Research in the medical laboratory provides opportunities both for the seasoned professional and also those in the early stages of their career. The work reported below focuses first on guidance provided to aid the establishment of defined reference intervals, and second reflects on the implementation and use of large, whole-mount sections and their value in the histopathological assessment of colorectal cancer.

Ferritin and vitamin B_{12} reference intervals: a modified approach

Establishing defined reference intervals in the laboratory can prove difficult. Here, Agata Sobczyńska-Malefora, Nadia Munim, Martin Crook, Dominic Harrington and Alexander Katayev provide some guidance.

Age and/or gender-specific population-based reference intervals (RIs) are rarely available or are difficult to establish in clinical laboratories. With an increased focus on the between-method standardisation and harmonisation of test results, the development of universal RIs for standardised and harmonised assays may help laboratories to improve patient care.

Both serum ferritin and serum vitamin B_{12} concentrations vary with age and gender, yet unified RIs are often applied.

Agata Sobczyńska-Malefora[*†], Nadia Munim[*], Martin A Crook[‡], Dominic J Harrington[*§], and Alexander Katayev[¥].

[*]Nutristasis Unit, Viapath, St Thomas' Hospital, London; [†]Faculty of Life Sciences & Medicine, King's College London; [‡]Departments of Clinical Biochemistry and Metabolic Medicine, University Hospital Lewisham and Guy's and St Thomas' NHS Foundation Trust, London; [§]Division of Women's Health, School of Medicine, King's College London, London, UK; and [¥]Laboratory Corporation of America Holdings, Elon NC, USA.

Table 1. An example of a representative, selected report (total B_{12}, age group 60+ years).

Size of data	5514
Number of outliers	258
Maximum error threshold	0.465
Maximum error	0.464
Data in linear range (%)	90.487
Start cut point	2.702
End cut point	93.189
RI	[9.059, 13.91]
Regression	y = (0.051)x + (8.932)
Box-Cox	c=0, λ=0.2
Inversed RI	[175.789, 773.72]
Confidence interval (CI)	[153.958, 200.028], [701.429, 851.851]
Data in calculated RI (%)	83.079
Data above the upper limit of calculated RI (%)	12.786
Data below the lower limit of calculated RI (%)	4.135
Mean of all data	521.011
Median of all data	390
SD of all data	482.695
Mean (linear region)	399.597
Median (linear region)	370
SD (linear region)	156.253
Mode (linear region)	258
Mode (linear region, transformed)	10.181

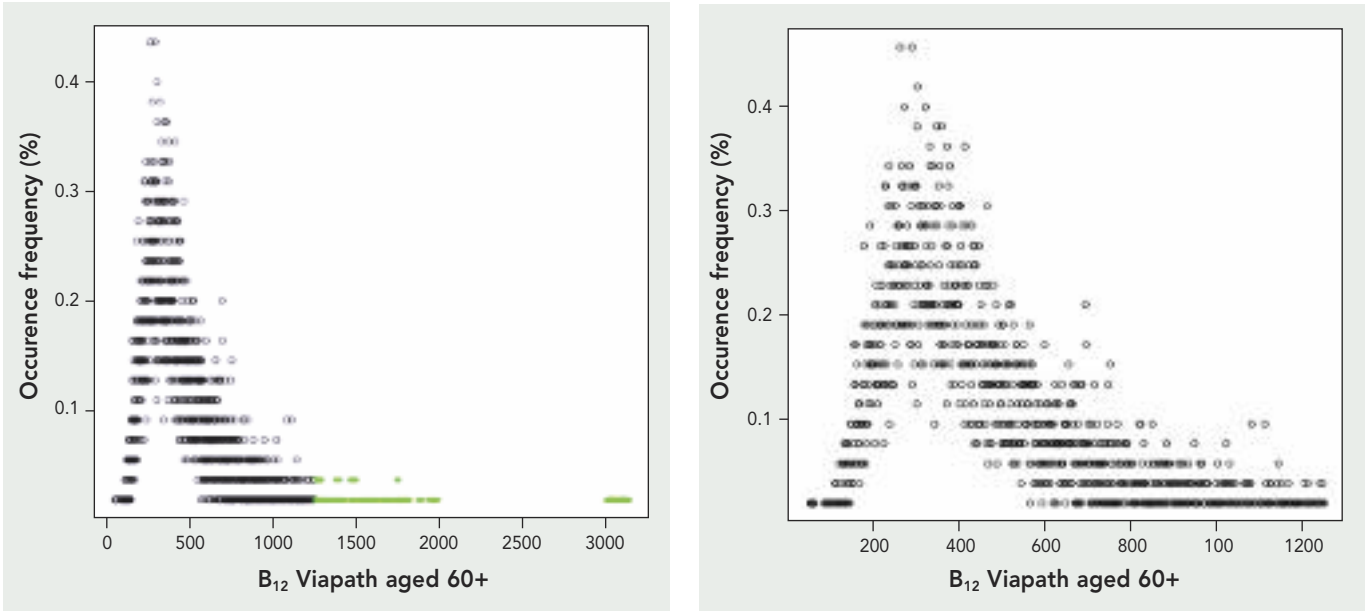

Fig 1. An example of a dot-plot showing **a)** 'good' data (black dots) and outliers (green dots), and **b)** the same dot-plot with the outliers removed. Data for total B_{12}, age group 60+ years.

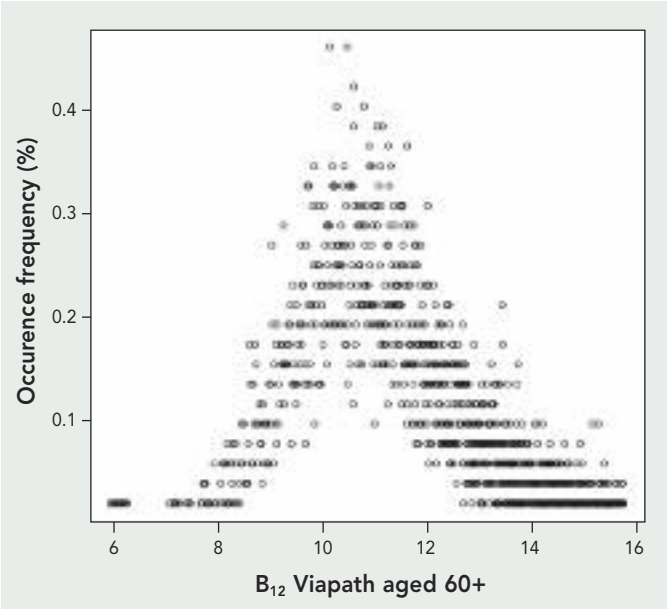

Fig 2. An example of a dot-plot (no outliers) after Box-Cox transformation (total B_{12}, age group 60+ years).

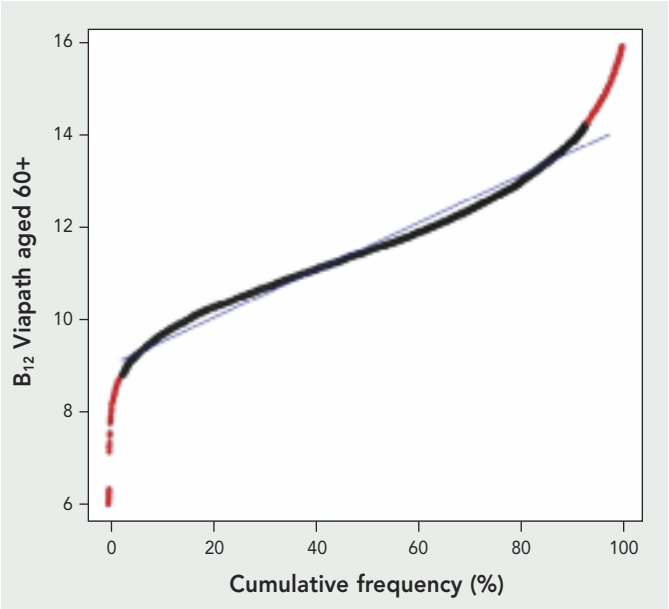

Fig 3. An example of cumulative frequencies (dots) and regression line (total B_{12}, age group 60+ years).

Both lower and upper limits for these markers are clinically important, as low values suggest deficiency leading to anaemia, and high values may reflect iron overloading/acute phase (ferritin) or abnormalities in vitamin B_{12} binding proteins (eg as seen in some cancers [B_{12}]). Therefore, accurate and subgroup-specific RIs should be applied.

The aim of the present study is to establish RIs for ferritin and B_{12} using a modified Hoffmann's approach.[1]

Modified Hoffmann's method

In 1963, Hoffmann described a simple, indirect method of calculating RIs using existing patient data from a laboratory database, named the 'probability paper method'.[2] Later, the first computerised software based on Hoffmann's approach was developed.[3]

In brief, Chauvenet's criteria were used for the detection of outliers (Fig 1a). Following the removal of outliers (Figs 1b and 2), the cumulative frequency of each test result was determined. Values from the linear portion of the cumulative frequency graph were used for computing the best fitting linear regression equation, $\gamma i = \alpha^* \chi + \beta + \varepsilon i$ (Fig 3). The RIs were then determined from the linear regression equation following extrapolation of the preceding curve, and calculated (for x=2.5% and 97.5%): $RImin = \alpha^* 2.5 + \beta$, $RImax = \alpha^* 97.5 + \beta$.

When the source data distribution is significantly skewed, a Box-Cox transformation may be applied (Fig 2), with back transformation after the linear portion is calculated from the transformed data. In this work, a fully computerised and validated method, with new functions and algorithms added, was used.

Methodology

All ferritin results processed between August 2014 and July 2015, and B_{12} results processed between January and June 2013 on the *i*2000SR (Abbott Diagnostics) from a population served by Guy's and St Thomas' hospitals in London, UK, were used to calculate RIs. Data were partitioned in accordance with literature-based knowledge about gender/ age-related differences in these markers.

Results

The RIs with percentage of values below and above the cut-offs are shown in Table 1. Owing to low sample numbers, separate RIs for the 0–12 months age group for ferritin and for the 0–5 years age group for B_{12} were not calculated (Table 2).

Table 2. Age- and/or gender-related RIs for serum ferritin and total B_{12}.

Ferritin					Total B_{12}				
Partition group by gender/age (y)	Data size	RIs (ng/mL)	% below lower limit	% above upper limit	Partition group by age (y)	Data size	RIs (ng/L)	% below lower limit	% above upper limit
M/1–5	845	9–70	5.2	30.0	0–19	720	224–1001	5.7	10.8
F/1–5	488	10–73	3.3	32.2	6–19	624	218–878	5.6	10.6
M/6–11	899	14–85	3.1	28.5	20–59	11641	194–829	4.8	10.1
F/6–11	802	13–74	3.5	38.6	60	5514	176–774	4.1	12.8
M/12–19	1122	17–143	3.5	31.7					
F/12–19	1760	7–75	3.7	22.9					
M/20–55	9767	34–314	6.8	15.4					
F/20–55	25823	9–102	5.7	13.9					
M/56+	9360	25–503	7.6	12.0					
F/56+	11575	19–262	6.8	15.3					

St Thomas' Hospital, London, home to the Nutristasis Unit, a leading laboratory for the measurement of endogenous vitamins in body fluids and tissues.

A combined RI for B_{12} for the 0–19 years age group was calculated (inclusive of 96 patients aged 0–5 years)..

Discussion

The RIs for serum ferritin and B_{12}, calculated using a modified Hoffmann's approach, are consistent with RIs established using harmonised methods and may serve as universal RIs for other laboratories using the same methodology. They incorporate variations related to age, gender, method and the population being tested. The variations in upper limits for ferritin are of particular interest in view of iron overloading and deserve further investigations. Application of these RIs can assist with a better assessment of iron and vitamin B_{12} status.

References

1 Katayev A, Fleming JK, Luo D, Fisher AH, Sharp TM. Reference intervals data mining: no longer a probability paper method. *Am J Clin Pathol* 2015; **143** (1): 134–42.

2 Hoffmann RG. Statistics in the practice of medicine. *JAMA* 1963; **185**: 864–73.

3 Katayev A, Balciza C, Seccombe DW. Establishing reference intervals for clinical laboratory test results: is there a better way? *Am J Clin Pathol* 2010; **133** (2): 180–6.

Colorectal cancer: can whole-mount sections facilitate tumour staging?

Alicia Philo undertakes an investigation into the implementation and use of large, whole-mount sections to study colorectal cancer in a routine histopathology laboratory and enhance diagnostic accuracy.

In England and Wales, colorectal cancer (CRC) was the fourth leading cause of mortality in both males and females in 2015.[1] Personalised diagnosis for patients with CRC is increasingly being utilised, and therefore methods to enhance diagnosis and facilitate histopathologist reporting is a topic of current interest. For this reason, use of whole-mount sections (WMS) was introduced in the histopathology laboratory at Hereford County Hospital.

Whole-mount sections permit a full cross section of tumour and bowel wall[2] to be examined. This small study was designed to determine whether or not their use can increase lymph node (N) yields, and the identification of lymphovascular invasion (LVI) in comparison to conventional sections (CS). The use of WMS has also been examined in relation to their correlation with MRI images and tumour staging.

In brief, the aims of the study are to: i) investigate whether or not adverse features are more readily identified from patients using WMS in comparison to CS; ii) determine whether all adverse features recorded in the final pathology report can be identified on WMS; and iii) investigate any correlation between pathology and MRI staging.

Methodology

Two groups of patients with CRC were identified; the first group was studied utilising WMS, and the second group utilising CS (Fig 1). Both groups were statistically analysed to identify whether or not the use of WMS enhanced CRC diagnosis, and if more adverse tumour features could be observed using WMS compared to CS.

In addition, the investigation also considered adverse features and tumour (T) staging observed in the WMS to see if they correlated with the final pathology report. Pathological and magnetic resonance imaging (MRI) staging were observed for correlation using weighted Cohen's kappa (κ_w), and receiver operating characteristic (ROC) curves. The histopathologists were also given questionnaires to complete to capture their views on the use of WMS in the diagnosis of CRC.

Results

Thirty patients each were recruited into the WMS and the CS groups. The groups comprised approximately two-thirds male, one-third female, with a mean age of 69 and 73 for the WMS and CS groups, respectively. The average number of WMS obtained per specimen was 1.9.

Statistical analysis demonstrated no significant difference between lymph node yields obtained in the WMS and CS groups, with similar results observed for LVI identification. Increased turnaround times were demonstrated for WMS compared to CS.

Of the adverse features recorded in the final histopathology report, 67% were observed in the WMS (Fig 2), and 90% of T stages correlated with the final report (κ_w =0.500 for T staging, κ_w =0.250 for N staging).

The ROC curves (Fig 3) demonstrated good accuracy for T2, N+ and N–, while T3 displayed poor accuracy. The questionnaire responses from histopathologists indicated that they felt WMS enhanced their CRC diagnosis, and more WMS blocks would be beneficial to observe the most adverse features.

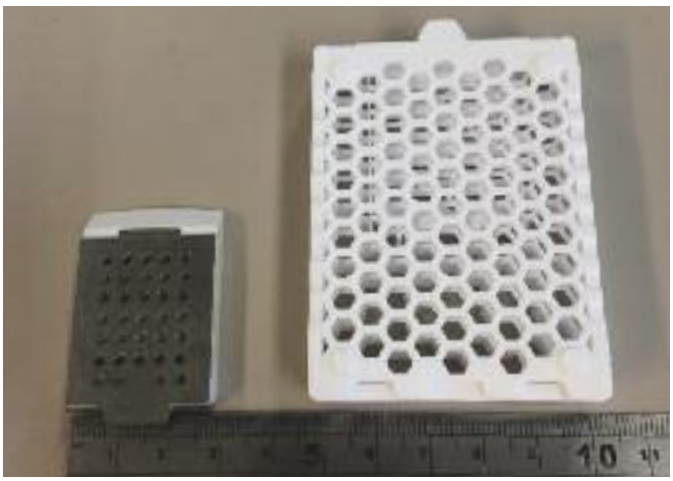

Fig 1. Comparison of conventional tissue cassettes (left) and whole-mount section cassettes (right).

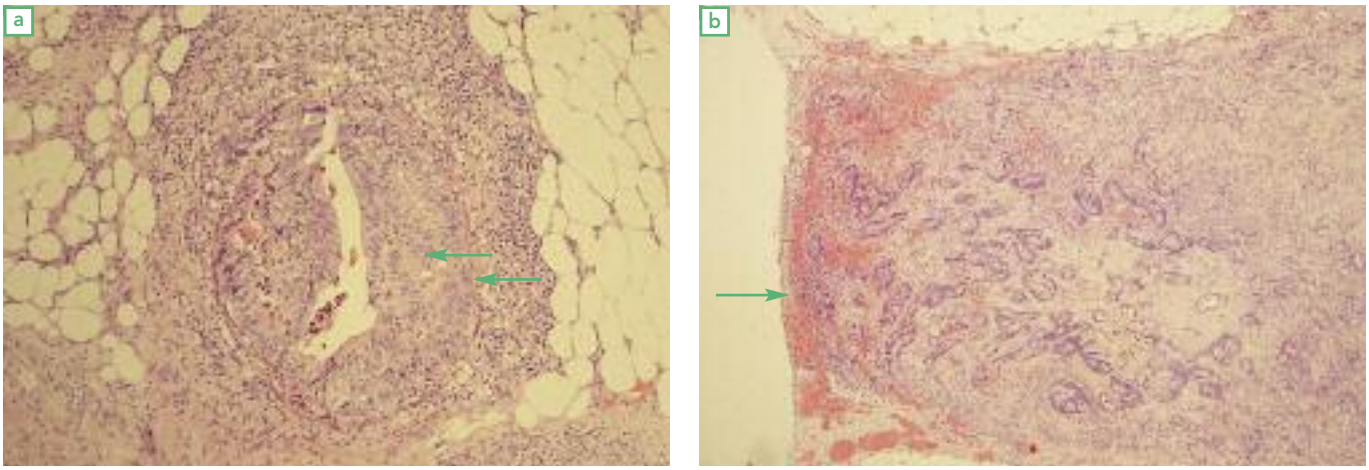

Fig 2. Whole-mount sections showing **a)** vascular invasion by tumour, and **b)** serosal involvement by tumour (haematoxylin and eosin [H&E] staining).

Key points from the study

- There was no significant increase in adverse features observed in the WMS group; however, specimen type, patient age, and therapy should be considered.
- Increased turnaround times were observed in the WMS group.
- The 67% correlation between WMS to final histopathology report suggests that WMS blocks were not being taken from the most adversely affected area of colon.
- Assessment of T3 tumours showed poor correlation with MRI staging, perhaps as a result of desmoplastic reaction or tumour progression between the time of MRI and surgery.
- Three WMS blocks were found to be optimal for reporting CRC.
- The implementation of WMS proved successful at Hereford County Hospital, due to enhancement of reporting found by histopathologists, in particular in cases that had received neoadjuvant therapy.
- Liaison between radiologists and histopathologists occurs regularly following the study to review WMS cases with the radiology report.

References

1 Office for National Statistics. *Mortality Statistics – Deaths Registered in England and Wales* 2016 (www.ons.gov.uk).
2 Wang Z, Zhou ZG, Wang C *et al.* Microscopic spread of low rectal cancer in regions of mesorectum: pathologic assessment with whole-mount sections. *World J Gastroenterol* 2004; **10** (20): 2949–53.

Further reading

- Conti J, Thomas G. The role of tumour stroma in colorectal cancer invasion and metastasis. *Cancers (Basel)* 2011; **3** (2): 2160–8.
- Denham LJ, Kerstetter JC, Herrmann PC. The complexity of the count: considerations regarding lymph node evaluation in colorectal carcinoma. *J Gastrointest Oncol* 2012; **3** (4): 342–52.
- Shen SS, Haupt BX, Ro JY, Zhu J, Bailey HR, Schwartz MR. Number of lymph nodes examined and associated clinicopathologic factors in colorectal carcinoma. *Arch Pathol Lab Med* 2009; **133** (5): 781–6.

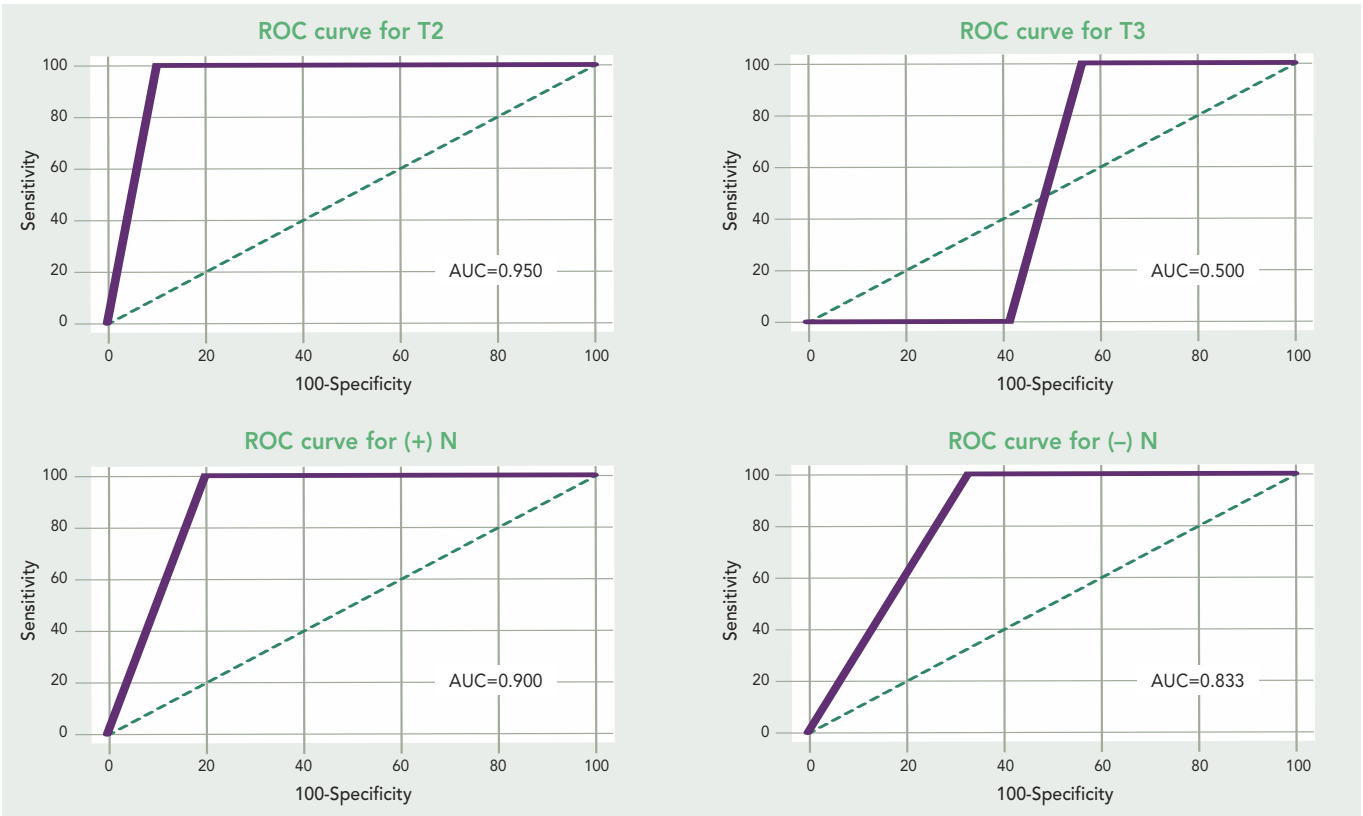

Fig 3. Receiver operating characteristic curves for T2, T3, N+ and N– TNM staging.