Active-B12 (Holotranscobalamin)
Utility and Routine Use
Active-B12 assays

**Abbott AxSYM**
Launched 2006

**Abbott ARCHITECT**
Launched October 2011 (USA December 2011)

**Axis-Shield ELISA**
Launched May 2012 (USA in progress)

**User Countries:**
- USA  
- Switzerland  
- Korea  
- UK  
- Germany  
- Finland  
- Sweden  
- Australia  
- Netherlands  
- Turkey  
- Czech Republic  
- Canada

Validation in  
- Hungary, Slovakia, Poland, Italy, New Zealand, Ukraine
How is Active-B12 used?

- Reflex test following indeterminate Total B12 result
- Direct replacement for Total B12

Clinical conditions
- Investigation of suspected pernicious anaemia alongside GPC Ab and IF Ab
- Gastroenterology patients
- Pregnancy
Correlation of Total B12 with Active-B12

$n = 468$
Spearman correlation $r = 0.74$
Correlation of Total B12 with Active-B12

n=468 patients

314 results (67%) are immediately indeterminate by Total B12.

Using common 150pmol/L Total B12 cut-off, 180 patients (38%) would be considered deficient by Active-B12.
Introduction of Active-B12 as reflex

<table>
<thead>
<tr>
<th>Total B12 test</th>
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<tbody>
<tr>
<td>&lt;150pmol/L</td>
</tr>
<tr>
<td>150 – 300 pmol/L</td>
</tr>
<tr>
<td>&gt;300pmol/L</td>
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</table>

- Likely deficient
- Resolve with Active-B12
- Unlikely deficient*

* Due to false positives and false negatives, all total B12 results could be confirmed with Active-B12.
Introduction of Active-B12 as front-line test

Subjects at risk of B12 deficiency

- Active-B12 <35pmol/L
  - Likely deficient

- Active-B12 ≥35pmol/L
  - Unlikely deficient*

* Renal patients should be further investigated
Advantages over Total B12

- Fewer grey-zone results – less additional testing
- No IF Ab interference – significant failing in all Total B12 assays
- Cost savings – less Ab testing or MMA testing when used in algorithms
- Lab throughput – faster than Total B12
“Siemens Healthcare Diagnostics has confirmed that the presence of Intrinsic Factor Blocking Antibody (IFBA) in some patients can result in a false and significant elevation in the reported concentration of vitamin B12 on the Dimension Vista® System. Siemens’ data indicate that the elevation is highly variable, and in some cases, values below the cutoff for B12 deficiency (i.e. 254 pg/mL) were reported as greater than 2000 pg/mL. All vitamin B12 results obtained from the Dimension Vista® System are impacted by this issue, starting in January 2008”

Failures of Cobalamin assays in Pernicious Anemia Carmel and Agrawal, NEJM 2012 367;4
Spurious Elevations of Vitamin B12 with Pernicious Anemia Yang and Cook, NEJM 2012 366;18
Highly complex denaturation step

<table>
<thead>
<tr>
<th>Total B12 assay</th>
<th>Active-B12 assay</th>
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<tbody>
<tr>
<td>Typically 2-3 reagents for multiple pre-treatment steps:</td>
<td>No pre-treatment:</td>
</tr>
<tr>
<td><strong>High pH</strong></td>
<td>Specific monoclonal binds HoloTC – no cross-reactivity for ApoTC or HoloHC.</td>
</tr>
<tr>
<td>Denatures TC and HC to release B12 for capture</td>
<td></td>
</tr>
<tr>
<td><strong>Reductant</strong></td>
<td></td>
</tr>
<tr>
<td>Denature anti-IF autoantibodies</td>
<td></td>
</tr>
<tr>
<td><strong>Cobinamide</strong></td>
<td></td>
</tr>
<tr>
<td>Blocking agent to prevent B12 re-binding to TC/HC that renatures</td>
<td></td>
</tr>
<tr>
<td><strong>KCN</strong></td>
<td></td>
</tr>
<tr>
<td>rIF capture has different affinities for different forms of B12, KCN converts all B12 to single form.</td>
<td></td>
</tr>
<tr>
<td>Detect B12 bound to rIF</td>
<td>Detect bound HoloTC</td>
</tr>
</tbody>
</table>
Throughput improvement

- Denaturation can be up to 15 minutes on some platforms
- No pre-analytical denaturation for Active-B12 – specific monoclonal for Holotranscobalamin.
- Subsequent impact on instrument throughput:
  - Abbott Architect Total B12 – 100 tests per hour
  - Abbott Architect Active-B12 – 200 tests per hour
Active-B12 in PA investigation – laboratory cost savings

- Lab algorithm reflexed all Total B12 results below 190pmol/L (258pg/mL) to GPC Ab and IF Ab tests
- New algorithm reflexes all Total B12 results below 190pmol/L (258pg/mL) to Active-B12
- Active-B12 results >35pmol/L are deemed non-deficient
- Results <35pmol/L are sent for Ab tests
- 50% reduction in samples sent for expensive Ab tests
Published evaluations – Netherlands

n=360 samples collected across 5 hospitals

<table>
<thead>
<tr>
<th>MMA cutoff µM</th>
<th>AUC Total B12</th>
<th>AUC HoloTC</th>
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<tbody>
<tr>
<td>&gt;0.32</td>
<td>0.63</td>
<td>0.70</td>
</tr>
<tr>
<td>&gt;0.45</td>
<td>0.70</td>
<td>0.78</td>
</tr>
<tr>
<td>&gt;0.77</td>
<td>0.73</td>
<td>0.92</td>
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“HoloTC has a better diagnostic accuracy than vitamin B12 and can replace the existing vitamin B12 assay as a primary screening test in patients suspected of vitamin B12 deficiency”
Published evaluations – Korea

n=184 patients:
n=45 where B12 requested (Alzheimers, stomach cancer, Parkinsons)
n=139 normocytic or macrocytic anaemia

“On the basis of these results, we concluded that the holo-TC levels may be more suitable than serum vitamin B12 levels to obtain information about vitamin B12 status. If the serum vitamin B12 level is between approximately 151-300 pmol/L, holoTC alone or in combination with the serum vitamin B12 is likely to be more useful than serum vitamin B12 levels alone to indicate vitamin B12 status”
"In conclusion, determination of HoloTC concentrations may be used as a complementary diagnostic strategy to avoid the development of pathological conditions (macrocytic anaemia or neurological disease) before symptoms emerge, and should also be used for large scale screening of subjects at latent risk of cobalamin deficiency."

<table>
<thead>
<tr>
<th>HoloTC</th>
<th>Total B12 &lt; 140</th>
<th>Total B12 ≥ 140</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 40</td>
<td>79</td>
<td>84</td>
</tr>
<tr>
<td>≥ 40</td>
<td>54</td>
<td>84</td>
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n=250 where Total B12 < 221 pmol/L
Protocol offers the test to every patient with Total B12 <200pmol/L

“….From our experience reviewing these cases, Active B12 has advantage over total B12 in assessing B12 status of an individual. Active B12 was helpful in excluding B12 deficiency falsely ‘identified’ by low total B12 such as in pregnancy. It is also helpful in confirming true B12 deficiency missed by a normal total B12 in patients such as those with iron deficiency and hypothyroidism.”

“Active B12 has fulfilled our expectation in being a robust assay that improves the assessment of vitamin B12 status. It provides reassurance to us that we have contributed to improved patient care. The routine availability of this assay differentiates our laboratory as a progressive provider of quality pathology.”
HoloTC is offered routinely with a cut-off of 32pmol/L

“Active-B12 appears to be a better predictor of disturbed B12-dependent metabolism than Total B12.”

“Accepting MMA >0.45µmol/L as a reference standard for Vitamin B12 deficiency the Active-B12 assay demonstrates a better sensitivity and specificity in detecting Vitamin B12 deficiency than the Total B12 assay in a mixed collection of diagnostic samples.”

“For the detection of B12-deficiency Active-B12 can replace Total B12 as a first-line diagnostic aid; no reason for combination with Total B12.”
“Primary interest is the development and application of novel markers of vitamins to improve patient care.”

“The majority (up to 80%) of serum vitamin B12 is not bio-available. Current assays measure total vitamin B12 which leads to a grey area where deficient patients can be missed - there is a poor correlation between circulatory total B12 and B12 status at the tissue level. Conversely patients can inappropriately be classified to a deficient state with the inconvenience and expense of long term supplementation regimes.”

“The Nutristasis Unit will be providing this new nutritional marker from September and GSTS will be the first to offer it outside of research environment in the UK.”
“Conventional tests for Vitamin B12 measure total serum Vitamin B12, not metabolically active B12. It is better understood now that total B12 levels are not as clearly correlated with clinical symptoms as they might be. Clinically significant vitamin B12 deficiency can occur, and B12 status misclassified, even with Total Vitamin B12 levels apparently within normal range. Measuring Active B12 is diagnostically more accurate for detecting B12 deficiency that requires therapy”

“It is fair to say that Active B12 and Total B12 do show good agreement at the extremes (ie “very deficient” or “not at all deficient”) but there is a large grey zone of indeterminate range between normal and abnormal which is likely to be misclassified if total serum B12 alone is relied upon. It is therefore expected that by testing with Active B12, findings will be less in number, but more clinically relevant. All Vitamin B12s will be replaced by Active B12 whether requested as a single test, or requested with red cell or serum folate”
Further information

www.active-b12.com

- Publications
- Clinical Utility
- Case Studies