Assay verification and the Adoption of Procalcitonin Guided Antibiotic Stewardship.

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INTRODUCTION

Overuse of antibiotics treatments and the continuous emergence of multidrug resistant organisms has posed a threat to public health. A lack of diagnostic and prognostic biomarkers for bacterial infection has led to inappropriate use of antibiotics, increased opportunity for antibiotic resistance, toxic side effects of antibiotics to patients which consequently increases mortality and is a cost burden on the NHS.

Within secondary care, worry over clinical deteriorations paired with an attempt to balance risk of poor clinical outcome results in over-prescription of antibiotics (Jee et al., 2018). Worry of patients potentially having an underlying and undiagnosed infection consequently results in a further increase in bacterial resistance (Tsangaris, I. et. al, 2009) whilst simultaneously increasing the risk of unnecessary prolonged exposure to antibiotics which can inadvertently affect a patient's overall clinical outcome. A reliable and immediate way of mitigating these challenges is the introduction of a rapid diagnostic and prognostic test – procalcitonin (PCT) assay.

Procalcitonin (PCT) is a 116 amino acid peptide that belongs to the calcitonin (CT) family and plays a role in the homeostasis of calcium (Vijayan et al., 2017).Under normal physiological conditions, healthy individuals have low levels of PCT (<0.05ng/ml) and elevated levels of PCT are reported in patients with bacterial infections (Assicot et al., 1993).

PCT has been reported to be a superior pro-inflammatory biomarker with favourable biokinetics over CRP, for early diagnosis and monitoring of bacterial infections. PCT is detectable within 4-6 hours of onset of bacterial infection and peaks within 24 hours; in contrast to CRP which gradually rises and peaks within 48 hours of infection (László et al., 2015). PCT has a half-life of 24 to 35 hours thus immediately indicating efficacy of antibiotics treatment and has been evidenced to have a high negative predictive rule out value (99% at a cut-off value of 0.2 ng/ml) (Pierrakos and Vincent, 2010) thus making it an ideal marker to rule out sepsis. Currently within the UK, PCT has not been approved for use parallel to clinical judgement for procalcitonin-guided antibiotic stewardship as the data is insufficient.

The intended use of this verification and use of assay is to aid in assistance that the presumed infection is specifically bacterial, prompting for a more accurate diagnosis and tailored appropriate antibiotic treatment plan, which is the fundamental principle of antibiotic stewardship.

METHODS

1. **Measurement of Trueness** - 11 Weqas EQA samples were assayed for Accuracy and evidenced using slope and intercept and Bias as well as calculation of trueness bias was done using Bland Altman plot.
2. **Method Comparison** - 31 samples were analysed for PCT method comparison. The Pearson's correlation co-efficient was used for PCT quantification method agreement and linearity between MKUH Access DXI800 and Morecombe Bay Hospital's DXI800.
3. **Measurement of Precision** - Mean, standard deviation and cv% of 10 replications of 3 levels of Randox Speciality IQC.
4. **Linearity**:- The Limit of detection (LoD), and the Limit of Quantification (LoQ) was performed to achieve a cv <20% and LoQ quoted by Beckman of 0.02ng/ml.

RESULTS

Measurement of Trueness

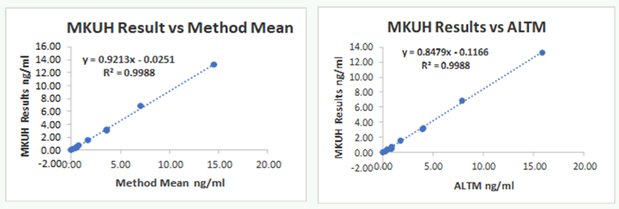
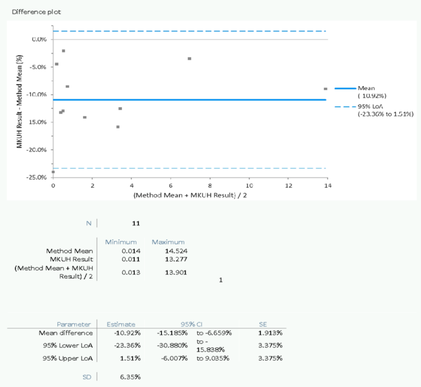
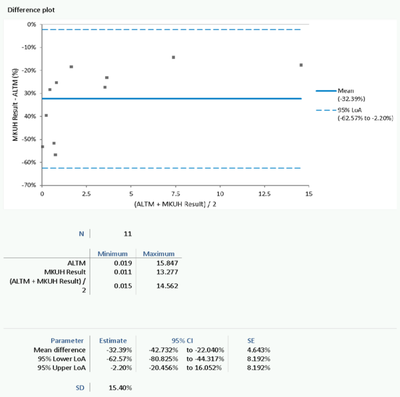


Fig1: Correlation between MKUH results against WEQAS EQA Method mean and ALTM showing correlation co-efficient and intercept

The method gave a correlation co-efficient of 0.998 against the ALTM and 0.998 against the current immunoassay method mean demonstrating good performance of the assay.



Bland Altman plot was used for compare method mean against MKUH access PCT method. There was a negative bias of 10.92%.



A significantly higher negative bias of 32.39% was seen on the ALTM vs MKUH results. Negative bias can be explained by method difference. The WEQAS and RIQAS EQA samples processed do not have results for Access PCT as it is not a part of their scheme. The ALTM is for BRAHMS PCT methods and results used where referenced as a nonspecific immunoassay.

Fig 2: Bland-Altman plots showing bias between MKUH Access PCT method vs EQA Method Mean and MKUH Access PCT method vs EQA ALTM

Method Comparison

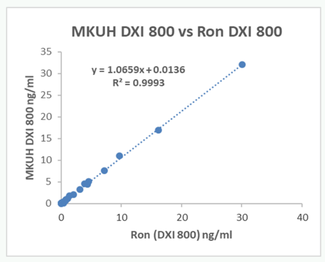
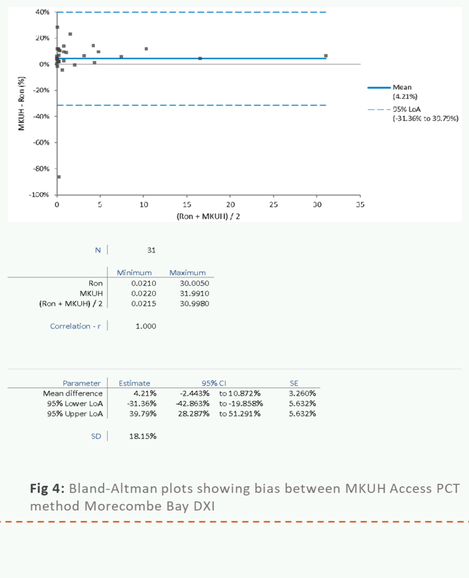


Fig3: Correlation between MKUH DXI vs Morecombe Bay DXI (RON) showing correlation co-efficient and intercept

Access DXI 800 instrument vs Morecombe Bay Access DXI800 (Ron) gave a correlation co-efficient of 0.999, slope obtained of 1.065 with an intercept of 0.01ng/ml demonstrating good correlation of the assays on the same platform



MKUH DXI 800 vs Morecombe Bay DXI 800 (Ron) resulted in a positive bias on MKUH DXI of 4.21% which is 0.022ng/ml higher than Morecombe bay DXI and within acceptable limits

Fig 4: Bland-Altman plots showing bias between MKUH Access PCT method Morecombe Bay DXI

Measurement of Precision

Intra- Batch QC1	QC2	QC3		Inter- Batch QC1	QC2	QC3
1.436	3.471	29.083		1.34	3.61	27.20
1.369	3.43	29.453		1.46	3.51	29.97
1.402	3.513	29.069		1.45	3.50	26.95
1.389	3.613	29.175		1.39	3.57	25.23
1.335	3.477	29.021		1.48	3.58	26.78
1.378	3.421	30.282		1.37	3.54	26.87
1.395	3.507	30.476		1.37	3.37	26.13
1.408	3.483	31.659		1.44	3.63	29.01
1.357	3.556	29.061		1.60	4.05	30.01
1.255	3.678	30.053		1.71	3.95	31.57
1.37	3.51	29.73	**Mean**	1.46	3.63	27.97 **Mean**
0.05	0.08	0.87	**SD**	0.12	0.21	2.04 **SD**
3.64	2.29	2.94	**CV**	7.88	5.78	7.28 **CV**

Fig5: Tables to show Intra and inter batch Mean, CV and as measurement of precision.

The desirable specification for measurement of precision for PCT is <10% across all ranges.

The Intra-batch imprecision quoted by Beckman is ≤6.0% CV at concentrations ≥0.150 ng/mL. The intra-batch CV's are all acceptable.

The inter-batch CV quoted by Beckman is 8.0% CV at concentrations ≥0.150 ng/m and the inter- batch CVs tabulated are within both the Beckman range and acceptable.

Linearity

A limited Linearity serial dilution study was performed using Level 3 IQC for top linearity and Level 1 IQC for the lower linearity.

Dilution	Expected	Measured
x2	45	45.631
x4	22.5	23.535
x8	11.25	10.806
x16	5.625	5.068
x32	2.825	2.48
x8 *	0.171	0.185
x16	0.086	0.085
x32	0.043	0.040
x64	0.021	0.018
x128	0.011	0.008
x256	0.005	0.003

* Level 1 IQC linearity

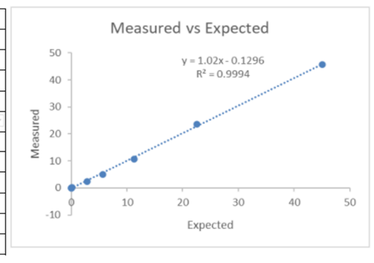


Fig 6 : Table and graph to show expected vs measured dilutions and linearity correlation between the measured and expected result.

The correlation coefficient for PCT is very good at 0.999 and the slope obtained was 1.02 with an intercept of 0.13ng/ml

Replicate	Result ng/ml
1	0
2	0
3	0
4	0
5	0
6	0
7	0

Fig7 : Limit of detection results of 7 replicates of saline

Limit of Detection (LoD) was measured by assaying saline repeatedly and gave an acceptable result of 0ng/ml. LOD quoted by Beckman is 0.01ng/ml (Beckman Coulter, 2019a).

Dilution	Expected Value	Measured Value	Δ%
x4	0.343	0.398	-16.204
x8	0.171	0.185	-8.029
x16	0.086	0.085	0.730
x32	0.043	0.040	6.569
x64	0.021	0.018	15.912
x128	0.011	0.008	25.255

Fig 8: LoQ expected and measured results with % difference CV

Limit of Quantification (LoQ) was performed using a low IQC sample which was serially diluted below the 20% CV LOQ quoted by Beckman of 0.02ng/ml (Beckman Coulter, 2019a) and value obtained was 0.018ng/ml at CV of 15.9% which is acceptable and within the Beckman quoted limits.

CONCLUSION

The use of PCT and the clinical interpretation of concentrations relies on assay sensitivity. The cut off value adopted that rules out suspected bacterial infection and discouragement of antibiotic treatment is <0.25ng/ml (Schuetz et al., 2019) whilst further monitoring being encouraged. The access PCT method passed with a limit of detection of 0ng/ml and limit of quantification was 0.018ng/ml at CV of 15.9% which is acceptable and within the Beckman quoted limits - < 20% CV at 0.02 ng/mL (Beckman Coulter, 2019a).

Owing to Access assay sensitivity, PCT can be used as a prognostic biomarker to guide continuation, de-escalation, and importantly discontinuation and discouragement of treatment thus aiding in Antibiotic Stewardship. However, the bias evidenced between two measurements on the same samples, using a similar assay but different machines, evidenced that PCT is not a suitable diagnostic marker for sepsis.`

References

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