COMPARISON OF BECKMAN COULTER AMH ELISA GEN II NEW AND OLDER PROTOCOL FOR AMH SERUM DETERMINATION

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OBJECTIVES

In June 2013, Beckman Coulter identified that the monoclonal capture antibody used in AMH ELISA Gen II may interfere with complement present in freshly drawn samples and may generate results 30 % lower than expected. The new protocol (NP) used a 1/6 premix dilution of freshly drawn serum and calibrators to reduce complement interference. AMH values obtained using the NP did not always correlate with antral follicle count. To elucidate if the premix removed all EIA inhibitors, we compared AMH values obtained with NP and old protocol (OP) using fresh and aged serum specimen.

MATERIALS AND METHODS

17 serum samples from non-pregnant women were analyzed. Collection, handling and processing of samples were conducted as per manufacturer's recommendations. Plasma samples were separated in 2 aliquots using different conditioning: fresh serum (FS) stored at -20°C for 7 days and aged serum (AS) kept at room temperature (RT) for 5 days. Testing was performed as per OP or NP manufacturer instructions, in duplicata, the same day.

RESULTS

To verify if the conversion factor provided by Beckman Coulter was appropriate, we compared the testing results of NP and OP performed the same day. The % of difference between the NP and OP was calculated for serum kept at -20°C. As shown in table 1, the mean% difference corresponds to the conversion factor but the standard deviation is wide.

It has been suggested that keeping samples at RT could degrade the complement. Therefore, we compared the OP and NP using two different sample conditioning (FS and AS). Table 2 shows the mean of samples in different conditions. The difference between OP and NP was for samples kept at for 5 days at RT (AS).

In addition, we lost signal when samples were kept 5 days at room temperature with NP. However, we gained signal when samples were kept at RT and using the OP.

Table 1 : AMH concentration comparison between NP and OP for frozen serum

New Protocol (NP)	Old Protocol (OP)	% difference
-20°C frozen samples (FS)	-20°C frozen samples (FS)	(NP vs. OP)
C (ng/ml)	C (ng/ml)	
1,981	1,498	+24,38
2,047	1,368	+33,17
1,865	0,965	+48,26
15,370	7,515	+51,11
13,400	8,527	+36,37
10,110	6,103	+39,63
1,436	0,722	+49,72
6,009	4,534	+24,55
9,424	4,184	+55,60
1,319	0,754	+42,84
6,725	4,417	+34,32
2,448	1,442	+41,09
3,370	2,136	+36,62
1,981	1,423	+28,17
4,593	3,134	+31,77
	Mean (SD) (NP – OP)	+38,51 ± 9,63

Table 2 : AMH mean concentrations comparison between NP and OP for fresh serum and aged serum

Sample conditioning	NP (Mean values)		OP (Mean values)		% difference
	C (ng/ml)		C (ng/ml)		
AS	4,209		3,375		-19,86
FS AS	4,828	4,209			-11,50
FS AS			2,866	3,375	+15,68

AS : Aged serum FS : Fresh serum

DISCUSSION

Table 1 shows that specimen dilution minimizes the complement effect. However, the correction factor based on the average % difference between NP and OP doesn't reflect the large SD probably due to the wide variation of complement levels in humans.

Considering that both dilution (NP) and RT conditioning completely nullify the complement effect, the second line of table 2 shows AMH degradation when samples were kept at RT. The third line of table 2 shows an increase in the immunoreactivity since samples are not diluted (OP) and there is always complement interference. When considering this, we should obtain an increase of 38,51 % instead of the 15,68 % obtained which is explained by the combination of complement degradation at RT, complement interference and AMH degradation at RT.

The NP using samples kept at -20°C is probably the best compromise to minimize complement interference from fresh samples and minimize AMH degradation but may not reduce all ELISA interfering factors present in fresh serum. Development of an international AMH standard is urgently needed to properly assess the ovarian reserve, guide commercial assays calibration and better define interfering factors on AMH serum determination.