JOURNAL OF INTERNATIONAL CRISIS AND RISK COMMUNICATION RESEARCH
ISSN: 2576-0017
2024, VOL 7, NO S9

A Review Laboratory Advancements in Thyroid Stimulating Receptor Autoantibodies Assays: Diagnostic and Clinical Applications

Suhail Ali Alsahabi [1], Hind Hassan Daghriri [2], Rawan Abdulrahman Almutairi [3], Ageel Mohammed Alrbaee [4], Abdulellah Fatini Abdulah Bin Khamees [5],Latifah Ahmed Salem Bin Hubaysh [6], Mohammad Suliman Nasser Al muhaini [7], Khaled Abdullah Alghamdi [8], Hussain Mohammad Issa Zughibi [9], Adnan Ahmed Alssaygh [10], Hind Ahmed Makrami [11], Mohammed Saleh Alshahrani [12], Ghaleb Abu Talib Hussain Alnoman [13], Idris Hamoud Arar Zaeri [14], Bassam Abdulgani D Niaz [15].

[1]Laboratory, Almuzahmiah General Hospital, Ministry of Health, Kingdom of Saudi Arabia. suhailali.sm11@gmail.com
[2]phlebotomist, Almuzahimiah Health Care Center Ministry Of Health. Kingdom of Saudi. HhDaghriri@moh.gov.sa
[3]laboratory. Almuzahimiah general hospital. Ministry of health, Kingdom of Saudi Arabia.rawamu980@gmail.com
[4]Laboratory, Almuzahmiah General Hospital, Ministry of Health, Kingdom of Saudi. ageel999@hotmail.com
[5]Medical Laboratory, Regional blood bank-Riyadh, Ministry of Health,Kigdom of Saudi Arabia. aboriyad2222@yahoo.com
[6]Medical Laboratory, Regional Blood Bank-Riyadh, Ministry of Health, kingdom of Saudi Arabia. lbinhubaish@gmail.com
[7]Medical Laboratory, Regional laboratory-Riyadh, Ministry of Health, Kingdom of Saudi Arabia. abusliman990@gmail.com
[8]Medical Laboratory, Regional laboratory-Riyadh, Ministry of Health, Kingdom of Saudi Arabia. kghamdi356@gmail.com
[9]Medical Laboratory, Regional laboratory-Riyadh, Ministry of Health, Kingdom of Saudi Arabia. hzghibi1390@gmail.com
[10]laboratory. Almuzahimiah General Hospital, Ministry of Health, Kingdom of Saudi. Arabia.abumohanad1@hotmail.com
[11]Laboratory Specialist, Riyadh Regional Laboratory, Ministry of Health, Kingdom of Saudi Arabia. hind998@hotmail.com
[12]Laboratory Specialist, Riyagh, Ministry of Health, Kingdom of Saudi Arabia. msm484711@gmail.com
[13]Operations Technician, Alhema primary healthcare, jazan, Ministry of Health, Kingdom of Saudi Arabia. Galeb110@hotmail.com
[14]Laboratory Technician, Al Iman General Hospital, Ministry of Health, kingdom of Saudi Arabia. 11edres111@gmail.com
[15]Medical Laboratory Specialist, Ministry of Health Riyadh Branch, Ministry of Health, kingdom of Saudi Arabia. bniaz@moh.gov.sa

Suhail Ali Alsahabi, Hind Hassan Daghriri, Rawan Abdulrahman Almutairi, Ageel Mohammed Alrbaee, Abdulellah Fatini Abdulah Bin Khamees, Latifah Ahmed Salem Bin Hubaysh, Mohammad Suliman Nasser Al Muhaini, Khaled Abdullah Alghamdi, Hussain Mohammad Issa Zughibi, Adnan Ahmed Alssaygh, Hind Ahmed Makrami, Mohammed Saleh Alshahrani, Ghaleb Abu Talib Hussain Alnoman, Idris Hamoud Arar Zaeri, Bassam Abdulgani D Niaz.

ABSTRACT

Thyroid-stimulating hormone receptor autoantibodies (TRAbs) play a crucial role in the pathophysiology of autoimmune thyroid diseases, particularly Graves' disease (GD). TRAbs can be classified into stimulating (S-TRAb), blocking (B-TRAb), and apoptotic (A-TRAb) autoantibodies based on their biological functions. Currently, two major categories of TRAb assays are utilized in clinical practice: immunoassays (TBII and IMMULITE 2000 TSI) and bioassays (TSAb). While TBII assays are widely used, they cannot differentiate antibody functionalities. The IMMULITE 2000 TSI assay offers improved sensitivity but lacks specificity. TSAb bioassays provide unparalleled sensitivity and functionality assessment, making them valuable for precise S-TRAb detection. However, variability in assay thresholds, differences in kit sources, and challenges in standardization limit their clinical applicability. Incorporating S-TRAb into standardized diagnostic frameworks and addressing assay inconsistencies are crucial for improving diagnostic precision. Beyond GD, S-TRAb assays exhibit potential in diagnosing related conditions such as Graves' ophthalmopathy, fetal and neonatal hyperthyroidism, dysthyroid optic neuropathy, and acute hyperthyroid myopathy. Further research is needed to explore the diagnostic utility, treatment guidance, and management implications of S-TRAb in these conditions. Standardization and consistency in assay methodologies are essential for establishing TSAb bioassays as reliable diagnostic tools for GD and other thyroid-related disorders.

Keywords: Thyroid stimulating receptor autoantibodies, TRAb, laboratory diagnosis

Introduction

In the late 20th century, various studies established that Thyroid-stimulating hormone receptor autoantibodies (TRAbs) interact with a distinct binding site on the Thyroid-stimulating hormone receptor (TSHR) molecule (W. B. Kim et al., 1996) and demonstrated its heterogeneity through analyses involving TSHR-luteinizing hormone/chorionic gonadotropin receptor chimeras. It was shown that homogeneous epitopes are located at the extracellular N-terminus, while heterogeneous epitopes extend beyond this region. TRAbs can be classified into three types based on their biological functions: stimulating (S-TRAb), blocking (B-TRAb), and apoptotic (A-TRAb) autoantibodies, each recognizing different epitopes (Kotwal & Stan, 2018). Among these, S-TRAb plays a pivotal role in thyroid autoimmune diseases and is particularly associated with the diagnosis and management of Graves' disease (GD). Functionally, both S-TRAb and TSH activate the cyclic adenosine monophosphate (cAMP) signalling pathway, leading to thyroid stimulation independent of the normal TSH feedback mechanism (Davies et al., 2020). In contrast, B-TRAb has an opposing biological action and can potentially lead to hypothyroidism. A-TRAb, also referred to as a neutral antibody, neither stimulates nor inhibits TSHR functional activity but plays a key role in signalling processes and can induce apoptosis in thyroid cells.

Currently, two major categories of TRAb assays are utilized in clinical practice: immunoassays and bioassays. Immunoassays are further divided into two subtypes: the TSH-R binding inhibitory immunoglobulin (TBII) assay, which blocks the binding of TSH to its receptor, and the Immulite 2000 thyroid-stimulating immunoglobulins (TSI) assay, developed in 2015 by Frank et al. using a novel double antibody sandwich method for detecting S-TRAb in serum (Allelein et al., 2019).

Bioassays, on the other hand, are cell-based assays designed to specifically detect S-TRAb and differentiate among the various types of TSHR antibodies (stimulating, blocking), often referred to as TSHR-stimulating antibodies (TSAb) or TSI. It is worth noting that different assays use varying terminologies for TSHR antibodies (TSHR-Abs).

Each of these methods has limitations. TBII assays do not distinguish between stimulating, blocking, and apoptotic antibodies. The TSI assay, though innovative, has limited specificity in certain contexts and requires further evaluation due to its relatively recent development. The TSAb bioassay, while precise, is time-intensive and necessitates specialized laboratory resources, which limits its broader clinical use. Despite previous studies providing an overview of the clinical utility of immunoassays in detecting TRAbs for thyroid disorders such as GD, there remains a lack of systematic comparisons among the three assay types, particularly with respect to the TSAb bioassay. This review aims to systematically explore the principles and advancements of TBII, TSI, and TSAb bioassays, assessing their potential, benefits, and limitations in accurately diagnosing GD and differentiating it from other disorders, thereby aiding precision in clinical management.

2. Assays for TRAb Detection

2.1. TSH-R Binding Inhibitory Immunoglobulin (TBII) Assay

The TBII assay is based on the principle that TSHR-Ab from patient samples competes with TSH for binding to the TSH receptor. Patient-derived TRAb inhibits the interaction between TSH and TSHR preparations. The earliest iteration of the TRAb assay utilized particulate thyroid tissue derived from Graves' disease patients and employed 125I-labeled bovine TSH. However, the diagnostic sensitivity of this method was limited due to non-specific interference from serum immunoglobulins in patient samples.

2.1.1. First- and Second-Generation TBII

The first-generation TBII, developed in the 1990s, used a liquid-phase method for TRAb detection. It incorporated recombinant human TSHR, detergent, and polyethylene glycol (PEG) to improve diagnostic specificity by employing detergent-solubilized TSHR preparations and 125I-labeled bovine TSH (Barbesino & Tomer, 2013). The assay was calibrated to the Medical Research Council (MRC B65/122; LATS) standard. However, PEG precipitation of free 125I-labeled TSH reduced sensitivity and specificity.

In the late 1990s, monoclonal antibodies against TSHR enabled immobilization of native porcine TSHR and recombinant human TSHR on solid surfaces such as plastic tubes or enzyme-linked immunoassay (ELISA) plates while preserving receptor functionality. This led to the development of second-generation TBII assays, which used solid-phase TRAb reagents. Calibrated to the International Reference Preparation (IRP) from the National Institute for Biological Standards and Control (NIBSC 90/672), these assays adopted the "IU/L" unit instead of "U/L" (Allelein et al., 2019). While these assays simplified operation, they continued to face challenges with sensitivity and specificity.

2.1.2. Third-Generation TBII

Both first- and second-generation TBII assays relied on bovine TSH as a tracer for competition with TRAb. The third-generation TBII introduced a monoclonal human thyrotropin antibody, M22, replacing bovine TSH in both liquid- and solid-phase assays. The assay measured how patient serum TRAb inhibited the interaction between M22 and immobilized porcine TSHR (Jansen et al., 2023). Clinical sensitivity significantly improved in this generation, increasing from 88.6% in the first generation to 97.4%. Furthermore, the automated M22-based immunoassay demonstrated enhanced sensitivity (85.5% vs. 72.6%) and reduced assay time compared to the second-generation method, benefiting both GD patients and non-GD individuals.

Over the past five decades, TBII assays have been widely used to identify TRAbs, consistently demonstrating exceptional analytical and clinical utility across diverse laboratory settings. Currently, widely adopted TRAb assays based on the third-generation TBII include Elecsys® Anti-TSHR (Roche, Germany), ELISA RSR TRAb Fast (RSR Limited, UK), and Kronus (Star Idaho, USA). These assays utilize an immunocompetitive reaction between patient antibodies and recombinant TSHR conjugated to a human monoclonal antibody. Despite their widespread use, TBII assays remain limited to reporting the presence or concentration of TRAb, without providing functional insights into antibody activity or efficacy (T. Liu et al., 2022).

2.2. IMMULITE 2000 Thyroid-Stimulating Immunoglobulin (TSI) Assay

The IMMULITE 2000 TSI assay employs a recombinant thyrotropin receptor chimera within a bridging technology framework. This automated, dual cycle chemiluminescence immunoassay minimizes interference from blocking antibodies, thereby enabling more specific detection of thyrotropin antibodies. Originally designed to directly quantify TSI using a non-competitive immunoassay, this method addresses the limitations of TBII in differentiating stimulating, blocking, and apoptotic autoantibodies. Despite these advancements, its relatively short development period necessitates continued evaluation in clinical practice.

2.3. Bioassays for TSHR-Stimulating Antibodies (TSAb) or Thyroid-Stimulating Immunoglobulin (TSI)

2.3.1. Conventional Bioassays

TSAb is also known as a long-acting thyroid stimulator (LATS) due to its extended action compared to TSH in bioassay procedures (Rodríguez Zapata, 1983). Cell-based bioassays validate antibodies that interact with human TSHR, detecting TSAb specifically while differentiating among TSHR antibody types (stimulating or blocking), thus establishing their role as TSAb bioassays (Jansen et al., 2023).

The first-generation TSAb bioassays utilized the FRTL-5 cell line derived from Fischer rat thyroid cells (Hinds et al., 1981). The second-generation bioassay advanced by employing cell lines transfected with the human TSHR gene, with CHO cells being the most common choice. This generation introduced improved cell selection techniques and incorporated cAMP immunoassays. The third-generation

TSAb bioassays further optimized cell lines through genetic modification, enabling detection within a single day. CHO-TSHR cells were genetically engineered to include cAMP-dependent reporter genes and multiple cAMP response elements within their promoter region. The fourth-generation bioassay, utilizing the CHO-K1 cell line and Mc4 receptor chimera, culminated in the development of Thyretain™ TSI, the first FDA-approved in vitro assay for diagnosing TSAb (Lytton et al., 2010).

Despite these advancements, conventional bioassays face several limitations, such as the requirement for overnight cell culture under sterile conditions, which complicates routine implementation in clinical laboratories (Furmaniak et al., 2020). Additionally, reporter gene testing extends the duration compared to direct cAMP measurement, as it involves intracellular signal transduction, nuclear transport, transcription, and translation. Conventional bioassays typically require approximately 21–22 hours to complete, limiting their applicability in laboratory settings.

2.3.2. Bioassays Employing Novel Biosensors

Recently, novel biosensors have enabled real-time monitoring of cAMP dynamics in live cells, accelerating advancements in the clinical application of TSAb for diagnosing and managing thyroid diseases. To address the limitations of conventional methods, Naohiro Araki et al. developed an advanced aequorin TSAb assay that combines human TSHR expression with cAMP-gated calcium channels and aequorin. This innovative bioassay can be completed in just 4 hours, eliminating the need for sterile cell culture (Araki et al., 2015).

Subsequently, the GloSensor cAMP biosensor (Promega, Madison, WI) was introduced, which measures TSAb using luciferase activity as an indicator of intracellular cAMP levels. To enhance the workflow of the Thyretain™ TSI bioassay, Miao LY et al. developed the Turbo™ TSI bioassay. This method employs a CHO-K1 stable cell line expressing both the Mc4 chimeric TSHR used in the Thyretain™ bioassay and the GloSensor™ cAMP biosensor, enabling homogeneous, real-time TSAb measurement in serum samples at room temperature within 1 hour (Miao et al., 2022).

Although these bioassays continue to evolve, standardization and consistency remain challenges due to significant variability in cut-off values across different techniques. This highlights the need for further evaluation and improvement.

3. Clinical Relevance and Applications of TRAb

3.1. TRAb Measurement in the Diagnosis of GD

3.1.1. Comparison of TBII, TSI, and TSAb Bioassay in GD Diagnosis

Approximately 80% of hyperthyroidism cases can be diagnosed by monitoring thyroid hormone levels, high-sensitivity TSH testing, thyroid iodine uptake, and clinical manifestations. However, diagnostic methods such as iodine scanning and uptake may not be feasible during pregnancy or lactation. In these scenarios, TRAb measurement proves valuable for detecting autoimmune thyrotoxicosis prior to clinical or biochemical manifestations. This diagnostic approach is recommended by the 2018 European Thyroid Association's Guidelines for the Treatment of Graves' Hyperthyroidism.

S-TRAb is predominantly detected using the Siemens IMMULITE 2000 TSI immunoassay analyzer, whereas TRAb measurement is performed using the Roche Cobas 8000 analyzer with TBII competition methods (J. J. Kim et al., 2019). The TSI assay offers significant advantages in distinguishing GD from other thyroid disorders, particularly in differentiating GD from Hashimoto's thyroiditis (HT). Additionally, TSI demonstrates greater sensitivity than TBII (98.1% vs. 94.0%) in the initial diagnosis of GD (Tong et al., 2021), enabling early disease detection, remission monitoring, and recurrence assessment. However, TSI's lack of specificity poses challenges. Tozzoli et al. reported a 20% false-positive rate for the TSI assay in patients with HT. Similarly, Tong et al. found that the TSI assay had a higher false-positive rate in autoimmune thyroiditis (21.7% vs. 10.8%) compared to the TRAb assay (Tong et al., 2021). Although the IMMULITE 2000 TSI assay has demonstrated high specificity through strong correlation with TSAb bioassays compared to TBII (87.21% vs. 12.9%), recent findings indicate its inability to distinguish between antibody types. This underscores the importance of TSAb bioassays for precise S-TRAb detection.

3.1.2. Advantages of TSAb Bioassay Over TSI and TBII

Research by Stan et al. revealed that TSAb bioassays exhibit higher sensitivity (97%) than TBII (86%) in identifying early-onset GD, facilitating timely disease management (Stan et al., 2022). Additionally, TSAb bioassays demonstrate superior sensitivity in quantifying low-concentration serum anti-TSHR-Ab compared to TBII. Consequently, these cell-based bioassays have been increasingly investigated for their clinical value in TSAb detection. Allelein et al. reported positive TSAb bioassay results in 93% of GD patients, regardless of disease duration, with the bioassay proving more reliable and sensitive than immunoassays.

In 10% of cases where immunoassays failed to detect S-TRAb, TSAb bioassays provided accurate results, highlighting their enhanced sensitivity and clinical significance. For instance, Kwon et al. demonstrated that TSAb bioassays accurately predicted GD relapse during drug withdrawal, unlike TBII (Kwon et al., 2016). Liu et al. established S-TRAb as a prognostic marker for methimazole (MZ) response. Kahaly et al. observed that positive TSAb bioassay results predicted relapse in MZ nonresponders or post-therapy relapses (Kahaly et al., 2020). Recently, Baek et al. proposed a new approach using a cut-off value of 66.5% for TSAb bioassays to predict GD recurrence. Results below this threshold were associated with a 46.63% reduction in GD recurrence two years post-therapy discontinuation (Baek et al., 2022). Employing a TSAb bioassay with this threshold is anticipated to significantly mitigate GD recurrence risk.

3.1.3. Challenges in Bioassay Implementation

A notable challenge in the clinical application of TSAb bioassays lies in the significant variability observed in determining the TSAb positive threshold. The variability can be attributed to several factors, including ethnicity, sample size, disease state, criteria for defining Graves' disease (GD), treatment approaches, assay methodologies, and the origin of assay kits (T. Liu et al., 2022). These inconsistencies complicate the establishment of TSAb bioassays as a standardized diagnostic tool for

GD and other thyroid conditions. Consequently, it is essential to incorporate autoimmune antibodies, such as S-TRAb, into diagnostic protocols and establish unified standards for their measurement and interpretation. Standardization is critical to ensuring the accuracy and reliability of TSAb bioassays in diagnosing GD and other thyroid-related disorders, thereby enhancing their clinical application.

3.2. Other Clinical Applications of S-TRAb

3.2.1. Graves' Ophthalmopathy (GO)

The pioneering work of Feliciello et al. established that TSHR expression extends beyond thyroid follicular cells (Feliciello et al., 1993). This finding was further corroborated by immunohistochemical studies demonstrating increased TSHR expression in orbital tissues associated with GO. The detection of S-TRAb has since emerged as a valuable tool in diagnosing GO, including atypical presentations such as unilateral GO or cases without hyperthyroidism. Research has highlighted S-TRAb as a functional biomarker closely associated with GO activity, with serum S-TRAb levels aiding in identifying patients with active disease (Hoang et al., 2022). Furthermore, S-TRAb titers correlate with the degree of inflammation in GO, providing prognostic value through continuous antibody monitoring. In addition to serving as an independent risk factor for GO, S-TRAb levels are predictive of disease severity and progression. Studies also suggest that TSAb bioassays exhibit greater sensitivity (100%) compared to traditional immunoassays (87%) in distinguishing between mild and moderate-to-severe GO cases. This distinction has profound clinical implications, allowing for tailored management strategies to mitigate severe inflammation while avoiding unnecessary treatments in mild cases. Monitoring S-TRAb titers facilitates the identification and tracking of GO progression, ensuring optimal patient outcomes.

3.2.2. Fetal/Neonatal Hyperthyroidism and Graves' Disease in Pregnancy

Maternal transmission of S-TRAb through the placenta has long been recognized as a potential cause of neonatal hyperthyroidism, a relationship confirmed by various TRAb assays. As a member of the immunoglobulin G class, S-TRAb crosses the placenta and can influence fetal thyroid function. Elevated maternal S-TRAb levels during the second trimester are a precise predictor of neonatal hyperthyroidism. Research has investigated maternal thyroid autoimmune antibodies as alternative markers for assessing fetal thyroid function. Notably, neonatal thyrotoxicosis may occur even in mothers with normal thyroid function, especially those with a history of radioactive iodine treatment or thyroid surgery during pregnancy (Luz et al., 2020). Approximately 1–5% of neonates born to mothers with hyperthyroidism history develop fetal or neonatal thyrotoxicosis. A TRAb titer exceeding 6 IU/L has been linked to an increased risk of such complications. If maternal TSAb levels remain elevated beyond 6 IU/L in the second trimester, heightened vigilance is warranted for the development of fetal goiter and hyperthyroidism. Unlike gestational Graves' disease (GD), gestational transient thyrotoxicosis (GTT) is characterized by elevated free T4, decreased TSH, and elevated TRAb levels, with S-TRAb remaining within the normal range. Moreover, GTT-related abnormalities typically resolve postpartum without pharmacological intervention. Thus, distinguishing between gestational GD and GTT through S-TRAb measurement facilitates a more rational therapeutic approach.

3.2.3. Dysthyroid Optic Neuropathy (DON)

Dysthyroid optic neuropathy (DON), a severe manifestation of thyroid-associated ophthalmopathy, involves optic nerve dysfunction and can lead to significant visual impairment. Despite the development of diagnostic algorithms, standardized methods for diagnosing DON remain lacking (Sio et al., 2024). Early diagnosis and intervention are critical to preventing irreversible vision loss and minimizing treatment-related adverse effects. A cross-sectional study by K. A. Ponto et al. was the first to demonstrate that serum S-TRAb levels, measured using an FDA-cleared cell-based bioassay, could serve as a biomarker for diagnosing early-onset DON (Ponto et al., 2015). This finding is pivotal in identifying patients who, despite lacking acute inflammatory symptoms, require immediate treatment. Further research is needed to explore the diagnostic utility, treatment guidance, and management implications of S-TRAb in DON.

3.2.4. Acute Hyperthyroid Myopathy (ATM)

Acute hyperthyroid myopathy (ATM), also referred to as acute hyperthyroid encephalopathy or bulbar paralysis, represents a rare but severe neuromuscular complication associated with GD (Fu et al., 2023). Patients with ATM often exhibit elevated cerebrospinal fluid S-TRAb levels, which are associated with rapid disease progression and can be life-threatening in severe cases (Y. Liu et al., 2022). Liu et al. demonstrated that S-TRAb induces abnormal activation and polarization of BV-2 microglial cells, triggering an inflammatory cascade potentially relevant to ATM pathogenesis. However, further studies are required to elucidate the precise effects of S-TRAb on the central nervous system.

Conclusion

Thyroid-stimulating hormone receptor autoantibodies (TRAbs) play a pivotal role in the pathophysiology of autoimmune thyroid diseases, particularly Graves' disease (GD). Over the decades, laboratory assays, including TBII, IMMULITE 2000 TSI, and TSAb bioassays, have significantly advanced, enabling enhanced detection and characterization of TRAb types. While TBII remains widely utilized, it lacks the ability to differentiate antibody functionalities. IMMULITE 2000 TSI offers improved sensitivity but struggles with specificity, underscoring the importance of TSAb bioassays, which provide unparalleled sensitivity and functionality assessment.

Despite these advances, the variability in assay thresholds, differences in kit sources, and challenges in standardization limit their clinical applicability. Furthermore, TSAb bioassays demand specialized laboratory setups, complicating routine usage. Incorporating S-TRAb into standardized diagnostic frameworks and addressing assay inconsistencies are crucial to improving diagnostic precision. Beyond GD, S-TRAb assays exhibit potential in diagnosing related conditions such as Graves' ophthalmopathy, fetal and neonatal hyperthyroidism, dysthyroid optic neuropathy, and acute hyperthyroid myopathy, reinforcing their clinical relevance.

References

Allelein, S., Diana, T., Ehlers, M., Kanitz, M., Hermsen, D., Schott, M., & Kahaly, G. J. (2019).

Comparison of a Bridge Immunoassay with Two Bioassays for Thyrotropin Receptor Antibody Detection and Differentiation. *Hormone and Metabolic Research*, *51*(6), 341–346. Scopus. https://doi.org/10.1055/a-0914-0535

Araki, N., Iida, M., Amino, N., Morita, S., Ide, A., Nishihara, E., Ito, M., Saito, J., Nishikawa, T., Katsuragi, K., & Miyauchi, A. (2015). *Rapid Bioassay for Detection of Thyroid-Stimulating Antibodies Using Cyclic Adenosine Monophosphate-Gated Calcium Channel and Aequorin*. https://doi.org/10.1159/000371740

Baek, H.-S., Lee, J., Jeong, C.-H., Lee, J., Ha, J., Jo, K., Kim, M.-H., Cho, J. H., Kang, M. I., & Lim, D.-J. (2022). The Prediction Model Using Thyroid-stimulating Immunoglobulin Bioassay For Relapse of Graves' Disease. *Journal of the Endocrine Society*, *6*(5), bvac023. https://doi.org/10.1210/jendso/bvac023

Barbesino, G., & Tomer, Y. (2013). Clinical utility of TSH receptor antibodies. *Journal of Clinical Endocrinology and Metabolism*, *98*(6), 2247–2255. Scopus. https://doi.org/10.1210/jc.2012-4309

Davies, T. F., Andersen, S., Latif, R., Nagayama, Y., Barbesino, G., Brito, M., Eckstein, A. K., Stagnaro-Green, A., & Kahaly, G. J. (2020). Graves' disease. *Nature Reviews Disease Primers*, *6*(1). Scopus. https://doi.org/10.1038/s41572-020-0184-y

Feliciello, A., Ciullo, I., Fenzi, G. F., Bonavolontà, G., Porcellini, A., & Avvedimento, E. V. (1993). Expression of thyrotropin-receptor mRNA in healthy and Graves' disease retro-orbital tissue. *The Lancet*, *342*(8867), 337–338. Scopus. https://doi.org/10.1016/0140-6736(93)91475-2

Fu, S.-E., Liang, X.-H., Tang, Z.-P., Kuang, Y.-Q., Qiu, C.-C., Liu, X.-F., Yang, H.-Y., Huang, Z.-X., Qin, Y.-F., Ma, Y., & Luo, Z.-J. (2023). Acute Thyrotoxic Myopathy Combined with Neck Pain: A Case Report. *Neuro Endocrinology Letters*, *44*(7), 427–431. Scopus.

Furmaniak, J., Sanders, J., Sanders, P., Miller-Gallacher, J., Ryder, M. M., & Rees Smith, B. (2020). Practical applications of studies on the TSH receptor and TSH receptor autoantibodies. *Endocrine*, *68*(2), 261–264. Scopus. https://doi.org/10.1007/s12020-019-02180-9

Hinds, W. E., Takai, N., Rapoport, B., Filetti, S., & Clark, O. H. (1981). Thyroid-stimulating immunoglobulin bioassay using cultured human thyroid cells. *Journal of Clinical Endocrinology and Metabolism*, *52*(6), 1204–1210. Scopus. https://doi.org/10.1210/jcem-52-6-1204

Hoang, T. D., Stocker, D. J., Chou, E. L., & Burch, H. B. (2022). 2022 Update on Clinical Management of Graves Disease and Thyroid Eye Disease. *Endocrinology and Metabolism Clinics of North America*, *51*(2), 287–304. Scopus. https://doi.org/10.1016/j.ecl.2021.12.004

Jansen, H. I., Gohy, H. G., Boelen, A., Bisschop, P. H., Hillebrand, J. J., & Heijboer, A. C. (2023). Stability of TSH receptor antibody concentrations and comparability of its immunoassays. *Clinica Chimica Acta*, *548*. Scopus. https://doi.org/10.1016/j.cca.2023.117505

Kahaly, G. J., Diana, T., Kanitz, M., Frommer, L., & Olivo, P. D. (2020). Prospective Trial of Functional Thyrotropin Receptor Antibodies in Graves Disease. *The Journal of Clinical Endocrinology & Metabolism*, *105*(4), e1006–e1014. https://doi.org/10.1210/clinem/dgz292

Kim, J. J., Jeong, S.-H., Kim, B., Kim, D., & Jeong, S. H. (2019). Analytical and clinical performance of newly developed immunoassay for detecting thyroid-stimulating immunoglobulin, the Immulite TSI assay. *Scandinavian Journal of Clinical and Laboratory Investigation*, *79*(6), 443–448. Scopus. https://doi.org/10.1080/00365513.2019.1658216

Kim, W. B., Cho, B. Y., Park, H. Y., Lee, H. K., Kohn, L. D., Tahara, K., & Koh, C.-S. (1996). Epitopes for Thyroid-Stimulating Antibodies in Graves' Sera: A Possible Link of Heterogeneity to Differences in Response to Antithyroid Drug Treatment *. *Journal of Clinical Endocrinology and Metabolism*, *81*(5), 1758–1767. Scopus. https://doi.org/10.1210/jcem.81.5.8626830

Kotwal, A., & Stan, M. (2018). Thyrotropin Receptor Antibodies—An Overview. *Ophthalmic Plastic and Reconstructive Surgery*, *34*(4), S20–S27. Scopus. https://doi.org/10.1097/IOP.0000000000001052

Kwon, H., Kim, W. G., Jang, E. K., Kim, M., Park, S., Jeon, M. J., Kim, T. Y., Ryu, J.-S., Shong, Y. K., & Kim, W. B. (2016). Usefulness of measuring thyroid stimulating antibody at the time of antithyroid drug withdrawal for predicting relapse of graves disease. *Endocrinology and Metabolism*, *31*(2), 300–310. Scopus. https://doi.org/10.3803/EnM.2016.31.2.300

Liu, T., Zhang, X., Long, L., Zhou, L., Chen, J., Li, M., Gao, Y., Zhou, X., Han, X., & Ji, L. (2022). Clinical evaluation of an automated TSI bridge immunoassay in the diagnosis of Graves' disease and its relationship to the degree of hyperthyroidism. *BMC Endocrine Disorders*, *22*(1), 218. https://doi.org/10.1186/s12902-022-01114-3

Liu, Y., Yang, H., Liang, C., Huang, X., Deng, X., & Luo, Z. (2022). Expression of functional thyroid-stimulating hormone receptor in microglia. *Annales d'Endocrinologie*, *83*(1), 40–45. https://doi.org/10.1016/j.ando.2021.11.009

Luz, I. R., Martins, J. R., Jerónimo, M., Caetano, J. S., Cardoso, R., Dinis, I., & Mirante, A. (2020).

Neonates born to mothers with graves' disease: 15 year experience of a pediatric endocrinology department. *Acta Medica Portuguesa*, *33*(7), 483–490. Scopus. https://doi.org/10.20344/amp.12279

Lytton, S. D., Li, Y., Olivo, P. D., Kohn, L. D., & Kahaly, G. J. (2010). Novel chimeric thyroid-stimulating hormone-receptor bioassay for thyroid-stimulating immunoglobulin. *Clinical and Experimental Immunology*, *162*(3), 438–446. Scopus. https://doi.org/10.1111/j.1365-2249.2010.04266.x

Miao, L. Y., Kim, H. J., Whitlatch, K., Jaiswal, D., Navarro, A., Egan, R., & Olivo, P. D. (2022). A rapid homogenous bioassay for detection of thyroid-stimulating antibodies based on a luminescent cyclic AMP biosensor. *Journal of Immunological Methods*, *501*. Scopus. https://doi.org/10.1016/j.jim.2021.113199

Ponto, K. A., Diana, T., Binder, H., Matheis, N., Pitz, S., Pfeiffer, N., & Kahaly, G. J. (2015). Thyroid-stimulating immunoglobulins indicate the onset of dysthyroid optic neuropathy. *Journal of Endocrinological Investigation*, *38*(7), 769–777. Scopus. https://doi.org/10.1007/s40618-015-0254-2

Rodríguez Zapata, M. M. (1983). LATS and other antibodies against the TSH receptor. *Medicina Clinica*, *80*(4), 177–181. Scopus.

Sio, S. W. C., Chan, B. K. T., Aljufairi, F. M. A. A., Sebastian, J. U., Lai, K. K. H., Tham, C. C. Y., Pang, C. P., & Chong, K. K. L. (2024). Diagnostic methods for dysthyroid optic neuropathy: A systematic review and analysis. *Survey of Ophthalmology*, *69*(3), 403–410. https://doi.org/10.1016/j.survophthal.2023.11.009

Stan, M. N., Algeciras-Schimnich, A., Murthy, V., Thapa, P., & Araki, N. (2022). Diagnostic Utility of a New Assay for Thyroid Stimulating Immunoglobulins in Graves' Disease and Thyroid Eye Disease. *Thyroid*, *32*(2), 170–176. Scopus. https://doi.org/10.1089/thy.2021.0299

Tong, M., Ding, J., Huang, B., Chen, J., Wei, X., Li, Z., Shu, J., Hu, Z., Jiang, X., & Sheng, H. (2021). Evaluation of the application of TSH receptor stimulating autoantibodies and the optimization of detection strategy in Graves' disease. *Clinica Chimica Acta*, *521*, 34–39. Scopus. https://doi.org/10.1016/j.cca.2021.06.017