

# TSH Immunoassay



## OVERVIEW

Luminex® incorporates a proprietary process to internally dye polystyrene microspheres with two spectrally distinct fluorochromes. Using precise ratios of these fluorochromes, an array is created consisting of 100 different microsphere sets – each with its own characteristic spectral address. Each microsphere can possess a different reactant on the surface. Since microsphere sets can be distinguished by their spectral addresses, they can be combined to allow up to 100 different analytes to be measured simultaneously in a single reaction vessel. A third fluorochrome coupled to a reporter molecule quantifies the biomolecular interaction that has occurred at the microsphere surface. Microspheres are interrogated individually in a rapidly flowing fluid stream as they pass by two separate lasers in the Luminex 100™ analyzer. High speed digital signal processing classifies the microsphere based on its spectral address and quantifies the reaction on the surface in a few seconds per sample. We utilized this platform to develop a third-generation immunoassay for TSH.

Measurement of TSH (Thyroid Stimulating Hormone or Thyrotropin) is useful in screening for both hyperthyroidism, where TSH levels may be very low, and hypothyroidism, where TSH levels may be high. To date, no single commercial assay is available that exhibits both the exquisite sensitivity and broad dynamic range required for screening for both conditions. Using the xMAP® system, we developed a TSH immunoassay that combines the third-generation sensitivity and the dynamic range necessary for practical use as an analytical tool.

## MATERIALS AND METHODS

>> Preparation of Assay Reagents: Anti-TSH monoclonal antibodies were coupled to xMAP microspheres using the recommended Luminex procedure. The microspheres were activated using 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC) and N-hydroxy-sulfosuccinimide (Sulfo-NHS) in 0.1 M sodium phosphate buffer, pH 6.3 and the antibody was coupled to activated microspheres in 0.05M 2-(N-morpholino) ethanesulfonic acid

(MES) buffer, pH 5.0. Detection anti-TSH monoclonal antibodies were biotinylated using EZ-Link-Sulfo-NHS-LC-Biotin (Pierce). The TSH calibration material was prepared in horse serum to mimic human samples.  
>> Reagent Selection: Antibodies were screened for sensitivity by coupling thirteen different monoclonal antibodies to xMAP microspheres and testing with 10 biotinylated detection monoclonal antibodies. From these results, OEM Concepts antibody, clone # 057-11003 was selected as the capture antibody and Medix Biochemica antibody, clone # 5403, and a second antibody were selected as detection antibodies.

Utilizing this antibody combination, we developed a 20 minute TSH assay with a sensitivity of <0.01 µIU/mL (Table 1).

In addition to sensitivity and speed, we sought to develop a TSH assay exhibiting broad dynamic range. Using additional bead sets, we included two more anti-TSH antibodies (OEM Concepts antibody, clone # 204-12252, and Medix Biochemica antibody, clone # 5401) that had different affinities than the original capture antibody.

Figure 1 shows that by using 3 different antibody-bead sets, the assay had a dynamic range greater than 5 orders of magnitude (<0.01 to > 1000 µIU/mL).

### Assay Components:

- >> Capture antibodies
  - 2.5 µg antibody coupled per 10<sup>6</sup> beads
  - 1000 beads for each of the 3 capture antibody bead sets
  - PBS-BSA (1% BSA) buffer containing 2% polyethylene glycol (MW 15,000)
- >> Detection antibodies
  - 20 µg/mL each
  - PBS-BSA (1% BSA)

**Table 1. Low-end Calibration Curve for TSH Assay**

TSH Calibrators (µIU/mL)	Average Median Fluorescent Intensity* (MFI)	SD 4 reps
0.08	524	112
0.04	275	50
0.02	148	38
0.01	93	8
0.005	77	28
0.0025	39	9
0.00	27	14

\*Uncoated bead fluorescence subtracted

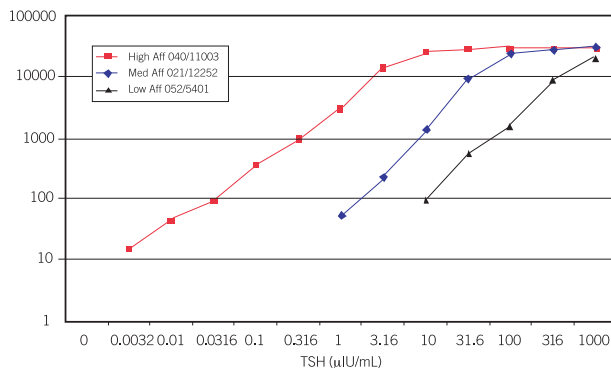


Figure 1

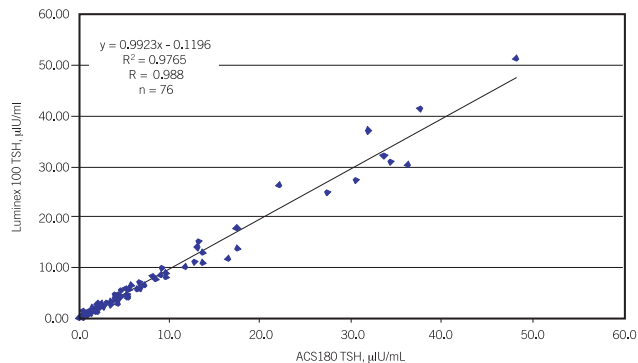


Figure 2

Table 2. Intra-Instrument Assay Precision

Bead	Antibody Affinity	Bio-Rad Controls	Expected µIU/mL	Observed µIU/mL	Std Dev	% CV
040	High Affinity	Control 1	0.48	0.64	0.03	5.39
021	Med Affinity	Control 2	5.00	4.20	0.22	5.24
052	Low Affinity	Control 3	28.00	21.00	1.39	6.64

Table 3. Results for Samples below Limit of Detection for ACS 180

Samples (µIU/mL)	ACS180 TSH Conc (µIU/mL)	xMAP TSH Co
CPL 26	<0.1	0.05
CPL 27	<0.1	0.06
CPL 28	<0.1	2.15
CPL 32	<0.1	0.02
CPL 36	<0.1	0.02
CPL 37	<0.1	0.05
CPL 38	<0.1	0.03
CPL 39	<0.1	0.02
CPL 41	<0.1	0.02

>> Wash buffer – PBS-BSA (1% BSA)  
 >> Streptavidin-R-Phycoerythrin (100 µg/mL) - in PBS-BSA (1% BSA)  
 >> Assay Protocol: Add 25 µL of patient sample (undiluted serum) to a well. Add 10 µL of capture antibody-coupled beads and incubate 10 minutes at 40°C. Add 10 µL of biotinylated detection antibodies and incubate 5 minutes at 40°C. Add 10 µL of streptavidin-R-phycoerythrin (SA-RPE) and incubate 5 minutes at 40°C. Transfer to a filter plate and wash 2 times with 200mL wash buffer. Resuspend in 110 µL wash buffer and read on the Luminex 100 with the PMT set to 800 mV.

## RESULTS

Bio-Rad Controls were used to assess intra-assay precision with this xMAP TSH Assay. Ten replicates of each of the three Bio-Rad controls were measured on one plate (Table 2).

>> Assay Limit of Detection: Fifteen replicates of the zero calibrator were used to

assess the limit of detection for this xMAP TSH Assay. Defining the LDD as the mean of the zero plus 3 SD, the LDD for this TSH assay was 0.006 µIU/mL.

>> Assay Correlation: Clinical samples were obtained from Clinical Pathology Laboratories (Austin, TX) with assigned values for TSH determined on the ACS 180. A total of 96 samples were tested with this TSH Assay. Reportable results were obtained for 89 samples of which 80 were within the dynamic range of the reference ACS 180 TSH Assay (Figure 2).

Four samples were excluded from this correlation because there was a large difference in the values of the two assays. The cause of the discrepancies is unknown and is under investigation. Nine samples were below the limit of detection for the reference assay, but were reportable with our xMAP TSH Assay (Table 3). The result for one sample was at variance with the reference value. These data demonstrate that our TSH assay correlates with the

ACS 180 and is significantly more sensitive.

## CONCLUSIONS

These results demonstrate the use of xMAP technology to develop a rapid, simple, third generation TSH assay with the combined sensitivity and sufficiently large dynamic range to be useful as a clinical assay for TSH.

The xMAP TSH Assay:

- >> Sensitive – 0.006 µIU/mL
- >> Simple – requires only one wash step
- >> Rapid – complete in 20 minutes
- >> Accurate – correlates with the ACS 180 TSH Assay (r<sup>2</sup> = 0.976)
- >> Practical – broad dynamic range (>1000 µIU/mL)

Call toll-free 1-888-219-8020 in North America, call 1-512-219-8020 outside of North America, visit [www.luminexcorp.com](http://www.luminexcorp.com), or email [info@luminexcorp.com](mailto:info@luminexcorp.com) for more information.

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