Harmonization of TSH immunoassays based on glycoengineered TSH as new calibrator
The « TSH Testing » project: 2011-2014

Aim of the study:

- Understand and solve current discordances
- Find a method to measure blood TSH on a molar basis
- Improve accuracy and define a therapeutic threshold

Background:

Sialylated TSH but not pitTSH is a good mimic of serum TSH in 20 existing assays

Polymorphism of TSH results from variable glycosylation and clearance rate

Short-lived TSH (liver receptors)

Long-lived TSH (hypothyroidism)

International Standard 3\textsuperscript{rd} IS 81/565 (pituitary TSH)

serumTSH

sialic acid NeuAc

>12 specific epitopes
Outline:

1. Production of glycoengineered TSH (rgTSH)
2. Epitope-defined strategy
3. Evaluation of glycoengineered TSH in pilot studies
4. Clinical validation: preliminary data
1- Production of glycoengineered TSH (rgTSH) (1/2)

Lectin- \textit{(red)} and immuno-staining of engineered cells

\textit{ST6}-expressing cells are robust for bioproduction at high cell density
1- Production of glycoengineered TSH (2/2)

TSH glycoprofile is different among calibrators: only rgTSH contains sialic acid

pituitary TSH  
(3rd IS pitTSH 81/565)

recombinant TSH  
(1st IRP recTSH94/674)

glycoengineered TSH  
(rgTSH)
To identify common epitopes:

- 2 antigenic regions
- with MIR and cluster II with overlapping epitopes
- and 1 remote cluster with overlapping epitopes
3- Group A assays (2/5)

Group A assays target the Main Immunogenic Region as most existing routine assays.

Pilot study: 84 serum samples and 3 calibrations

Group A assays are discordant and TSH values are dependent upon the targeted epitopes

(Andréi et al., manuscript in preparation)
In contrast to Group A, Group B assays behave similarly over the 0.5-30mIU/L range.
3- rgTSH allows harmonization of both Groups A and B assays (4/5)

Applicable to most if not all assays

(Andréi et al., submitted)
3- **rgTSH allows mass calibration (5/5)**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Slope</th>
<th>$R^2$</th>
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</thead>
<tbody>
<tr>
<td><strong>3rd IS pitTSH 81/565</strong>&lt;br&gt;Group A</td>
<td>#A1 47.244</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>#A2 30.084</td>
<td>0.9989</td>
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<tr>
<td></td>
<td>#A3 32.689</td>
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<tr>
<td></td>
<td>#B1 46.572</td>
<td>0.8958</td>
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<tr>
<td></td>
<td>#B2 40.128</td>
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<td></td>
<td>#B3 42.052</td>
<td>0.9957</td>
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<tr>
<td></td>
<td>#B4 32.396</td>
<td>0.9976</td>
</tr>
<tr>
<td><strong>1st IRP recTSH 94/674</strong>&lt;br&gt;Group A</td>
<td>#A1 13.923</td>
<td>0.9983</td>
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<tr>
<td></td>
<td>#A2 11.944</td>
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<td>#A3 13.215</td>
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<td>#B1 16.271</td>
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<td>#B2 15.190</td>
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<td>#B3 15.439</td>
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<tr>
<td></td>
<td>#B4 11.775</td>
<td>0.9976</td>
</tr>
</tbody>
</table>

*Linear correlation between rgTSH and international standards*

Slopes represent the ng/mlU factor of conversion

*(Andréi et al., submitted)*
4- Ongoing clinical study

1363 serum samples

• Recruited 2012-2015

• All TSH levels measured by IRMA

• 4 assays

• 2 calibrations (3rd IS pitTSH 81/565 and rgTSH)

Preliminary data:
→ Higher specificity and sensitivity of the 4 assays tested
→ Performances are higher than IRMA

(Andréi et al., manuscript in preparation)
Conclusions

*Glycoengineered TSH provides harmonization*

- In large agreement with the IS preparation
- For a wide panel of assays
- With unlimited supply of material

*Assays must target defined epitopes to*

- Deliver similar TSH values
- Be calibrated on a mass basis
- Display high performances
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