Assay of TSH receptor stimulating immunoglobulins using paramagnetic microbeads as solid phase

Sigrid Bräth, Ingrid Büsselmann & Ulrich Loos

IFCC 2015 Paris

KreLo GmbH Medical Diagnostics, Sedanstr. 14, D-89077 Ulm, Germany (www.krelo-med.de)

Figure 1: GD patient with hyperthyroidism, goiter and exophthalmos (written consent).

BACKGROUND

Graves' disease (GD) is caused by thyroid stimulating immunoglobulins (TSI) directed against the thyrotropin receptor (TSHR). Assays for the diagnosis of GD detect TSH receptor autoantibodies indirectly by competition with TSH or monoclonal antibodies. Here a Bridge Assay is presented for direct detection of TSI using a TSHR chimera in which the capture TSHR is anchored on paramagnetic microbeads enabling high through-put on automates.

METHODS

The capture receptor is constructed as a chimeric human TSHR (CTR) where aa residues 261-329 in the extra cellular domain (ECD) are replaced with aa residues from rat LH/CG receptor and fused with a proprietary protein (patent filed). Fixed to paramagnetic microbeads this CTR construct binds one arm of the TSI. The second arm bridges to a detection CTR constructed from aa 21-261 and fused with secretory alkaline phosphatase. All experiments in this feasibiity study were performed manually with simple lab scale equipment and a 12-tube magnet.

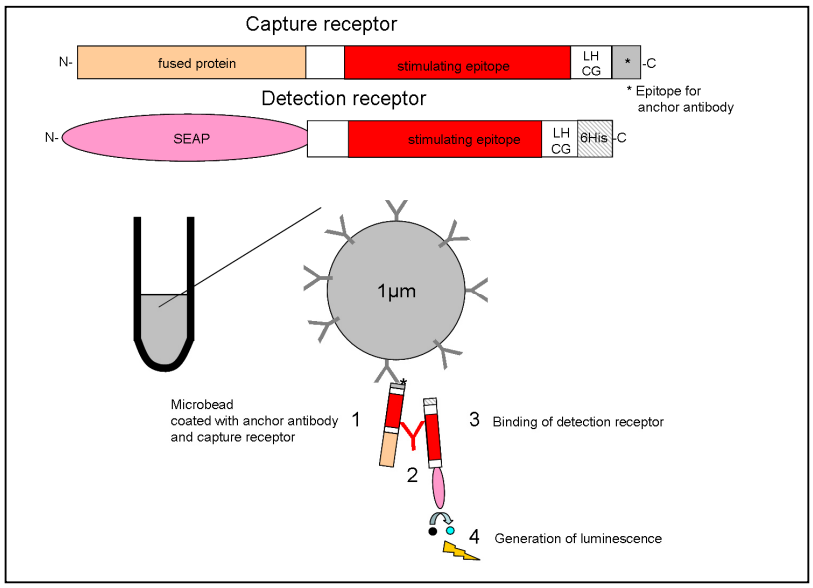

Figure 2: Priniciple of Bridge Assay using paramagnetic microbeads. The capture receptor chimera is coated on the surface of the microbead via a monoclonal antibody. The signal receptor is constructed from the chimeric ECD of the receptor and N-terminally fused SEAP.

RESULTS

ROC analysis of 190 samples (136 GD positive, 54 GD negative) showed a sensitivity of 94.1%, a specificity of 98.8% and a cut-off of 1.5 U/L with an AUC of 0.981. Analytical and functional sensitivity were determined at 1.1 U/L, with a working range up to 50 U/L, a mean between-run precision of 15.8% and a within-run precision of 4.0%. Due to manual assay performance, all data were tested for outliers (generalized ESD test with α=0.05 and Tukey). The GD negative group included 30 apparently healthy individuals and 20 patients with other, non-GD related diagnoses (14 Hashimoto, 4 Struma, 1 Thyroiditis de Quervain, 1 clinically normal patient with positive TRAb). Four patients with hypothyroidism and positive TRAb had negative results. Both receptors are secreted in cell culture supernatant realizing comfortable production. The new capture receptor yielded very good stability data (functionality and half life) at 4°C (up to 12d / up to 15d) and at 37°C (3h / 4.5h) as well as after drying (at least 4 weeks). The lyophilized detection receptor has a proven stability for >2 years.

CONCLUSIONS

The assay shows excellent sensitivity and specificity and a cut-off comparable to current high through-put assays. Further improvement of technical statistics (sensitivity and specificity) is expected by means of establishment of this prototype on fully automated machines. Together with the good stability data, these results suggest interesting possibilities for high through-put systems.

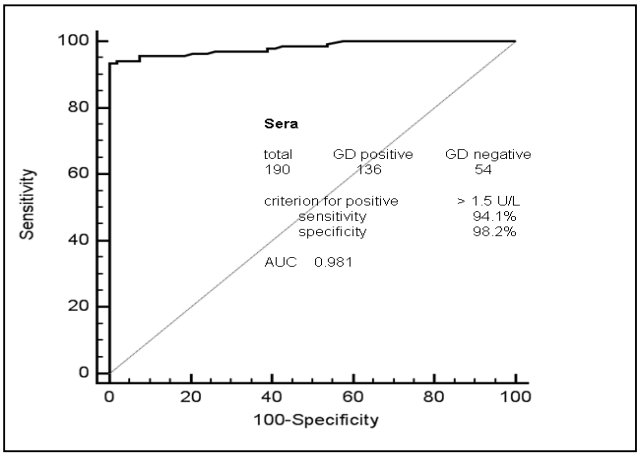

Figure 3: ROC plot analysis of 190 sera in total (136 GD positive, 54 GD negative).

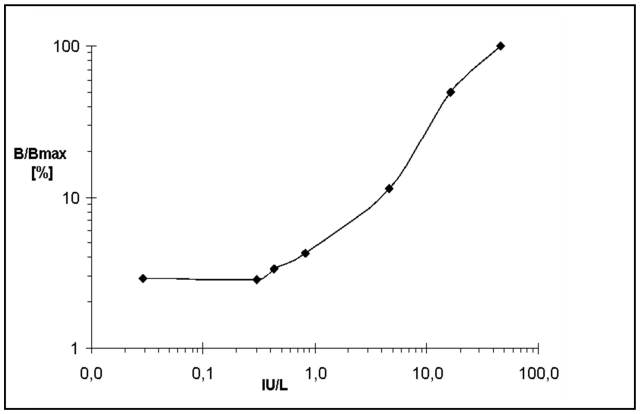

Figure 4: Typical standard curve, calibrated against WHO90/972, plotted as percentages of bound autoantibodies relative to binding of maximum calibrator (% B/Bmax) .

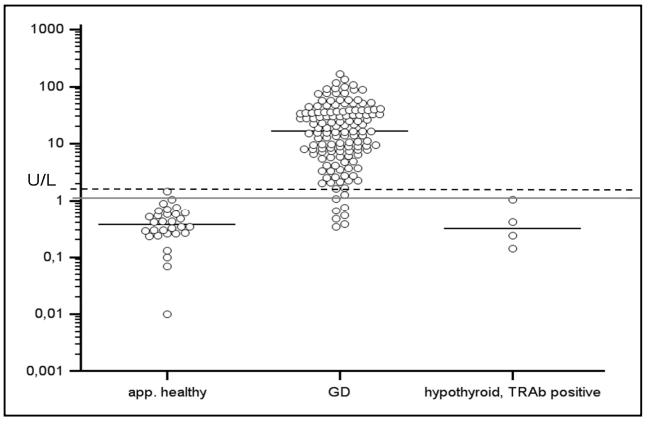

Figure 5: Scatter plot showing values of 30 apparently healthy individuals (median 0.37 U/L) and 136 GD positive patients (median 16.45 U/L). Four samples were from clinically and biochemically hypothyroid patients who were positive for TRAb in a commercial competition assay. Solid black lines show the median values; the broken black line indicates the cut-off, the solid grey line the functional sensitivity.

	Capture receptor coated on microbeads		Detection receptor, lyophilized	
	functionality	half life	functionality	half life
4°C	up to 12 d	15 d	≥ 2 years	≥ 2 years
37°C	3 h	4.5 h	3 h	~ 10h
dried and stored at 4°C	≥ 4 weeks	≥ 4 weeks, extrapolated > 1 year	–	

Table 1: Stability data of capture and detection receptor.