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ELISA 2.0 Inhalable Allergens – Validated Performance Parameters

	HOUSE DUST MITE							STORAGE MITES	ANIMAL					
Allergen:	**Der p 1**	**Der p 1a**	**Der p 23**	**Der p 2**	**Der f 1**	**Der f 2**	**Blo t 5**	**Tyr p 2**	**Fel d 1**	**Fel d 4**	**Can f 1**	**Mus m 1**	**Ory c 3**	**Rat n 1**
Linearity (R^2)[1]	1.0	1.0	0.998	0.999	0.999	0.999	0.999	0.999	0.999	1.0	1.0	1.0	0.999	1.0
Range (ng/ml)[2]	100-0.78	50-1.56	50-0.10	12.5-0.10	25-0.39	62.5-0.98	7.5-0.12	250-7.81	25-0.20	5-0.04	25-0.39	25-0.10	20-0.16	50-0.39
Limit of Quantification[3]														
LLOQ (ng/ml)[3a]	0.39-1.56	0.39-1.56	0.391.56	0.10-0.40	0.20-0.39	0.49-1.95	0.12-0.23	1.95-7.80	0.20-0.39	0.02-0.31	0.39	0.10-0.20	0.08-0.31	0.20-0.39
ULOQ (ng/ml)[3b]	50-25	50-6.25	25-12.5	25-6.25	25-12.5	250-62.5	30-3.75	500-250	25-12.5	5	25	25-12.5	20-5	50-25
Accuracy (% Recovery)[4]														
Intra-assay (n=9)[4a]	98-117%	82-129%	87-129%	83-132%	88-118%	73-118%	84-130%	84-132%	96-129%	93-106%	83-103%	91-113%	92-126%	85-129%
Inter-assay (n=54)[4b]	103%	107%	101%	104%	106%	88%	105%	108%	113%	97%	91%	103%	104%	102%
Precision (%CV)[5]														
Intra-assay (n=9)[5a]	4-13%	1-23%	2-25%	4-14%	5-15%	1-12%	1-16%	3-14%	4-13%	4-8%	5-18%	5-9%	2-14%	6-12%
Inter-assay (n=54)[5b]	8%	11%	8%	7%	10%	7%	8%	6%	9%	6%	9%	8%	6%	10%

	COCKROACH			POLLENS						MOLDS		CANNABIS
Allergen:	**Bla g 1**	**Bla g 2**	**Bla g 5**	**Per a 7**	**Bet v 1 EP**	**Phl p 5**	**Amb a 1**	**Lol p 1**	**Cry j 1**	**Alt a 1**	**Asp f 1**	**Can s 3**
Linearity (R^2)[1]	1.0	0.999	0.999	0.999	0.999	1.0	1.0	0.994	1.0	1.0	1.0	1.0
Range (ng/ml)[2]	50-0.39	100-0.39	125-1.95	12.5-0.20	50-0.39	250-0.98	100-0.78	125-7.81	50-0.78	25-0.10	40-0.31	62.5-0.49
Limit of Quantification[3]												
LLOQ (ng/ml)[3a]	0.20-0.78	0.39-1.56	0.98-7.81	0.19-0.39	0.39	1.95-0.98	1.56-0.78	15.63-3.91	0.78-0.39	0.40-0.10	0.16-0.63	0.24-0.49
ULOQ (ng/ml)[3b]	50-25	50-25	250-125	50-6.25	100-50	250-62.5	200-25	250-125	50-25	25-6.25	40-20	62.5
Accuracy (% Recovery)[4]												
Intra-assay (n=9)[4a]	71-108%	92-113%	77-129%	70-121%	82-120%	85-120%	90-108%	81-112%	94-106%	90-115%	78-115%	88-127%
Inter-assay (n=54)[4b]	94%	101%	102%	99%	100%	103%	100%	96%	100%	99%	95%	106%
Precision (%CV)[5]												
Intra-assay (n=9)[5a]	3-9%	5-15%	3-16%	2-15%	1-9%	7-10%	6-12%	7-16%	6-16%	3-11%	4-22%	2-19%
Inter-assay (n=54)[5b]	6%	10%	8%	6%	7%	9%	8%	12%	10%	6%	12%	11%

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Validation Notes:

1. **Linearity** is the mean R^2 of six ELISA plates for control curves generated using 4-parameter logistic fit.
2. **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.
3. **Limit of Quantification**
   - 3a. LLOQ - The lowest concentration points of six control curves with a recovery of 70-130% and %CV <15, expressed as a range.
   - 3b. ULOQ - The highest concentration points of six control curves with a recovery of 70-130% and %CV <15, expressed as a range.
4. **Accuracy**
   - 4a. Intra-assay - The range of average percent recovery of samples A, B, and C run in triplicate from six ELISA plates (n=9).
   - 4b. Inter-assay - The overall average percent recovery of samples A, B, and C run in triplicate from six ELISA plates (n=54).
5. **Precision**
   - 5a. 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).
   - 5b. Inter-assay - The overall average percent coefficient of variation of samples A, B, and C run in triplicate from six ELISA plates (n=54).
6. Bet v 1 ELISA 2.0 EP has been cross-validated against the candidate Ph. Eur. Method for Bet v 1 determination.