

## ELISA 2.0 – Validated Performance Parameters

### Inhaled Allergens:

Allergen:	Der p 1	Der f 1	Mite Group 2	Fel d 1	Fel d 4	Can f 1	Mus m 1	Rat n 1
<b>Linearity (R<sup>2</sup>)<sup>1</sup></b>	1.0	0.999	0.999	0.999	1.0	1.0	1.0	1.0
<b>Range (ng/ml)<sup>2</sup></b>	100-0.78	25-0.39	12.5-0.10	25-0.20	5-0.04	25-0.39	25-0.10	12.5-0.20
<b>Limit of Quantification<sup>3</sup></b>								
<i>LLOQ (ng/ml)<sup>3a</sup></i>	1.56-0.39	0.39-0.20	0.40-0.10	0.39-0.20	0.31-0.02	0.39	0.20-0.10	0.39-0.20
<i>ULOQ (ng/ml)<sup>3b</sup></i>	50-25	25-12.5	25-6.25	25-12.5	5	25	25-12.5	50-25
<b>Accuracy (% Recovery)<sup>4</sup></b>								
<i>Intra-assay (n=9)<sup>4a</sup></i>	98-117%	88-118%	83-132%	96-129%	93-106%	83-103%	91-113%	85-129%
<i>Inter-assay (n=54)<sup>4b</sup></i>	103%	106%	104%	113%	97%	91%	103%	102%
<b>Precision (%CV)<sup>5</sup></b>								
<i>Intra-assay (n=9)<sup>5a</sup></i>	4-13%	5-15%	4-14%	4-13%	4-8%	5-18%	5-9%	6-12%
<i>Inter-assay (n=54)<sup>5b</sup></i>	8%	10%	7%	9%	6%	9%	8%	10%

Allergen:	Bla g 1	Bla g 2	Bla g 5	Bet v 1	Bet v 1 EP <sup>6</sup>	Phl p 5	Alt a 1	Asp f 1
<b>Linearity (R<sup>2</sup>)<sup>1</sup></b>	1.0	0.999	0.999	1.0	0.999	1.0	1.0	1.0
<b>Range (ng/ml)<sup>2</sup></b>	50-0.39	100-0.39	125-1.95	12.5-0.39	50-0.39	250-0.98	25-0.10	40-0.31
<b>Limit of Quantification<sup>3</sup></b>								
<i>LLOQ (ng/ml)<sup>3a</sup></i>	0.78-0.20	1.56-0.39	7.81-0.98	0.39	0.39	1.95-0.98	0.40-0.10	0.16-0.63
<i>ULOQ (ng/ml)<sup>3b</sup></i>	50-25	50-25	250-125	25-12.5	50-100	250-62.5	25-6.25	40-20
<b>Accuracy (% Recovery)<sup>4</sup></b>								
<i>Intra-assay (n=9)<sup>4a</sup></i>	71-108%	92-113%	77-113%	79-107%	82-120%	85-120%	90-115%	78-115%
<i>Inter-assay (n=54)<sup>4b</sup></i>	94%	101%	102%	94%	100%	103%	99%	95%
<b>Precision (%CV)<sup>5</sup></b>								
<i>Intra-assay (n=9)<sup>5a</sup></i>	3-9%	5-15%	4-12%	4-14%	1-9%	7-10%	3-11%	4-22%
<i>Inter-assay (n=54)<sup>5b</sup></i>	6%	10%	8%	8%	7%	9%	6%	12%

## Food Allergens:

Allergen:	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Native Bos d 5	Shrimp Tropomyosin
<b>Linearity (R<sup>2</sup>)<sup>1</sup></b>	1.0	0.999	0.999	1.0	1.0	0.999
<b>Range (ng/ml)<sup>2</sup></b>	1000-31.25	125-0.98	62.5-0.49	25-0.05	12.5-0.10	25-0.20
<b>Limit of Quantification<sup>3</sup></b>						
<i>LLOQ (ng/ml)<sup>3a</sup></i>	31.25-15.63	3.91-0.49	1.95-0.49	0.20-0.05	0.78-0.10	0.39-0.10
<i>ULOQ (ng/ml)<sup>3b</sup></i>	1000	250-31.25	62.5-31.25	25-12.5	25-12.5	25-6.25
<b>Accuracy (% Recovery)<sup>4</sup></b>						
<i>Intra-assay (n=9)<sup>4a</sup></i>	82-124%	80-116%	74-127%	105-113%	84-120%	95-115%
<i>Inter-assay (n=54)<sup>4b</sup></i>	109%	101%	97%	108%	103%	108%
<b>Precision (%CV)<sup>5</sup></b>						
<i>Intra-assay (n=9)<sup>5a</sup></i>	3-12%	7-14%	3-11%	7-13%	4-9%	4-14%
<i>Inter-assay (n=54)<sup>5b</sup></i>	8%	10%	5%	8%	6%	7%

- Linearity** is the mean R<sup>2</sup> of six ELISA plates for control curves generated using 4-parameter logistic fit.
- Range** is the average usable range of control curves from six ELISA plates that have a value of 70-130% of the expected concentration, with %CV < 15 between duplicate points.
- Limit of Quantification**
  - LLOQ - The lowest concentration points of six control curves with a recovery of 70-130% and %CV < 15, expressed as a range.
  - ULOQ - The highest concentration points of six control curves with a recovery of 70-130% and %CV < 15, expressed as a range.
- Accuracy**
  - Intra-assay - The range of average percent recovery of samples A, B, and C run in triplicate from six ELISA plates (n=9).
  - Inter-assay - The overall average percent recovery of samples A, B, and C run in triplicate from six ELISA plates (n=54).
- Precision**
  - Intra-assay - The range of average percent coefficient of variation of samples A, B, and C run in triplicate from six ELISA plates (n=9).
  - Inter-assay - The overall average percent coefficient of variation of samples A, B, and C run in triplicate from six ELISA plates (n=54).
- Bet v 1 ELISA 2.0 EP has been cross-validated against the candidate Ph. Eur. Method for Bet v 1 determination.

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