

More confidence in the follow-up of thyroid cancer patients

Thyroglobulin and mini-recovery:
revealing all possible hTg assay interferences

Thyroglobulin as a serum marker in thyroid carcinoma ... facing the challenge

Differentiated thyroid carcinoma (DTC) is the most common endocrine cancer worldwide with an ever-increasing prevalence. The initial treatment consists of total thyroidectomy usually followed by ablation of thyroid remnants with radioiodine. For a reliable and effective monitoring of patients for recurrent or persistent disease, circulating thyroglobulin (hTg) is used as a biochemical marker. As thyroid cells are the only source of hTg, it is highly specific for DTC and recommended for use as biomarker by international clinical practice guidelines.^{1,2,3}

hTg assay interference

The main challenge for any thyroglobulin determination is assay interference. The following different sources of interference have been described which can lead to either false positive or false negative hTg results:^{3,4,5}

- Tg auto-antibodies
- Heterophilic antibodies
- High-dose hook effect

For a confident DTC follow-up, it is essential to identify all suspicious hTg results, regardless of the source of the interference.

Anti-Tg testing

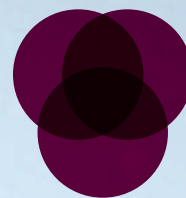
The current established method to rule out assay interference is a separate test for anti-Tg auto-antibodies. Anti-Tg positive samples are discarded and the hTg results are dismissed. hTg results from anti-Tg negative samples are trusted.

However, it is not possible to assess viability of an hTg result for an individual patient based on the anti-Tg result alone.^{2,3,4}

Thermo Scientific
B·R·A·H·M·S KRYPTOR compact PLUS (REF106172)



B·R·A·H·M·S hTg sensitive KRYPTOR mini-recovery providing the solution



hTg mini-recovery concept

In the unique Thermo Scientific™ B·R·A·H·M·S™ hTg sensitive KRYPTOR™ mini-recovery concept, an individual amount of Thyroglobulin is added to the sample, depending on the original sample hTg concentration (fig. 1a). Low amounts of hTg will be added to samples with low hTg concentrations, higher amounts of hTg to samples with higher concentrations.

This added Thyroglobulin will be affected by potential interference factors in the same way as the sample hTg (fig. 1b).

The rate by which the additional hTg can be recovered is equivalent to the level of interference. The optimal reference range for an unaffected mini-recovery is 80-120%.^{6,7}

Therefore the mini-recovery concept shows any possible interference.

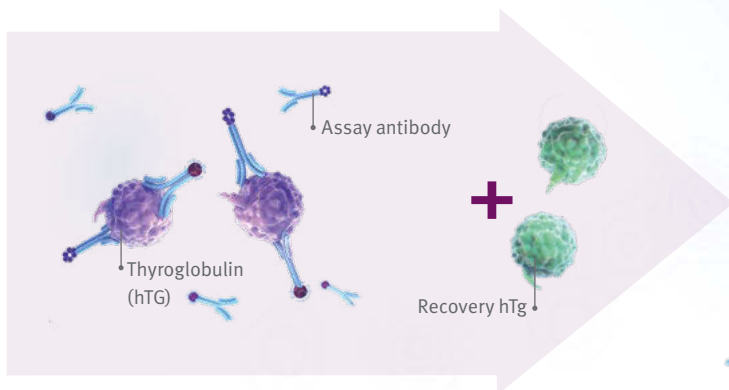


Figure 1: hTg assay

Figure 1a: Mini-recovery measurement

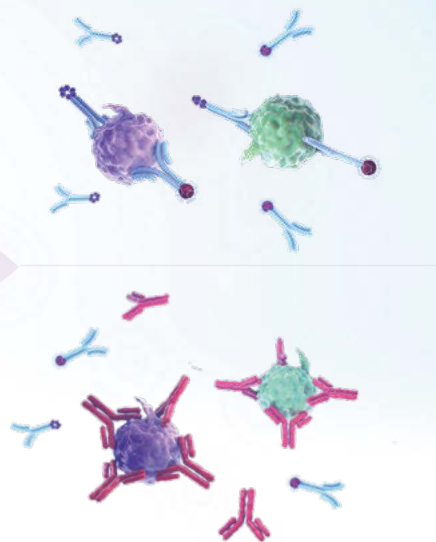
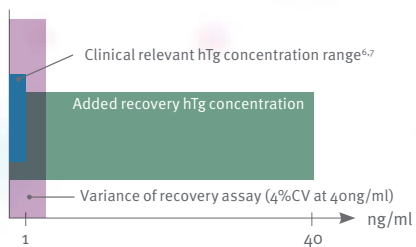


Figure 1b: Recovery with interference



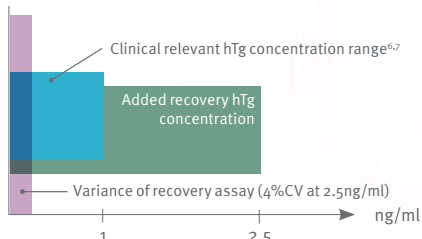
Conventional recovery

The variance of the assay (imprecision) obscures the clinically relevant concentration range.



Mini-recovery on KRYPTOR

With the mini-recovery the assay variance (imprecision) is considerably smaller in relation to the clinically relevant concentration range.



A universal amount of added hTg is not suitable for patients with low hTg concentrations because clinically relevant interference will be concealed by the imprecision of the assay.^{6,7}

The B·R·A·H·M·S KRYPTOR platform is able to run the mini-recovery as a reflex test and decides autonomously the amount of recovery hTg which is suitable for a given patient sample.

Figure 2: Different recovery concepts at low sample hTg concentration levels.

B·R·A·H·M·S hTg sensitive KRYPTOR mini-recovery detecting hTg auto-antibody interference

Interfering hTg auto-antibodies

hTg auto-antibodies are blocking the epitopes for the assay antibodies. This leads to false low or false negative hTg results.⁸

Mini-recovery:

Added recovery-hTg will be bound by the auto-antibodies, therefore the observed hTg concentration of the sample-recovery mixture will be lower than expected.

In this case the hTg result should be discarded due to a disturbed recovery rate.

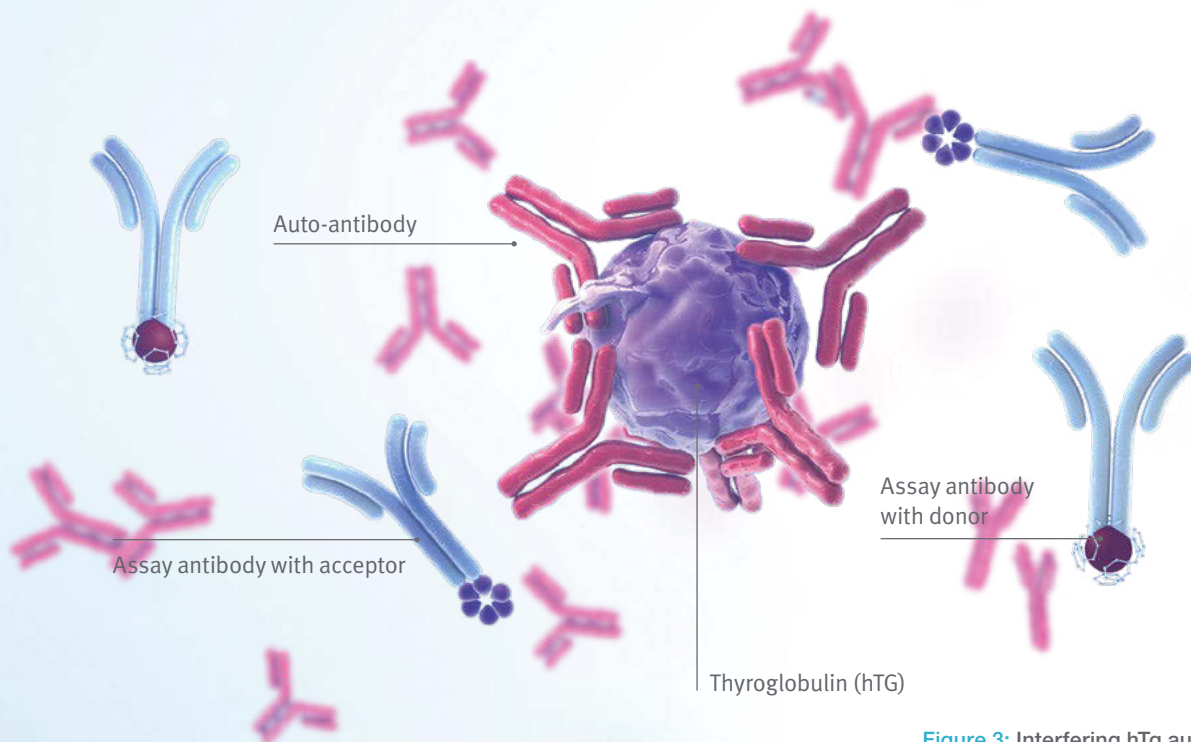
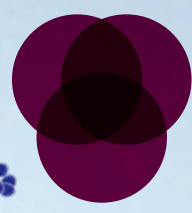


Figure 3: Interfering hTg auto-antibodies

hTg result verified by:	Verification result:	Conclusion:
Anti-Tg assay	positive	hTg result will be discarded ✓
Mini-recovery	disturbed	hTg result will be discarded ✓



Non-interfering hTg auto-antibodies

hTg auto-antibodies bind to different epitopes of the hTg molecule than the assay antibodies. Verification of the hTg result through conventional Tg auto-antibody determination would falsely render the hTg result invalid.⁷

Mini-recovery:

Auto-antibodies will bind to the added recovery hTg without affecting the assay antibodies. Observed hTg concentration in the sample-recovery mixture will be as expected. The hTg result is valid.

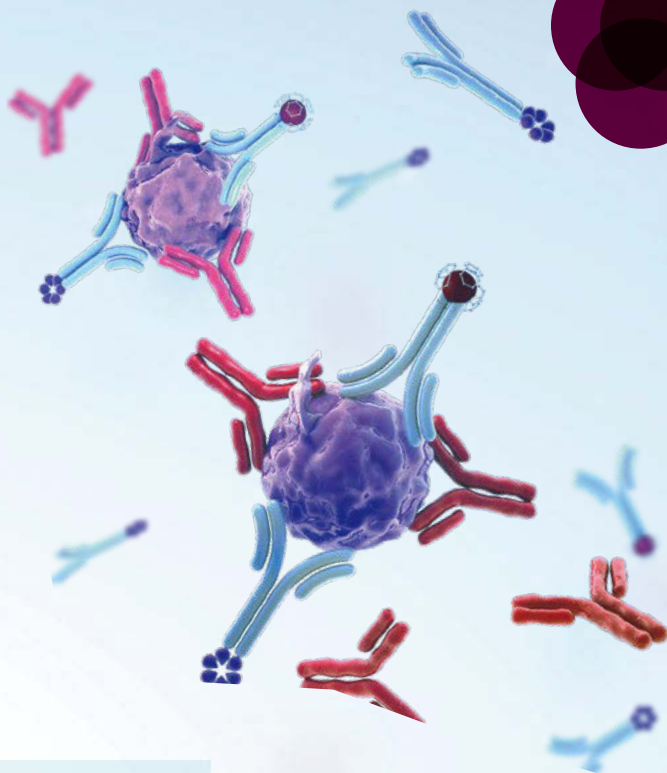


Figure 4: Non-interfering hTg auto-antibodies

hTg result verified by:	Verification result:	Conclusion:
Anti-Tg assay	positive	hTg result will falsely be discarded ❌
Mini-recovery	unaffected	hTg result is considered valid ✅

Undetectable hTg auto-antibodies

Patient hTg auto-antibodies are blocking the hTg epitopes for the assay antibodies, but are undetectable with an anti-Tg assay.

A conventional verification approach by Tg auto-antibody measurement can ultimately lead to a false negative result in this case.⁹

Mini-recovery:

Added recovery-hTg will be bound by the auto-antibodies to a certain degree as well. The observed hTg concentration of the sample-recovery mixture will be lower than expected.

The hTg results needs to be discarded due to disturbed recovery rate.

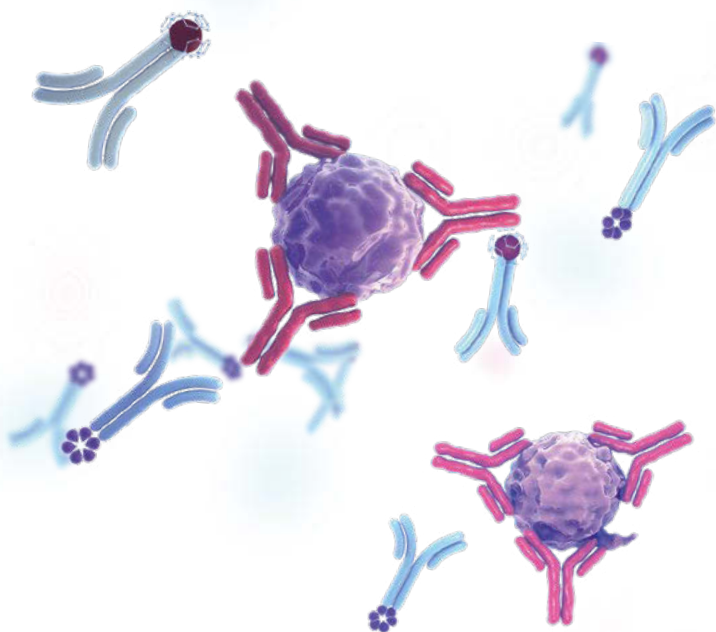


Figure 5: Undetectable hTg auto-antibodies

hTg result verified by:	Verification result:	Conclusion:
Anti-Tg assay	negative	hTg result is falsely considered valid ❌
Mini-recovery	disturbed	hTg result will be discarded ✅

B·R·A·H·M·S hTg sensitive KRYPTOR mini-recovery ... detecting other interferences

Heterophilic antibodies

Interfering heterophilic antibodies are found in 2-5% of the patients. These antibodies may lead to either false positive hTg results or false negative hTg results. ^{4,5,9}

Mini-recovery:

Adding recovery buffer will dilute the sample and also the concentration of the heterophilic antibodies. Non-linear dilution behavior will result in a disturbed recovery rate. ⁴

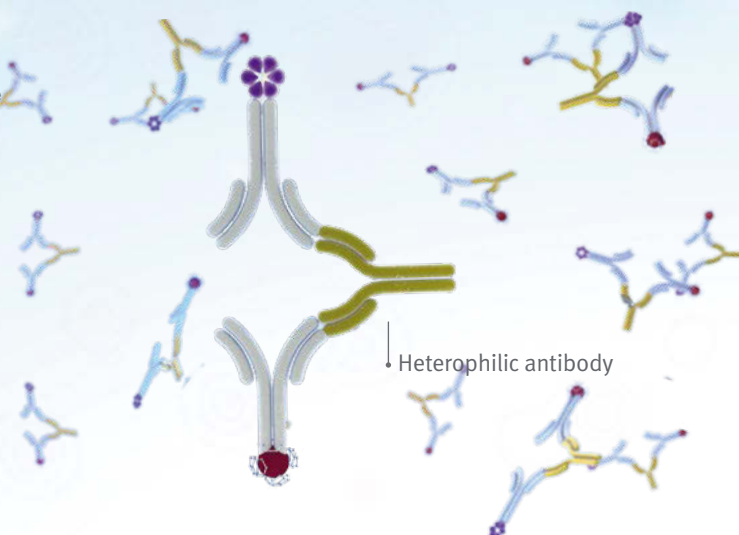
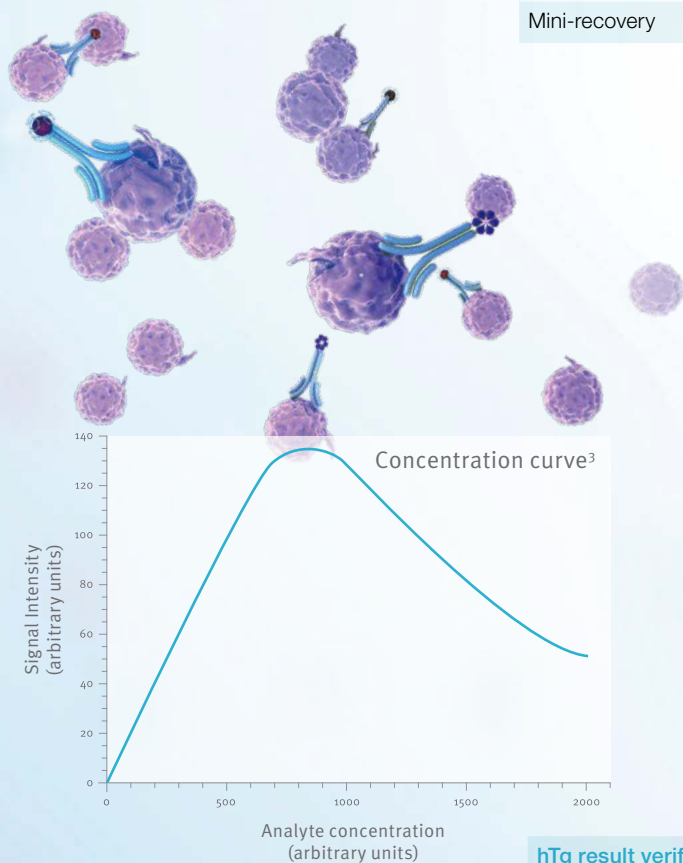


Figure 6: Heterophilic hTg auto-antibodies (false positive variant)

hTg result verified by:	Verification result:	Conclusion:	
Anti-Tg assay	negative	hTg result is falsely considered valid	✗
Mini-recovery	disturbed	hTg result will be discarded	✓



High-dose hook effect

This effect is named after the characteristic shape of the concentration curve at extremely high hTg concentrations. The applied assay antibodies get sequestered by excess antigen. The formation of sandwich-complexes to detect hTg becomes less likely which leads to false low hTg results. ^{3,10}

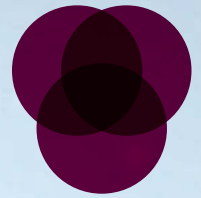
Mini-recovery:

Addition of recovery buffer will dilute the hTg saturated sample so that formation of sandwich complexes increases. The non-linear dilution behavior will result in a disturbed recovery rate.

Figure 7: High-dose hook effect

hTg result verified by:	Verification result:	Conclusion:	
Anti-Tg assay	negative	hTg result is falsely considered valid	✗
Mini-recovery	disturbed	hTg result will be discarded	✓

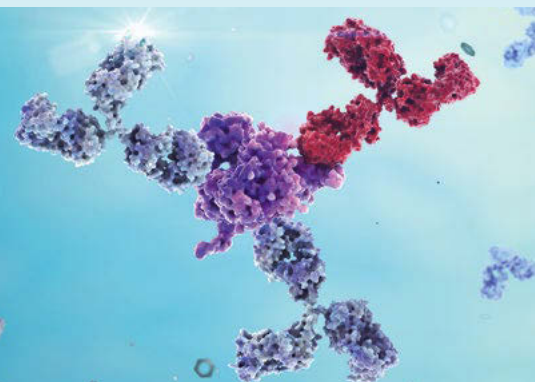
Summary



- The Thermo Scientific B·R·A·H·M·S hTg sensitive KRYPTOR assay is able to reliably detect hTg concentrations at levels relevant for the follow-up DTC and therapy control
- The Thermo Scientific B·R·A·H·M·S hTg sensitive KRYPTOR mini-recovery concept offers an additional recovery approach especially for patients with low hTg levels
- In combination with mini-recovery Thermo Scientific B·R·A·H·M·S hTg sensitive KRYPTOR shows the highest detection rate of recurrent disease⁷
- All assay interferences can be detected, not only those caused by hTg auto-antibodies

	hTg assay verification	
	by mini-recovery	by anti-Tg test
Interfering anti-Tg auto-antibodies	possible	possible
Non-interfering anti-Tg auto-antibodies	possible	not possible
Undetectable anti-Tg auto-antibodies	possible	not possible
Heterophilic antibodies	possible	not possible
High-dose hook effect	possible	not possible

The use of Thermo Scientific B·R·A·H·M·S KRYPTOR hTg sensitive with mini-recovery guarantees the most efficient and effective classification of “valid” and “invalid” Tg results in therapy follow-up of differentiated thyroid carcinoma by showing all possible interferences.



Thermo Scientific B·R·A·H·M·S hTg and anti-Tg assays

Thermo Scientific B·R·A·H·M·S **Tg-pluS** RIA (with mini-recovery)
 Thermo Scientific B·R·A·H·M·S **Tg-pluS** LIA (with mini-recovery)
 Thermo Scientific B·R·A·H·M·S **hTg** sensitive KRYPTOR (with **mini-recovery**)

Thermo Scientific B·R·A·H·M·S **anti-Tg_n** RIA
 Thermo Scientific B·R·A·H·M·S **anti-Tg_n** LIA
 Thermo Scientific B·R·A·H·M·S **anti-Tg_n** KRYPTOR

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