

**Validation of Bet v 1 ELISA 2.0 EP (EDQM version) Based on Recovery of Bet v 1
From Spiked Birch Pollen Matrix Samples**

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Below is a summary of InBio's validation of the EDQM version of the Bet v 1 ELISA 2.0 assay (Bet v 1 ELISA 2.0 EP). The data includes measurements of a birch pollen extract (Sample 1) spiked with EDQM rBet v 1 CRS (Samples 2-4) and EDQM rBet v 1 CRS run controls (RC 1-3).

The results of nine spike recovery experiments are provided in the following tables. Table 1 shows the average recovery from all plates: total Bet v 1 recovery ranged from 91-95%; spike recovery ranged from 89-92%. Tables 2-9 are individual results from each plate run by a single operator, and Table 10 shows results from a second operator. Total Bet v 1 recovery from all plates ranged from 84-105%; spike recovery from all plates ranged from 75-112%.

Table 1.

	Total expected Bet v 1 content* [ng/ml]	Total measured Bet v 1 content [ng/ml]	Total recovery [%]	Expected spike rBet v 1 content [ng/ml]	Measured spike sample – Sample 1 [ng/ml]	Spike recovery [%]
Sample 1	30	28.2	94			
Sample 2 (spike)	80	73.7	92	50	45.5	91
Sample 3 (spike)	67.5	61.7	91	37.5	33.5	89
Sample 4 (spike)	55	51.3	93	25	23.1	92
RC 1	50	46.5	93			
RC 2	37.5	35.4	94			
RC 3	25	23.7	95			

*Bet v 1 concentration in birch pollen extract was determined prior to starting the spike recovery experiments.

Table 2.

	Total expected Bet v 1 content [ng/ml]	Total measured Bet v 1 content [ng/ml]	Total recovery [%]	Expected spike rBet v 1 content [ng/ml]	Measured spike sample – Sample 1 [ng/ml]	Spike recovery [%]
Sample 1	30	28.4	95			
Sample 2 (spike)	80	74.0	93	50	45.6	91
Sample 3 (spike)	67.5	60.2	89	37.5	31.8	85
Sample 4 (spike)	55	52.0	95	25	23.6	94
RC 1	50	47.8	96			
RC 2	37.5	35.5	95			
RC 3	25	24.1	96			

Table 3.

	Total expected Bet v 1 content [ng/ml]	Total measured Bet v 1 content [ng/ml]	Total recovery [%]	Expected spike rBet v 1 content [ng/ml]	Measured spike sample – Sample 1 [ng/ml]	Spike recovery [%]
Sample 1	30	28.0	93			
Sample 2 (spike)	80	67.9	85	50	39.9	80
Sample 3 (spike)	67.5	58.3	86	37.5	30.3	81
Sample 4 (spike)	55	48.3	88	25	20.3	81
RC 1	50	46.9	94			
RC 2	37.5	35.1	94			
RC 3	25	23.4	94			

Table 4.

	Total expected Bet v 1 content [ng/ml]	Total measured Bet v 1 content [ng/ml]	Total recovery [%]	Expected spike rBet v 1 content [ng/ml]	Measured spike sample – Sample 1 [ng/ml]	Spike recovery [%]
Sample 1	30	27.7	92			
Sample 2 (spike)	80	73.4	92	50	45.7	91
Sample 3 (spike)	67.5	63.1	93	37.5	35.4	94
Sample 4 (spike)	55	51.6	94	25	23.9	96
RC 1	50	45.6	91			
RC 2	37.5	34.0	91			
RC 3	25	22.1	88			

Table 5.

	Total expected Bet v 1 content [ng/ml]	Total measured Bet v 1 content [ng/ml]	Total recovery [%]	Expected spike rBet v 1 content [ng/ml]	Measured spike sample – Sample 1 [ng/ml]	Spike recovery [%]
Sample 1	30	29.4	98			
Sample 2 (spike)	80	75.2	94	50	45.8	92
Sample 3 (spike)	67.5	61.4	91	37.5	32.0	85
Sample 4 (spike)	55	51.3	93	25	21.9	88
RC 1	50	47.0	94			
RC 2	37.5	35.6	95			
RC 3	25	23.6	94			

Table 6.

	Total expected Bet v 1 content [ng/ml]	Total measured Bet v 1 content [ng/ml]	Total recovery [%]	Expected spike rBet v 1 content [ng/ml]	Measured spike sample – Sample 1 [ng/ml]	Spike recovery [%]
Sample 1	30	29.6	99			
Sample 2 (spike)	80	67.3	84	50	37.7	75
Sample 3 (spike)	67.5	61.8	92	37.5	32.2	86
Sample 4 (spike)	55	49.2	89	25	19.6	78
RC 1	50	43.7	87			
RC 2	37.5	36.1	96			
RC 3	25	23.1	92			

Table 7.

	Total expected Bet v 1 content [ng/ml]	Total measured Bet v 1 content [ng/ml]	Total recovery [%]	Expected spike rBet v 1 content [ng/ml]	Measured spike sample – Sample 1 [ng/ml]	Spike recovery [%]
Sample 1	30	26.2	87			
Sample 2 (spike)	80	79.0	99	50	52.8	106
Sample 3 (spike)	67.5	63.8	95	37.5	37.6	100
Sample 4 (spike)	55	54.3	99	25	28.1	112
RC 1	50	49.1	98			
RC 2	37.5	36.8	98			
RC 3	25	22.5	90			

Table 8.

	Total expected Bet v 1 content [ng/ml]	Total measured Bet v 1 content [ng/ml]	Total recovery [%]	Expected spike rBet v 1 content [ng/ml]	Measured spike sample – Sample 1 [ng/ml]	Spike recovery [%]
Sample 1	30	26.1	87			
Sample 2 (spike)	80	72.4	91	50	46.3	93
Sample 3 (spike)	67.5	60.0	89	37.5	33.9	90
Sample 4 (spike)	55	51.2	93	25	25.1	100
RC 1	50	47.8	96			
RC 2	37.5	34.7	93			
RC 3	25	24.9	100			

Table 9.

	Total expected Bet v 1 content [ng/ml]	Total measured Bet v 1 content [ng/ml]	Total recovery [%]	Expected spike rBet v 1 content [ng/ml]	Measured spike sample – Sample 1 [ng/ml]	Spike recovery [%]
Sample 1	30	30.0	100			
Sample 2 (spike)	80	81.7	102	50	51.7	103
Sample 3 (spike)	67.5	62.9	93	37.5	32.9	88
Sample 4 (spike)	55	54.9	100	25	24.9	100
RC 1	50	42.2	84			
RC 2	37.5	36.0	96			
RC 3	25	26.3	105			

Table 10.

	Total expected Bet v 1 content [ng/ml]	Total measured Bet v 1 content [ng/ml]	Total recovery [%]	Expected spike rBet v 1 content [ng/ml]	Measured spike sample – Sample 1 [ng/ml]	Spike recovery [%]
Sample 1	30	28.0	93			
Sample 2 (spike)	80	72.1	90	50	44.1	88
Sample 3 (spike)	67.5	63.5	94	37.5	35.5	95
Sample 4 (spike)	55	49.0	89	25	21.0	84
RC 1	50	48.0	96			
RC 2	37.5	34.7	93			
RC 3	25	23.7	95			