How accurate is your Sclerostin measurement?

Comparison between three commercially available sclerostin ELISA kits.

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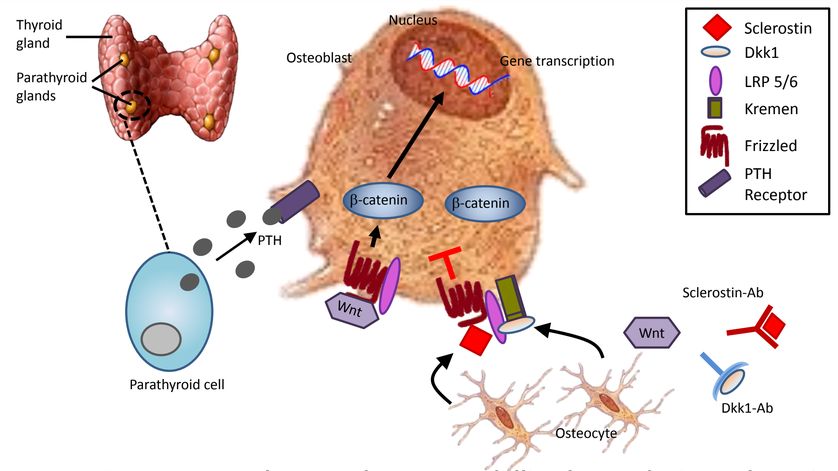
Introduction

Sclerostin (SOST), an osteocyte-secreted soluble antagonist of the Wnt/β-catenin signalling pathway

- is a potent inhibitor of osteoblastogenesis. Mutations in the SOST gene are associated with loss or decrease of sclerostin (Sclerosteosis[1,2], van Buchem disease[3,4]),
- is a regulator of the skeletal anabolic action of PTH[5-6],
- is a potential treatment for osteoporosis - anti-sclerostin antibodies are being investigated as potential therapeutic molecules for osteoporosis[7-9],

Measurement of circulating sclerostin is therefore of utmost importance for the diagnosis of bone disorders and therapy effectiveness.

We compared the levels of circulating sclerostin measured using ELISA kits from three different providers: Biomedica (Vienna, Austria), R&D Systems (Abingdon, UK) and TecoMedical (Sissach, Switzerland).



Osteocytes orchestrate bone remodelling by producing sclerostin which inhibits bone formation by osteoblasts

Adapted from Lippuner et al., Swiss Med Wkly. 2012;142:w13624

1-Methods

Description of kits used:

	BIOMEDICA	R&D Systems	TECO
ELISA kit cat#	BI-20492	DSST00	TE1023HS
Standard range	0- 240 pmol/L	31.3-2000 pg/mL 1.3-88 pmol/L	0-3 ng/mlL 0-132 pmol/L
LOD **LLOQ**	3.2 pmol/L 7.5pmol/L	N/A 1.74 pg/mL (7.66 pmol/L)	0.008 ng/ml (0.35 pmol/L) 0.01 ng/ml (0.44 pmol/L)
Sample type	Serum / EDTA or Hep Plasma	Serum / EDTA or Hep Plasma	Serum / EDTA or Hep Plasma
plated coating	polyclonal goat anti human SOST antibody	monoclonal anti human SOST antibody	Streptavidin
Antibody	monoclonal mouse anti human SOST antibody – biotin	polyclonal anti human SOST antibody -HRP	polyclonal anti human SOST Biotin+ monoclonal anti human SOST-HRP
Conjugate	streptavidin-HRPO	hydrogen peroxide	
Substrate	TMB	TMB	TMB
Incubation time /T°C	21.5hrs / RT	4.5hrs / RT	4.5hrs / RT
Sample volumes (µL)	20	50	25

Samples:

- 46 serum randomized samples from healthy volunteers (aged 17-32yrs)
- 27 matching EDTA-plasma samples
- Kits were used as per manufacturer's instructions.

Results are given in pmol/L using a conversion factor of 44 from ng/mL to pmol/L. Values are given in mean ± SD. Statistical analysis was carried out using SPSS.

2-Assay Characteristics

- **Inter-assay precision:**

Six EDTA samples were run in two independent experiments in both assays. A serum pool was run 8 times on two different plates.
Biomedica: mean at 54 pmol/L (n=16), CV 5%; mean at 154 pmol/L, CV 5%.
Teco: mean at 23.8 pmol/L (n=8), CV 2.8%.
R&D Systems: mean at 9 pmol/L, (n=8), CV 3.9%.

- **Intra-assay imprecision:** Plasma: as mean of CVs of samples run in duplicates . Serum pool was run 8 times. See table 1, results expressed as mean CV ± SD.
- **Linearity:** We assessed the linearity of the assay by diluting samples (n=2) 1:2; 1:4 and 1:8 using the sample diluent provided with the kit. Sample percentage recovery after dilution was estimated. See table 1.
- **Recovery**: Spiked recovery (%) was determined by adding a known quantity of sclerostin to samples with different levels of endogenous sclerostin. See table 1.

	Intra-assay (%CV ± SD)		Linearity (% ± SD)		Recovery (% ± SD)	
	EDTA	SERUM	EDTA	SERUM	EDTA	SERUM
Biomedica	7.3 ± 6.2	8.9 ± 11.2	149.5 ± 32.1*	142.7 ± 29.8*	104.0 ± 8.7	93.4 ± 7.1
TECO	2.7 ± 2.5	2.7 ± 2.6	101.8 ± 8.6	98.6 ± 7.0	102.4 ± 10.2	103.4 ±2.1
R&D Systems	7.0 ± 5.4	25.8 ± 5.8	73.26 ± 9.9	125.9 ± 23.9	94.5 ± 2.6	100.7 ± 9.9

* SPSS, different from other kits, p<0.05

Table 1: Intra-assay imprecision, linearity and spiked recovery obtained from the 3 kits tested.

3-EDTA plasma samples

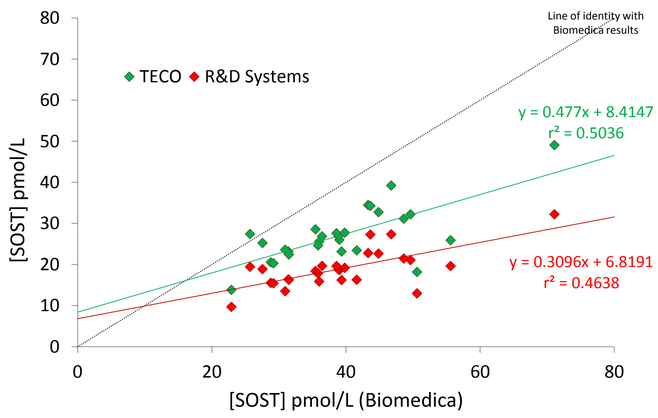


Fig. 2: Chart of plasma [SOST] obtained with R&D Systems and TECO vs Biomedica.

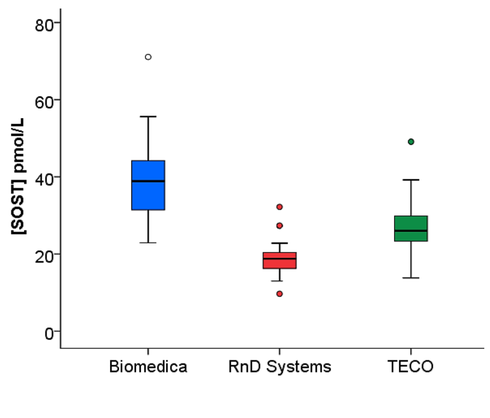


Fig. 3: Box plot showing distribution of [SOST] in plasma obtained with the 3 kits.

- Higher [SOST] obtained with Biomedica (29.5% and 50.6% on average vs TECO and RnD Systems)

- Biomedica:	39.4 ± 10.3 pmol/L	(18.2 pmol/L)	Mean [SOST] in healthy donors as quoted by manufacturers
- R&D Systems:	19.0 ± 4.7 pmol/L	(18.7 pmol/L)	
- TECO:	27.2 ± 6.9 pmol/L	(32.1 pmol/L)	

4-Serum samples

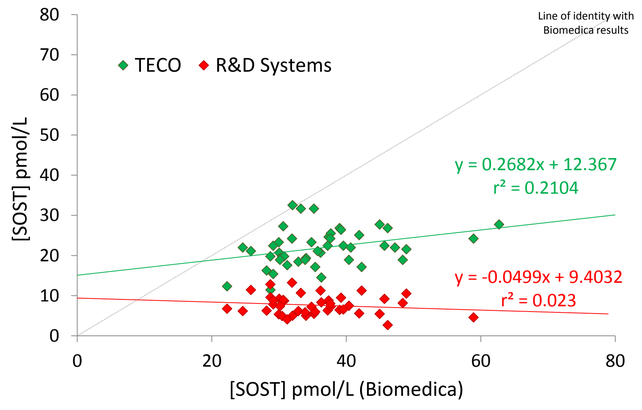


Fig. 4: Chart of serum [SOST] obtained with R&D Systems and TECO vs Biomedica.

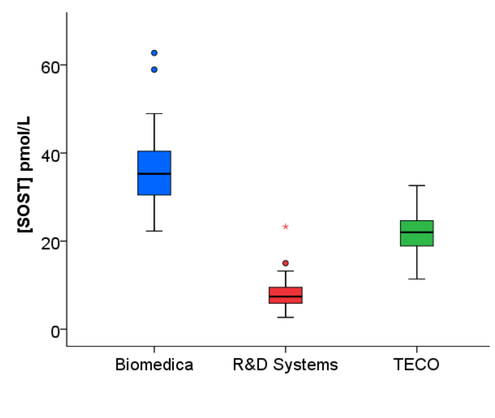
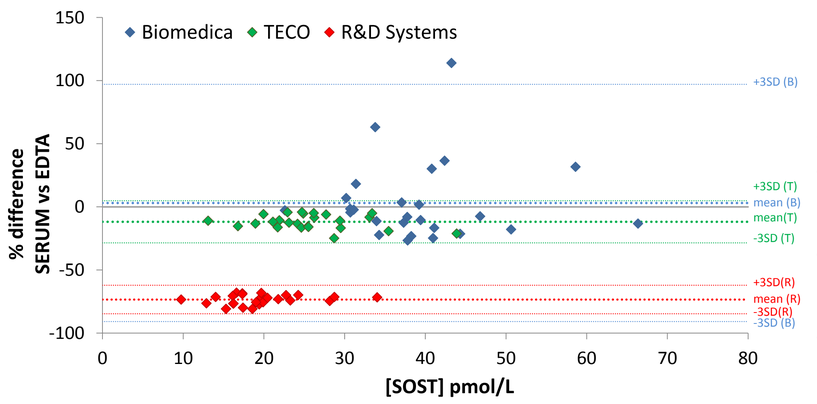


Fig. 5: Box plot showing distribution of [SOST] in serum obtained with the 3 kits.

- Higher [SOST] obtained with Biomedica (40% and 78% on average vs TECO and R&D Systems)

- Biomedica:	36.5 ± 8.3 pmol/L	(24.1 pmol/L)	Mean [SOST] in healthy donors as quoted by manufacturers
- R&D Systems:	8.2 ± 3.5 pmol/L	(7.6 pmol/L)	
- TECO:	21.9 ± 4.7 pmol/L	(26.8 pmol/L)	

5-EDTA vs SERUM



Bland-Altman plot showing the differences in [SOST] between EDTA and Serum samples

Both serum and EDTA samples were from the same patients.

- Serum [SOST] were lower
- R&D systems gave the highest difference.
- Low correlation between serum and EDTA [SOST] when using Biomedica

Conclusions

- Serum [SOST] were higher using Biomedica by up to 62pmol/L (p<0.0001)
- EDTA plasma [SOST] higher using Biomedica by up to 32pmol/L (p<0.0001)
- Except for Biomedica, Serum and plasma [SOST] were also significantly different (p<0.0001 and p<0.03 for R&D and TECO respectively).

The TECO assay demonstrated less variability between duplicates (2.6±2.4 % and 7.3±6.2% and 7.0±5.4% vs Biomedica and R&D respectively). A dilution study showed that the Biomedica kit over-recovered diluted samples by up to 60%.

The variability in values generated from Biomedica, R&D Systems and TECO assays has raised questions regarding the accuracy and specificity of the assays (e.g. antibodies used, interference with the matrix or other proteins). To determine the source of variation between the three kits, specificity experiments are being conducted using external sources of sclerostin.

Measurement of SOST may be invaluable to understand the mechanism by which osteocytes regulate bone turnover, however, until the issues mentioned above are resolved, care should be taken when interpreting the results.

References

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