

# Separation-free immunoassay for hTSH with femtomolar sensitivity

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## BACKGROUND

Measurement of hTSH has traditionally been used to reflect the sensitivity of new immunoassay techniques. We developed a sensitive method for hTSH using separation-free ArcDia™ TPX assay technique and nanoparticle tracer (NPT).

## METHODS

The NPT was prepared by coating fluorescent nanospheres with monoclonal antibodies (Koskinen 2007). Each nanosphere contains several hundreds of fluorophores, while conventional molecular tracer (MT) has only few fluorophores per tracer antibody. In addition, assay buffer composition and the used antibody clones were optimized. The performance of the NPT method was compared to a corresponding method which employs MT.

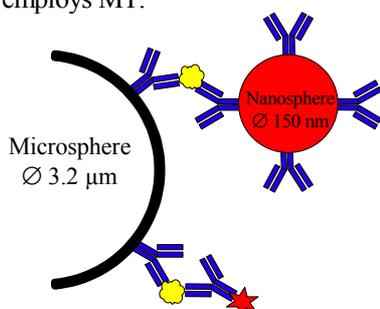


Figure 1. Binding schemes of the methods using NPT (upper) and MT.

The methods follow immunometric binding principle where three-component immunocomplexes are formed on the solid-phase surface in proportion to the analyte concentration (Figure 1). Thus, the tracer concentrates on the surface of the microspheres. The fluorescence from the surface of the individual microspheres is measured separation-free with ArcDia™ TPX Plate Reader (Figure 2, ref. Koskinen 2004).

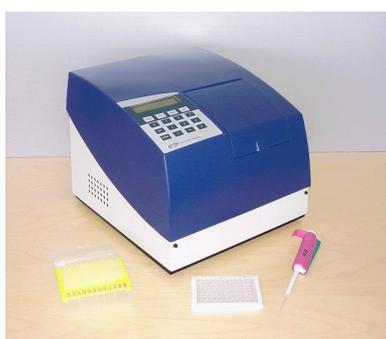


Figure 2. ArcDia™ TPX Plate Reader

## RESULTS

Performance figures of the hTSH methods are shown in Table 1, and the dose-response curves of the methods are shown in Figure 3. The NPT method showed lowest limit of detection of 0.01 mIU/L (20 fM). Compared to the MT method the NPT method showed 20-fold higher sensitivity, and improved precision. Furthermore, the NPT method showed wide dynamic range and excellent linearity ( $R^2 \geq 0.996$ ).

Table 1. Performance figures. LLD, lowest limit of detection (zero control + 3SD). FS, functional sensitivity (concentration  $CV \leq 20\%$ ). CV, intra-assay imprecision at the concentration of 0.5 mIU/L.

	NPT metod	MT method
LLD	0.01 mIU/L	0.2 mIU/L
FS	0.05 mIU/L	0.5 mIU/L
CV	8%	20%

### References:

Koskinen, J.O. et al. (2004) Fluorescent nanoparticles as labels for immunometric assay of C-reactive protein using two-photon excitation assay technology. *Anal. Biochem.* **328**:210-218.

Koskinen J.O. et al. (2007) A Rapid separation-free method for influenza A and B virus antigen detection using TPX assay technique and dry-chemistry reagents. *JCM*, in review.

Performance of the TPX technique is independent of the reaction volume. When applying 1  $\mu$ L reaction volumes the detection limit amounts to 10,000 molecules being one of the most sensitive techniques reported – still, the technique is separation-free.

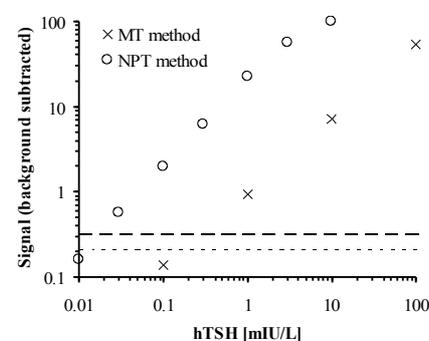


Figure 3. Dose-response curves for hTSH methods employing molecular tracer (3SD, dashed line) and nanoparticle tracer (3SD, dotted line).

## CONCLUSIONS

The use of nanoparticle tracer improved sensitivity of the hTSH method by an order of a magnitude. The method is expected to allow screening of thyroid dysfunctions in the primary care.

The new ArcDia™ TPX technique allows development of simple and cost-efficient random access analyzer for point-of-care testing of immunochemistry and clinical chemistry analytes with comparable performance to the conventional laboratory methods.

Implementation of such diagnostic products for point-of-care testing would ensure correct diagnosis, and enable rapid treatment decisions. This would improve patient care and cost-efficiency of the health care system.