Immunosuppressant PT; Results and Methodologies examined.

Karen Morgan, LGC

Aim: To investigate trends in the results submitted by participants: methodologies and sample type

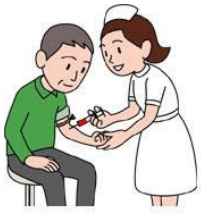
- **Objectives:**
- To identify whether there has been any shift in methodologies used by participants.
- To identify variations in how participants report results for zero spike samples.
- To investigate participant results submitted for patient pools for methodology variation and potential bias.

HISTORY

- **ASI involved in Immunosuppressant Drug (ISD) PT schemes since 1983. LGC acquired the ASI scheme in Oct 2016 having produced samples for 6 years previous. Prof. David Holt an adviser**
- Samples consist of spikes and pooled patient samples
- Method related assigned values
- Results from this scheme presented.
- **Other schemes are available (including CAP, and others)**

Why do TDM of Immunosuppressant Drugs (ISD)

- The target range is narrow.
- Consequences severe if target range is missed; too high- (drug toxicity and/or over-immunosuppresion (excessive risk of infection and malignancies)), or graft function impairment or loss if too low.
- Toxicodynamic effects can be difficult to distinguish from disease.
- The dose/exposure relationship is highly variable inter/intra-individually . i.e. Patient specific.
- Compliance- adherence is critical.



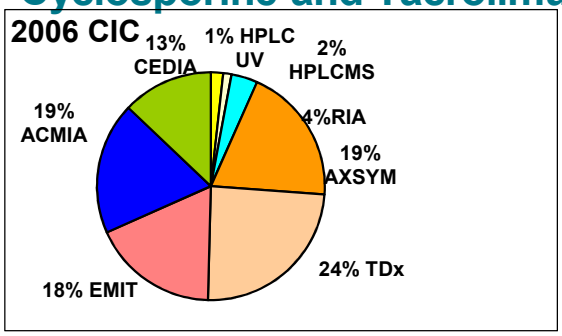
Methodologies Used

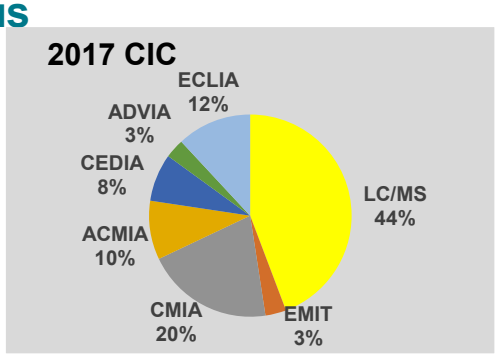
ISD PT: Methods

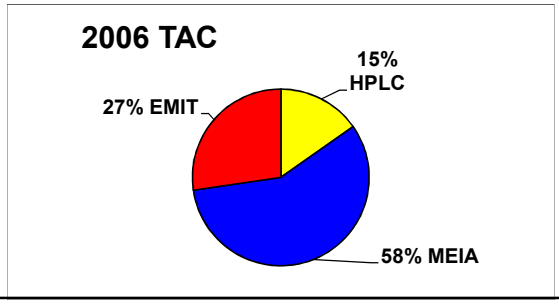
- There have been significant method advances. LCMSMS is seen as a reference method. Isotope-labelled internal standard are considered the Gold Standard.
- However in a Survey in 2013:
  - > 62% of laboratories used ascomycin for TAC assays and also sometimes for SIR (29%) and CIC (6%) which are structurally less related.
  - > Stock solutions used for preparation of calibrators and also QC by 34% of laboratories.
  - > 25% of laboratories used serial dilutions for calibrator production.
- LCMSMS- Different sample preparation procedures between laboratories. Therefore potential for significant variation in results between laboratories.

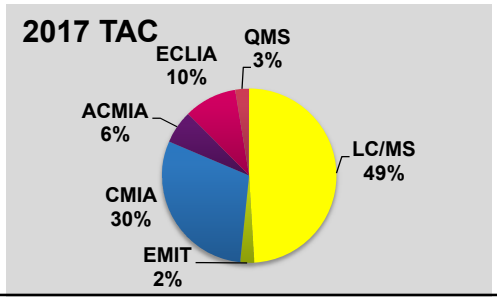
- Immunoassay based systems: IVD certified or FDA cleared commercial tests must state the guideline followed for method validation.
- Each laboratory should have a validation plan including: LLOQ, ULOQ, storage conditions, assay precision and accuracy, specificity to the parent drug and interferences....
- Advances seen in technologies – develop methods with lower LOQ. Validation of new assays. Certified reference materials to calibrate LC assays.

Cyclosporine and Tacrolimus









Comparison of methods used

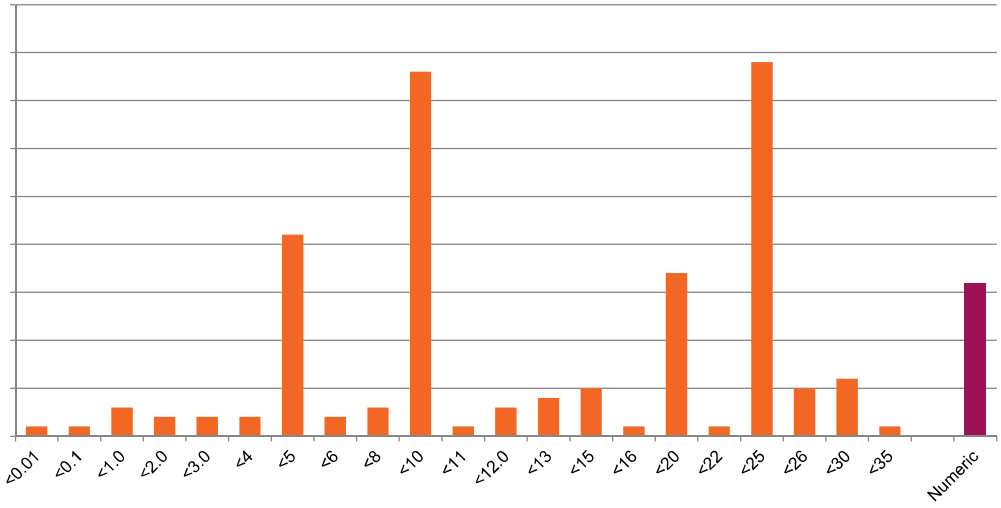
- **Issues:**
- Other- combination of methods- assessed by method.
- Low number of results by method- cannot be assessed statistically

Zero spikes

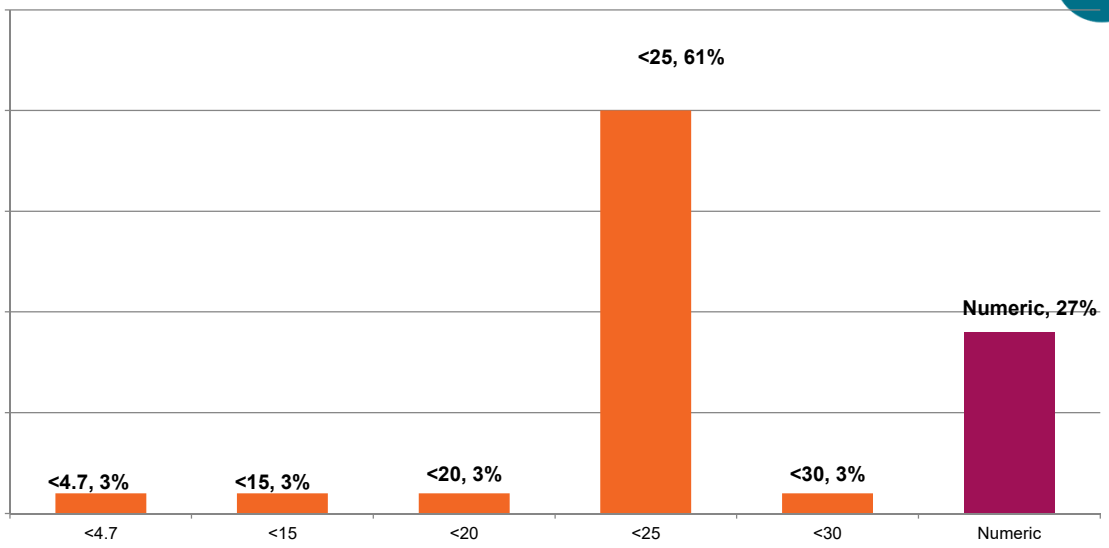
How to report zero spikes

- **Zero spike sample:**
- No parent drug has been added to the blood.
- The blood has been pre-screened.
- Each laboratory should know their own LOD or LOQ. If the result obtained is lower than this then it should be reported as a < value.
- The < value can be assessed in certain situations.
- Noted that in some instances an actual value may be entered – reading straight off the machine. These cannot be assessed if fall in the < range.
- Cyclosporine used as example of data submitted, 3 methods only as examples.

Cyclosporine LCMS information for zero spike, Round 394 A (µg/L), Numeric (9%) 0.1 to 18 , (range <0.01 to <35.0 (<25 is 22%))

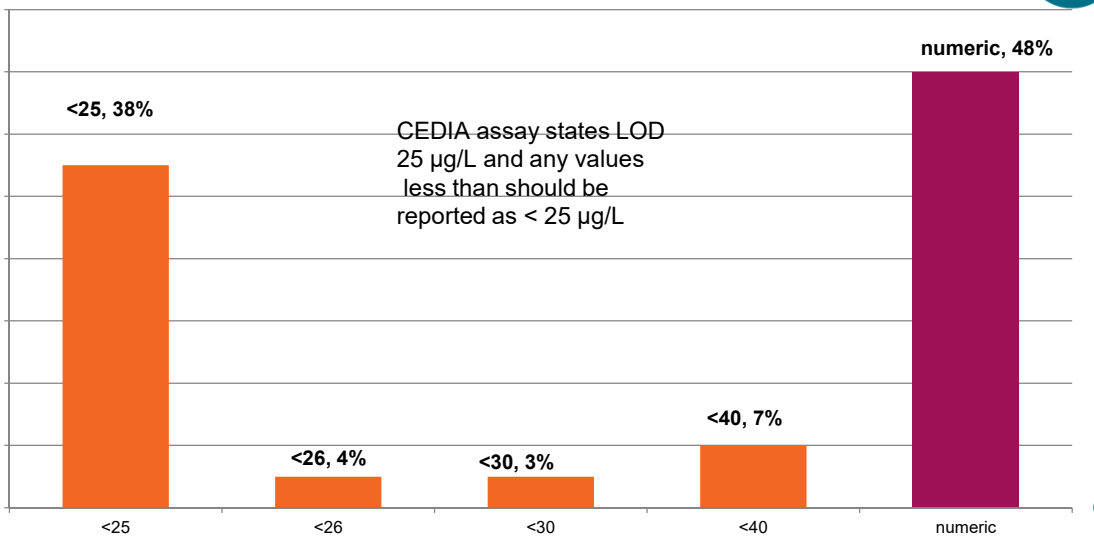


Cyclosporine ACMIA zero spike, µg/L, Numeric 0.1 to 7.3 µg/L, (range <4.7 to <30)



13

Cyclosporine CEDIA zero spike information, µg/L, Numeric 1 to 53 µg/L, (range <25 to <40)



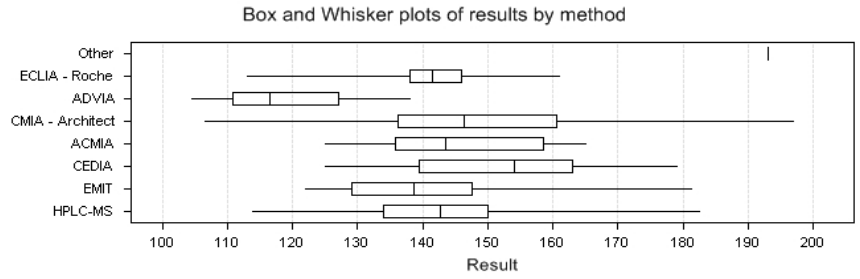
14

Patient Pools

Method specificity

- Target ranges used for TDM for the ISDs are for the parent drug.
- Therefore, analytical methods need to be specific for the parent drug determination.
- If metabolites are present, assay cross reactivity should be known.
- Cross-reactivity with drug metabolites (or non-separation) may lead to an overestimation of the drug concentration.

Patient pools: Cyclosporine

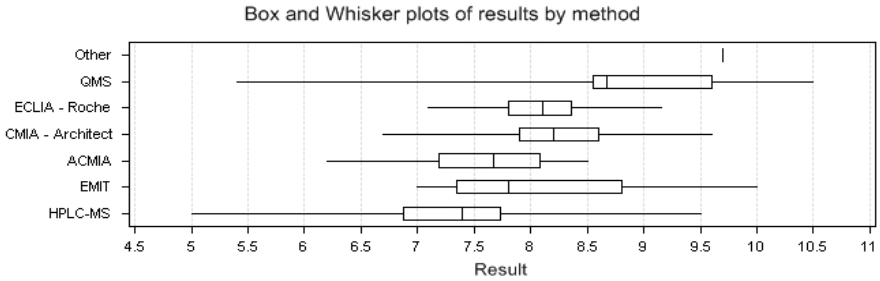


- CIC Round 399B.
- LCMS Median 143 µg/L
- Number of results:

LCMS 175	EMIT 11	CEDIA 25	ACMIA 31
CMIA 81	ADVIA 11	ECLIA 46	Other 1

- Result pattern typical of all pools

Patient pools: Tacrolimus

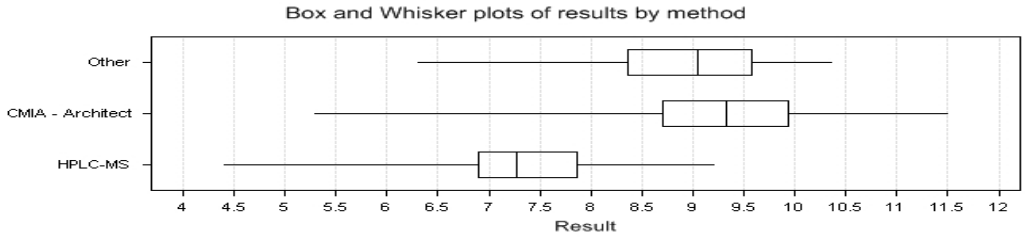


- TAC 399A
- LCMS Median 7.4 µg/L,
- Number of results:

LCMS 190	EMIT 10	ACMIA 24	
CMIA 115	ECLIA 38	QMS 10	Other 1

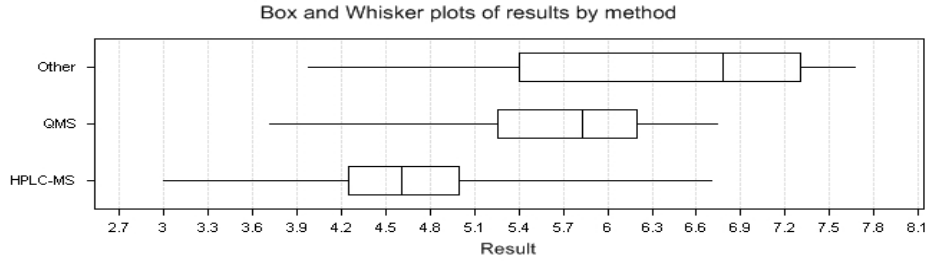
- Result pattern typical of all pools- suggests positive bias for certain assays to metabolites

Patient Pools: Sirolimus



- SIR 398B
- LCMS Median 7.28 µg/L,
- Number of results:
- LCMS 160 CMIA 53 Other 15
- Other: ECLIA, ACMIA and unknown
- Result pattern typical of all pools- suggests positive bias for certain assays to metabolites.

Patient Pools: Everolimus



- EVE 398 B
- LCMS Median 4.61µg/L,
- Number of results:
- LCMS 136 QMS 32 Other 15
- Other: ECLIA and unknown
- Result pattern typical of all pools- suggests positive bias for certain assays to metabolites.

Patient Pools: MPA

- None so far this year prior to this presentation being produced.

References and Thank you:

Professor David Holt, ASI

Assuring the Proper Analytical Performance of Measurement procedures for Immunosuppressant Drug Concentrations in Clinical Practice...., Seger et al. Ther Drug Monit, Vol 38, No 2, April 2016 p170- 189

Thank you for listening.

Any questions