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## PEARLS OF LABORATORY MEDICINE

### **Thyroid Function Testing**

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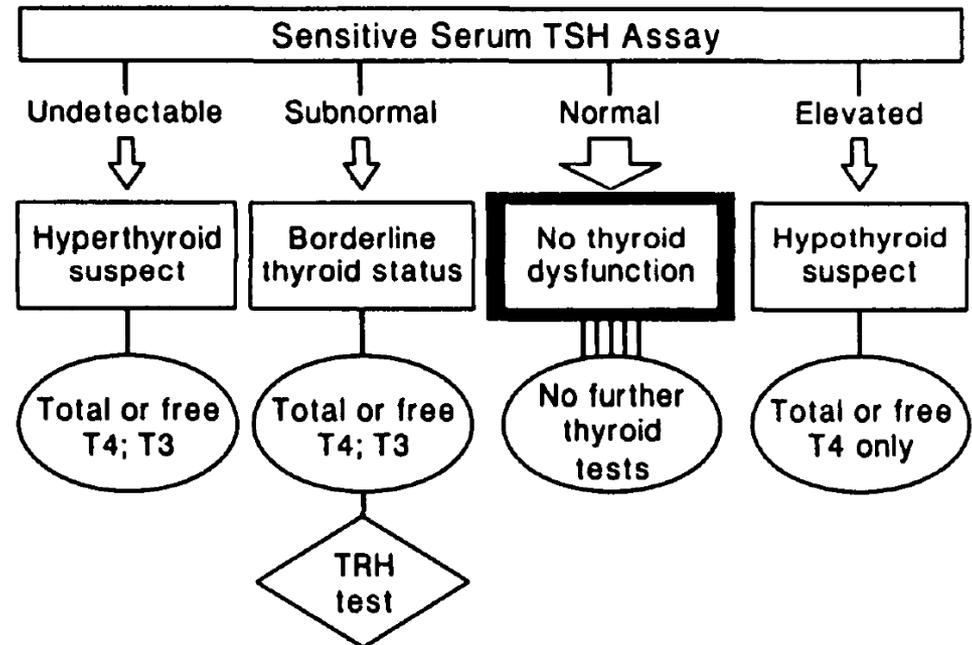
# Evolution of Thyroid Function Testing

- Basal Metabolic Rate (1900-1950s)
  - Oxygen consumption when at rest
  - Indirect measure of thyroid hormone concentration, many contributors, difficult to standardize “at rest”
- Protein-Bound Iodine (1960s-1970s)
  - Precipitate thyroid carrier proteins, measure iodine
  - Variable recovery, poor specificity, interference from inorganic iodide (drugs, smoking)
- Total T4 Competitive Immunoassay (1965)
- FT4 Index (1970s-late 1990s)
  - Free T4 recognized as biologically active fraction
- FT4 by Equilibrium Dialysis (mid 1980s)
- TSH Assay Improvement (mid-late 1980s)
  - Facilitated by development of monoclonal antibodies (1984 Nobel Prize)
- “One-Step” Automated FT4 Immunoassay (late 1980s - early 1990s)



# Initial Evaluation of Thyroid Function

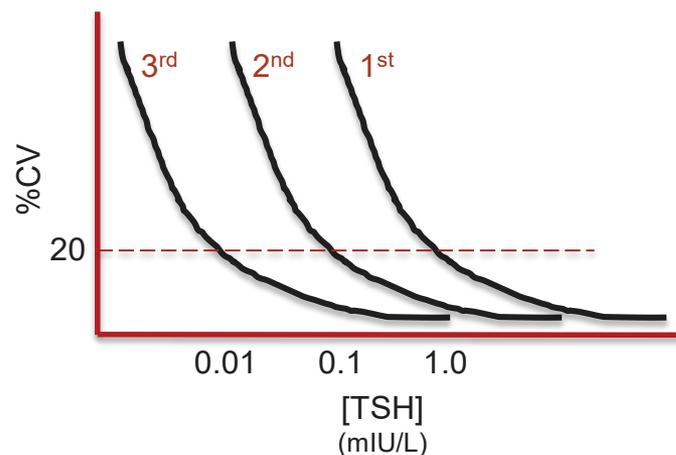
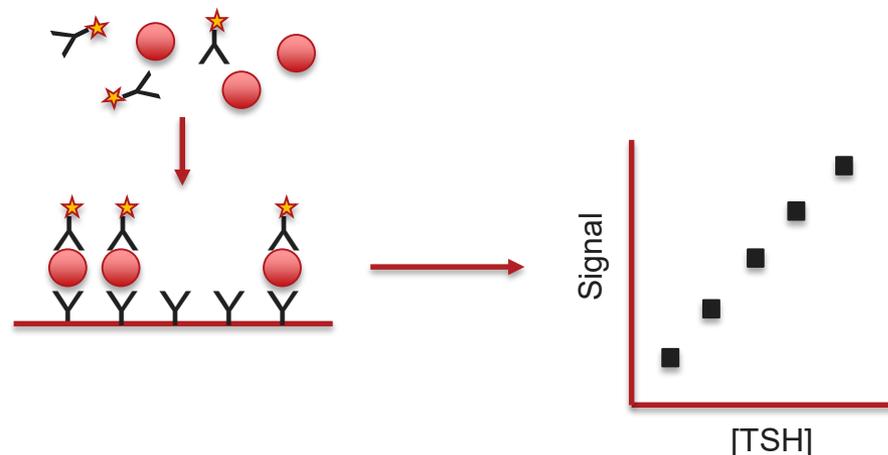
- TSH measured as initial screening test
- Thyroid disease unlikely if TSH within reference interval
- FT4 measured to confirm thyroid disease if TSH outside reference interval
  - FT4 preferred over total T4



Klee GG and Hay ID, J Clin Endocrinol Metab 1987;64:461

# TSH Measurement

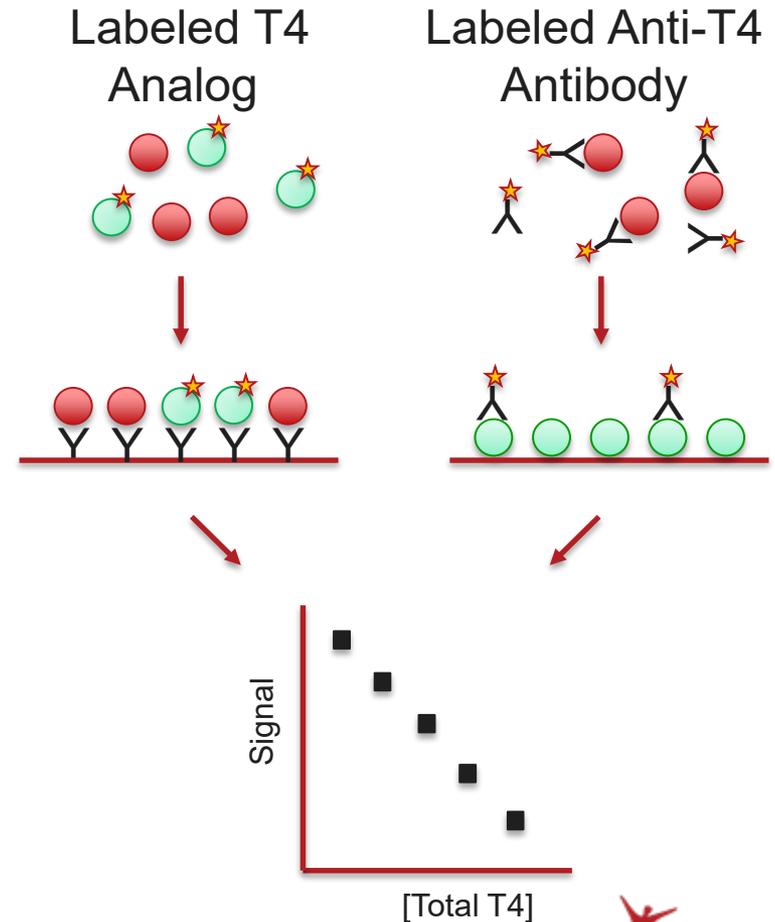
- Two site “sandwich” immunoassay
- Signal directly proportional to concentration
- Analytical sensitivity: lowest concentration at which 20% CV can be achieved
  - 1<sup>st</sup> generation: 1.0 mIU/L
  - 2<sup>nd</sup> generation: 0.1 mIU/L
  - 3<sup>rd</sup> generation: 0.01 mIU/L





# Total T4 Measurement

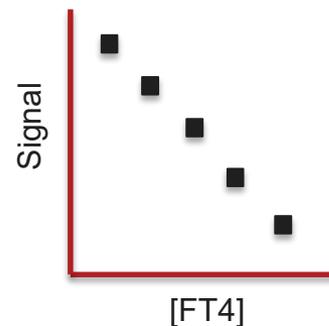
- T4 displaced from binding protein
  - 8-anilino-1-naphthalene-sulfonic acid (ANS)
- Total T4 measured by competitive immunoassay
  - Signal inversely proportional to T4 concentration





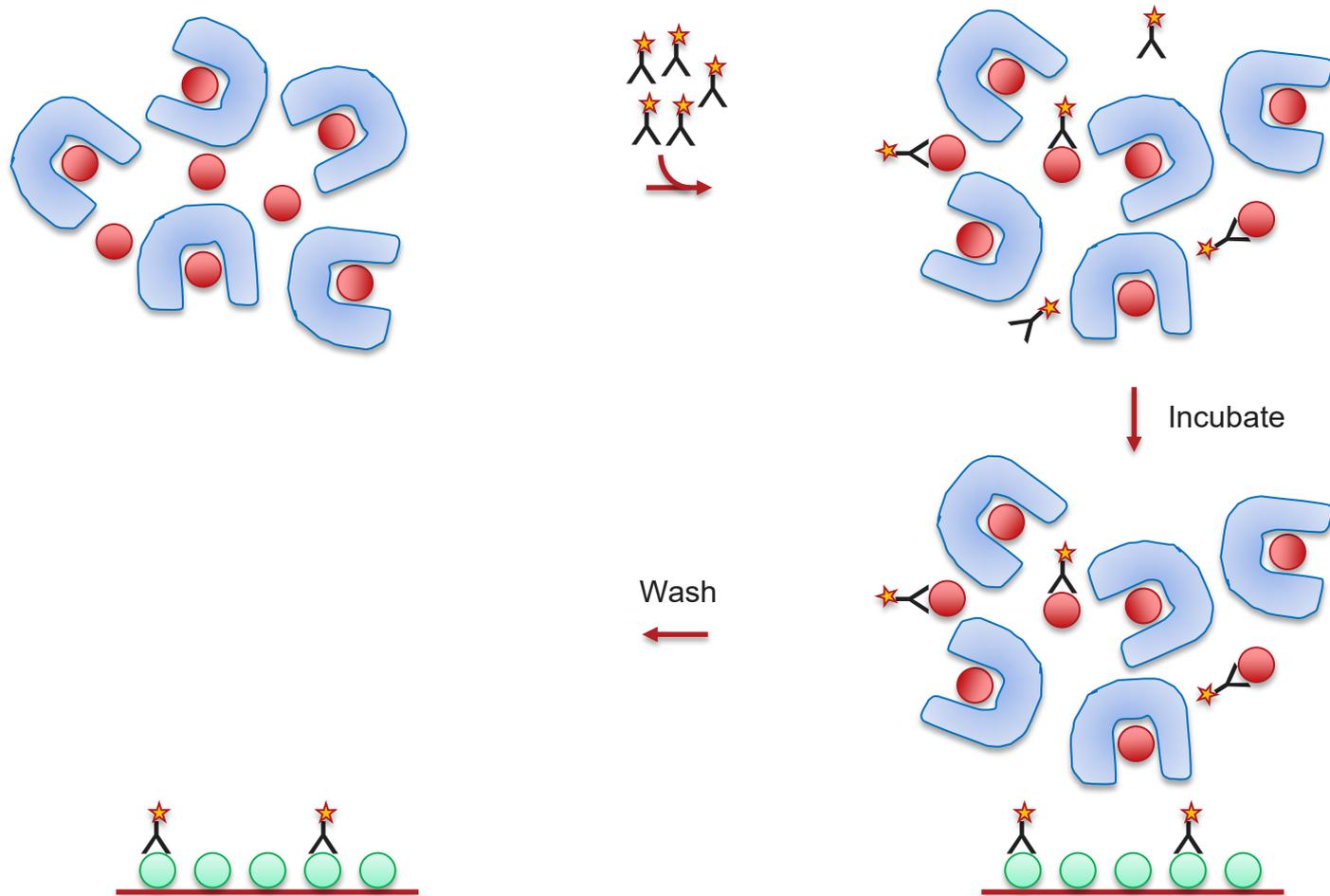
# Hospital Laboratory FT4 Measurement

- No separation of free and protein-bound T4
  - Exogenous T4 analog or antibody disrupts free/protein-bound T4 equilibrium
  - Estimate of FT4 concentration
- Correlate well with reference methods if patient sample and assay calibrators have similar T4 protein-binding capacity
  - Atypical T4 binding protein concentration or affinity = inaccurate results
- One-step competitive immunoassay
  - Signal inversely proportional to FT4 concentration



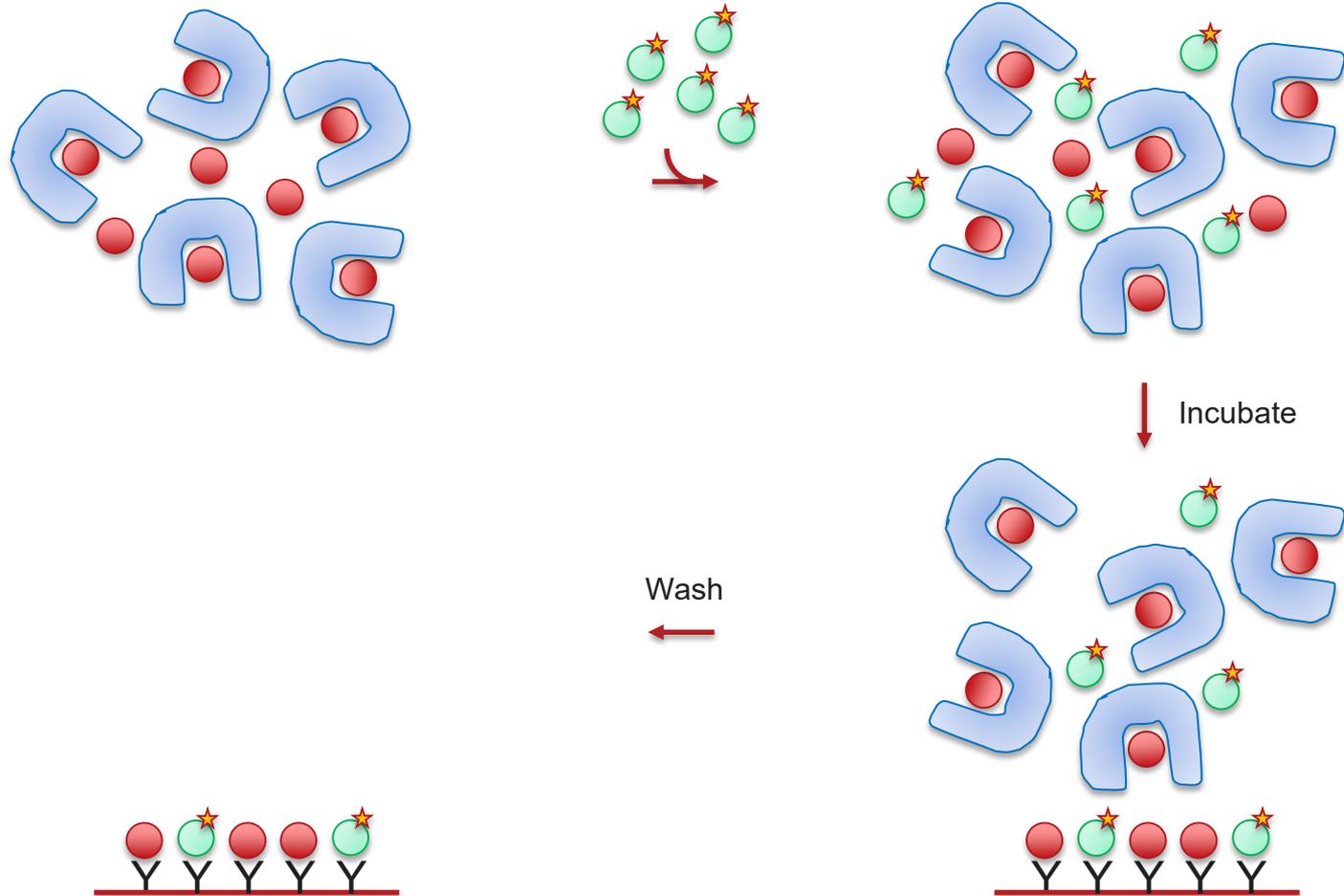


# FT4 Method 1: Labeled Anti-T4 Antibody





# FT4 Method 2: Labeled T4 Analog

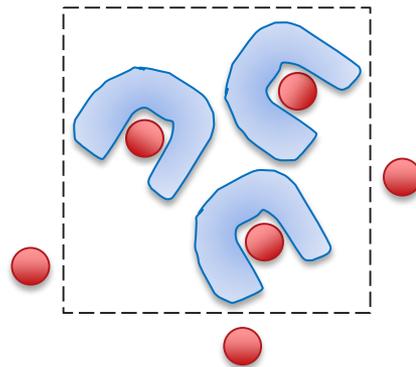




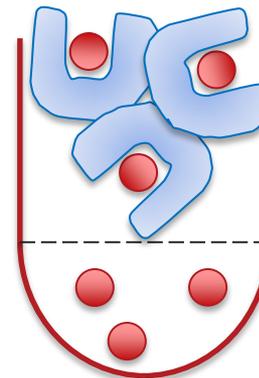
# Reference Laboratory FT4 Measurement

- Physically separate free from protein-bound T4
- Measure free T4 following separation
  - Mass spec
  - Immunoassay
- Most accurate but time/labor-intensive
  - Performed if altered T4 binding protein affinity/concentration suspected

Equilibrium Dialysis



Ultrafiltration

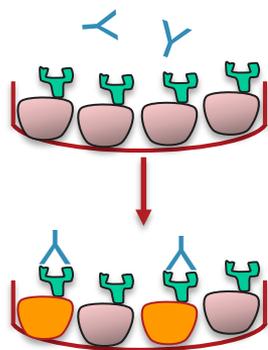


# Anti-TSHR Ab Measurement

- Autoantibody stimulation of the TSH receptor
- Confirm autoimmune etiology in Graves' Disease patients

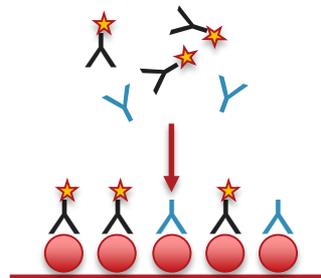
## Bioassay

- Manual, time-intensive
- Specific for activating antibodies
- Signal directly proportional to Ab concentration



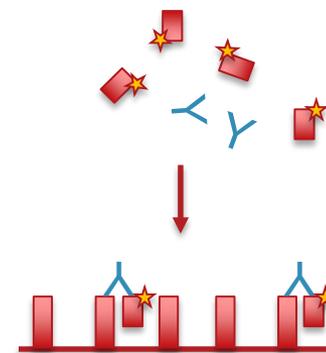
## Competitive Immunoassay

- Automated
- Not specific for activating antibodies
- Signal inversely proportional to Ab concentration



## Bridge Immunoassay

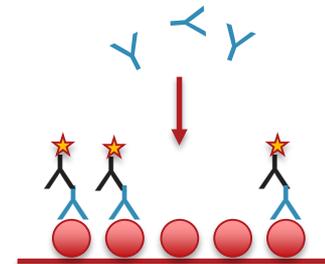
- Automated
- Specific for activating antibodies
- Signal directly proportional to Ab concentration





# Anti-TPO/Anti-Tg Ab Measurement

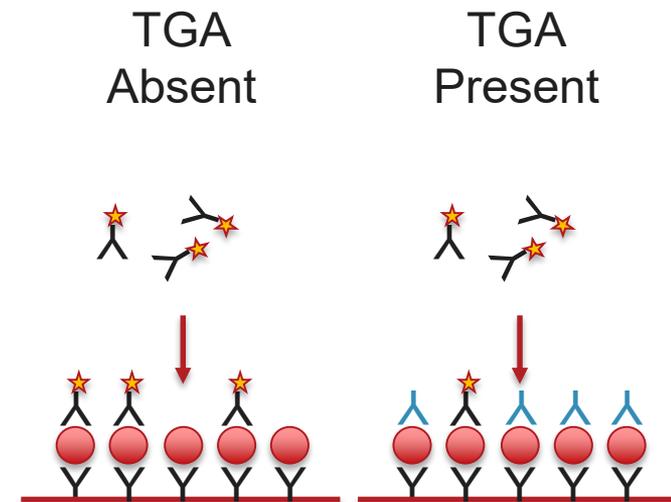
- Hashimoto thyroiditis: cell-mediated autoimmune destruction of thyroid gland
- Anti-thyroperoxidase and/or anti-thyroglobulin antibodies typically present
  - Confirm autoimmune etiology in patients with hypothyroidism
  
- TPO/Tg attached to solid phase
  
- Patient antibody detected using labeled anti-human antibody
  
- Immunometric assay
  - Signal directly proportional to autoantibody concentration





# Thyroglobulin (Tg) Measurement

- Tumor marker in differentiated thyroid cancer (DTC)
- Immunometric “sandwich” assay or RIA
- Interference from thyroglobulin antibody (TGA)
  - 15-35% of DTC patients
  - Immunoassay unreliable if TGA present
    - Falsely low if noncompetitive immunometric
    - Falsely high if competitive RIA
  - Mass spectrometry methods not susceptible to TGA interference





# Summary

- TSH is the front-line screening test for thyroid disease
- If TSH is abnormal, free T4 should be measured to confirm disease
- Autoantibody measurement can help confirm autoimmune etiology





# References

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# Disclosures/Potential Conflicts of Interest

*Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:*

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- **Patents:** None declared



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