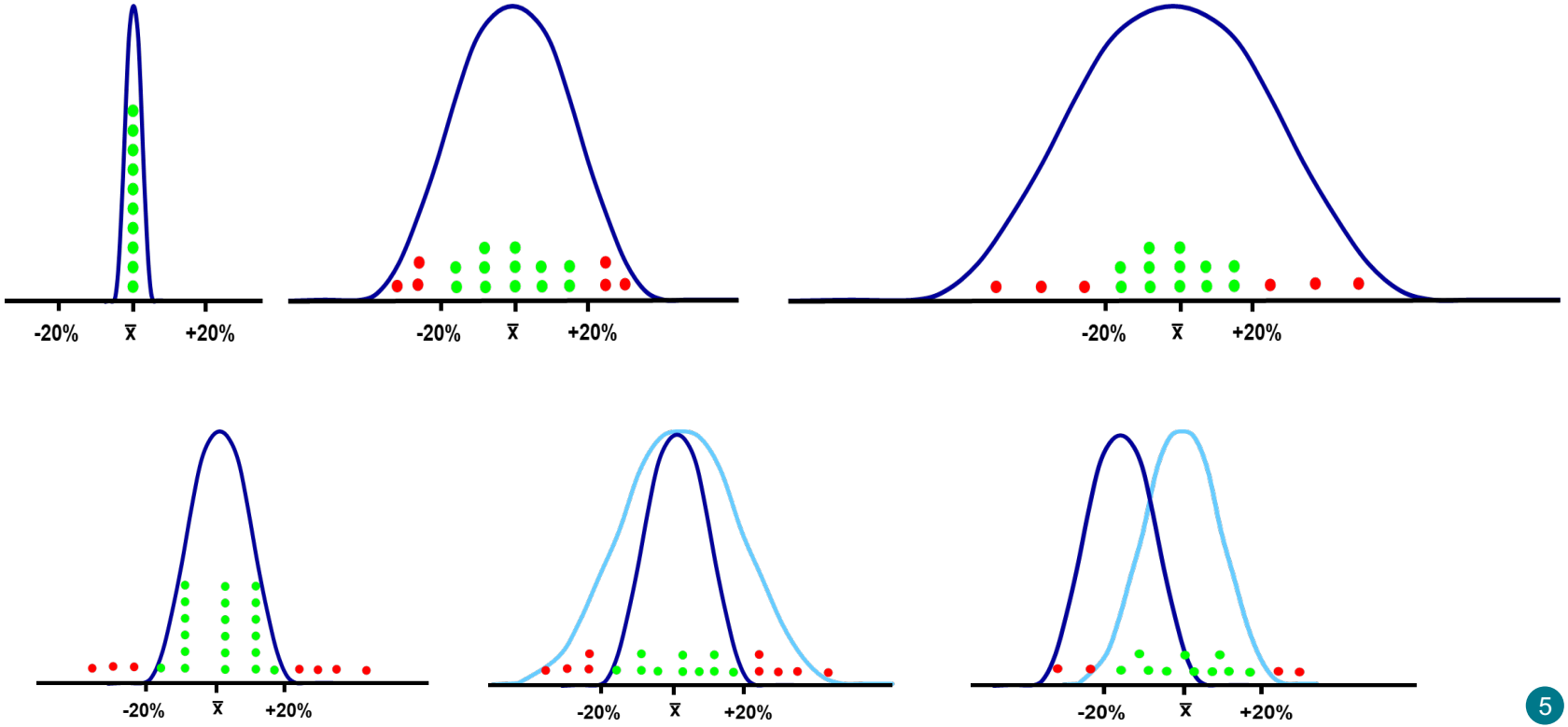
How do you demonstrate control

- The probability of all the analytes in a plex passing quality control simultaneously falls as the number of analytes goes up

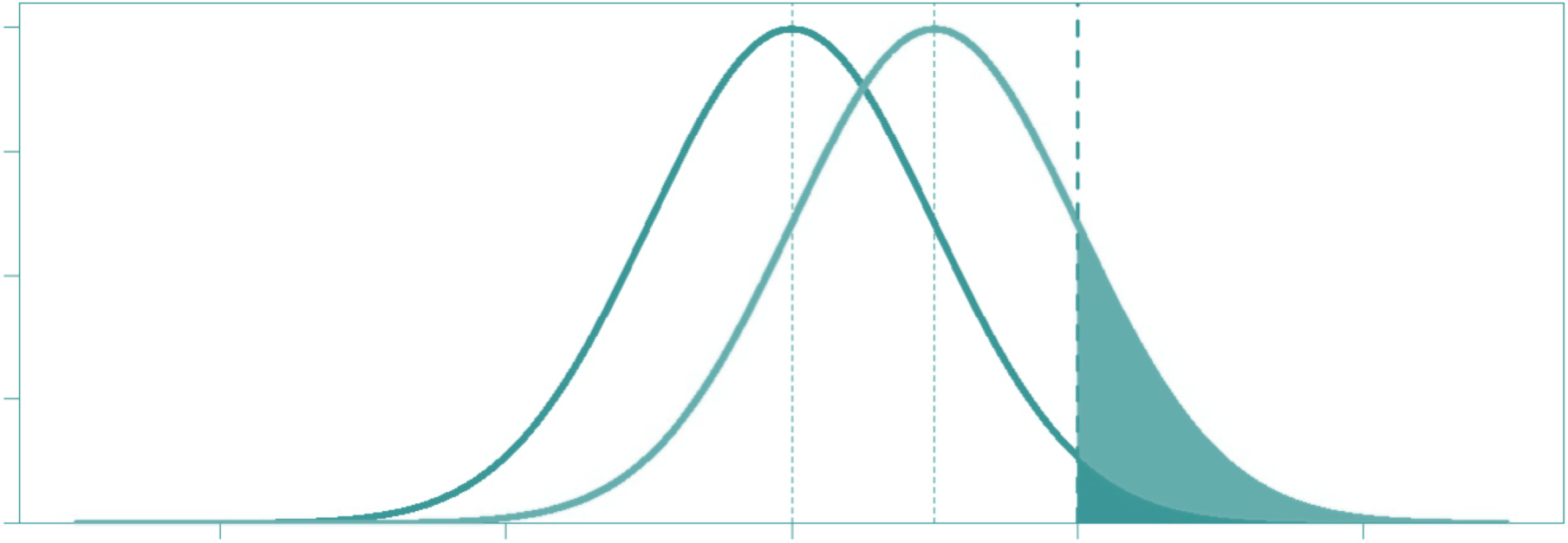
- This is worse for Biomarkers!

- This is exacerbated with poorly characterised assays

4-6-X, Validations and Characterising the assay

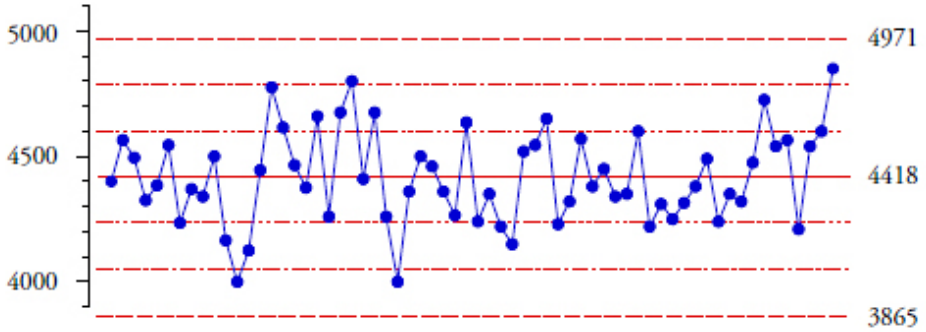


Compounding the Error



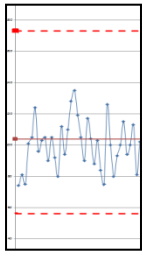
Clinical Diagnostic Approach – Confidence Limit Acceptance Criteria [1,2]

- QC Acceptance Criteria determined according to the performance of the assay
- Performance of the assay compared to the intended use of the Biomarker (TAE)



1) Levey S, Jennings ER. The use of control charts in the clinical laboratory. Am J Clin Pathol 1950;20:1059-1066.
2) Westgard JO, Barry PL, Hunt MR. A multi-rule Shewhart chart for quality control in clinical chemistry. Clin Chem 1981;27:493-501

Clinical Laboratory Validation



Bioanalytical validation

CLSI C62-A Validation

Local healthy population assessment & setting RIs

Ethics approval & disease state assessment

Outpatient preanalytics assessment

Additional Stability & Hospital population matrix assessment

Go Live

The Westgard Rules

1_{3S} ● >3 SD from target mean [A]

2_{2S} ● Last 2 > 2SD from target mean [A]

R_{4S} ● Difference between consecutive values >4SD [P]

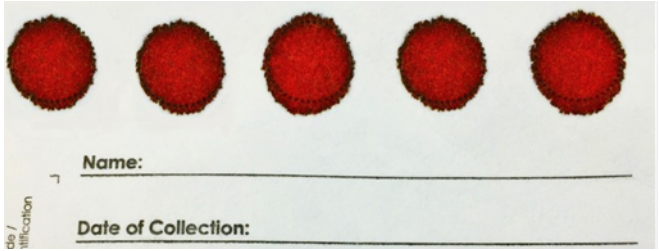
4_{1S} ● Last 4 >(Mean+ 1 SD) or <(mean – 1SD) [A]

10_{X} ● Last 10 on same side of mean [A]

No Westgard Rules for Multiplex Assays

2001-2004
Screening for phenylketonuria (PKU) &
Medium chain acyl-CoA dehydrogenase deficiency(MCADD)

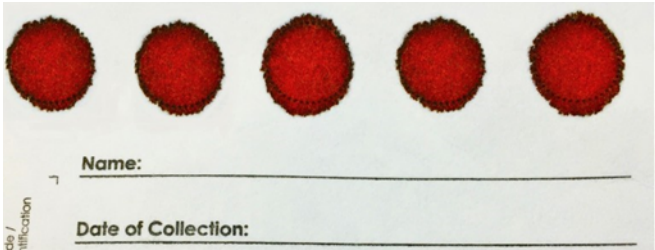
Phenylalanine and C8/C10 by LC-MS/MS



- **Fatty Acid Oxidation Disorders:**
- -Carnitine palmitoyltransferase I (CPTI) deficiency
- -Medium-chain 3-ketoacyl-CoA thiolase (MCKAT) deficiency
- -Dienoyl-CoA reductase deficiency
- -Short-chain acyl-CoA dehydrogenase (SCAD) deficiency
- -Medium/Short-chain 3-hydroxyacyl-CoA dehydrogenase (M/SCHAD) deficiency
- -Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency
- -Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency & trifunctional protein deficiency
- -Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency
- -Carnitine palmitoyl transferase type II (CPT-II) deficiency
- -Carnitine-acylcarnitine translocase (CACT) deficiency
- -Electron transfer flavoprotein (ETF) deficiency, ETF-dehydrogenase deficiency (multiple acyl-CoA dehydrogenase deficiency [MADD]; glutaric acidemia type II)
- **Organic Acid Disorders:**
- -Glutaryl-CoA dehydrogenase deficiency (glutaric acidemia type I)
- -Propionic Acidemia
- -Methylmalonic Acidemia
- -Isovaleric Acidemia
- -3-hydroxy-3-methylglutaryl-CoA carboxylase deficiency
- -3-Methylcrotonyl carboxylase deficiency
- -Biotinidase deficiency
- -Multiple carboxylase deficiency
- -Isobutyryl-CoA dehydrogenase deficiency
- -2-Methylbutyryl-CoA dehydrogenase deficiency
- -Beta-ketothiolase deficiency
- -Malonic aciduria
- -Ethylmalonic encephalopathy
- -Glutamate formiminotransferase deficiency (Formiminoglutamic aciduria)

3 Plex Gives Birth to a 22 Plex

- Free C0
- Acetyl C2:0
- Propionyl C3:0
- Butyryl C4:0
- Isovaleryl C5:0
- Malonyl C3DCA
- Hexanoyl C6:0
- Methylmalonyl C4:0DCA
- Glutaryl C5:0DCA
- Octanoyl C8:0
- Decenoyl C10:1
- Decanoyl C10:0
- C12:0
- Tetradecenoyl C14:1
- Myristoyl C14:0
- Palmitoyl C16:0
- 3OH Hexadecenoyl C16:1-OH
- 3OH Hexadecanoyl C16:0-OH
- Linoleoyl C18:2
- Oleoyl C18:1
- Stearoyl C18:0
- C18:1-OH
- C18:0-OH



What is happening?

Westgard multi-rules have a small percentage chance of failing a batch when there is nothing wrong with the assay

e.g 1_{3S} 99.7% CI = 0.3% false failure

Batch analysis removes the safety net – assay succeeds or fails – can't re-run individual components

Can't "reflex test" QCs or patients – often only know that a QC has failed when the batch is run

Mock 510k (Class II) submission[1]

"Lack of robust multivariate QC algorithms and guidelines for multiplexed protein array data rejection criteria are problematic"[2]

1) Regnier etal Clinical Chemistry (2010) 56:2 pg 165-171
2) Ellington etal Clinical Chemistry (2009) 55:6 pg1092-1099

Passing the 1_{3S} QC test

Multiplex No.	%Probability of pass 1 QC level	%Probability of pass 2 QC levels	%Probability of pass 3 QC levels
1	99.7	99.4	99.1
10	97.3	94.7	92.2
100	76.3	58.2	44.4
30,000	6×10^{-36}	4×10^{-71}	2×10^{-106}

For reference – the number of seconds since the universe began is ~ 4.3×10^{17}

- This is the best case scenario
- These are not true failures
- This will get worse if the mean and SD are not perfect

Steve Masters: Too Much Information? Quality Control for Emerging Multiplex Assays
https://www.youtube.com/watch?v=3QiFdUDIglg

4 Approaches

- **How is this data being used?**

1) Limited Plex

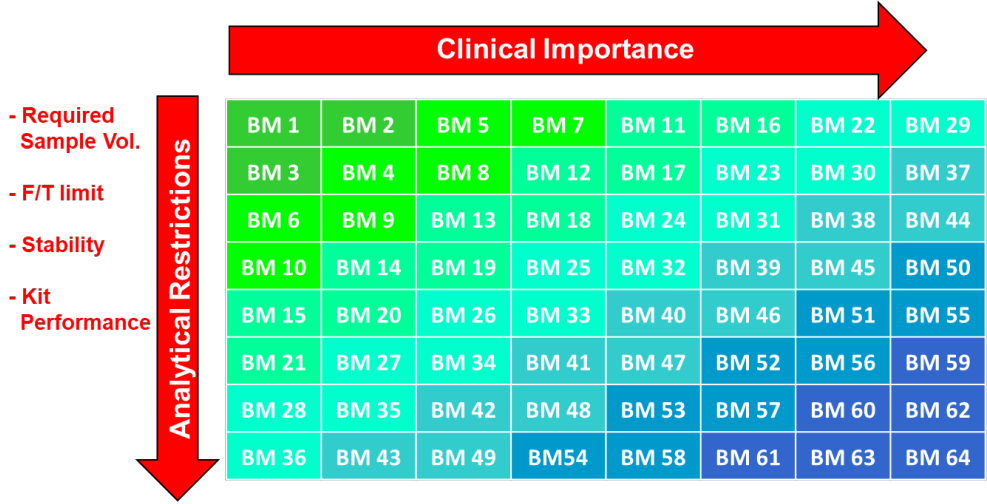
2) Analytical Performance Strategy

3) Pattern Approach

4) Panel Approach

Limited Plex Strategy

- Key BM versus “nice-to-haves”
- Reduce QC stringency where possible
  > Only do QCs on the ones that matter – the rest is what it is and guide on interpretation not control
- Strip out poor performers – redevelop/ troubleshoot as a single-plex



- Not always suitable
- Minimises the impact of the phenomenon rather than circumventing it.
  > IVDMIA: OVA 1 - Trimmed out 3 of 8 due to analytical reasons

Analytical Performance Strategy

~~CLSI~~

- Do not base acceptance criteria on the validation
  - e.g. what is the sort of change that would need to be seen to elicit a clinical intervention?
- Stretch criteria out as far as they can go based on the requirements at the clinical decision point
  - Screening where +ve confirmation is 50x biological variability
  - If 9SD before clinical action 4/5SD rule is appropriate
- + Limited Plex (key markers vs nice to haves)
  - CRO and Customer – need to convince cautious outsourcing departments
- Must have necessary analytical performance for this to work

Classify the plex

1) Assay Failure (sentinel QCs) vs component failure

2) Pattern or Panel

Pattern Based Approach

Many analytes – one (few) CoU

- Precision medicine

Power in multivariate analysis with complementary BM

- If a number of markers give you your answer do you need QC?

Large BM Index analysis

- Check the ability of the plex to withstand fliers and bias by deliberately perturbing components and assessing you ROC analysis to see if it is impacted

Inverse relationship between redundancy and QC stringency – if you have more BM then assess putting them in to build redundancy

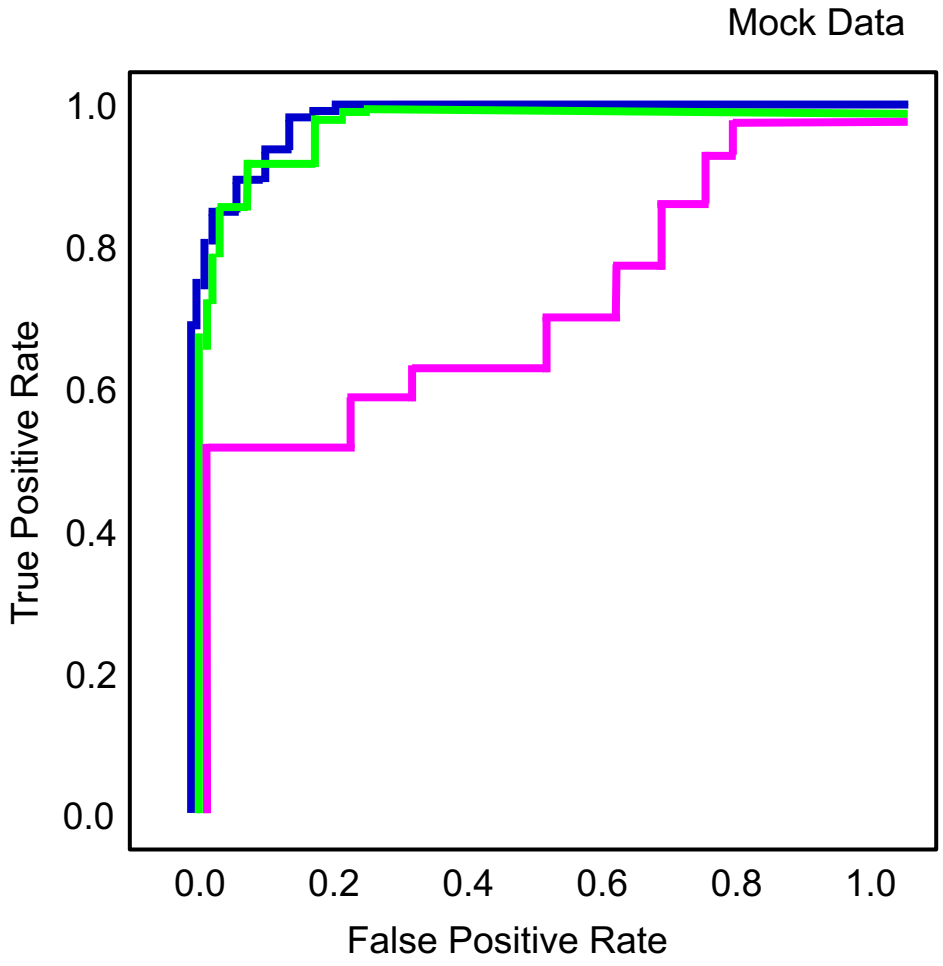
Analyte	QC pass	Group	Accept Data?
CA	✓	U	✓
DCA	✗	U	
CDCA	✓	U	
GCA	✓	G	✓
GDCA	✗	G	
GCDCA	✓	G	
TCA	✗	T	✓
TDCA	✗	T	
TCDCA	✓	T	

Stress-test plex's ability to withstand fliers, inaccuracy and bias by deliberately perturbing components

Assess ROC analysis to determine impact

Grey-zones/Bimodal decision points
(e.g. Metanephrines)

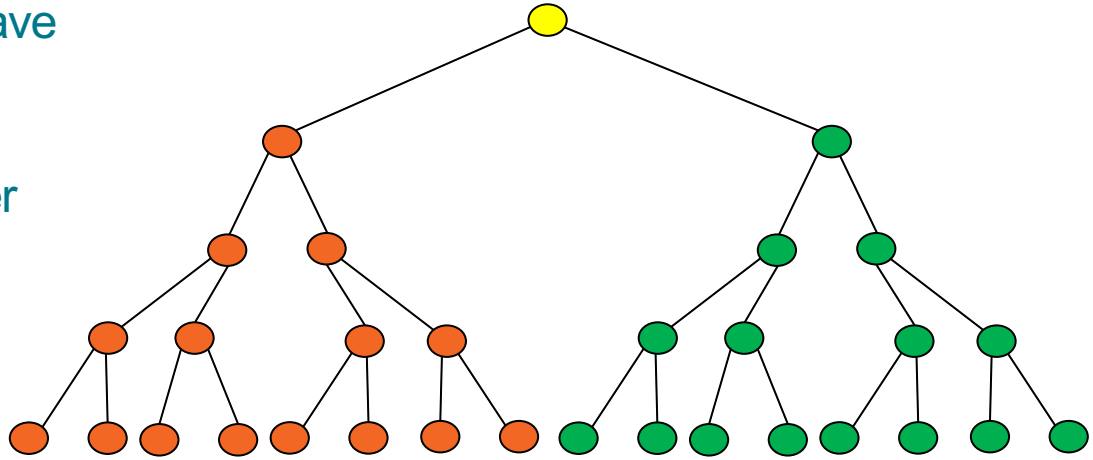
Build redundancy



Assess algorithm for component specific perturbation weakness

What impact does each point have on changing the outcome?

More redundant markers – lower impact of individual points



Panel Based Approach

- **Many analytes – Many CoU**
  > every analyte has a question attached (steroids, metanephrines)
- Sample limitations are driven by Paediatrics
- In Clinical Diagnostic settings this is often driven by Mass Spectrometry applications

- Assay Failure vs component failure
- F-test and T-test – multiple hypothesis testing with Bonferroni Correction

Analytes	N needed, 2 levels
1	10
10	14
100	18
1000	22
30,000	28

Masters etal in preparation

Conclusions

- **Traditional QC approaches do not work for Multiplex assays**
- Limited Plex and Analytical Performance Strategies can work
- Risk Assessments of assay failure vs component failure – possibility of sentinel QC?
- ↑Redundant markers ↓ QC stringency required but you will need bioinformatics
- Panels assays can benefit from traditional statistical assessments of an ↑ number of replicates of fewer QCs but may be of limited value in Bioanalysis

Questions?

Science
for a safer world