# Active $B_{12}$
# The Next Level of B12 Testing

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# Active $B_{12}$
## The Next Level of B12 Testing
## Course Objectives

At the end of the program, participants will be able to describe:

- The clinical presentation of patients with vitamin B12 deficiency.
- The physiology of B12 and the B12-binding proteins.
- The clinical utility of tests to diagnose B12 deficiency with special reference to active B12.

# Vitamin B12 Deficiency

**Scope:**

- History of B12 deficiency
- Prevalence of B12 deficiency
- Review of normal physiology
- Causes of B12 deficiency
- Clinical Manifestations of B12 deficiency
- Diagnosing B12 deficiency
- Holotranscobalamin (Active B12) and its usefulness

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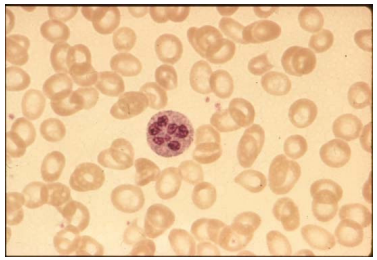
# Pernicious Anemia: Description *(Cabot, 1908)*

## ❑Anemia



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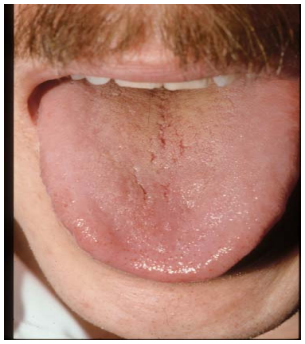
**associated with a diagnostic triad of:**

- **Sore tongue (glossitis)**



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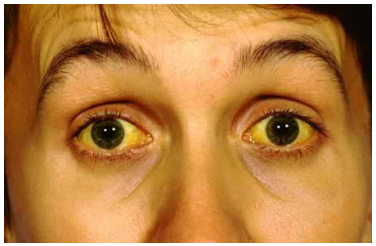
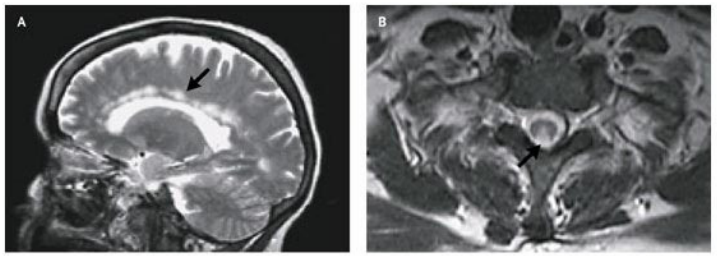
- **Jaundice**



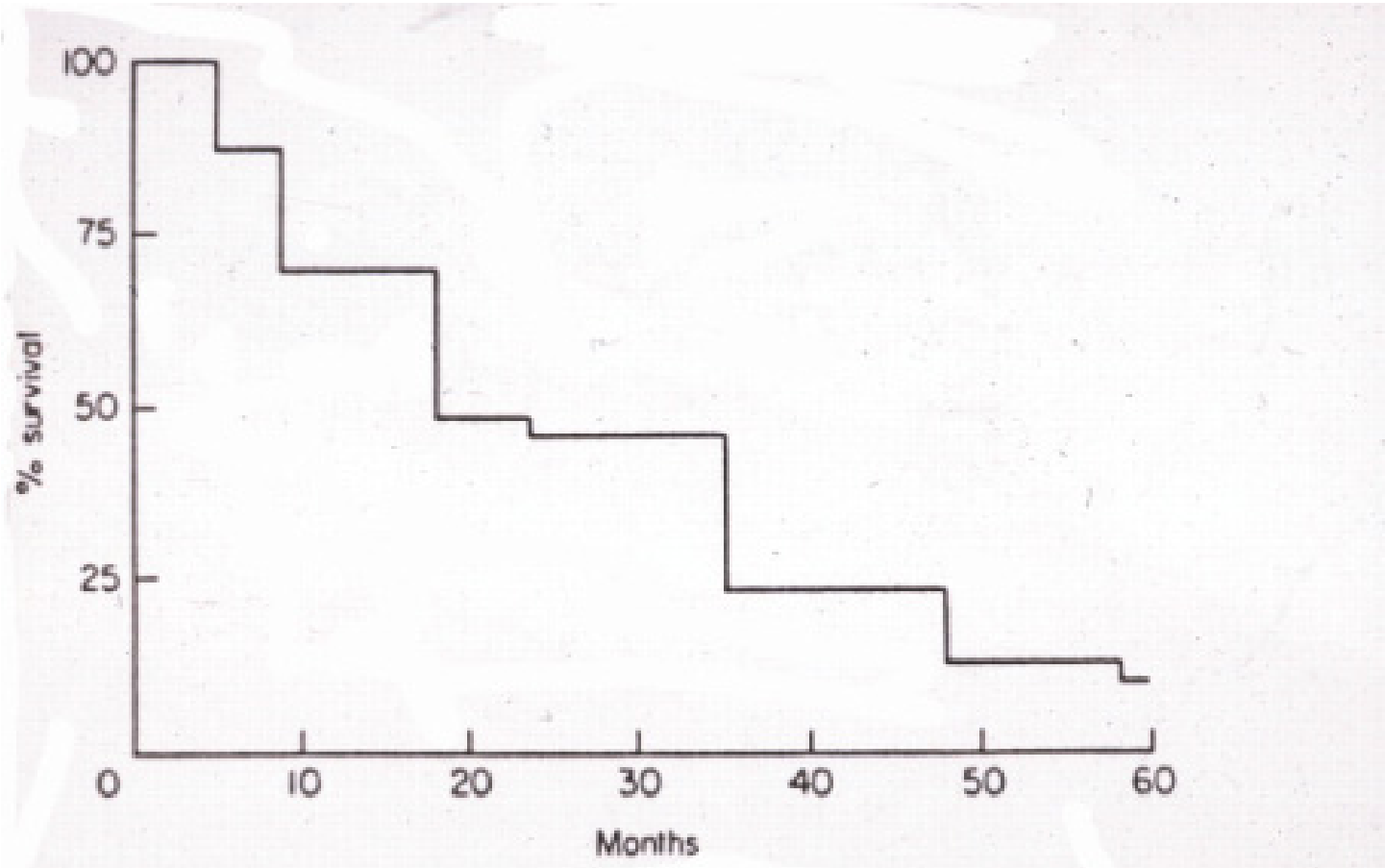
*www.patient.co.uk*

- **Spinal cord damage**




*New Engl J Med 348; 2208: 2003*

**Pernicious Anemia was an inevitably fatal disease prior to the Nobel Prize-winning discoveries of Minot, Murphy and Whipple**




**Kaplan-Meyer survival curve for 320 patients with pernicious anemia pre-1926 (Cabot 1908)**

# The Conquest of Pernicious Anemia & The Characterization of Vitamin B12

Karl Folkers

Minot, Whipple & Murphy Nobel Prize for Physiology & Medicine 1934 – "Cure of PA"

Karl Folkers and Lester Smith 1948 – Anti-pernicious anemia principle crystallized from liver; B12 named

Dorothy Hodgkin – Nobel Prize for Chemistry for studies on X-Ray crystallographic structure of B12 and proteins

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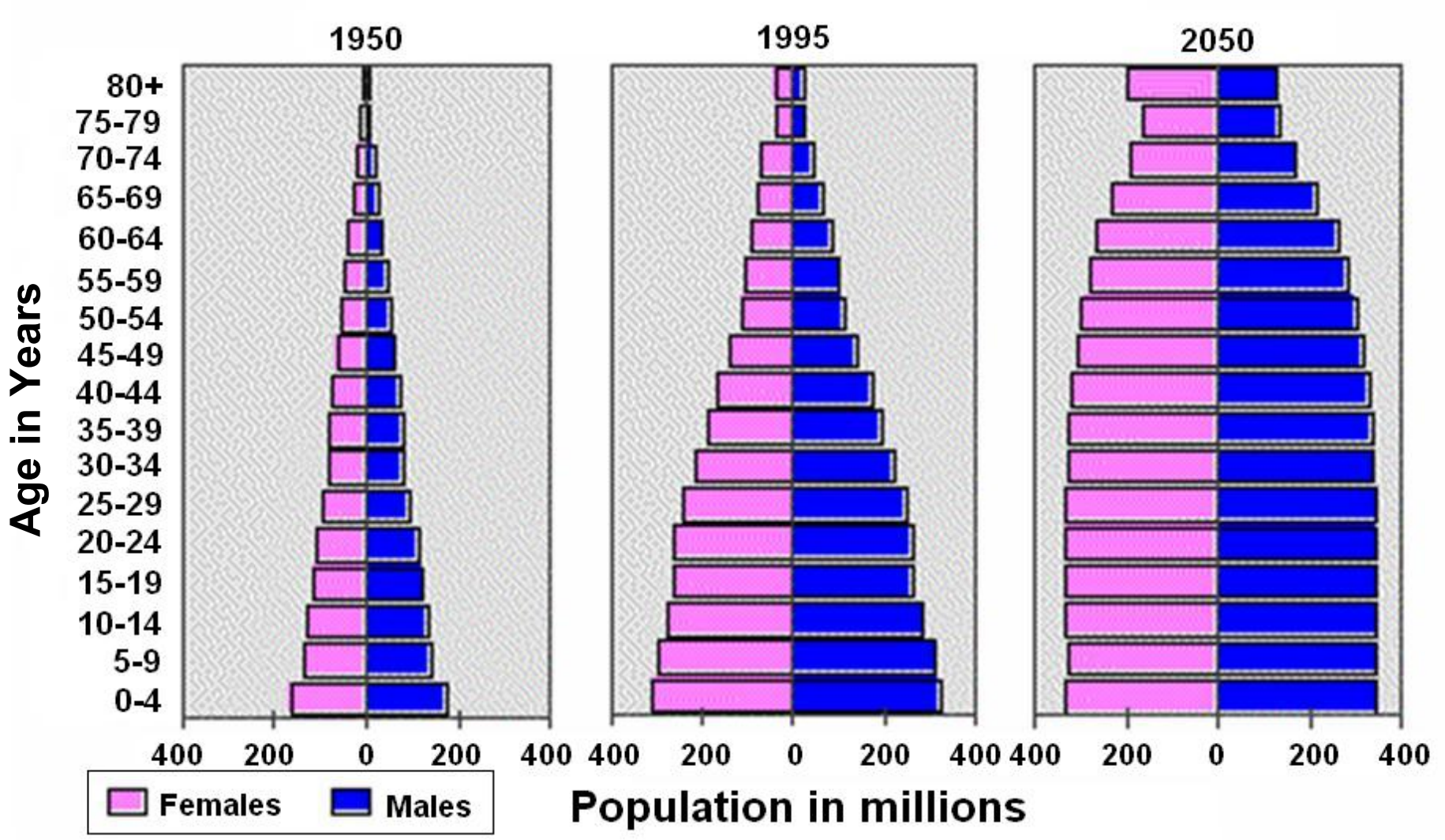
# Prevalence of B12 Deficiency in the United States

- **Adults age >65 years**
  - **2-3% have pernicious anemia**
  - **30-40% have food B12 malabsorption**

- **Sacramento Area Latino Study on Aging (SALSA)**
  - **Elderly Latinos, age >60 years**
  - **6.5% with total serum B12 <200 pg/ml**
  - **18% with total serum B12 200-300 pg/ml**

Green R, Miller JW: Clin. Chem. Lab. Med. 43:1048-51, 2005

# World Population by Age




Source: United Nations Data

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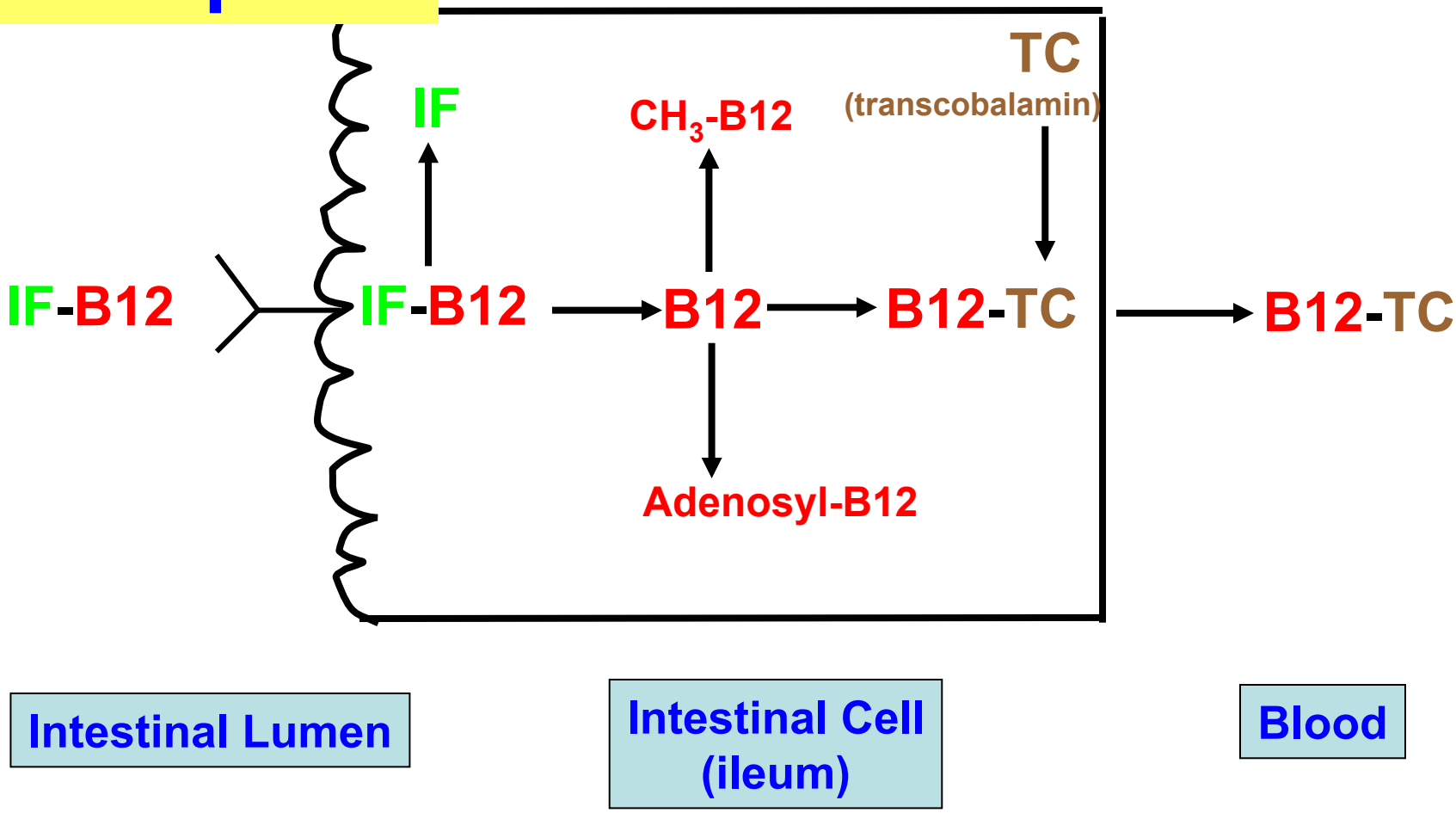
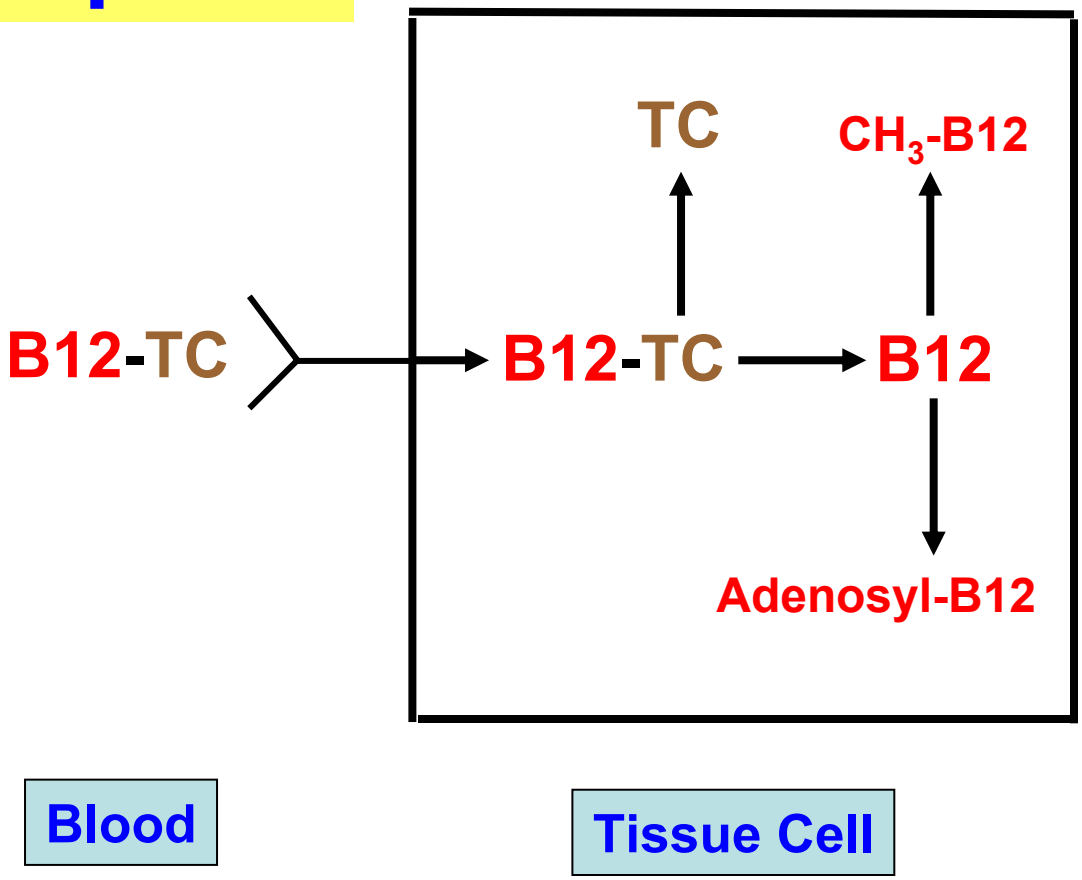
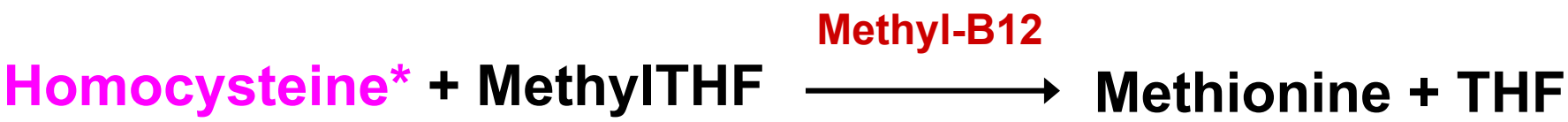
# Vitamin B12 Absorption



# Vitamin B12 Cellular Uptake



# Vitamin B12-Dependent Reactions





*Levels rise in B12 deficiency

# Plasma B12 Transport Proteins

| | Haptocorrin (TC I + III) | Transcobalamin (TC II) |
|---|---|---|
| **Source** | granulocytes | endothelial cells, gut |
| Transport functions | storage, excretion of B12 analogues, antimicrobial | cellular B12 uptake |
| **Binding specificity** | low specificity, binds B12 analogues | binds B12 with higher specificity |
| **Membrane receptors** | non-specific asialoglyco-protein receptors on hepatocytes | specific receptors on most cells |
| **Saturation** | high (mainly "holo") | low (mainly "apo") |
| Fraction of total B12 | 70-90% | 10-30% |
| **Plasma clearance** | slow ($t_{1/2} \approx 10$ days) | rapid ($t_{1/2} \approx 6$ min) |
| **Molecular Weight** | 60,000 | 38,000-45,000 |

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# Vitamin B12 Deficiency

**Causes:**

- Dietary deficiency (rare, primarily occurs in strict vegans and their offspring)
- **Malabsorption**
- Nitrous oxide (irreversibly oxidizes B12)
- Transcobalamin deficiency
- Genetic enzyme defects

# Causes of Vitamin B12 Malabsorption

- Atrophic Gastritis (achlorhydria or loss of stomach acid)
- Autoimmune production of IF or parietal cell antibodies (**pernicious anemia**)
- Gastrectomy
- Pancreatic insufficiency
- Bacterial overgrowth (*H. pylori*)
- *Diphyllobothrium latum* (fish tapeworm)
- HIV Infection
- Ileal disease or resection
- Selective Vitamin B12 Malabsorption –Immerslund-Gräsbeck Syndrome (Autosomal Recessive Megaloblastic Anemia (MGA1) –defects in *cub, amn.*

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# Symptoms and Signs of Vitamin B12 Deficiency

**Clinical Manifestations:**

- **Megaloblastic anemia**
- **Subacute combined degeneration (SACD) (demyelination with central and peripheral neuropathy, most notably in spinal cord)**
- **Gait ataxia**
- **Cognitive deficits (can be Alzheimer-like)**
- **Glossitis**
- **Increased risk of vascular disease, cancer, neural tube defects**
- **Osteopenia/osteoporosis**

# Patients in Whom to Suspect Possible B12 Deficiency

- Symptoms and signs of B12 deficiency
- Anemia with or without macrocytosis
- Neurological disturbances with or without anemia

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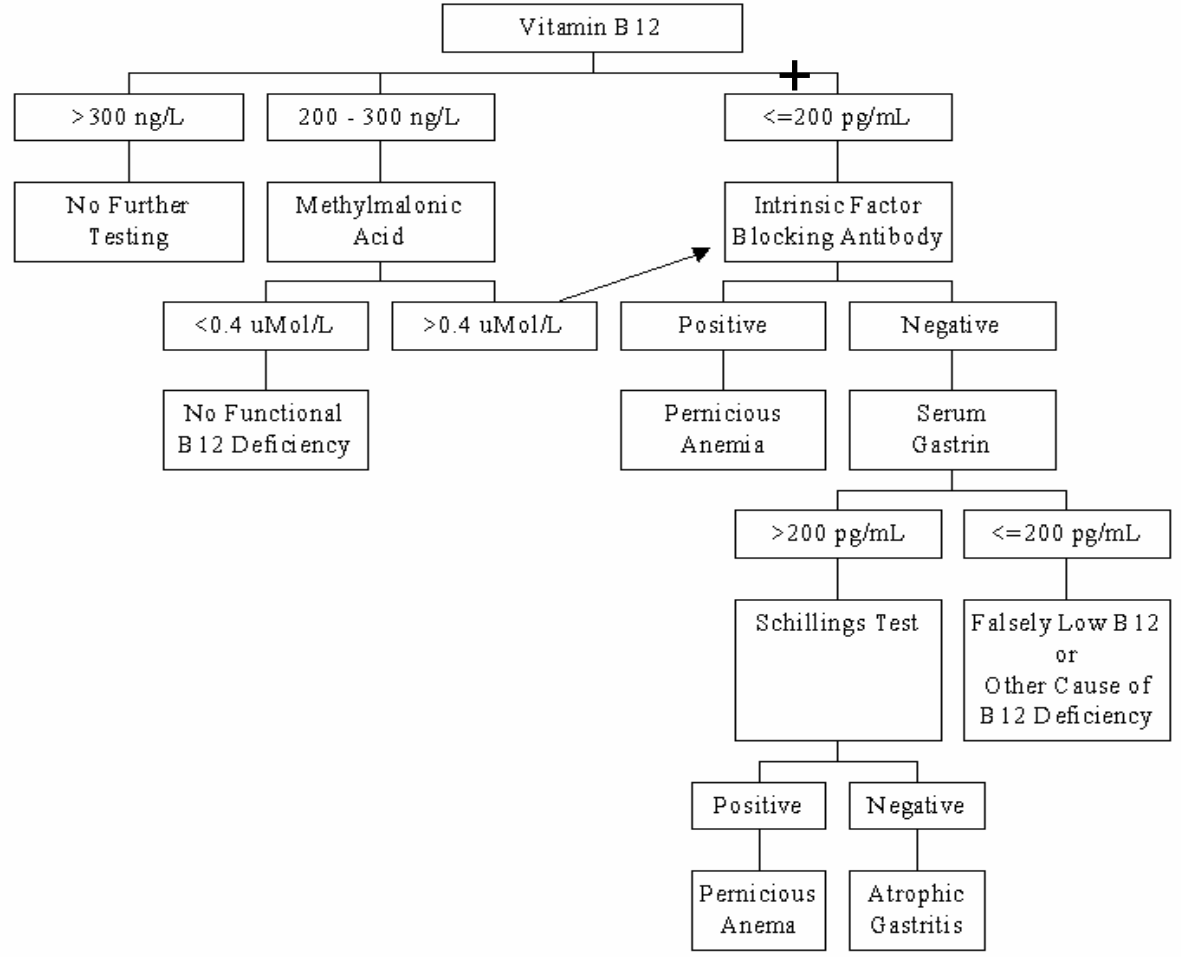
# Diagnosis of Vitamin $B_{12}$ Deficiency

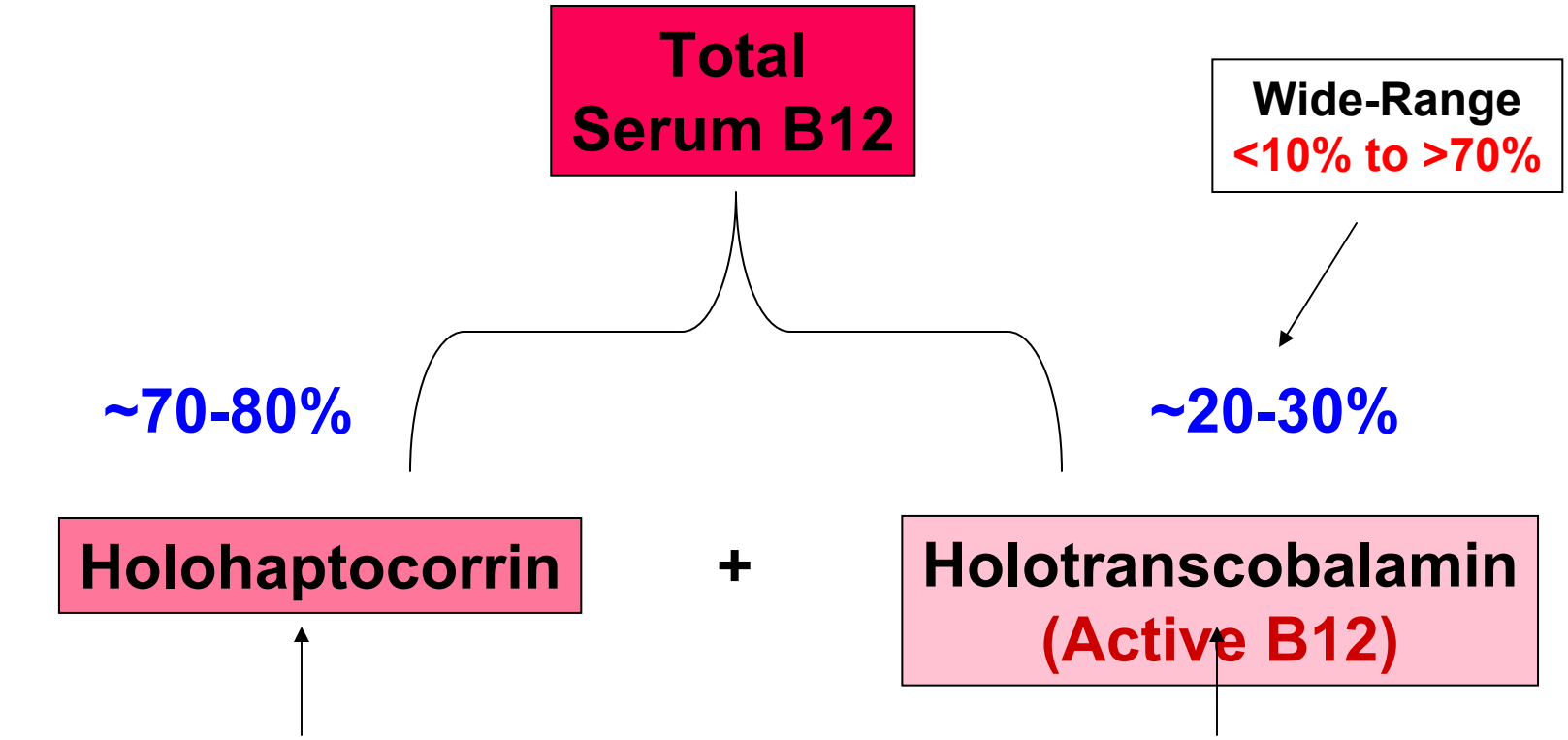
- Macrocytic megaloblastic anemia with or without neurological involvement.
- Atypical presentations (neurological syndrome without anemia or macrocytosis
- Low plasma B12 as an isolated lab finding
- Raised plasma metabolites (methylmalonic acid and homocysteine
- Low transcobalamin B12 (HoloTC) = “Active B12”

# Approach to the diagnosis of Pernicious Anemia




**Reference: Pernicious Anemia Cascade, Mayo Medical Laboratories, October 1998 (From Green & Kinsella, Current Concepts in the Diagnosis of Cobalamin Deficiency, Neurology 1995; 45: 1435-1440)**

# Assessing B12 Status




- Fraction of total B12 bound to haptocorrin.

- Fraction of total B12 bound to transcobalamin.
- Delivers B12 to all tissues.

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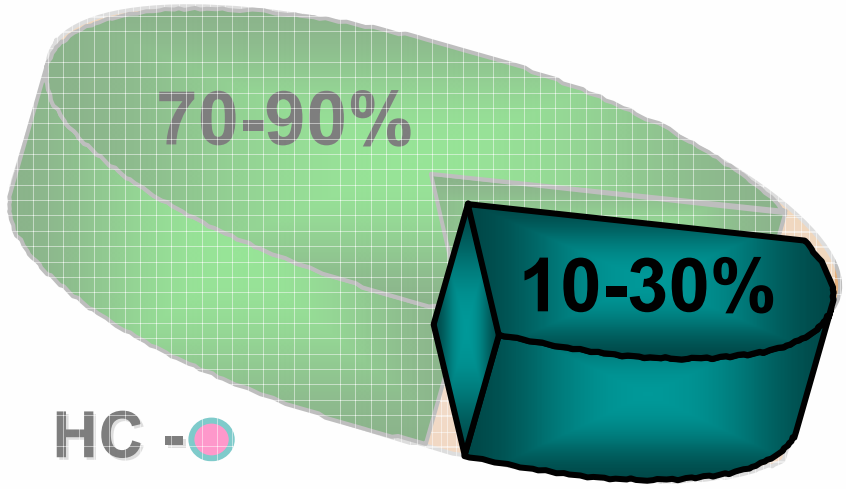
# Theoretical Advantages of HoloTC (Active B12) in the Diagnosis of B12 Deficiency

- TC delivers B12 to all tissues; haptocorrin does not. Except on the liver no cellular receptors exist for the $B_{12}$ carried by haptocorrin (HC)
- Genetic TC deficiency leads to life-threatening functional B12 deficiency; genetic haptocorrin deficiency is relatively benign.
- HoloTC has a short half-life (~6 min) and is therefore expected to fall early during states of B12 malabsorption.
- It can take months, even years, for a significant fall in HoloHC levels and so the more rapid decline in HoloTC (Active-B12) may be masked when measuring total serum B12

# Not all vitamin $B_{12}$ in serum is active




Around 20% of circulating $B_{12}$ is carried on transcobalamin.

HC -

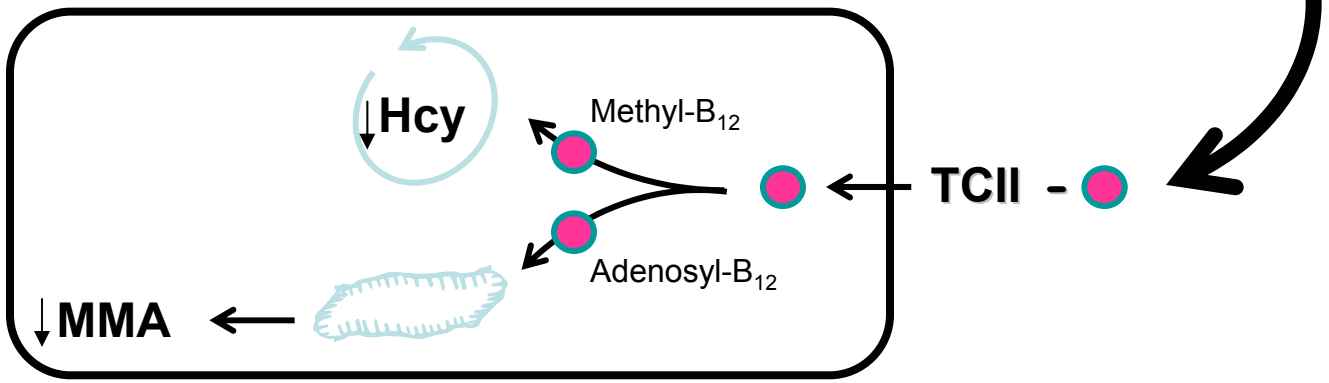
Holohaptocorrin (holoHC)

**Biologically inert**

TC -

Active-B12 (holotranscobalamin)

**Biologically active**



# Sequence of Changes in Developing B12 Deficiency*

1. **Early:** low holoTC (Active B12)

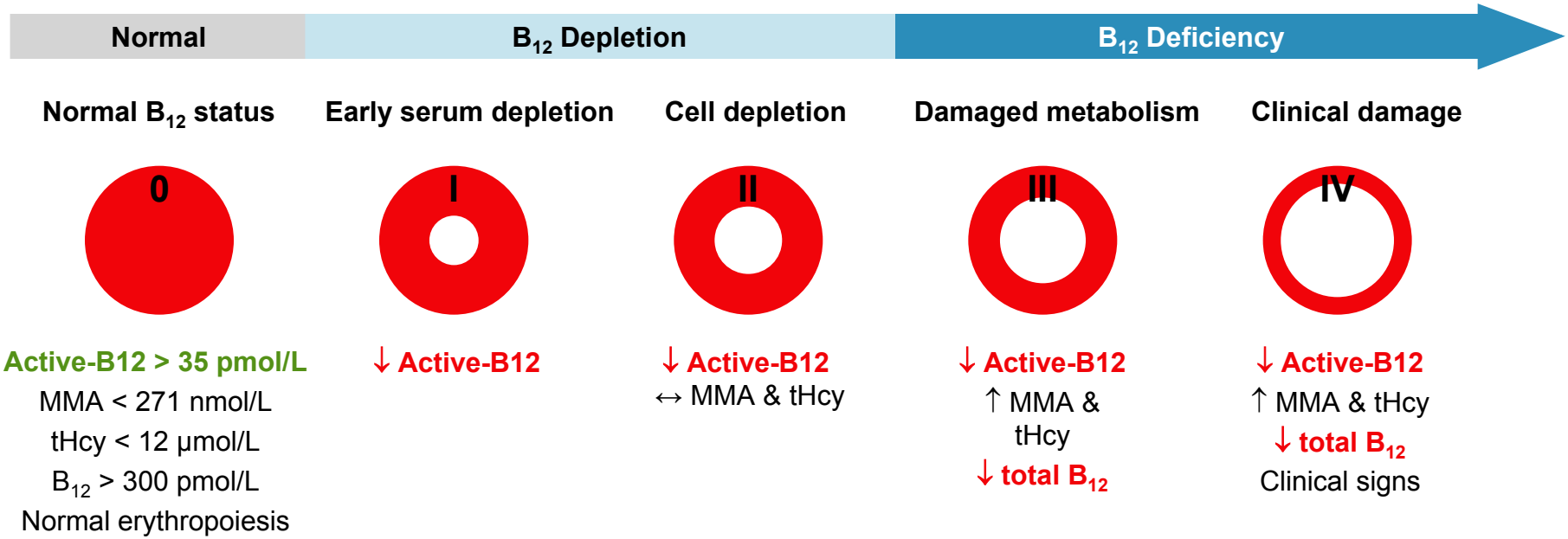
2. **Cellular:** low serum B12, depletion of body stores

3. **Metabolic:** increased Hcy and MMA

4. **Clinical:** macrocytic anemia, neurological impairment

**Victor Herbert 1987*

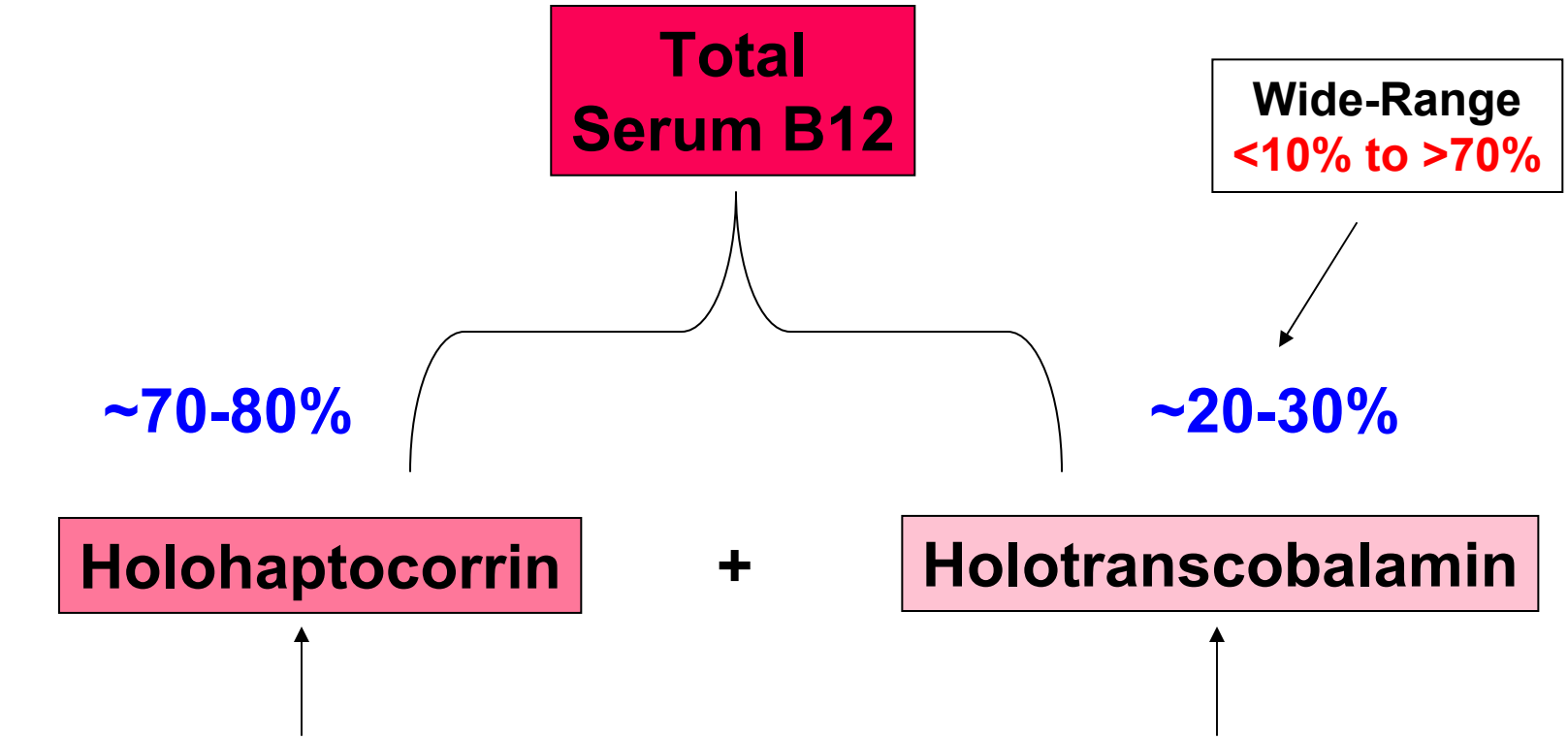
# How vitamin $B_{12}$ deficiency develops (hypothesis)




**Active-B12 levels react *early* in the process.**

1. Modified from V. Herbert, Am J Clin Nutr 1994

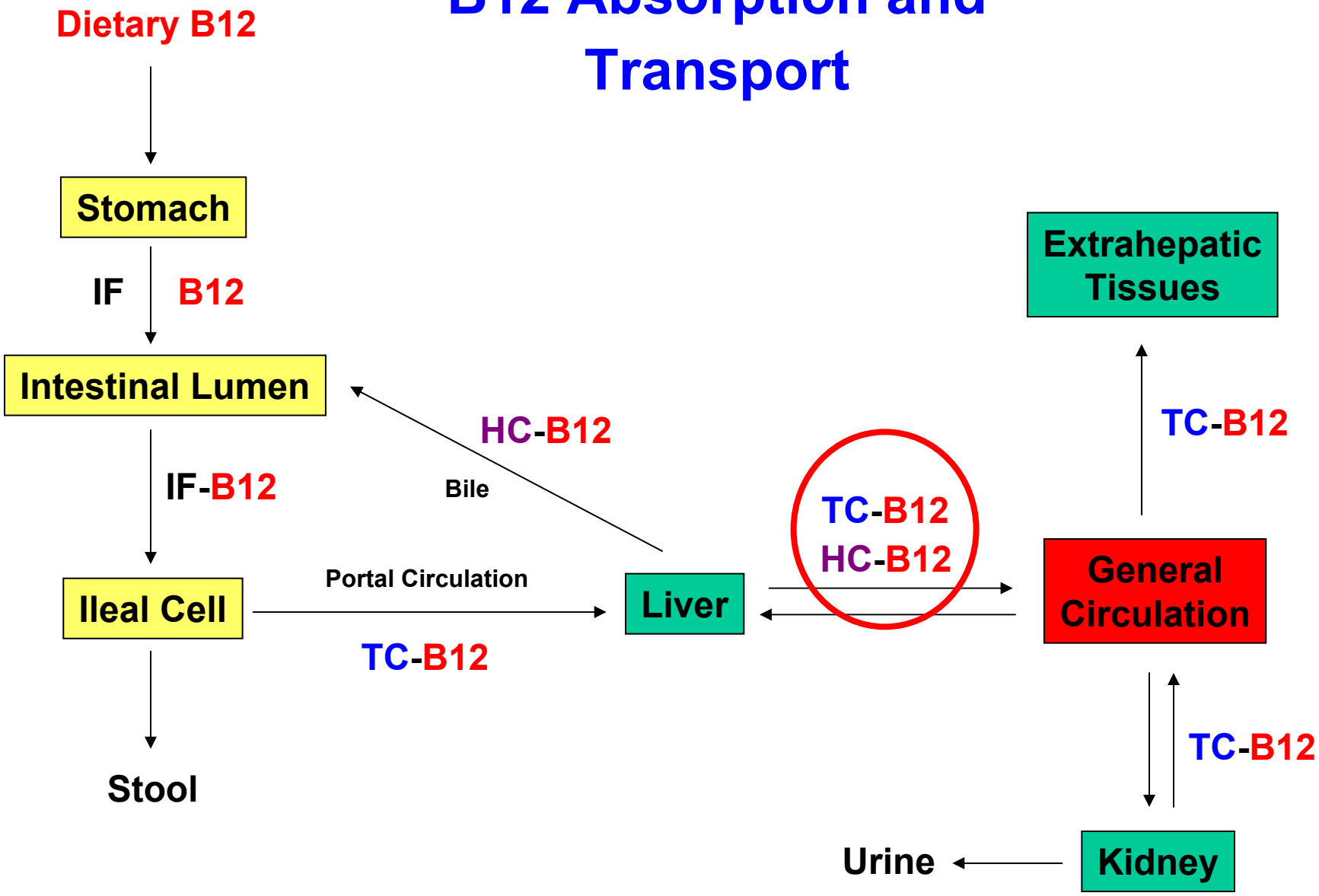
# Assessing B12 Status



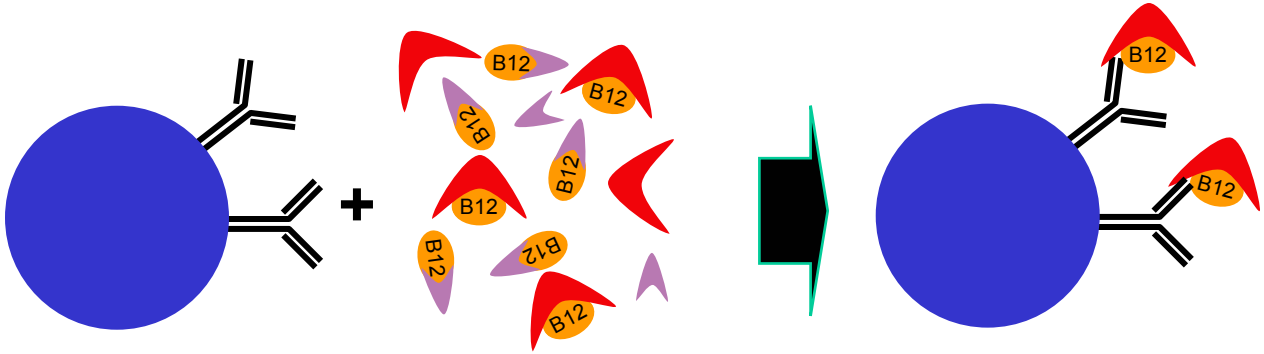

- Fraction of total B12 bound to haptocorrin.

- Fraction of total B12 bound to transcobalamin.
- Delivers B12 to all tissues.

# TC-B12 or Active B12 in B12 Absorption and Transport



# Active-B12 reaction schematics
# 2-Step sandwich MEIA

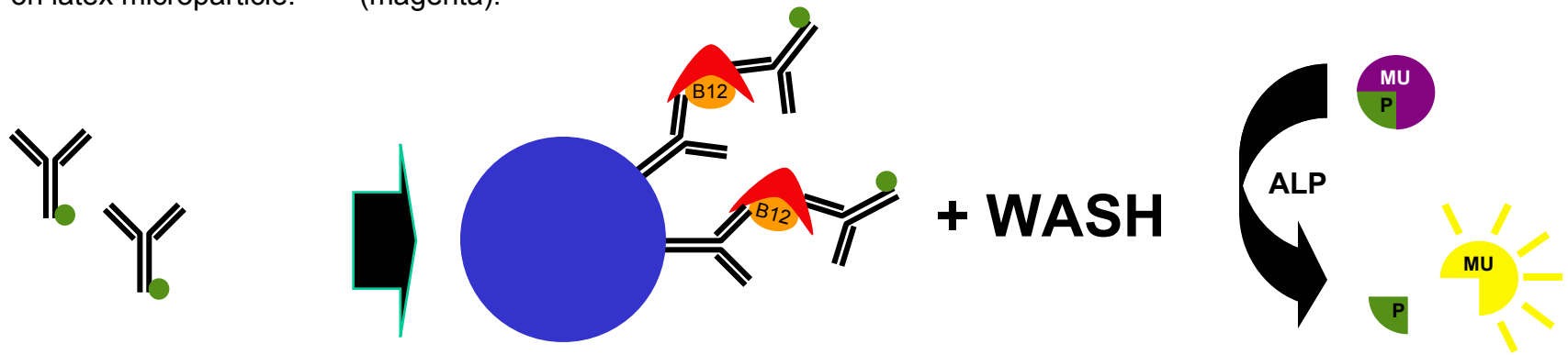



Active-B12 specific Mab (mouse, monocl.) immobilised on latex microparticle.

Sample B12 bound to transcobalamin (red) and haptocorrin (magenta).

Only $B_{12}$-TC (Active-B12) will bind to solid phase.

Suspension moved to glass fiber matrix and washed to remove unbound sample.




Anti-TC:ALP conjugate (mouse, moncl.) is added.

Conjugate binds to TC bound to solid phase.

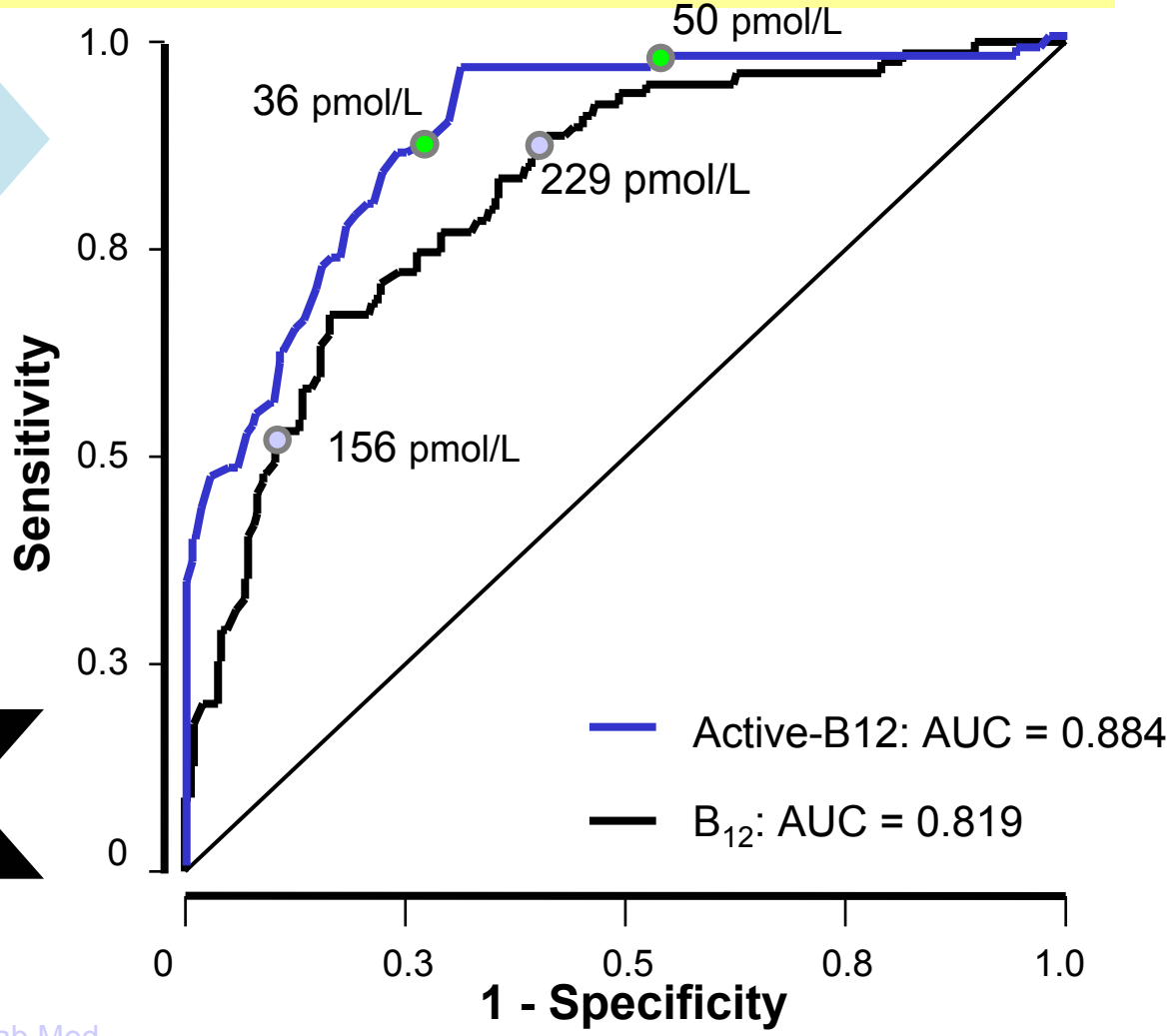
Unbound conjugate is removed.

Rate of fluorescent MU formation is directly proportional to [Active-B12] in sample.

# Active-B12 levels are low in patients with biochemical signs of vitamin $B_{12}$ deficiency

**$B_{12}$ deficiency defined by [9]:**

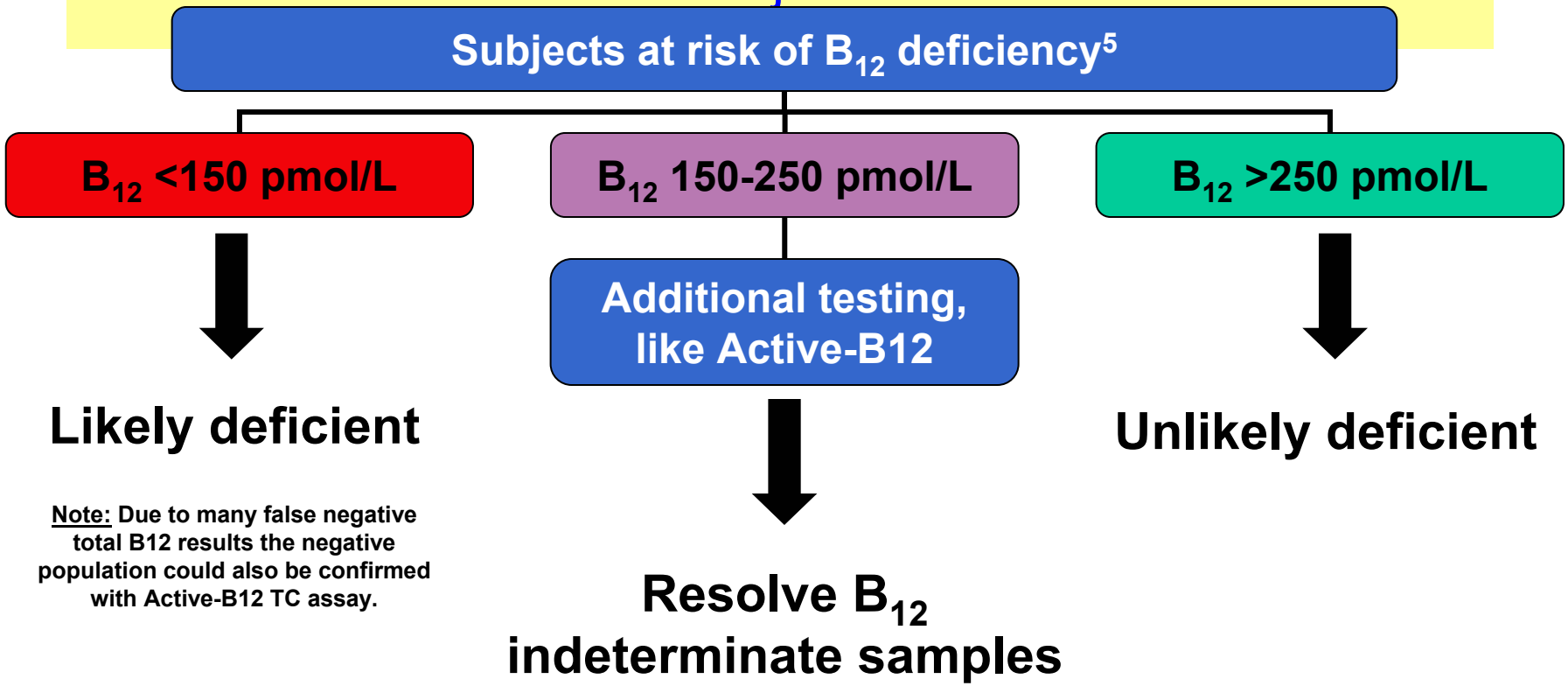
- **MMA >400 nmol/l and**
- **Normal renal function**




**Data suggests improved identification of $B_{12}$ deficient patients with Active-B12 compared to total serum $B_{12}$.**

Adapted from Herrmann *et al*. Clin Chem Lab Med. 2003,41,1478-88

# Recently Proposed Algorithm for $B_{12}$ Deficiency Subjects




Adapted from Schneede J., Scan J Clin Lab Invest 2003; 63: 369 - 376

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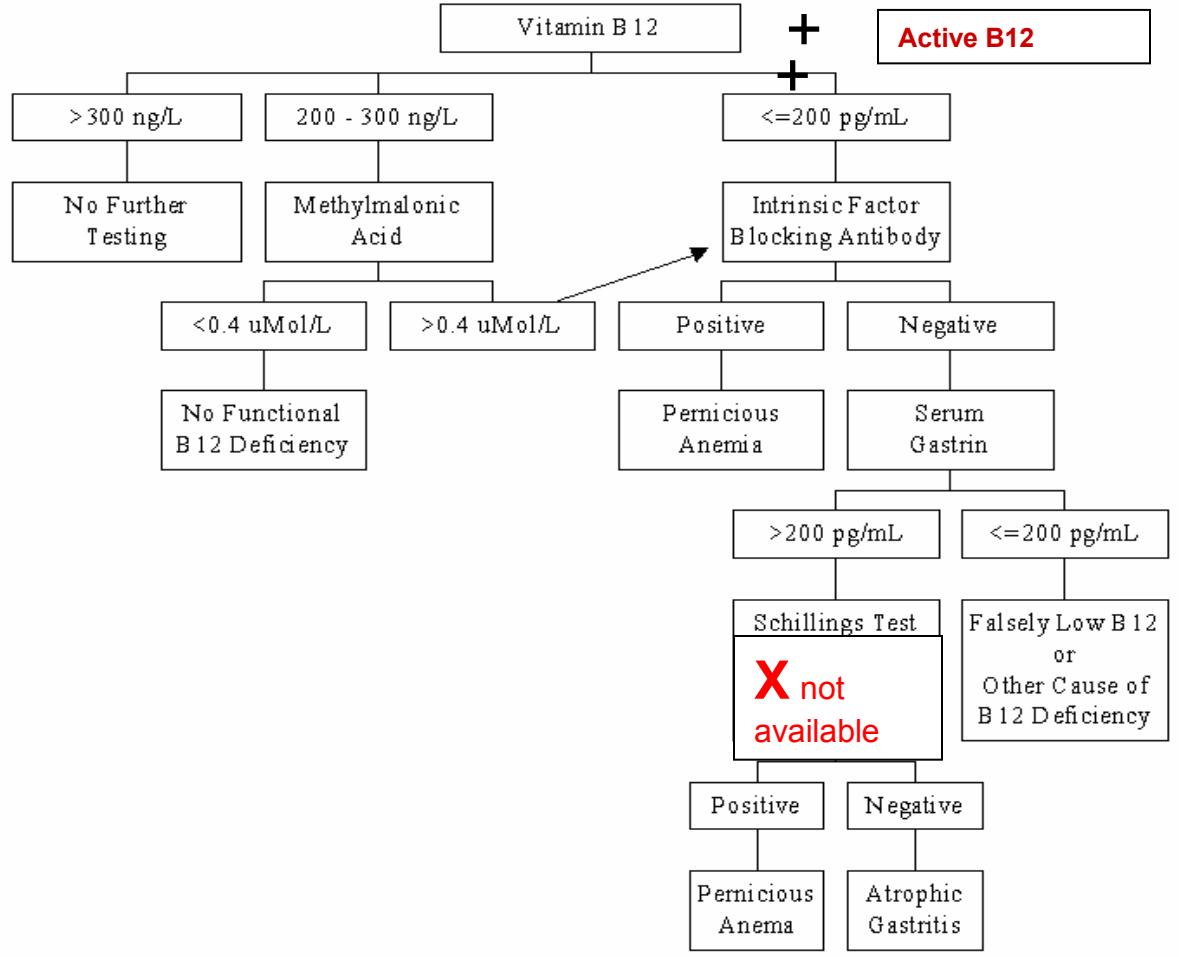


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