Major Allergen Measurements: Sources of Variability, Validation, Quality Assurance and Utility for Laboratories, Manufacturers and Clinics

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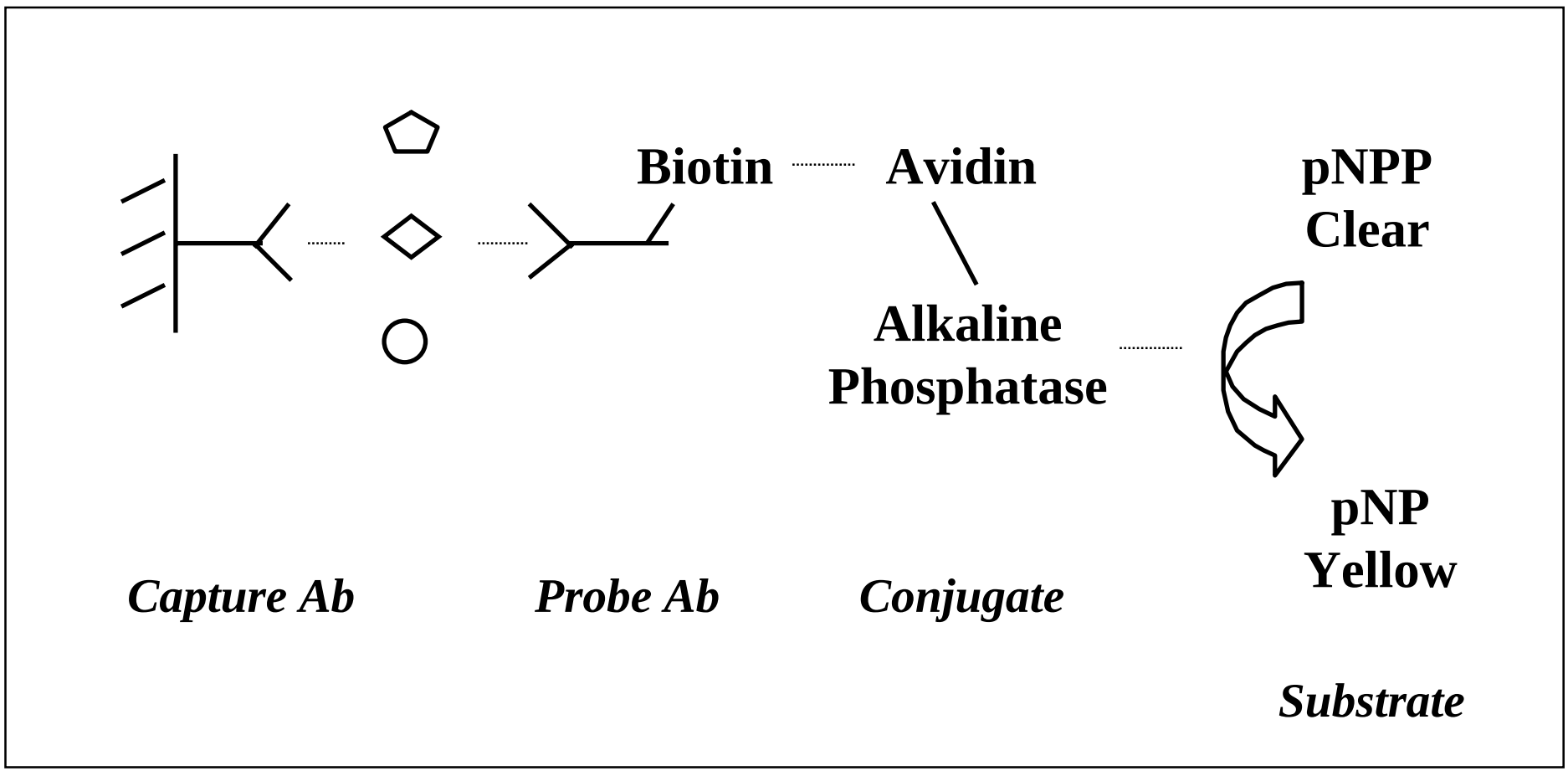
Overview

- **Major allergen levels are important indicators of extract consistency and quality**
- **Quantitative assays specific for major allergens are developed and performed in numerous laboratories**
- **Maintenance IT doses based on major allergen content (µg per injection) are recommended in position papers, workshops and seminars**

Major Allergen Assay Differences

- **Assay configuration or format**
  - **Double-Bind ELISA, ELISA Inhibition, RID, CRIE**
- **Antibody and allergen reagents**
- **Purified allergens, reference extracts and calibration procedures**
- **Assay conditions, performance characteristics and validation requirements**
  - **Sensitivity, specificity and linearity**
  - **Precision, reproducibility, accuracy, robustness and stability**

Double-Bind (Sandwich) ELISA



Critical Factors

- **Capture and probe antibody may recognize distinct major allergen sequences or 3D conformations**
- **Allergen molecules must be multivalent to produce positive reactions**
- **Nanogram-level sensitivities for many allergens**
- **Parallel dose responses for pure antigens, references and test extracts required for accurate test results**

Questions and Concerns

- **Intralaboratory vs. interlaboratory variability**
  - **Single and multiple (interactive) variables**
- **Precision and reproducibility of test results**
  - **Differences due to analyst, solid phase and reagent lots**
- **Qualification of new antibody/allergen references**
- **Comparability of multiple assay formats**
  - **One laboratory, same antibody and allergen reagents**
  - **Different laboratories, same or different assay reagents**

Variable: Analyst

- **3 Analysts**
  Primary + 2 alternates
- **5 Double-Bind ELISA assays**
  Alt a 1, Der f 1, Can f 1, Sol i 3, Ves 5
- **Optimal plates, reagents and incubations**
- **6 Independent evaluations of each assay**
  2 per analyst, 14-23 valid dilutions per analyte

Analyte	Extract	Mean conc. ± 1 SD (µg/mL)	%CV
Alternaria	Aq	24.1-37.5	21.8
Alt a 1	Glyc	13.1-15.0	7.0
D. farinae	Std	80.4-139	26.7
Der f 1	FD	123-184	19.9
Dog	Aq Ep	0.605-0.827	15.5
Can f 1	Glyc H/D	218-288	14.0
Fire ant	Aq	2.11-4.97	40.4
Sol i 3	Glyc	0.677-1.47	37.0
Y. jacket	Glyc lot 1	152-208	15.4
Antigen 5	Glyc lot 2	109-153	16.8

Variable: Microplate

- **3 Plates run by primary analyst**
  Validated plate + 2 alternates
- **5 Double-Bind ELISA assays**
  Alt a 1, Der f 1, Can f 1, Sol i 3, Ves 5
- **Optimal reagents and incubations**
- **6 Independent evaluations of each assay**
  2 per plate, 12-25 valid dilutions per analyte

Analyte	Extract	Mean conc. ± 1 SD (µg/mL)	%CV
Alternaria	Aq	20.9-34.3	21.8
Alt a 1	Glyc	9.8-17.0	7.0
D. farinae	Std	60.2-161	41.4
Der f 1	FD	135-175	12.9
Dog	Aq Ep	0.60-0.84	16.4
Can f 1	Glyc H/D	191-358	30.5
Fire ant	Aq	1.84-3.18	26.8
Sol i 3	Glyc	0.88-1.14	13.2
Y. jacket	Glyc lot 1	146-225	21.2
Antigen 5	Glyc lot 2	113-167	19.2

Variable: Coat Ab lot + BiAb dilution

Dog albumin Double-Bind ELISA assay

- **2 Lots of Coating antibody**
  Original + replacement
- **2 Dilutions of Biotinylated antibody**
- **2 Lots of 1:10 *w/v* Dog epithelia extract**

1° Analyst, Optimal reagents and incubations

BiAb dilution	Dog lot #	Mean conc. ± 1 SD (µg/mL) Coat Ab lot A	Coat Ab lot B	% of lot A
1:5,000	1	546-656	490-654	90-100
	2	259-645	289-589	91-112
1:1,000	1	726-1040	539-717	69-74
	2	645-959	382-764	59-80

Variable: Coat Ab source + Plate location

Dog Can f 1 Double-Bind ELISA assay

- **2 Sources of Coating antibody**
  Mouse monoclonal IgG + Rabbit polyclonal IgG

- **2 Plate locations for Test extracts**
  Left half of plate (columns 1-6) vs. right half (columns 7-12)

- **2 Dog extracts** **Epithelia + Hair/dander**

1° Analyst, Optimal reagents and incubations

Dog extract	Coat Ab source	Mean conc. ± 1 SD (µg/mL) Plate col. 1-6	Plate col. 7-12	% of col. 1-6
Epithelia	Mouse	0.60-0.81	0.56-0.78	93-96
	Rabbit	0.57-0.80	0.62-0.70	88-109
Dander	Mouse	215-287	179-283	83-99
	Rabbit	234-374	40-142	17-38

Variable: Coat Ab storage buffer

Egg white Double-Bind ELISA assay

- **2 Coating antibody storage buffers**
  Borate, pH 8.4 + Phosphate-buffered saline (PBS), pH 7.4

- **3 Egg extracts**
  Egg white + Whole egg + Egg yolk

- **1° Analyst, Optimal reagents and incubations**

Coat Ab storage buffer	Extract	Mean conc. (µg/mL)	% of Egg white µg/mL
Borate	Egg white	3770	100
	Whole egg	4585	122
	Egg yolk	3229	86
PBS	Egg white	2966	100
	Whole egg	1658	56
	Egg yolk	615	21

Variable: Assay format

- **Alternaria Alt a 1 assays**
- **Rabbit anti-Alt a 1 (11 kd hypoallergen fragment)**
- **4 Extracts from distinct *A. alternata* strains**
- **4 Assay formats**

  ELISA inhibition, Double-Bind ELISA,

  Radial immunodiffusion (RID), SDS-PAGE immunoblotting

Extract/ strain #	Rel. Potency vs. extract #1			Blot intensity
	ELISA inhib	DB ELISA	RID	30-35 kd
1	1.0	1.0	1.0	++
2	0.7	0.2	0.3	+/-
3	0.2	0.04	0.0	+/-
4	0.5	0.004	1.6	++++

Conclusions

- **Differences in major allergen values may be caused by assay differences as well as extract variations**
- **Accuracy of major Ag values (and IT dose ranges) are likely to vary from vendor-vendor and lab-lab**
- **Assay performance based on defined references, formats, cal/qual methods and lab-lab comparability**
- **Validated major Ag assays are well suited for consistency monitoring/standardization of extracts**