

## MARIA® for Inhalable Allergens - 1-plex Validated Performance Parameters

Allergen:	Mite								
	Der p 1	Der f 1	Group 2	Der p 23	Blo t 5	Fel d 1	Can f 1	Mus m 1	Rat n 1
<b>Linearity (R<sup>2</sup>)<sup>1</sup></b>	0.998	0.999	0.996	0.997	0.997	0.997	0.998	0.998	0.998
<b>Range (ng/ml)<sup>2</sup></b>	125-0.06	125-0.06	25-0.02	10-0.005	15-0.01	12.5-0.02	125-0.06	12.5-0.01	50-0.02
<b>Limit of Quantification<sup>3</sup></b>									
<i>LLOQ (ng/ml)<sup>3a</sup></i>	0.06-0.24	0.06	0.02-0.05	0.005-0.01	0.01	0.02	0.06-0.98	0.01	0.02-0.78
<i>ULOQ (ng/ml)<sup>3b</sup></i>	125	62.5-15.63	25-6.25	10-0.63	15-3.75	50-6.25	125	12.5	50
<b>Accuracy (% Recovery)<sup>4</sup></b>									
<i>Intra-assay (n=9)<sup>4a</sup></i>	100-124%	85-107%	107-112%	72-94%	77-113%	97-129%	104-120%	108-125%	103-120%
<i>Inter-assay (n=36)<sup>4b</sup></i>	112%	96%	109%	84%	99%	107%	112%	117%	109%
<b>Precision (%CV)<sup>5</sup></b>									
<i>Intra-assay (n=9)<sup>5a</sup></i>	4-10%	5-8%	3-8%	2-12%	3-15%	4-6%	4-7%	3-9%	4-5%
<i>Inter-assay (n=36)<sup>5b</sup></i>	7%	6%	6%	5%	7%	5%	6%	5%	4%

Allergen:	Guinea Pig							
	UP	Bla g 2	Alt a 1	Asp f 1	Bet v 1 EP	Phl p 5	Amb a 1	Lol p 1
<b>Linearity (R<sup>2</sup>)<sup>1</sup></b>	0.998	0.999	0.998	0.997	0.995	0.998	0.999	0.999
<b>Range (ng/ml)<sup>2</sup></b>	1000-0.49	125-0.06	25-0.05	20-0.01	100-0.05	100-0.20	100-0.05	500-1.0
<b>Limit of Quantification<sup>3</sup></b>								
<i>LLOQ (ng/ml)<sup>3a</sup></i>	0.49	0.06-0.49	0.02-0.05	0.02-0.31	0.05-0.78	0.05-0.78	0.05-0.10	0.49-1.95
<i>ULOQ (ng/ml)<sup>3b</sup></i>	1000-500	125	50-25	20-10	100	100-50	100	500
<b>Accuracy (% Recovery)<sup>4</sup></b>								
<i>Intra-assay (n=9)<sup>4a</sup></i>	91-125%	117-126%	87-106%	96-120%	75-125%	82-102%	82-121%	93-122%
<i>Inter-assay (n=36)<sup>4b</sup></i>	107%	122%	94%	112%	92%	93%	103%	107%
<b>Precision (%CV)<sup>5</sup></b>								
<i>Intra-assay (n=9)<sup>5a</sup></i>	1-12%	2-4%	3-6%	3-6%	1-13%	2-10%	2-9%	2-17%
<i>Inter-assay (n=36)<sup>5b</sup></i>	6%	4%	5%	3%	5%	5%	5%	7%

### **Validation Notes:**

1. **Linearity** is the mean  $R^2$  of four MARIA plates for control curves generated using 4-parameter logistic fit.
2. **Range** is the average usable range of control curves from four MARIA 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 four control curves with a recovery of 70-130% and %CV <15, expressed as a range.
  - 3b. ULOQ - The highest concentration points of four 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 four MARIA plates (n=9).
  - 4b. Inter-assay - The overall average percent recovery of samples A, B, and C run in triplicate from four MARIA plates (n=36).
5. **Precision**
  - 5a. Intra-assay - The range of average percent coefficient of variation of samples A, B, and C run in triplicate from four MARIA plates (n=9).
  - 5b. Inter-assay - The overall average percent coefficient of variation of samples A, B, and C run in triplicate from four MARIA plates (n=36).