McLeish, S., Costa, M., Burt, K. S., & Papasouliotis, K. (2017). *Analytical quality assessment and method comparison of an immunoassay for serum vitamin B12 and Folate measurement in dogs and cats*. Poster session presented at Proceedings 19th ESVCP-ECVCP Annual Congress, London, United Kingdom.

# Analytical quality assessment and method comparison of immunoassays for serum cobalamin and folate measurement in dogs and cats

**S. McLeish, M. Costa, K. Burt, K. Papasouliotis**
Diagnostic Laboratories, Langford Vets, University of Bristol, UK

## Introduction

- The TOSOH assays (TOSOH Bioscience) are two competitive enzyme immunoassays for the measurement of serum folate (FOL) and vitamin B12 (B12), originally labelled for use in human medicine.
- The analytical quality of this assay needs to be evaluated prior to use in cats and dogs.

## Objectives

- To assess the analytical performance of the TOSOH immunoassays for measurement of folate and vitamin B12 in cat and dog serum, by means of linearity, imprecision and recovery.
- To carry out a method comparison study on clinical samples, using the Immulite assays (Siemens Healthcare) as the reference method. These chemiluminescence immunoassays have been previously validated for use in cats and dogs.

## Materials and methods

- **Samples used:**

  → Linearity, imprecision, recovery studies: high/medium/low B12/FOL canine and feline pools were created from stored frozen serum.

  → Imprecision study: high/medium/low B12/FOL quality control materials (QCM) were also used.

  → Method comparison: fresh canine and feline serum was used.
- **Linearity:** High cat/dog B12/FOL pools were serially diluted with diluent buffer and FOL/B12 until the analyser could no longer detect the analyte. All data points were measured in duplicate.
- **Imprecision:** Assessed using each level of QCM and each pool.

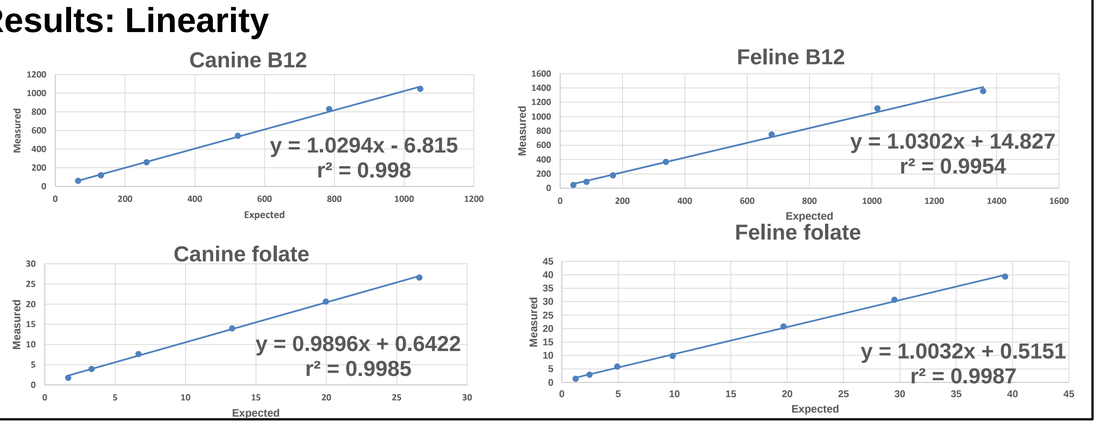
  → Within-run: samples analysed 10-13 times in a row, mean, SD and CV calculated. Between-run: samples analysed in duplicate, once daily for 10 to 15 days

  → For the QCM (between-run), bias and observed total error (TEobs) were also calculated.
- **Recovery:** High, medium and low pools were mixed with one another in equal proportions, [B12] and [FOL] measured in duplicate and recovery percentage calculated.
- **Method comparison:** Fresh serum samples from 39 dogs and 29 cats were tested on the reference (Immulite 2000) and TOSOH analysers (AIA-900) on the same day and results compared.

## Statistics

- Linearity was assessed by plotting measured vs. expected results.
- Imprecision was evaluated using intra- and inter-assay coefficients of variation (CVs, %), bias and TE(obs).
- For the method comparison, data were tested for Gaussian distribution, then correlation (Spearman for B12/Pearson for FOL) and regression analysis (Deming and Passing Bablok regression) were carried out. This was followed by Bland-Altman analysis.

## Results: Linearity




## Results: Imprecision

| | B12 | | | Folate | | |
|---|---|---|---|---|---|---|
| | QCM | Dog pools | Cat pools | QCM | Dog pools | Cat pools |
| **Within-run CV** | ≤ 2.8% | ≤ 4.1% | ≤ 9.3% | ≤ 9% | ≤ 5.2% | ≤ 2.9% |
| **Between-run CV** | ≤ 4% | ≤ 6% | ≤ 6.8% | ≤ 8% | ≤ 5.4% | ≤ 7.8% |
| **TE(obs)** | ≤ 13% | | | ≤ 31.4% | | |

→ TE(a) for B12: ≤ 30%[a] so TE(obs) ≤ TE(a). CV<15%[b,c]

→ TE(a) for Folate: ≤ 39%[a] so TE(obs) ≤ TE(a). CV<15%[b,c]

## Results: Recovery

The average recoveries were:

→ B12: 99% (dog), 100% (cat) *(ranges 97-101% and 95-106% respectively).*

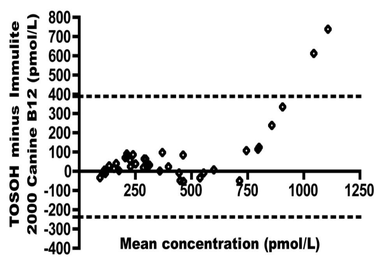
→ Folate: 101% (dog), 98% (cat) *(ranges 100-102% and 96-102 respectively)*

## Results: Method comparison

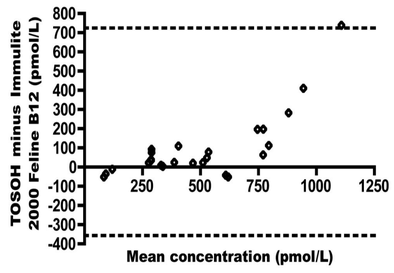
Ranges of sample concentrations and medians for the TOSOH and Immulite, and results of correlation and Deming/Passing Bablok regression:

| Analyte | TOSOH median | TOSOH range | Immulite median | Immulite range | Correlation r value | Slope estimate | 95% CI | y intercept estimate | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| **B12 canine** | 328 | 76 to >1476 | 291 | <111 to >738 | 0.98 (p<0.001) | 1.19 | 1.04 to 1.38 | -17.57 | -58.29 to 18.67 |
| **B12 feline** | 552 | 58 to >1476 | 499 | <111 to >738 | 0.97 (p<0.001) | 1.69 | 1.29 to 2.30 | -160.98 | -415.59 to 49.21 |
| **FOL canine** | 22.9 | 9 to >45.4 | 23.8 | 6.7 to >54.4 | 0.88 (p<0.0001) | 0.75 | 0.60 to 0.88 | 4.96 | 1.19 to 8.74 |
| **FOL feline** | 30.1 | 9.4 to >45.4 | 34 | 9.8 to >54.4 | 0.92 (p<0.0001) | 0.81 | 0.67 to 0.94 | 1.91 | -2.91 to 6.73 |

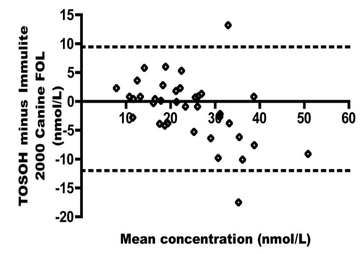
**Bland Altman plot B12 canine. Bias = 75 pmol/**L



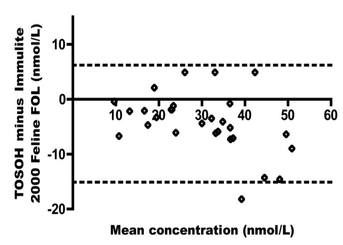

**Bland Altman plot B12 feline. Bias = 184 pmol/L**




**Bland Altman plot Folate canine. Bias = -1 nmol/L**




**Bland-Altman plot Folate feline. Bias = -4 nmol/L**




## Conclusions

- The TOSOH immunoassays were linear over a wide range of concentrations, with high $r^2$ values.
- The assays met acceptability criteria for imprecision and bias. Recovery results were excellent.
- Analysis of 95% CI for intercept and slope estimates indicated the presence of proportional and constant error for B12, and proportional error for FOL.
- Despite correlations being excellent (B12) or good (FOL), the TOSOH assays cannot be used interchangeably with the reference method and require specific reference intervals.

**Funding & Collaboration:** TOSOH The Chemistry of Innovation

[a] Ricos C. et al. Scand J Clin Lab Invest 1999;59:491-500.
[b] Guideline on bioanalytical method validation EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2
[c] US Department of Health and Human Services, FDA, CDER, CVM. (2001) Guidance for the Industry: Bioanalytical Method Validation.

Bristol Veterinary School