

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

**TJ Grier, PhD, DM Hazelhurst, BS,  
EA Duncan, BS, TK West, BS and RE Esch, PhD**

***Greer Laboratories, Inc.  
Lenoir, North Carolina USA***

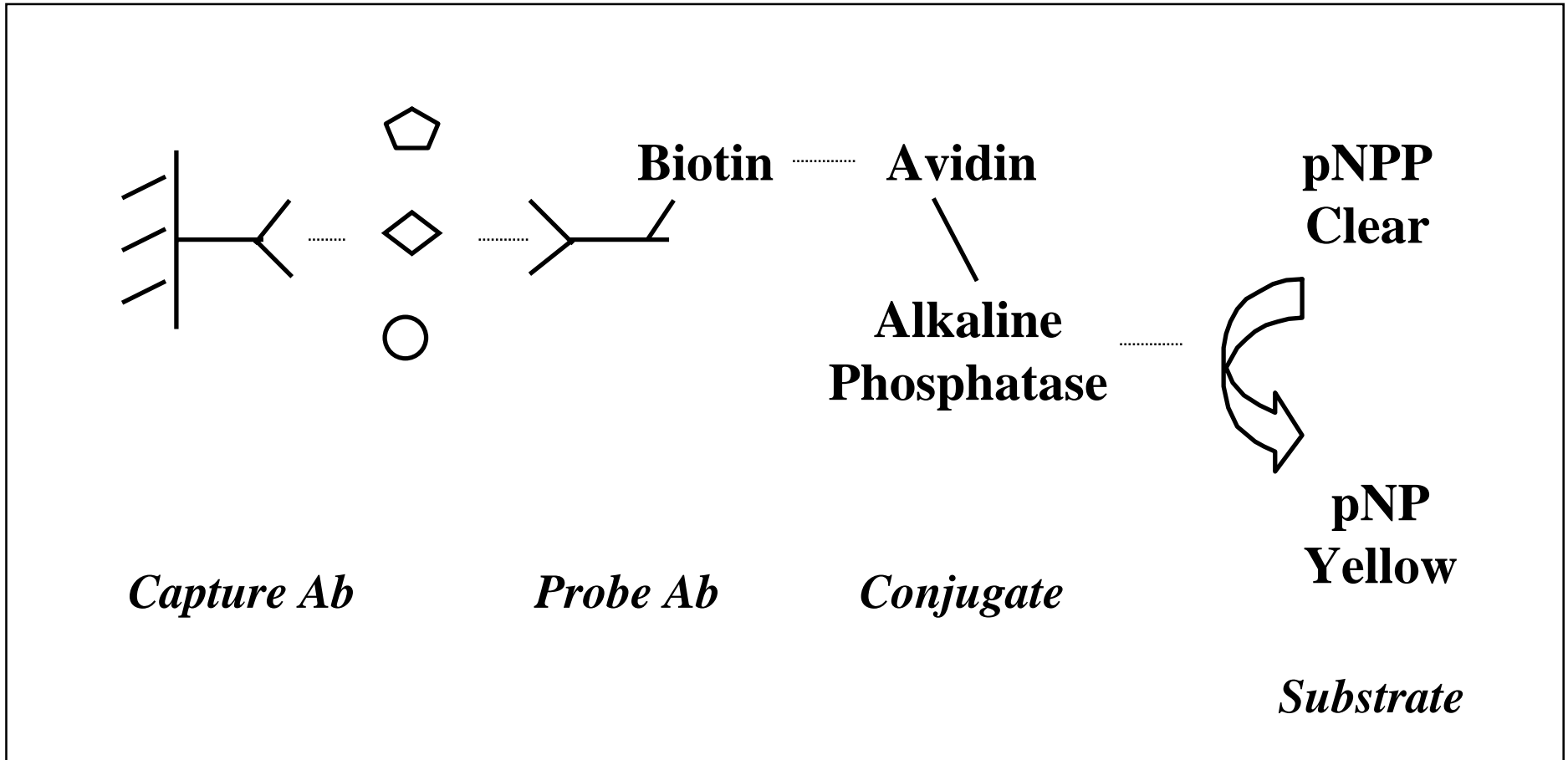
# *Overview*

- **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 ( $\mu\text{g}$  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

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

# *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

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

# ***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

<b>BiAb dilution</b>	<b>Dog lot #</b>	<b>Mean conc. <math>\pm</math> 1 SD (<math>\mu\text{g/mL}</math>)</b>		
		<b>Coat Ab lot A</b>	<b>Coat Ab lot B</b>	<b>% of lot A</b>
<b>1:5,000</b>	<b>1</b>	<b>546-656</b>	<b>490-654</b>	<b>90-100</b>
	<b>2</b>	<b>259-645</b>	<b>289-589</b>	<b>91-112</b>
<b>1:1,000</b>	<b>1</b>	<b>726-1040</b>	<b>539-717</b>	<b>69-74</b>
	<b>2</b>	<b>645-959</b>	<b>382-764</b>	<b>59-80</b>

# ***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**

<b>Dog extract</b>	<b>Coat Ab source</b>	<b>Mean conc. <math>\pm</math> 1 SD (<math>\mu\text{g/mL}</math>)</b>		
		<b>Plate col. 1-6</b>	<b>Plate col. 7-12</b>	<b>% of col. 1-6</b>
<b>Epithelia</b>	<b>Mouse</b>	<b>0.60-0.81</b>	<b>0.56-0.78</b>	<b>93-96</b>
	<b>Rabbit</b>	<b>0.57-0.80</b>	<b>0.62-0.70</b>	<b>88-109</b>
<b>Dander</b>	<b>Mouse</b>	<b>215-287</b>	<b>179-283</b>	<b>83-99</b>
	<b>Rabbit</b>	<b>234-374</b>	<b>40-142</b>	<b>17-38</b>

# *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**

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

# *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

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

# *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**