



Foutief verhoogd vitamine B12: Pitfalls in een immuno-assay

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Pitfalls in de Vitamine B12 assay



- **Aanleiding:**
 - **Hypersegmentatie en megaloblastair BM**
 - **Vitamine B12: > 738 pmol/l (RW: 130-700)**
 - **HcY: 69 μ mol/l (RW: <14)**
 - **MMA: 5,84 μ mol/l (RW: <0,45)**
 - **Holotranscobalamine: 4 pmol/l (21-117)**

Pitfalls in de Vitamine B12 assay



- **Aanleiding:**
 - **Hypersegmentatie en megaloblastair BM**
 - **Vitamine B12: > 738 pmol/l (RW: 130-700)**

Hoe kan dit?

- **Auto-antilichamen in hoge titer tegen IF**

Pitfalls in de Vitamine B12 assay



- **Achtergronden bij de immunoassay**
- **Wat is het probleem?**
- **Probleem ook bij andere assay's?**
- **Take home message**

Achtergronden bij de assay



Competitieve chemiluminescentie enzym immunoassay (Siemens Immulite 2500)

Stap 1: Alkalische denaturatie stap

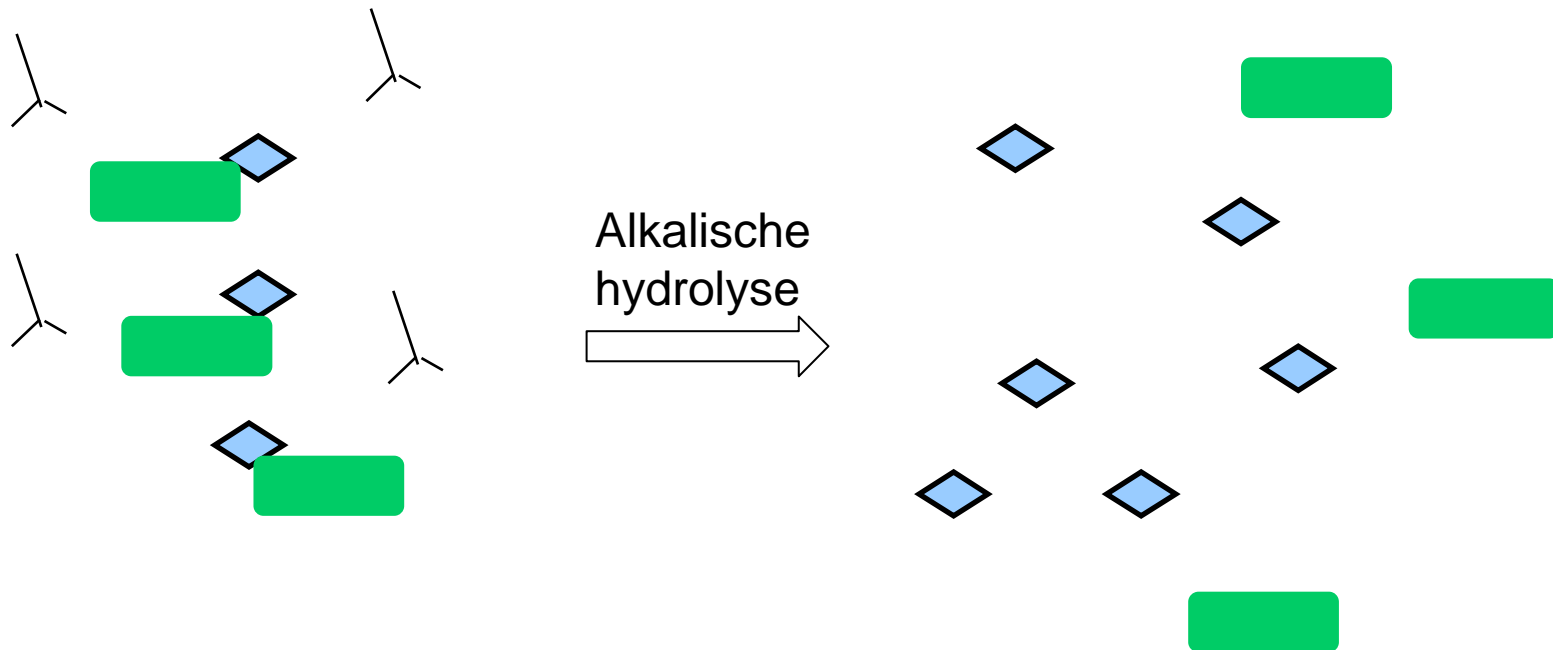
Stap 2: Coated Vit. B12 beads en hog IF

- **Competitie van Vit. B12 van patient met coated Vit. B12 voor hog IF**

Stap 3: AF- gebonden Anti-hog IF

Stap 4: Wassen en substraat toevoeging en meting

Achtergronden bij de assay: stap 1



Antilichamen, bijv. anti-IF

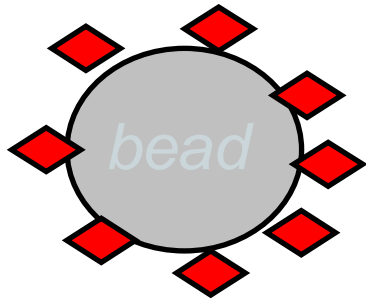


Endogeen drager eiwit



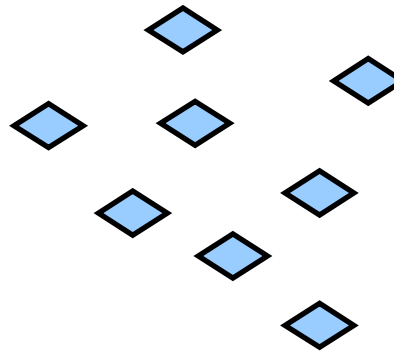
Vitamine B12

Achtergronden bij de assay: stap 2



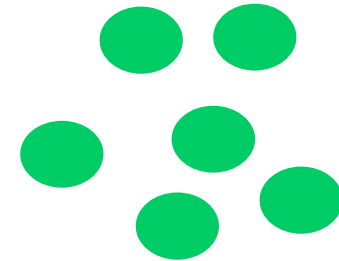
Vitamine B12 –
coated bead

+



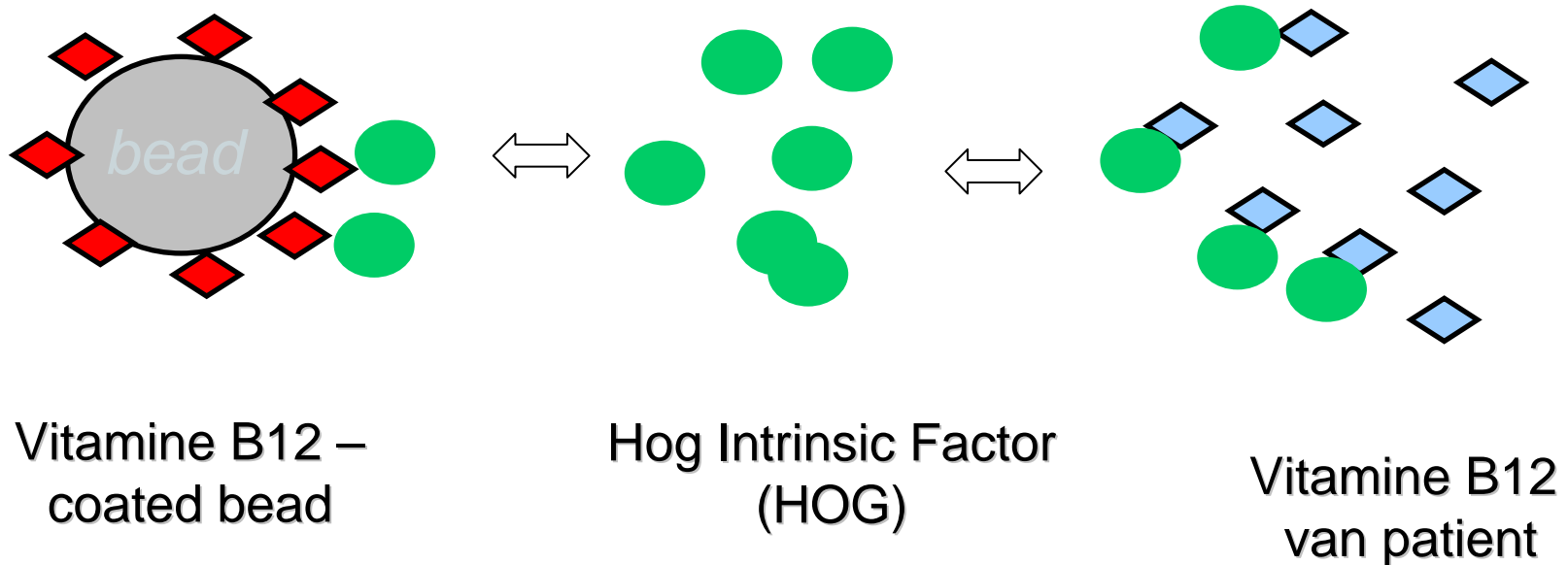
Vitamine B12
van patient

+



Hog Intrinsic Factor
(HOG)

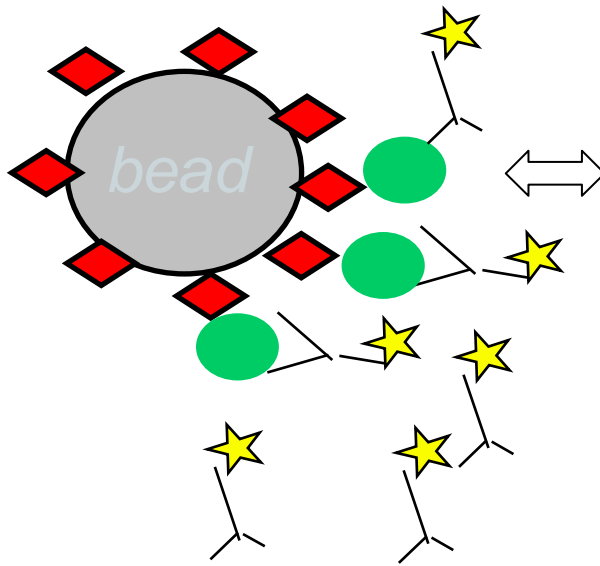
Achtergronden bij de assay: stap 2



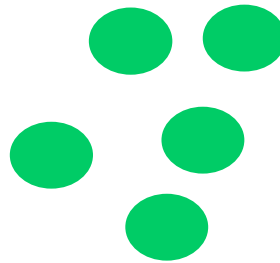
Competitie tussen endogeen en exogeen vitamine B12 voor HOG

Achtergronden bij de assay: stap 3

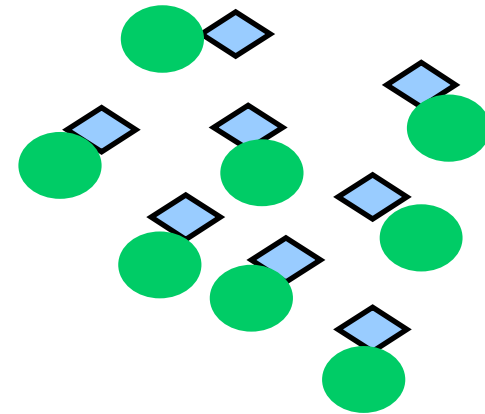
Vitamine B12 –
coated bead



Hog Intrinsic Factor
(HOG)

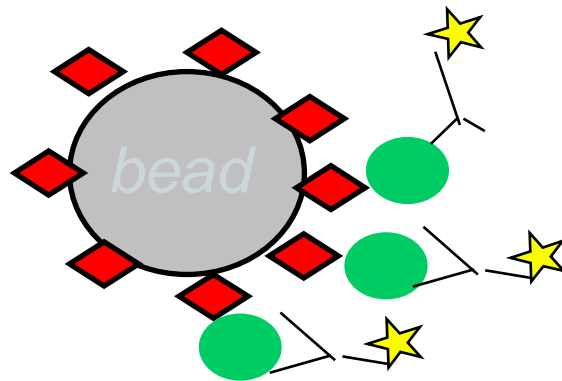


Vitamine B12
van patient



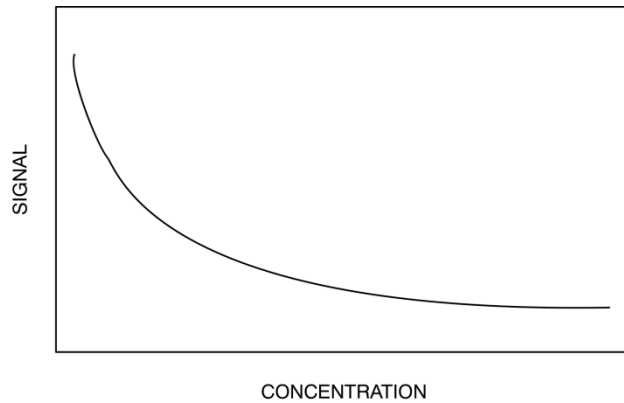
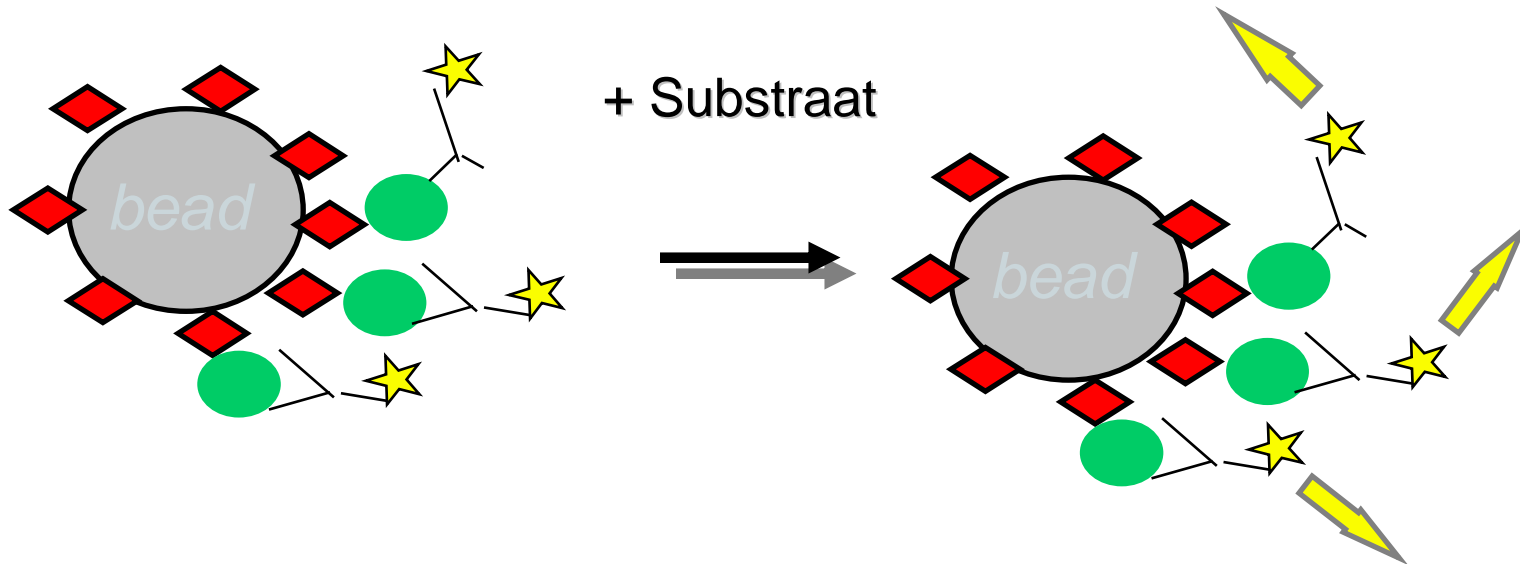
Vervolgens worden AF-gelabelde anti-IF antilichamen toegevoegd

Achtergronden bij de assay: stap 4



Ongebonden antigeen
en ongebonden gelabeld
antigeen wordt weggewassen

Achtergronden bij de assay: stap 4



De hoeveelheid geproduceerd licht
is omgekeerd evenredig met de
concentratie
antigeen in het monster

**Echter het gaat niet
altijd goed...**

Achtergronden bij de assay



High Measured Cobalamin (Vitamin B₁₂) Concentration Attributable to an Analytical Problem in Testing Serum from a Patient with Pernicious Anemia

Clinical Chemistry 52, No. 1, 2006

L. Thomas Vlasveld^{1*}
Jan W. van't Wout¹
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Ad Castel³

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³ Clinical Chemistry and Hematology
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The Hague, The Netherlands
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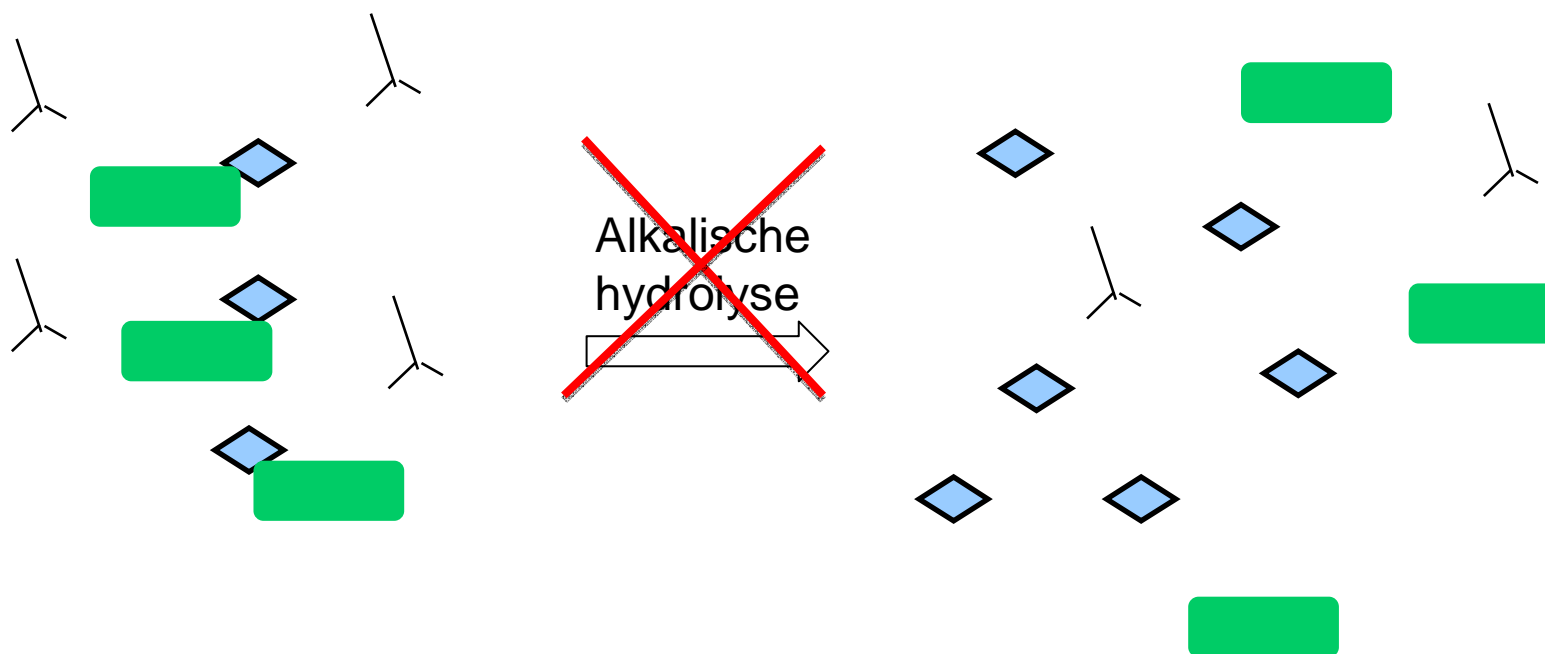
Clin Chem Lab Med 2011;49(1):105–109 © 2011 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2011.017

Introduction of a new cobalamin (vitamin B12) assay: lessons from a flawed validation study

André P. van Rossum¹, Jacqueline Klein
Gunnewiek², François M. Verheijen³, L. Tom
Vlasveld⁴, Ad Castel¹ and Michael A. Fouraux^{3,*}

over the entire measurement range, even when both assays
are produced by the same manufacturer.

Achtergronden bij de assay



Antilichamen, bijv. anti-IF

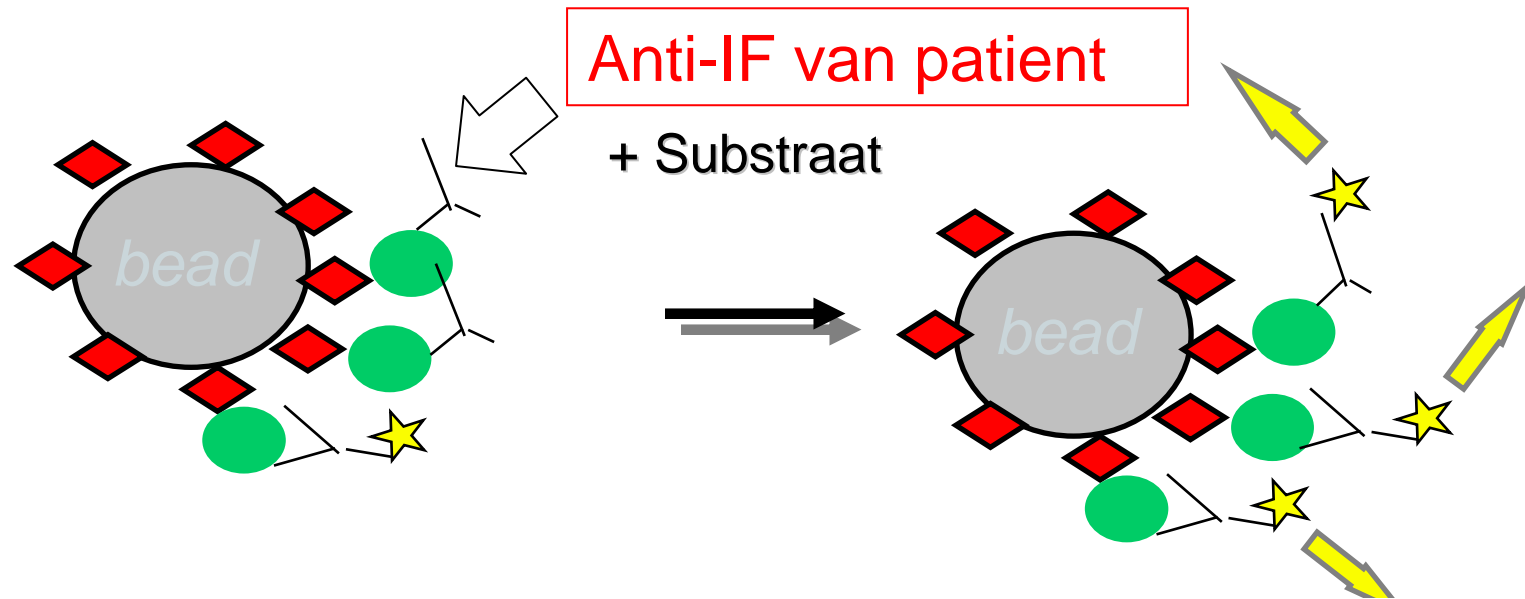


Endogeen drager eiwit



Vitamine B12

Pitfall bij alkalische hydrolyse



Echter deze alkalische hydrolyse stap zou zijn verbeterd (zie ook Clin Chem. 2006. Response to the letter of the editor). Eveneens zou deze stap nu worden gecontroleerd met QC monster met hoge titer anti IF.

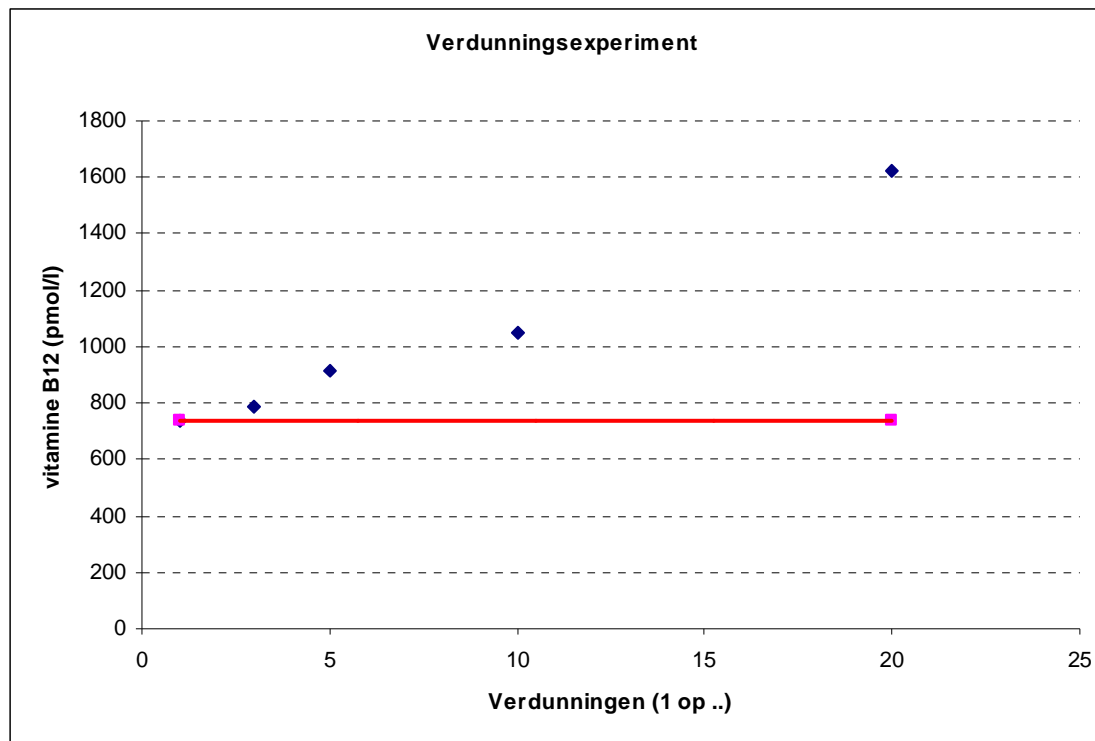
Pitfall bij de vitamine B12 assay



- **Wat is dan het geval?**
- **Uitsluiten interferentie (*Clin. Chem.*, 2008)**
 - **Verdunningsexperiment**
 - **Heterofiele blocking tubes (heterofiele antilichamen, eg. HAMA's)**
 - **Andere vitamine B12 assay's**
 - **PEG precipitatie**

Uitsluiten interferentie

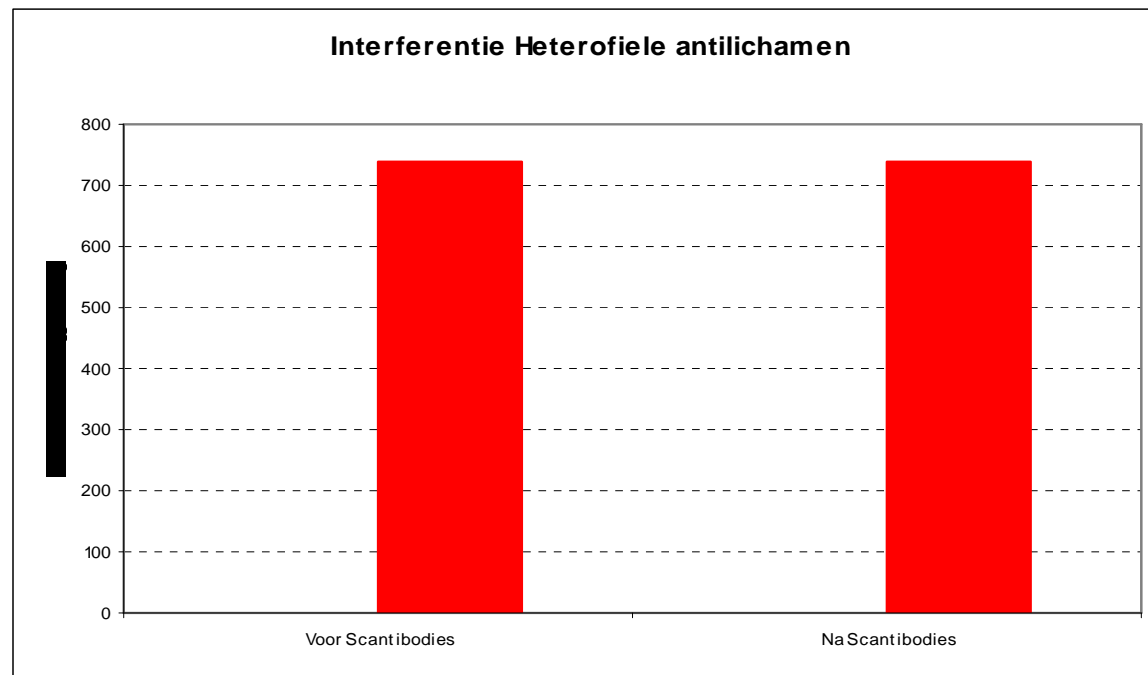
- Verdunningsexperiment



Let wel: assay laat niet lineaire uitverdunding zien. Aldus aanwijzing voor interferentie

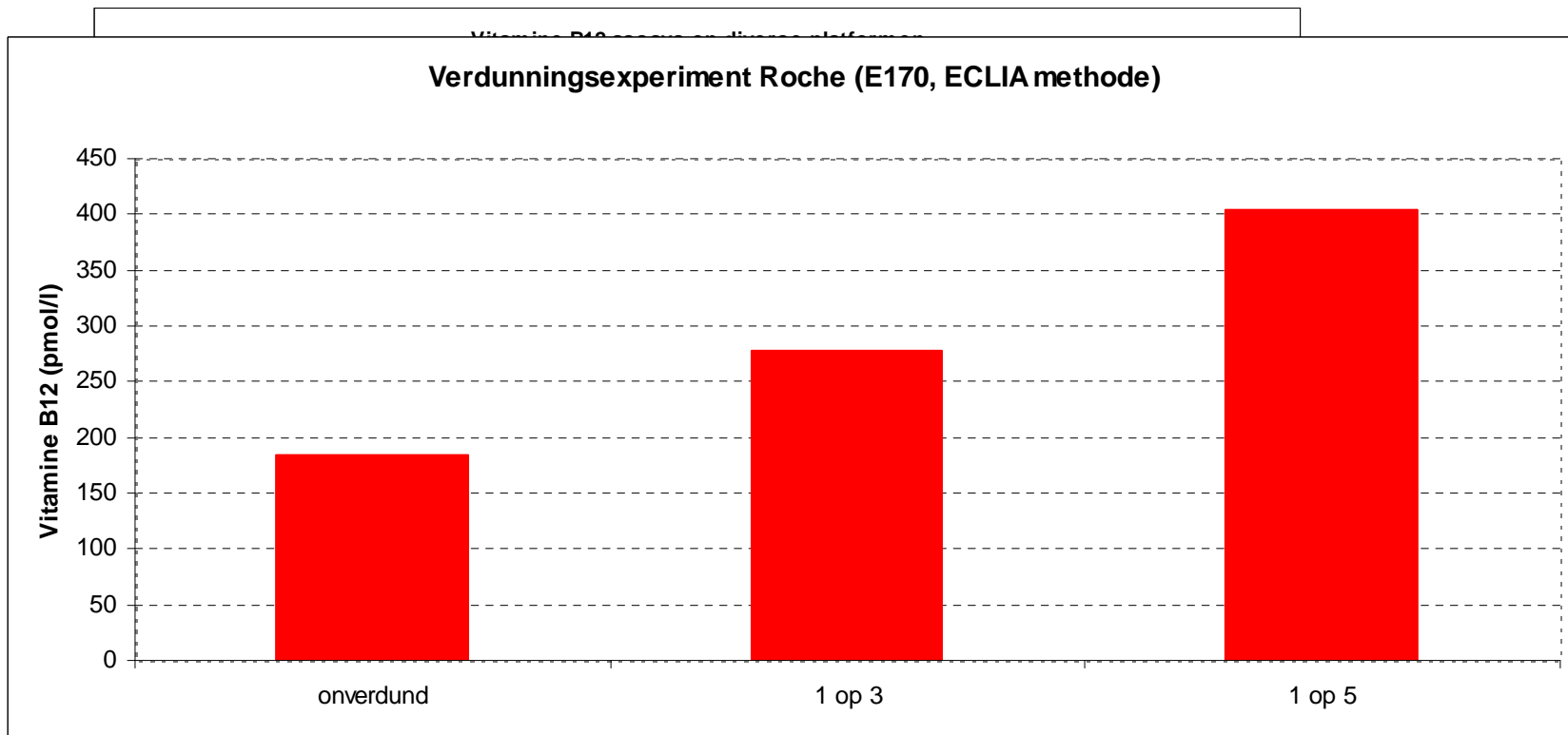
Uitsluiten interferentie

- Uitsluiten heterofiele antilichamen middels ..



Uitsluiten interferentie

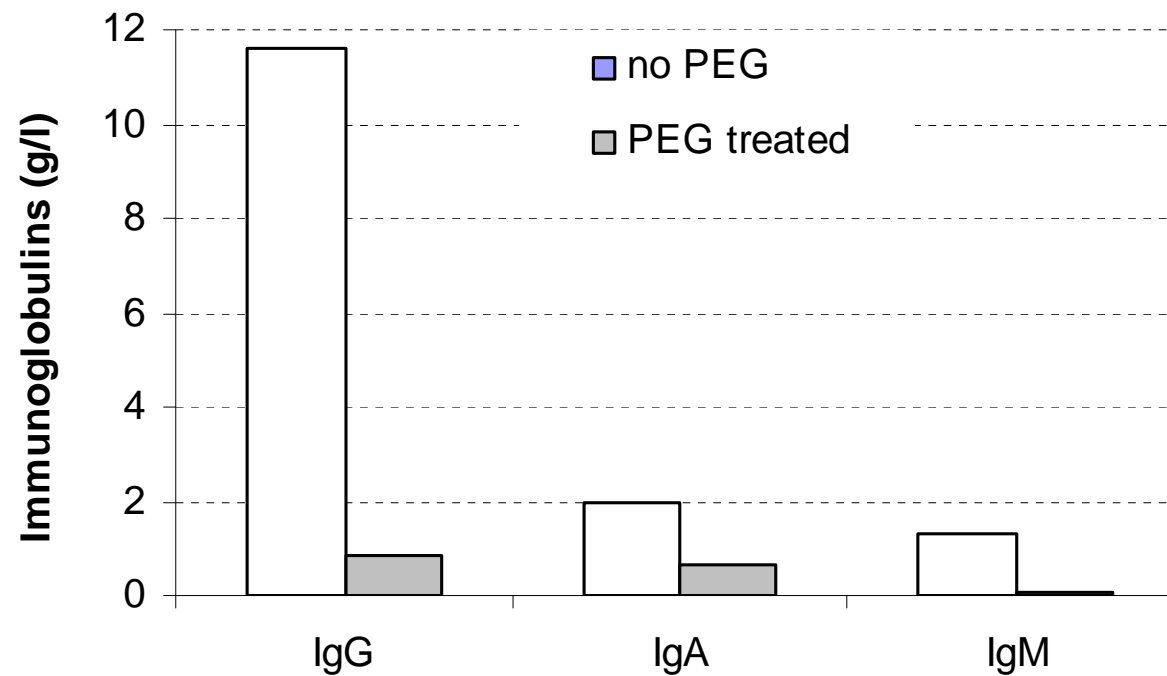
- Andere assay's



Let wel: Geen enkele assay liet een verlaagd totaal vitamine B12 zien en laat niet lineaire uitverdunding zien (Roche ECLIA assay).

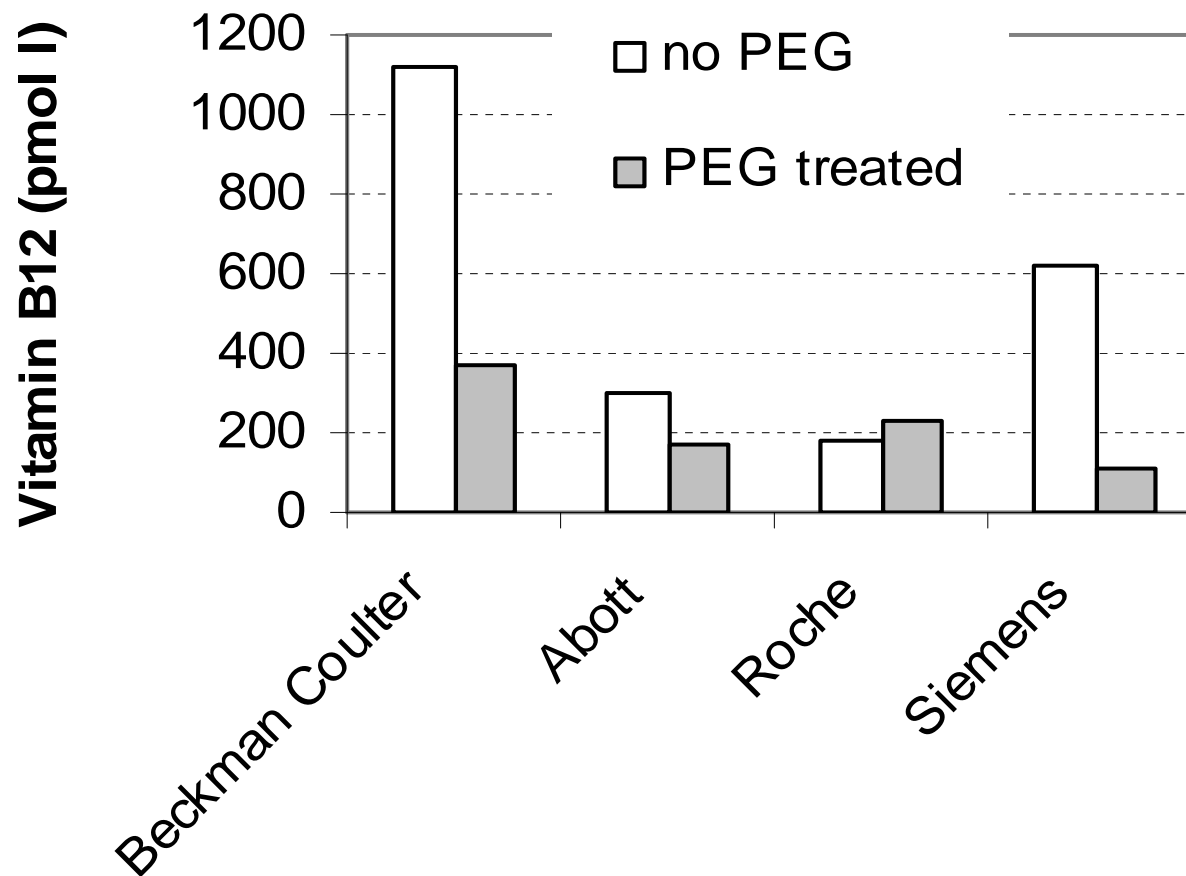
Uitsluiten interferentie

- PEG 6000 (25%) precipitatie (1 op 1)



Uitsluiten interferentie

- **PEG 6000 (25%) precipitatie**



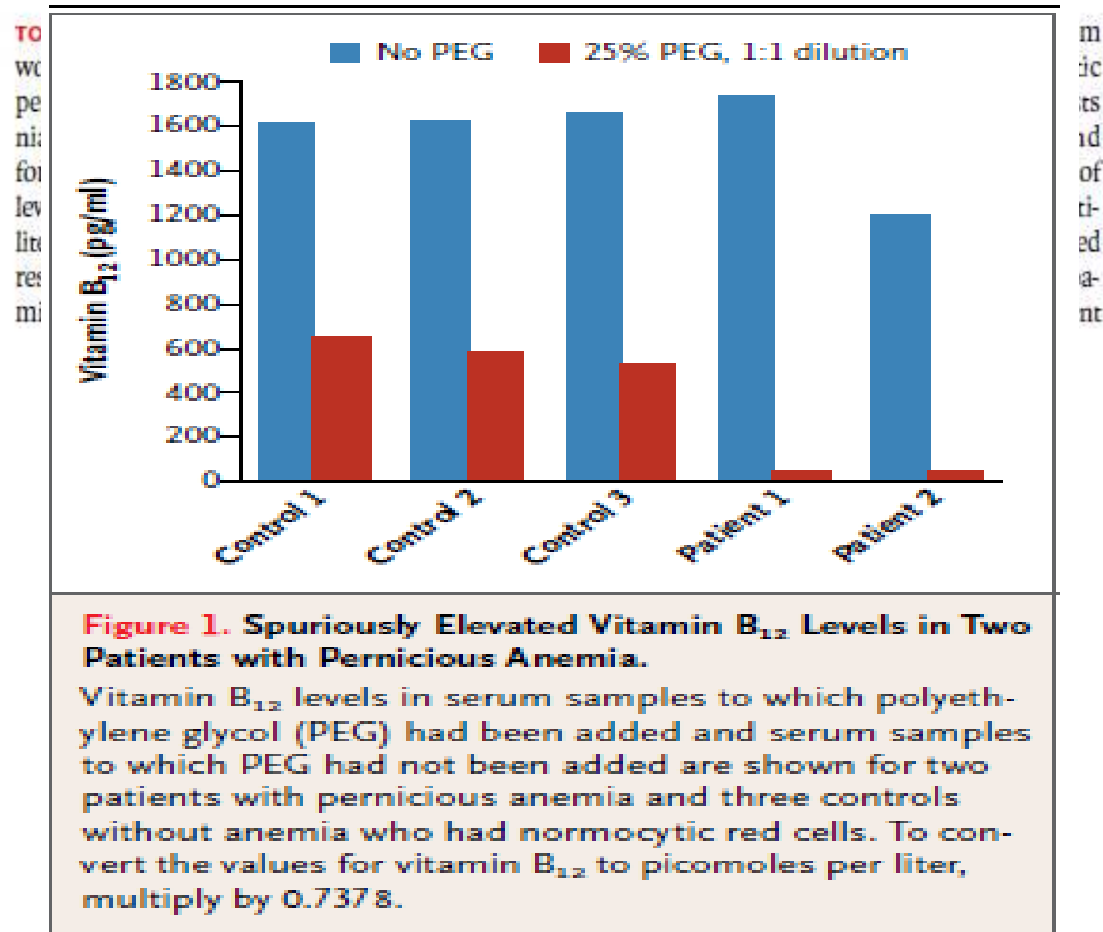
Interferentie in vitamine B12 assay



- **Vitamine B12 concentratie gaat niet naar deficiënte waarden na PEG precipitatie**
- **Analyse van monster na 1 op 1 PEG precipitatie laat echter nog steeds positieve titer zien na precipitatie**
- **Pas na herhaalde PEG precipitatie (1 op 4) is de antistoftiter naar zwak positief gedaald (echter niet meer meetbaar voor vitamine B12)**
- **Kortom, opnieuw foutief verhoogde vitamine B12 (op verschillende platformen) bij interferentie (zeer waarschijnlijk toch) als gevolg van auto antistoffen tegen intrinsic factor.**

Elders in de wereld...

Spurious Elevations of Vitamin B₁₂ with Pernicious Anemia



CORRESPONDENCE

THE NEW ENGLAND JOURNAL OF MEDICINE

Measurement of the active vitamin E₁₂, or holotranscobalamin, instead of total cobalamin,³ would circumvent the problem. Another solution would be to demand that producers of commercial diagnostic kits exchange hog intrinsic factor with a binding protein not recognized by autoantibodies against intrinsic factor.

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No potential conflict of interest relevant to this letter was reported.

1. Carmel R, Agrawal YP. Failures of cobalamin assays in pernicious anemia. *N Engl J Med* 2012;367:385-6. [Erratum, *N Engl J Med* 2012;367:976.]

2. Yang DT, Cook RJ. Spurious elevations of vitamin B₁₂ with pernicious anemia. *N Engl J Med* 2012;366:1742-5.

3. Nexø E, Hoffmann-Lücke E. Holotranscobalamin, a marker of vitamin E-12 status: analytical aspects and clinical utility. *Am J Clin Nutr* 2011;94:359S-365S.

DOI: 10.1056/NEJMc1210169

TO THE EDITOR: Carmel and Agrawal recently added 8 cases to the 11 previously reported in which the presence of intrinsic factor autoantibodies was associated with misleadingly normal cobalamin values. However, they incorrectly suggest that this phenomenon also explains my previous finding that neither normal levels of cobalamin nor normal levels of the cobalamin-dependent metabolite, methylmalonic acid (MMA), preclude the presence of cobalamin-responsive clinical disorders.^{1,2} In fact, when the data in my previous study were reanalyzed to include only those patients in whom intrinsic factor autoantibodies were not detected, 67% of the 39 patients with one low cobalamin level had a normal second value, 53% of the 74 patients with two normal cobalamin values had high MMA levels, and 75% of the 29 patients with a clinical response had consistently normal cobalamin values (with both cobalamin values >300 pg per milliliter in 61%), and MMA values were less than 376 nmol per liter in 54% of the 29 patients with a clinical response (unpublished data). In conclusion, "false" normal cobalamin values often result from intraindividual variation, and clinical responses to cobalamin therapy occur despite normal cobalamin and MMA values. Thus, there is still no "gold standard" for the diagnosis of cobalamin deficiency, and therapeutic trials are warranted when the clinical picture is consistent with this disorder.³

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No potential conflict of interest relevant to this letter was reported.

1. Solomon LR. Cobalamin-responsive disorders in the ambulatory care setting: unreliability of cobalamin, methylmalonic acid, and homocysteine testing. *Blood* 2005;105:978-85.

2. Solomon LR. Tests for cobalamin-responsive disorders are unreliable. *Blood* 2005;106:1137-8.

3. Green R. Unreliability of current assays to detect cobalamin deficiency: "nothing gold can stay." *Blood* 2005;105:910-1. DOI: 10.1056/NEJMc1210169

TO THE EDITOR: Carmel and Agrawal highlight the problems of using the results of the serum competitive-binding luminescence assay (CBLA) in diagnosing pernicious anemia (also previously reported by me¹) but fail to suggest the option of testing serum levels of holotranscobalamin² to detect cobalamin deficiency. This assay circumvents the problem of interference from intrinsic factor autoantibodies that characterizes the CBLA. In addition, the level of intrinsic factor autoantibodies that is most likely to cause interference has not been reported. Unless every CBLA is also assessed for intrinsic factor autoantibodies, and the level of serum cobalamin is assessed in relation to the level of intrinsic factor autoantibodies, the CBLA may have little meaning. The practicality and cost of such an approach are prohibitive, particularly in a high-volume, high-throughput routine diagnostic laboratory. The use of a widely and routinely used assay that may give a false negative result is particularly worrying. There are arguments for³ and against⁴ the use of holotranscobalamin, but the time is ripe to consider it as an alternative to cobalamin to screen for and diagnose cobalamin deficiency in a routine diagnostic laboratory setting.

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No potential conflict of interest relevant to this letter was reported.

1. Devalia V. Diagnosing vitamin B-12 deficiency on the basis of serum E-12 assay. *EMJ* 2006;333:385-6.

2. Heil SG, de Jonge R, de Rotte MC, et al. Screening for metabolic vitamin B12 deficiency by holotranscobalamin in patients suspected of vitamin B12 deficiency: a multicentre study. *Ann Clin Biochem* 2012;49:184-9.

3. Nexø E, Hoffmann-Lücke E. Holotranscobalamin, a marker of vitamin E-12 status: analytical aspects and clinical utility. *Am J Clin Nutr* 2011;94:359S-365S.

4. Carmel R. Holotranscobalamin: not ready for prime time. *Clin Chem* 2012;58:643-5.

DOI: 10.1056/NEJMc1210169

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Take home message...



Kortom, vitamine B12 assays hebben anno 2012 nog steeds (intrinsieke) zwakten!

Houd hier dus rekening mee!

En vraag/voeg bij twijfel altijd een HcY of MMA!

Vragen?