Comparison of an ELISA and a Single-Plex Bead-based Immunoassay (Luminex) for the Analysis of Thyroid Stimulating Hormone in rat serum

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INTRODUCTION
Thyroid-stimulating hormone (TSH) is a major regulator of the body metabolism. Secreted by the pituitary gland, it stimulates the thyroid gland to produce thyroxine (T₄) and triiodothyronine (T₃) which in turn stimulate the metabolism of many tissues. Varying levels of TSH are indicative of diseases such as hypothyroidism or hyperthyroidism, or pituitary tumor. It is thus a marker of importance for a disease state or response to various drugs, and has been selected as a key biomarker for toxicological studies. An ELISA and a single-plex bead-based immunoassays were compared when developing and validating a method for the quantification of TSH in rat serum.

MATERIALS AND METHODS
The following validation parameters were assessed using an ELISA (Alpco Diagnostics, catalog no. 56-TSHRT-E01) and a single-plex bead-based immunoassay kit (Millipore, catalog no. RTHYMAG-30K):

• Specificity/absorbency and sensitivity
• Parallelism (bead-based assay) and linearity of dilution (ELISA)
• Precision and relative accuracy

Endogenous level: Concentrations measured with the ELISA assay ranged between 2.70 to 7.02 ng/mL. Concentrations measured with the bead-based assay ranged from 2.70 to 7.00 ng/mL.

Precision and Relative Accuracy: In order to monitor the performance of the assay, precision and relative accuracy assessments of QC samples prepared with reference material in buffer (LLOQ, low, mid, high and ULOQ) were conducted.

Parallelism of the bead-based assay proved successful for 5-fold and 27-fold diluted samples. Parallelism assessment performed with the bead-based assay proved successful for 5-fold and 10-fold diluted samples.

Stability: Stability was validated with endogenous TSH for the bead-based assay, and with exogenously spiked TSH in rat serum for the ELISA. The following stabilities were validated with the ELISA assay: 6 hours 59 minutes at ambient RT, 26 hours 3 minutes in an refrigeration set to maintain 4°C, 4 freeze-thaw cycles in freezers set to maintain -20°C and -80°C, 30 days in a freezer set to maintain -20°C and 188 days in a freezer set to maintain -80°C. The following stabilities were validated with the bead-based assay: 21 hours 14 minutes at ambient RT in a refrigeration set to maintain 4°C, 4 freeze-thaw cycles in freezers set to maintain -20°C and -80°C, and 119 days in freezers set to maintain -20°C and -80°C.

Stability assessment demonstrated no interference effect of the matrix when tested with the ELISA or bead-based assay.

DISCUSSION
Working range: The curve range of the ELISA kit was from 2.50 to 43.00 ng/mL. The curve range of the bead-based assay was from 0.04 to 6.67 ng/mL. (Table 1)

Precision and Accuracy: To evaluate the validation parameters using the single-plex bead-based kit, normal rat sera were spiked with recombinant TSH material. To evaluate the validation parameters using the ELISA kit, normal rat sera were spiked with recombinant reference material in buffer (Luminex) for the analysis of Thyroid Stimulating Hormone in rat serum. The single-plex bead-based kit offered a higher sensitivity and was able to detect normal baseline levels in rat serum, ranging between 2.70 and 7.00 ng/mL. This allowed the assessment of validation parameters without the need to spike TSH reference material and the inclusion of a positive sample used as a QC to monitor the performance of the assay on a day-to-day basis. Furthermore, this method was proven sensitive enough to detect both increases or decreases in TSH concentrations that may occur as a test item related effect, or as the result of a disease state.

Endogenous level: Concentrations measured with the ELISA assay ranged between 2.70 to 7.02 ng/mL. Concentrations measured with the bead-based assay ranged from 2.70 to 7.00 ng/mL.

The selectivity assessment demonstrated no interference effect of the matrix when tested with the ELISA or bead-based assay.

Parallelism of linearity of dilution: Linearity assessment performed with the ELISA assay proved successful between 4.5-fold and 27-fold diluted samples. Parallelism assessment performed with the bead-based assay proved successful for 5-fold and 10-fold diluted samples.

Stability: Stability was validated with endogenous TSH for the bead-based assay, and with exogenously spiked TSH in rat serum for the ELISA. The following stabilities were validated with the ELISA assay: 6 hours 59 minutes at ambient RT, 26 hours 3 minutes in a refrigeration set to maintain 4°C, 4 freeze-thaw cycles in freezers set to maintain -20°C and -80°C, 30 days in a freezer set to maintain -20°C and 188 days in a freezer set to maintain -80°C. The following stabilities were validated with the bead-based assay: 21 hours 14 minutes at ambient RT in a refrigeration set to maintain 4°C, 4 freeze-thaw cycles in freezers set to maintain -20°C and -80°C, and 119 days in freezers set to maintain -20°C and -80°C.

Stability assessment demonstrated no interference effect of the matrix when tested with the ELISA or bead-based assay.

RESULTS

<table>
<thead>
<tr>
<th>STDQG ID</th>
<th>Concentration (ng/mL)</th>
<th>ELISA</th>
<th>Bead-based assay</th>
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<tbody>
<tr>
<td>STD 1</td>
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</tr>
<tr>
<td>STD 5</td>
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<td>5.00</td>
<td>4.90</td>
</tr>
</tbody>
</table>

Accuracy standard:
• *B: rat serum with exogenously spiked TSH
• T: rat serum with endogenous TSH

4 DISCUSSION

5 CONCLUSION/NOVEL ASPECT

All validation parameters met the acceptance criteria using both kits. In the absence of available samples with measurable or sufficiently high TSH concentrations, positive samples were generated by spiking blank rat sera with TSH reference material when using the ELISA. However, the sensitivity of the ELISA was not assessed in biologically meaningful samples, raising the concern of not being able to detect TSH variations in toxicological study samples. The single-plex bead-based kit offered a higher sensitivity and was able to detect normal baseline levels in rat serum, ranging between 2.70 and 7.00 ng/mL. This allowed the assessment of validation parameters without the need to spike TSH reference material and the inclusion of a positive sample used as a QC to monitor the performance of the assay on a day-to-day basis. Furthermore, this method was proven sensitive enough to detect both increases or decreases in TSH concentrations that may occur as a test item related effect, or as the result of a disease state.

In the absence of clear guidelines for biomarker assays, the generation of positive samples by spiking blank matrices with recombinant material is a widely used approach in the validation of assay kits when endogenous levels are not detectable. However, the generation of samples with measurable endogenous biomarker concentrations, by in vivo or in vitro simulation when needed, is the recommended approach in the development of biomarker assays.