

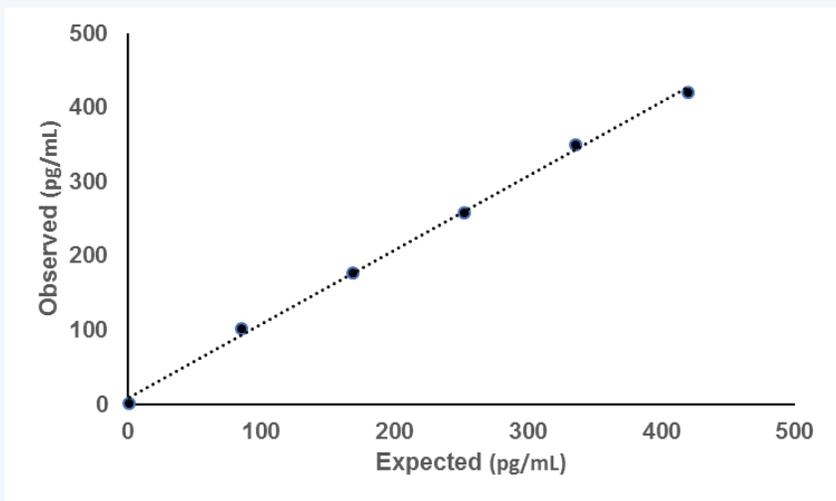
VALIDATION REPORT SUMMARY

The Salivary Oxytocin Assay SalivaLab Assay No.5115

This SalivaLab assay is approved for Research Use Only and has been validated to provide accurate results from normal populations by qualitative saliva in compliance with ISO 13485. All required parameters have met the applicable acceptance criteria for Salimetrics.

*Oxytocin is present at very low levels in saliva that are not reliably measured directly by traditional immunoassay without sample manipulation. The Salimetrics Oxytocin assay is based on a highly sensitive electro-chemiluminescence platform to improve sensitivity over colorimetric detection and requires testing samples in triplicate to gain confidence in values at the lower limit of detection.

Linearity: Admixtures of two saliva samples, one spiked with oxytocin, were prepared. Each sample was run as normal and both mixed in proportions to one another incrementally (7 proportions total). Recovery percentage was calculated by dividing the resultant predicted values by the admixture-based expected values. Results shown are representative of three separate experiments.



Intra Assay Precision: Saliva samples were pooled individually from 5 participants (keeping each participant's saliva separate) for sufficient volume to test 20 replicates of 5 different concentrations spanning the range of the standard curve.

Inter Assay Precision: Inter-assay precision was determined by measuring controls of varying concentrations (n=5) over three separate assay runs.

Intra Assay Precision			Inter Assay Precision		
N	Reported Average pg/mL	% CV	N	Reported Average pg/mL	% CV
15	4.9	33.8	15	28.2	7.9
15	16.3	23.5	15	60.9	1.5
15	27.0	13.1	15	196.3	2.3
15	53.0	11.5			

Assay Drift: Drift samples are ordered to reflect time of addition, Sample 1 plated first and sample 32 plated last. Signal percent difference was calculated to obtain drift.

Assay Drift		
Sample	Signal Mean	% Drift
1	3294	2
32	3354	

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Sample Dilution Recovery: To observe Sample Dilution Recovery, three saliva samples of different salivary oxytocin concentrations were diluted in Oxytocin Assay Diluent and assayed. Results showed that samples that diluted at 2, 4 and 8-fold with Oxytocin assay dilution obtained various recoveries, which ranged between 79% and 114%. The results indicate that saliva samples can be diluted with Oxytocin Assay Diluent, additional supplemental testing of a larger pool of samples is recommended to access Dilution Recovery beyond the x2 dilution.

Sample Dilution Recovery				
Saliva Sample	Dilution	pg/ml	% CV	% Recovery
1	Neat	825	7	
	x2	413	5	108
	x4	206	4	111
	x8	103	8	114
2	Neat	1087	5	
	x2	543	3	85
	x4	272	10	86
	x8	136	3	79
3	Neat	1021	2	
	x2	510	10	88
	x4	255	12	81
	x8	128	9	90

Functional Sensitivity: Functional sensitivity in saliva (LLOQ) defined as $CV \leq 30\%$ is 8 pg/mL. Average LLOD, conservatively defined as concentration corresponding to counts 20% lower than buffer blank, is 4 pg/mL (n = 11).

Spike and Recovery: Fifteen replicates of saliva samples spanning the physiologically relevant lower range of the standard curve were tested for spike/recovery. The 4.9 pg/mL sample is endogenous oxytocin to which indicated amounts of oxytocin was added. Values shown are representative of multiple independent experiments.

Spike and Recovery				
N	Reported Average pg/ml	Added pg/mL	Expected pg/ml	% Recovery
15	4.9	0	4.9	N/A*
15	16.3	10	14.9	109
15	27.0	20	24.9	108
15	53.0	40	44.9	118

Representative Data: Salivary oxytocin levels measured before and at various times after stimulus. Samples were frozen at -20°C immediately after collection.

