Harmonization of TSH immunoassays based on glycoengineered TSH as new calibrator

Meeting of JCTLM Members and Stakeholders
30 November - 1 December 2015

Sia Med'Xpress
GLYCOTECHNOLOGY AT WORK

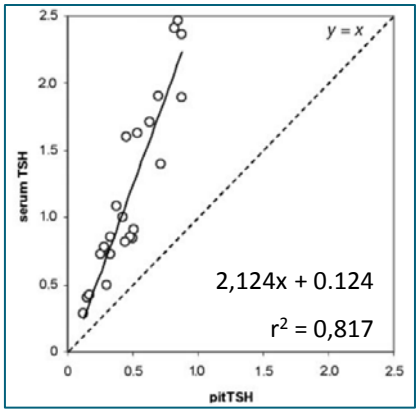
The « *TSH Testing* » project: 2011-2014

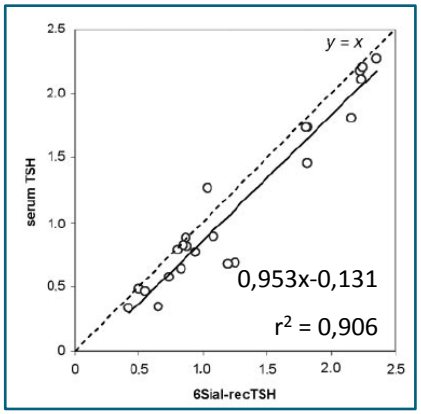
***Aim of the study*:**

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

Background:

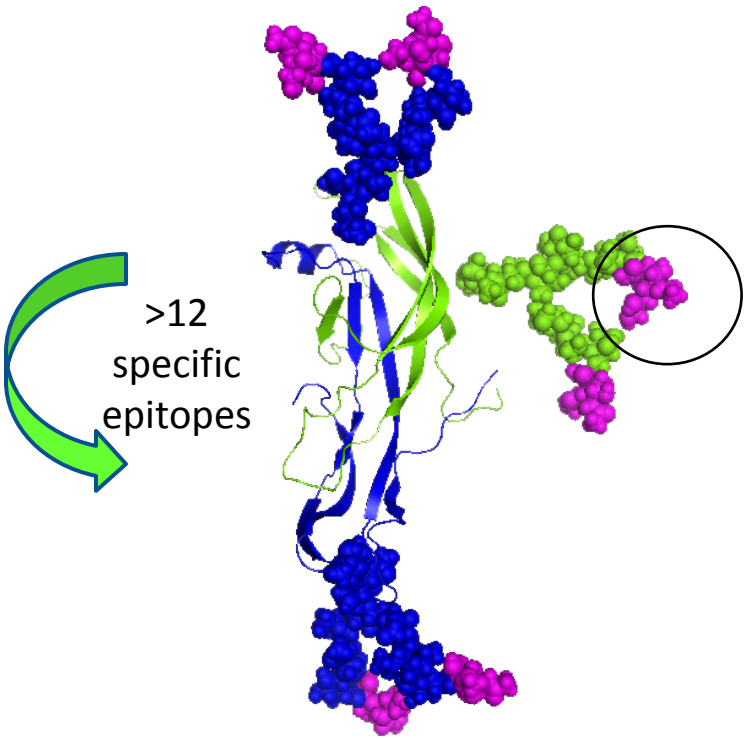
Sialylated TSH but not pitTSH is a good mimic of serum TSH in 20 existing assays





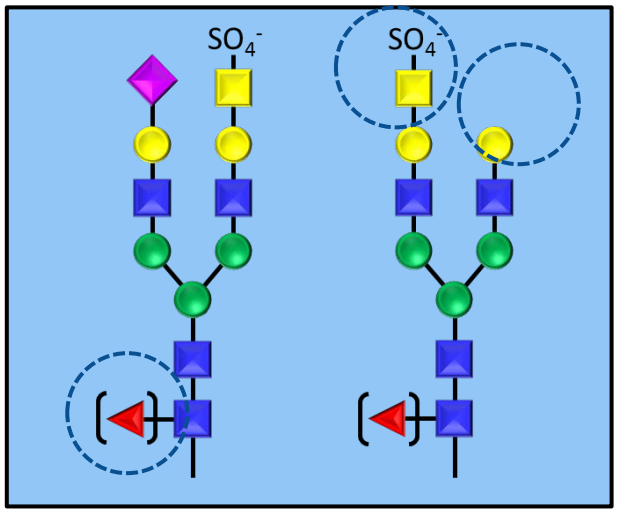
Donadio-Andrei et al.,
Clin Chem, 2006, 52(2):286-97

Polymorphism of TSH results from variable glycosylation and clearance rate



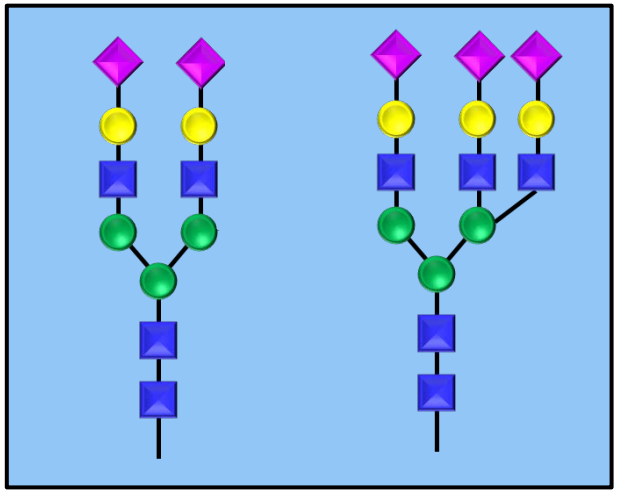
sialic acid
NeuAc

Short-lived TSH
(liver receptors)



International Standard
3rd IS 81/565 (pituitary TSH)

Long-lived TSH
(hypothyroidism)



serumTSH

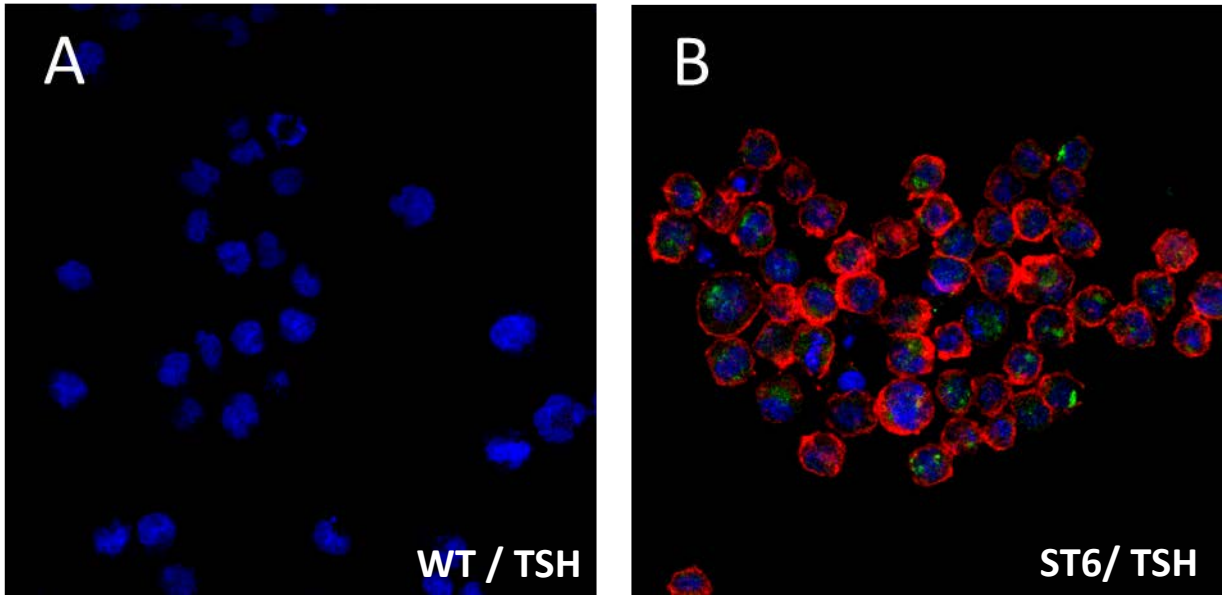
Outline:

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)

recombinant TSH (recTSH)

recombinant glycoengineered TSH (rgTSH)

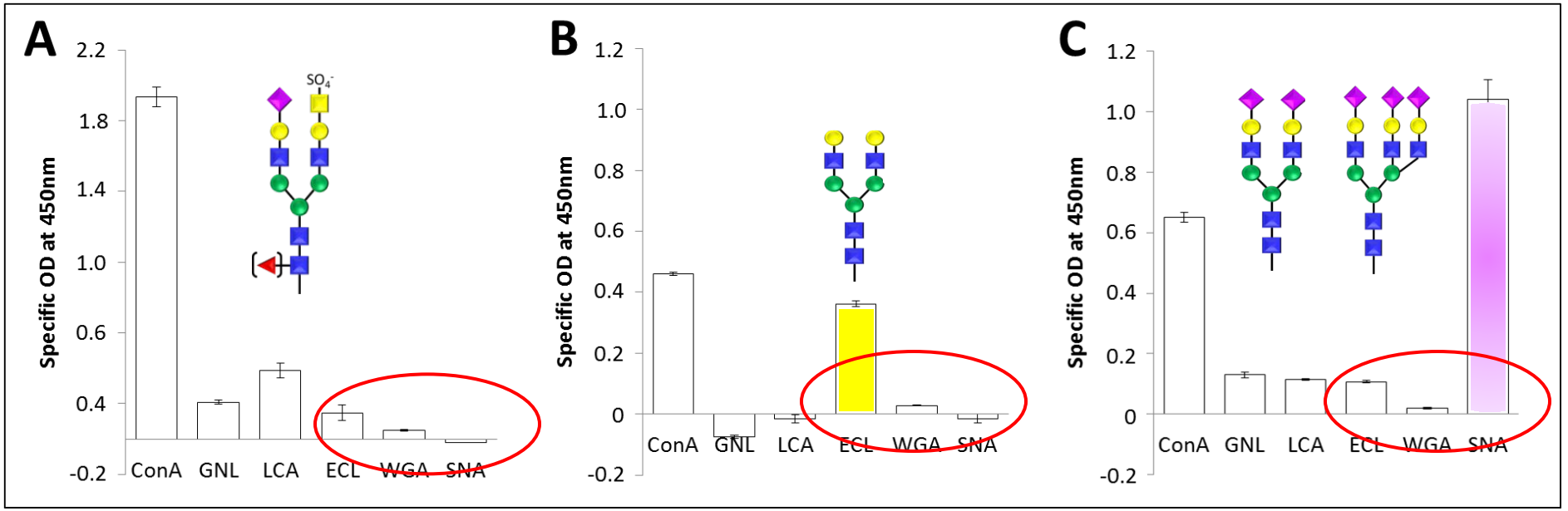


Lectin-*(red)* and immuno-staining of engineered cells

ST6-expressing cells are robust for bioproduction at high cell density

1- Production of glycoengineered TSH (2/2)

TSH *g*lycoprofile is different among calibrators: only rgTSH contains sialic acid



pituitary TSH

(3rd IS pitTSH 81/565)

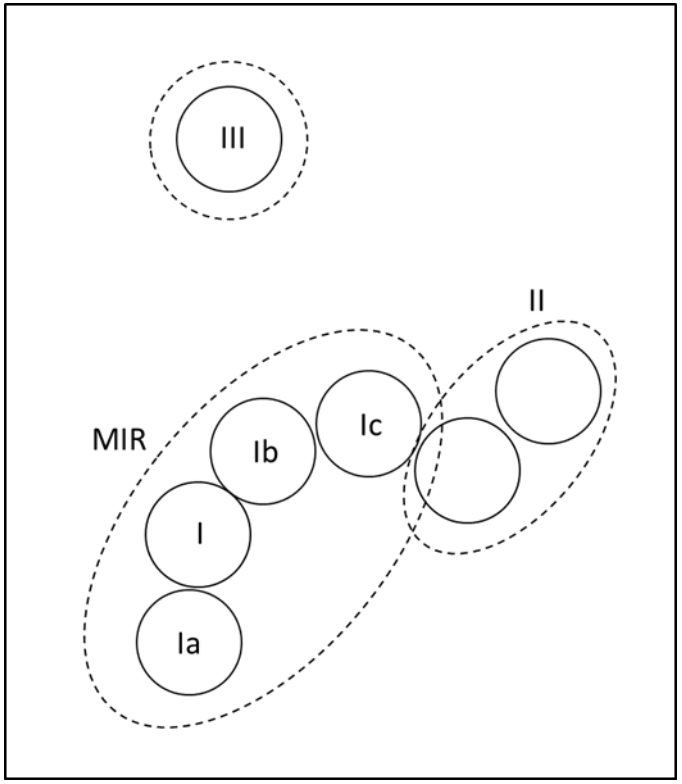
recombinant TSH

(1st IRP recTSH94/674)

glycoengineered TSH

(rgTSH)

2- Dual mapping of pituitary and rgTSH (1/5)



To identify common epitopes:

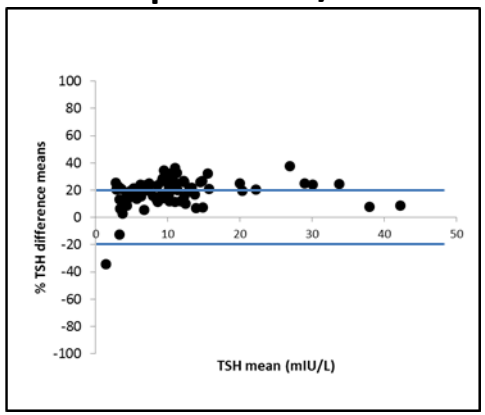
- 2 antigenic regions
- with MIR and cluster II with overlapping epitopes
- and 1 remote cluster with overlapping epitopes

3- Group A assays (2/5)

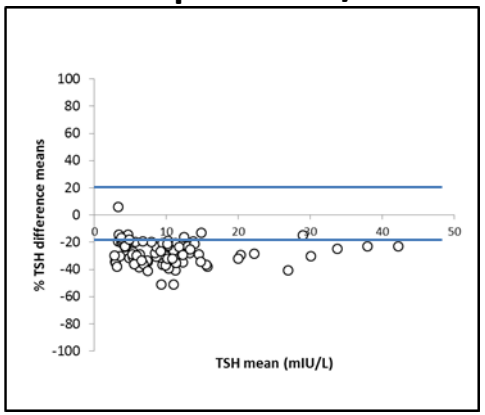
Group A assays target the Main Immunogenic Region as most existing routine assays

Pilot study : 84 serum samples and 3 calibrations

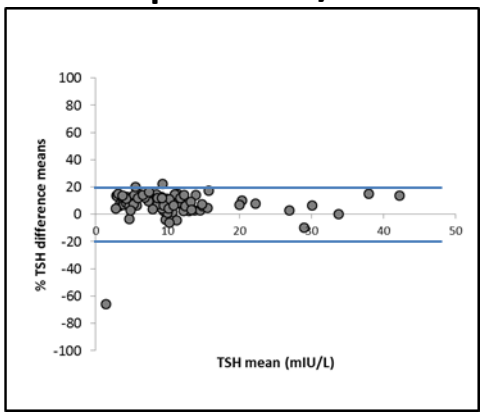
3rd IS pitTSH 81/565



3rd IS pitTSH 81/565



3rd IS pitTSH 81/565



(Andréi et al., manuscript in preparation)

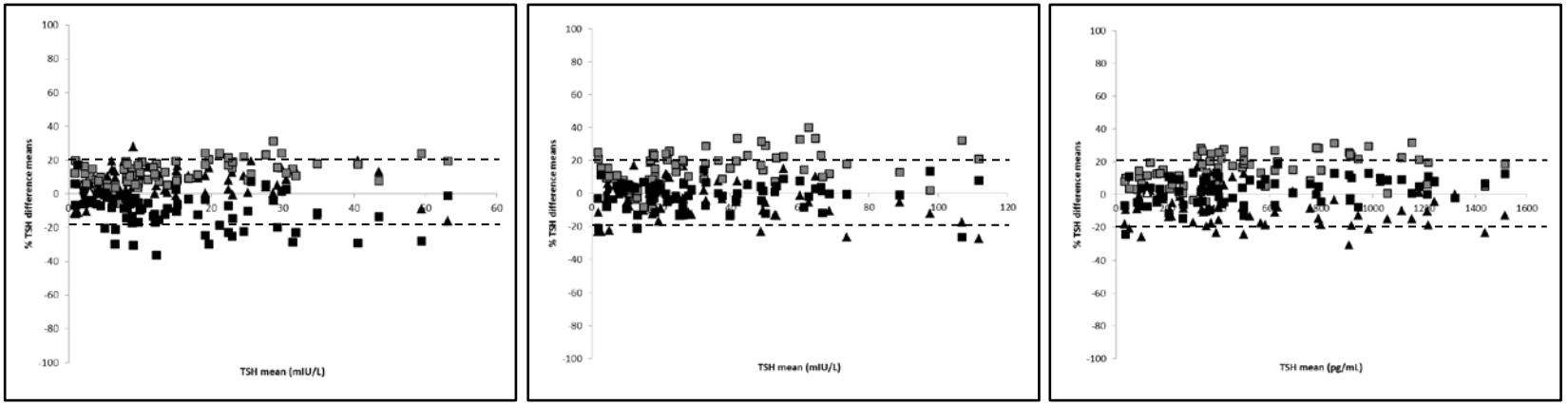
Group A assays are discordant
and
TSH values are dependent upon the targeted epitopes

3- Group B assays (3/5)

Pilot study: 73 serum samples and 3 calibrations

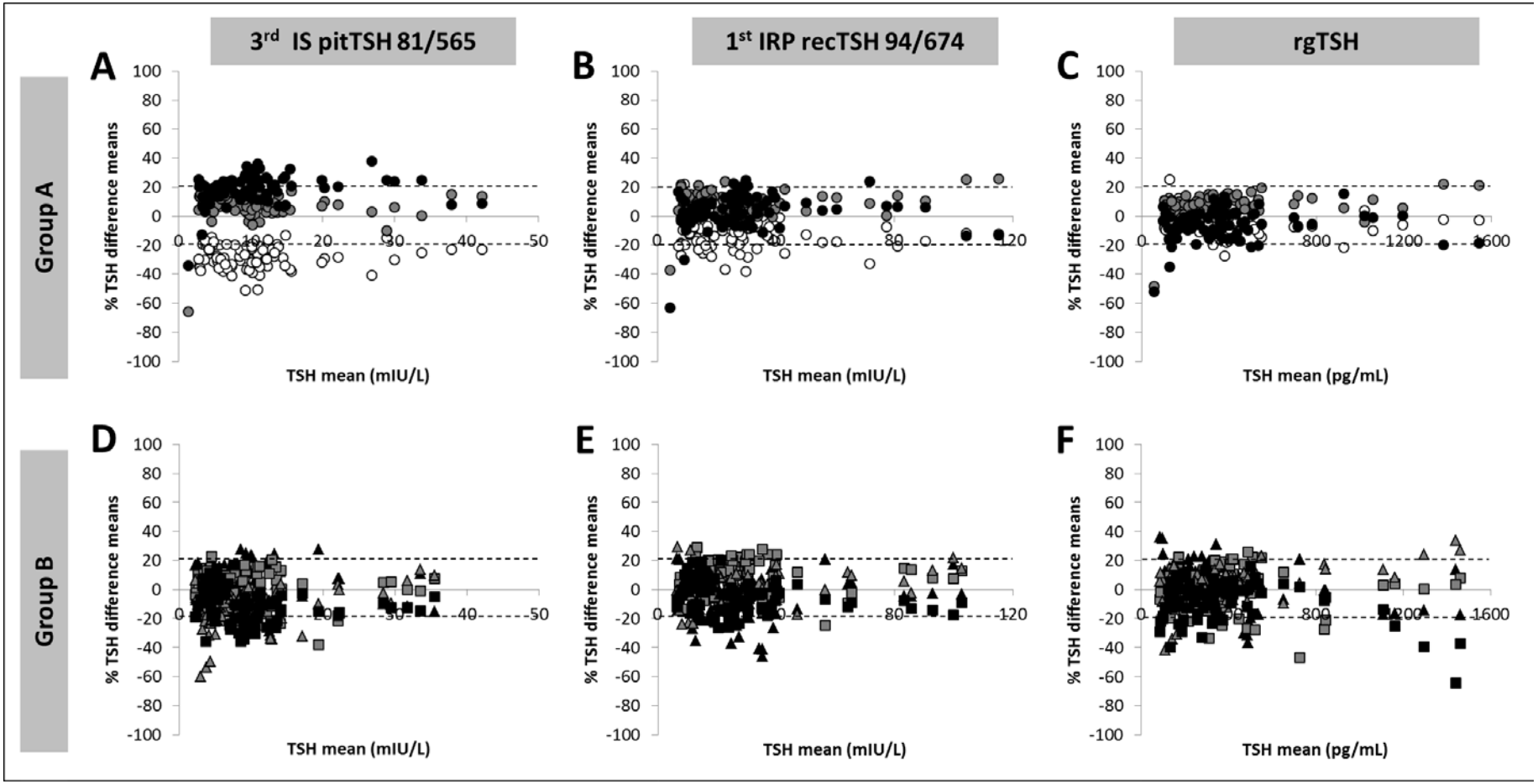
#B1, #B2, #B3

3rd IS pitTSH 81/565 | **1st IRP rTSH 94/674** | **rgTSH**



In constrast 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

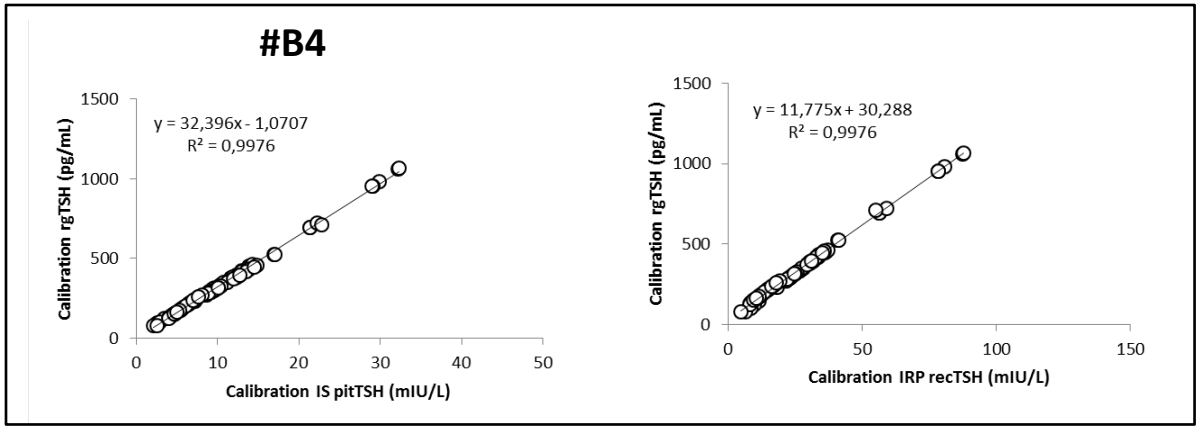
(Andréi et al.,submitted)

3- rgTSH allows mass calibration (5/5)

		Assay	Slope	R^2
3rd IS pitTSH 81/565	Group A	#A1	47.244	0.9999
		#A2	30.084	0.9989
		#A3	32.689	0.9992
	Group B	#B1	46.572	0.8958
		#B2	40.128	0.9942
		#B3	42.052	0.9957
		#B4	32.396	0.9976
1st IRP recTSH 94/674	Group A	#A1	13.923	0.9983
		#A2	11.944	0.9945
		#A3	13.215	0.9919
	Group B	#B1	16.271	0.9394
		#B2	15.190	0.9969
		#B3	15.439	0.9873
		#B4	11.775	0.9976

Linear correlation between rgTSH and international standards

Slopes represent the ng/mIU 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)

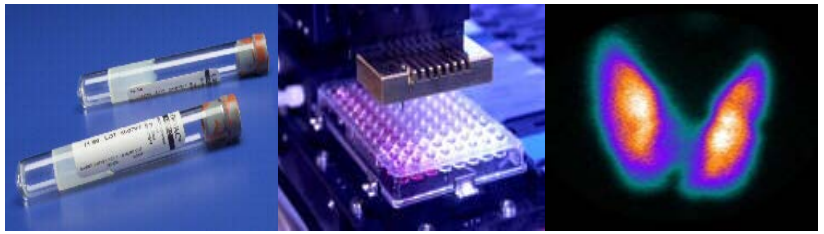
Conclusions

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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