Stephanie Filep
Director of Laboratory Services
700 Harris Street
Charlottesville
VA 22903
U.S.A.

TEL: (1) (434) 984 2304
FAX: (1) (434) 984 2709
www.inbio.com

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New Formulation of Amb a 1 ELISA 2.0 and MARIA®

Indoor Biotechnologies would like to inform customers of the transition from the traditional Amb a 1 ELISA format to ELISA 2.0 and MARIA®. The redeveloped assays provide increased sensitivity and dynamic range and improved quantification and standardization. Everything about the Amb a 1 assays is new: monoclonal capture antibody, biotinylated monoclonal detection antibody and an assay standard formulated from purified, natural Amb a 1. Effective immediately Indoor Biotechnologies will cease to manufacture the traditional Amb a 1 ELISA due to inconsistencies in subsequent polyclonal antibody production batches.

The original ELISA kit (Product Code EL-AM1) using a purified rabbit polyclonal capture antibody (PA-AM1), a biotinylated purified rabbit polyclonal detection antibody (BI-AM1) and Short Ragweed extract standard (ST-AM1 Lot# 32094) is being replaced by Amb a 1 ELISA 2.0 (EPC-AM1) and Amb a 1 MARIA®. Each kit comprises the following reagents:

ELISA 2.0

- 96 well polystyrene microtiter plate coated with mAb 2B6, anti-Amb a 1 (Product Code MA-2B6, Lot# 41118) and treated with stabilizing agent.
- Biotinylated mAb 4H7, anti-Amb a 1 (Product Code BI-4H7, Lot# 41102)
- Natural Amb a 1 standard (Product Code PST-AM1, Lot# 41088)
- Wash and assay buffer concentrates, TMB developing substrate and stop solution.

MARIA®

- mAb 2B6, anti-Amb a 1-coupled microspheres, bead region #46 (Product Code MS-AM1, Lot# 41098).
- Biotinylated mAb 4H7, anti-Amb a 1 (Product Code BI-4H7, Lot# 41102)
- Natural Amb a 1 standard (Product Code ST-AM1, Lot# 41088)
- Streptavidin R-phycoerythrin Conjugate (SAPE)

Complete method validations were performed for both new Amb a 1 immunoassays to determine parameters of linearity, range, limits of quantification and detection, accuracy and precision (REC-072 and REC-073). Although the newly-developed assays incorporate different antibody pairs, results obtained with the new Amb a 1 standard correlate well with the previous standard (Figure 1). A thorough evaluation of thirty-seven commercial Ragweed extracts were compared in the pAb Amb a 1 ELISA, mAb Amb a 1 ELISA 2.0 and MARIA® assays (Figure 2).

Advantages of Amb a 1 ELISA 2.0 and MARIA are as follows:

1) Continuity of Amb a 1 determinations using monoclonal antibody pairs with faster development time, decreased background signal and improved specificity.

2) Increased sensitivity. The sensitivity of ELISA 2.0 has increased by 2.5-fold compared to the pAb Amb a 1 kit, from 1.95ng/ml to 0.78ng/ml. The MARIA® assay offers ~40-fold increased sensitivity compared to the pAb Amb a 1 kit.
3) The use of natural Amb a 1 standard with protein content determined by amino acid analysis allows accordance with international standardization guidelines recommended by the WHO/IUIS Allergen Standardization Sub-committee. Previously, results were expressed as Units allergen per milliliter using the Short Ragweed extract standard (Lot# 32094) with estimates of 1U=1µg. Calculations using the natural Amb a 1 Standard Lot# 41088 result in a corrected conversion of 1U=0.40µg.

To re-emphasize, Indoor Biotechnologies will be discontinuing the traditional Amb a 1 ELISA effective immediately. The new ELISA 2.0 and MARIA® assays and services are available forthwith.

Indoor Biotechnologies is committed to providing the highest quality quantitative ELISA and MARIA® kits and continuously improving our immunoassay reagents based on the best available data and products. We recognize that changes in standards can be disruptive, especially for allergenic products manufacturers, and encourage you to contact us if you require any further information.

Sincerely,

Stephanie Filep, BS
Director of Laboratory Services

Figure 1: Comparison of Amb a 1 dose response curves with the pAb ELISA and Short Ragweed Extract ST-AM1 Lot# 32094 versus the mAb ELISA 2.0 and Natural Amb a 1 ST-AM1 Lot# 41088.

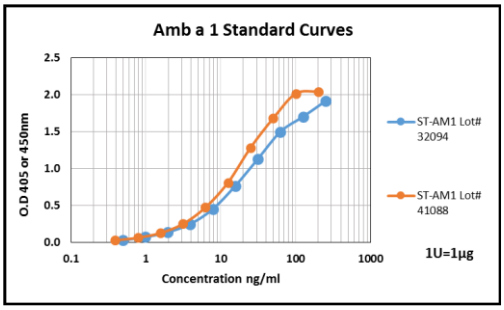
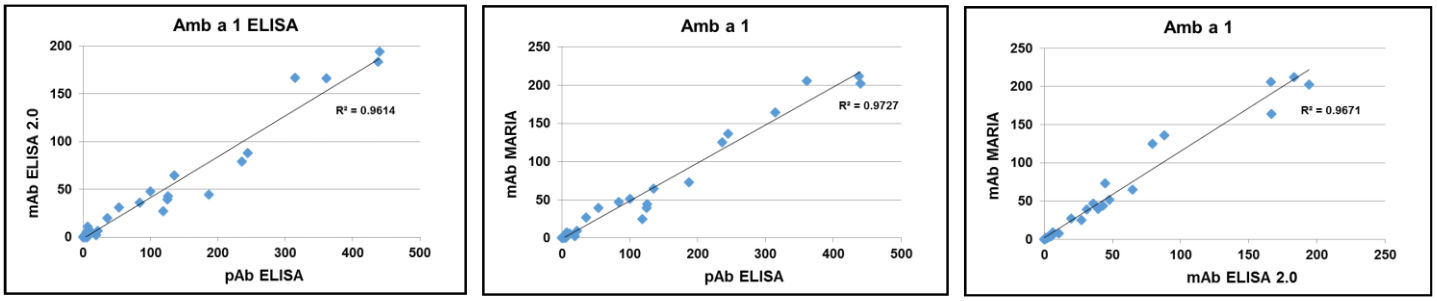


Figure 2: Comparison of Amb a 1 levels in Ragweed extracts using polyclonal ELISA, ELISA 2.0 and MARIA®.



NOTES:

1) Amb a 1 standard (ST-AM1, Lot# 41088) was prepared using natural Amb a 1 purified from Short Ragweed pollen (*Ambrosia artemisiifolia*) (Product Code NA-AAR1-1), with protein content determined by amino acid analysis. The Amb a 1 standard (ST-AM1, Lot# 32094) was formulated using Short Ragweed extracts and sub-standardized against the U.S. Food and Drug Administration radial immunodiffusion reference for Amb a 1, C14-Ras, which contains 30 Units Amb a 1/ml.

2) The Amb a 1 ELISA 2.0 and MARIA® assays were evaluated for potential cross-reactivity using commercially available ragweed extracts from U.S. manufacturers. The Amb a 1 assays were specific for Short Ragweed and showed slight reactivity to Western, Giant/Tall and False Ragweed as expected, but not Slender, Desert or Southern Ragweed.

3) When comparing the polyclonal ELISA with the new monoclonal ELISA 2.0, results for Short Ragweed and Ragweed Mix extracts were 2.7-fold higher in the polyclonal ELISA and 1.6-fold higher for Western, Giant/Tall and False Ragweed extracts.

4) Amb a 1 ELISA 2.0 was also tested for cross-reactivity to Mugwort extracts, natural Cry j 1 (Japanese Cedar tree pollen) and Japanese Cedar tree pollen extracts. These samples were not detectable in the assay.

Title: Amb a 1 MARIA Method Validation Record		**Record Date:** Feb. 28, 2018
Author: Bryan Smith	**Approved By:**	**Version:** 1.0

PURPOSE: To provide a final report form for MARIA 1-plex immunoassay validation results.

SCOPE: This report shall be completed by laboratory personnel conducting MARIA validation testing on any current or new immunoassays developed and manufactured at Indoor Biotechnologies, Inc.

CHANGE CONTROL: This report shall be updated as necessary per the established applicable change control procedure(s).

VALIDATION PARAMETERS:

Linearity
Range
Limits of Quantification and Detection
Accuracy
Precision

RESULTS:

MARIA 1-plex: Amb a 1; MRA-C1
Microspheres: Amb a 1 2B6 Beads; MS-AM1 # 41098
Allergen Standard: Amb a 1 Allergen Standard; ST- AM1 #41088
Biotinylated antibody: Biotin 4H7, anti- Amb a 1; BI-4H7 #41102
Streptavidin-Phycoerythrin: SAP-MRA # 1905131

Table 1: Assay Linearity

Plate #	Operator	Assay Day	R^2	P/F
1	**1**	**1**	0.9975	P
2		**2**	0.9973	P
3	**2**	**1**	0.9998	P
4		**2**	0.9999	P
Average = 0.9986				

*Pass acceptance criteria = a R^2 value equal to or greater than 0.99

Figure 1: Control Curve

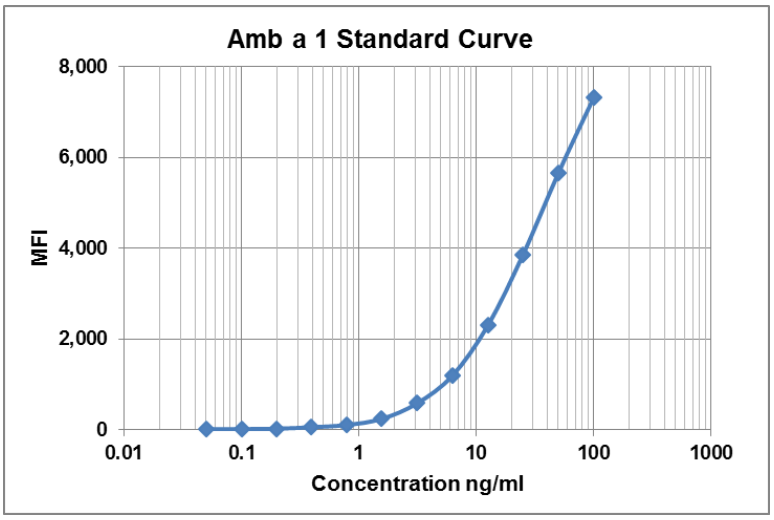


Table 2: Range

STD No.	Concentration (ng/ml)	Accuracy (mean %Recovery)	Precision (mean %CV)	Pass/Fail
S1	100	**105**	**7**	**P**
S2	50	**99**	**2**	**P**
S3	25	**102**	**4**	**P**
S4	12.5	**101**	**2**	**P**
S5	6.25	**100**	**5**	**P**
S6	3.13	**100**	**6**	**P**
S7	1.56	**99**	**11**	**P**
S8	0.78	**99**	**7**	**P**
S9	0.39	**105**	**8**	**P**
S10	0.2	**98**	**12**	**P**
S11	0.1	**98**	**6**	**P**
S12	0.05	**105**	**9**	**P**

*Pass acceptance criteria = % mean recovery between 70-130% **and** mean coefficient of variation $\leq$ 15%.

Assign each standard point a Pass or Fail (P/F) status in the right hand column of the table above. The range is assigned to the set of continuous points that pass the acceptance criteria. The upper and lower points of this acceptable range will be designated by two bold green lines.

Table 3: Limit of Quantification

Plate #	LLOQ	ULOQ	LOD
1	0.05	100	0.05
2	0.05	100	0.05
3	0.10	100	0.05
4	0.05	100	0.05

Table 4: Accuracy

Sample	n=	Accuracy: Mean % Recovery per Plate			
Intra-assay	**per plate**	**Plate 1**	**Plate 2**	**Plate 3**	**Plate 4**
Std. A – ng/mL	3	82	98	103	109
Std. B – ng/mL	3	95	101	114	121
Std. C – ng/mL	3	94	109	108	103
Inter-assay	**total**	**Accuracy: Mean % Recovery Between 4 Plates**			
Std. A – ng/mL	12	98			
Std. B – ng/mL	12	108			
Std. C – ng/mL	12	104			

*Pass acceptance criteria = % mean recovery between 70-130%

Table 5: Precision

Sample	n=	Precision: Mean % CV per Plate			
Intra-assay	**per plate**	**Plate 1**	**Plate 2**	**Plate 3**	**Plate 4**
Std. A – ng/mL	3	2	6	4	7
Std. B – ng/mL	3	9	4	8	2
Std. C – ng/mL	3	2	5	3	5
Inter-assay	**total**	**Accuracy: Mean % Recovery Between 4 Plates**			
Std. A – ng/mL	12	5			
Std. B – ng/mL	12	6			
Std. C – ng/mL	12	4			

*Pass acceptance criteria = mean coefficient of variation $\leq$ 15%

Revision

Date	Version	Description
Feb. 28, 2018	1.0	Original version

Title: Amb a 1 ELISA 2.0 Method Validation Record		Record Date: Mar. 6, 2018
Author: Bryan Smith	Approved By:	Version: 1.0

PURPOSE: To provide a final record form for ELISA 2.0 immunoassay validation results following SOP-036.

SCOPE: This record shall be completed by laboratory personnel conducting ELISA 2.0 validation testing on any current or new immunoassays developed and manufactured at Indoor Biotechnologies, Inc.

CHANGE CONTROL: This record shall be updated as necessary per the established applicable change control procedure(s) (see SOP-008).

VALIDATION PARAMETERS:

Linearity
Range
Limits of Quantification and Detection
Accuracy
Precision

RESULTS:

ELISA Kit: Amb a 1; EPC-AM1-1 and EPC-AM1-5
Pre-coated Plate: mAb 2B6, anti-Amb a 1 #41118 coated plate; EPL-AM1
Allergen Standard: Amb a 1 Allergen Standard; PST-AM1 #41088
Biotinylated antibody: Biotin 4H7, anti-Amb a 1; BI-4H7 #41102

*For raw data files refer to DATA (\\elisa\shares) (Y:)>data>ELISA 2.0 Method Validations>Amb a 1

Table 1: Assay Linearity

Plate #	R^2	P/F
1	1	P
2	1	P
3	1	P
4	1	P
5	0.999	P
6	0.999	P
	Average =	**1**

*Pass acceptance criteria = an R^2 value equal to or greater than 0.99

Figure 1: Representative Control Curve

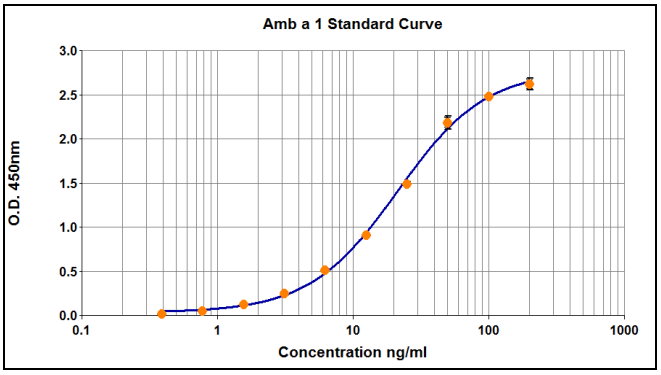


Table 2: Range

STD No.	Concentration (ng/ml)	Accuracy (mean %Recovery)	Precision (mean %CV)	Pass/Fail
1	200	**90**	**NA**	**F**
2	100	**105**	**9**	**P**
3	50	**98**	**9**	**P**
4	25	**102**	**4**	**P**
5	12.50	**98**	**2**	**P**
6	6.25	**103**	**7**	**P**
7	3.13	**103**	**5**	**P**
8	1.56	**102**	**8**	**P**
9	0.78	**89**	**12**	**P**
10	0.39	**NA**	**NA**	**F**

*Pass acceptance criteria = % mean recovery between 70-130% **and** mean coefficient of variation ≤15%.

Assign each standard point a Pass or Fail (P/F) status in the right hand column of the table above. The range is assigned to the set of continuous points that pass the acceptance criteria. The upper and lower points of this acceptable range will be designated by two bold black lines.

Table 3: Limit of Quantification

Plate #	LLOQ	ULOQ	LOD
1	0.78	100	1.56
2	1.56	200	0.78
3	0.78	100	0.78
4	1.56	200	0.78
5	0.78	50	0.78
6	1.56	25	0.78

Table 4: Accuracy

Sample	n=	Accuracy: Mean % Recovery per Plate					
Intra-assay	**Per Plate**	**Plate 1**	**Plate 2**	**Plate 3**	**Plate 4**	**Plate 5**	**Plate 6**
A	3	105	95	98	93	87	84
B	3	111	87	103	93	103	108
C	3	108	88	109	90	110	120
Inter-assay	**Total**	**Accuracy: Mean % Recovery Between 6 Plates**					**P/F**
A	18	94					**P**
B	18	101					**P**
C	18	104					**P**

*Pass acceptance criteria = % mean recovery between 70-130%

Table 5: Precision

Sample	n=	Precision: Mean % CV per Plate					
Intra-assay	**Per Plate**	**Plate 1**	**Plate 2**	**Plate 3**	**Plate 4**	**Plate 5**	**Plate 6**
A	3	7	7	8	6	13	10
B	3	3	6	11	6	6	11
C	3	7	4	7	13	7	15
Inter-assay	**Total**	**Precision: Mean % CV Between 6 Plates**					**P/F**
A	18	9					**P**
B	18	7					**P**
C	18	9					**P**

*Pass acceptance criteria = mean coefficient of variation $\leq$15%

Revision

Date	Version	Description
Mar. 6, 2018	1.0	Original version