

Potency Limits for Standardized Allergen Vaccines

Topics:

- Setting specifications (general)
- New CBER limits for lot release by ELISA (grass pollen and mite extracts)
- Comparing standardized and unstandardized
- Dropping protein content

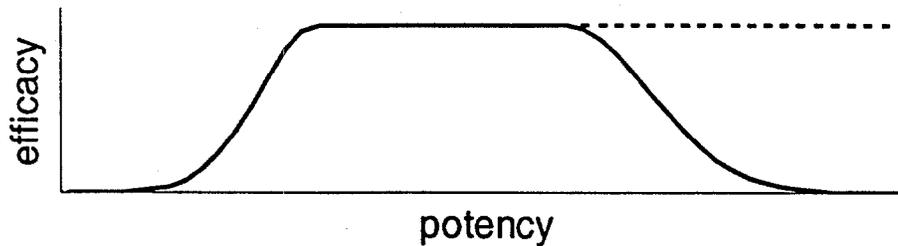
Underlying theme:

Need to balance

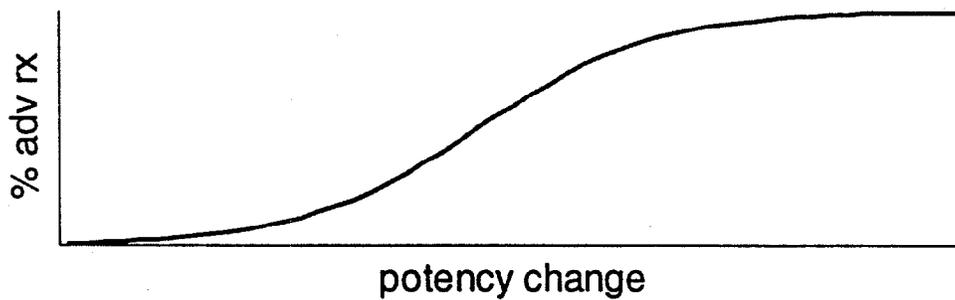
- manufacturer's risk (*rejecting an acceptable lot*)
- consumer's risk (*accepting a marginal lot*)

Basis for setting specifications for potency:

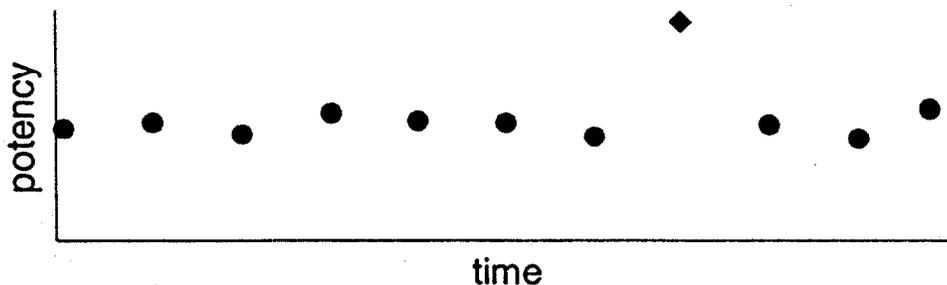
- Efficacy (*diagnostic or therapeutic*)



- Safety (*anaphylaxis upon bottle change following expiration or change of manufacturer*)



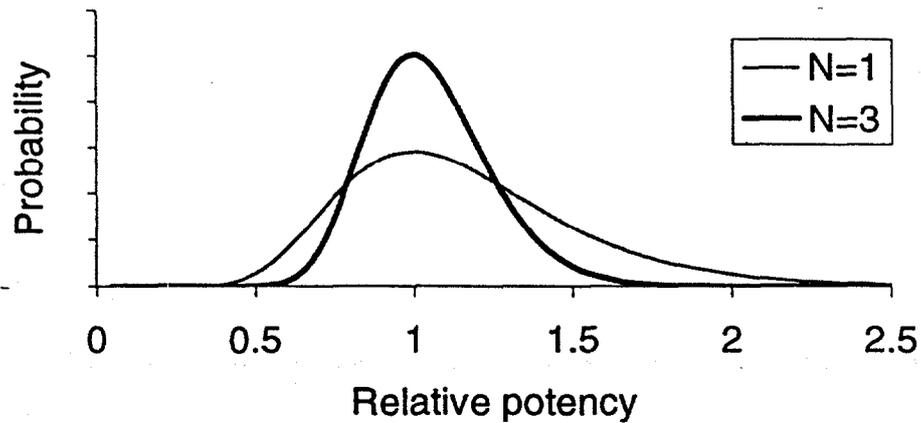
- Manufacturing Consistency (*might not know what's wrong, but something is different*)



Safety and Efficacy are critical (not flexible)
Manufacturing Consistency is more flexible

Assay variability a significant factor:

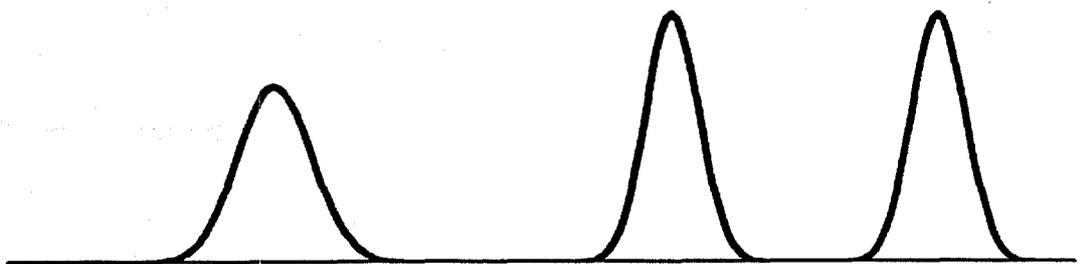
Current ELISA ($\sigma=0.1375$ in log RP)



(Improvement over RAST, where $\sigma=0.28$)

CBER currently rejects at 98% CI (0.654-1.530, when $N=3$) \rightarrow 2% of lots with (actual) $RP=1.0$ fail

Reality: Manufactured lots are not all $RP = 1.0$
 \rightarrow a broadened observed distribution of RP



$$\sigma_{obs}^2 = \sigma_{assay}^2 + \sigma_{sample}^2$$

Problem:

- RP of lot is “really” 0.7 (*many test result*)
- Manufacturer tests lot as 0.8 (N=3), and passes it
- CBER tests lot as 0.6, and fails it

Is this OK?

Need to:

1. Analyze clinical data for efficacy, safety
(*CBER trials primarily to establish standard*)
2. Validate ELISA at extreme points
(*original validation was at $RP=1.0$*)
3. Estimate variability of manufactured lots
(*412 grass/92 mite*)
4. Calculate average change in RP with bottle change to estimate safety risk
(*a straightforward statistics problem*)

Guidance for Reviewers

Potency Limits for Standardized Dust Mite and Grass Allergen Vaccines: A Revised Protocol

DRAFT GUIDANCE

ALSO:

The Determination of Equivalent Doses of Standardized Allergen Vaccines, J. Slater and R.W. Pastor, *J. Allergy and Clinical Immunology*, in press (March?)

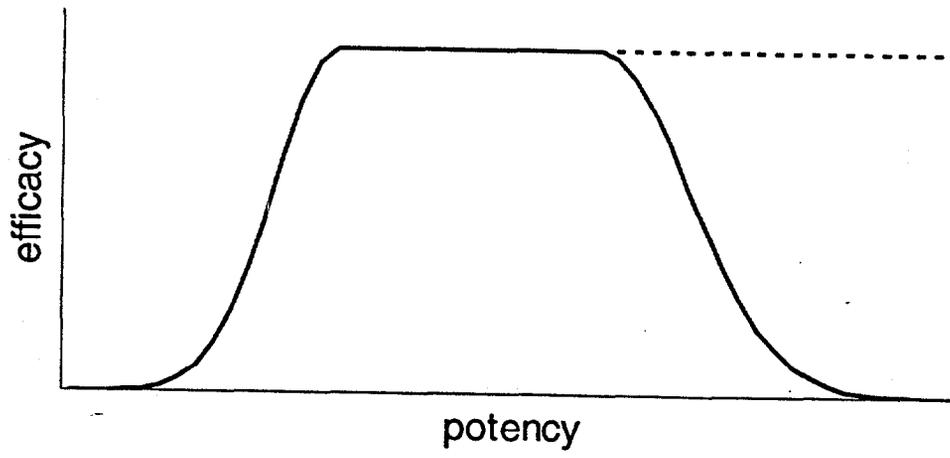
Statistical Considerations in the Establishment of Release Criteria for Allergen Vaccines, in *Proceedings of the 1999 Paul Ehrlich Symposium*, Jay E. Slater, Albert A. Gam, Maneesha D. Solanki, Suzann H. Burk, Faith M. May and Richard W. Pastor, in press.

Draft - Not for Implementation

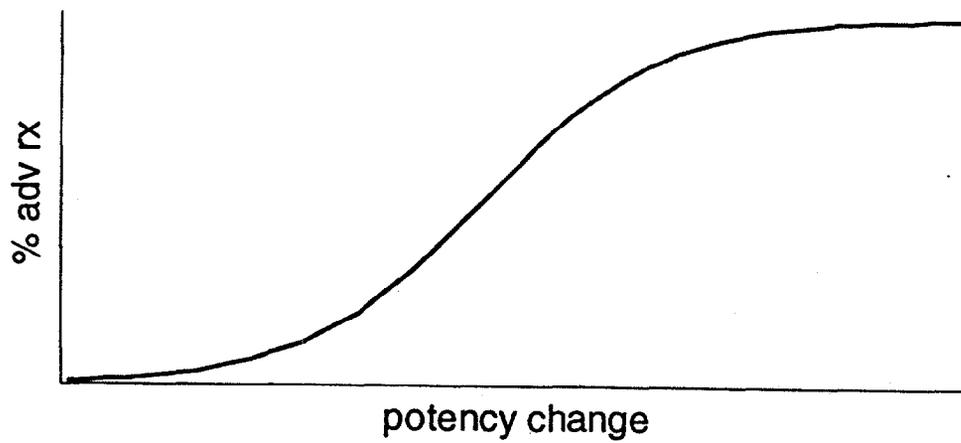
Allergen	Dose response endpoint(s)	Number of patients in active group	Dose range	Observations	Reference	Notes
Amb a 1	Systemic reactions	33	Up to 18.7 µg	7/15 patients undergoing the weekly regimen, and 10/18 patients undergoing the cluster regimen, experienced systemic reactions at doses ranging from 0.13 to 13.1 µg.	6 Van Metre, et al. 1984	Commercial lyophilized product, compared with purified reference allergens by RID. Placebo control.
Amb a 1	Antibody responses	51	Up to 93.5 µg	Threshold doses for antibody responses varied 1000-fold.	7 Creticos et al. 1984	Commercial aqueous extract. Standardization uncertain. No placebo or untreated control.
Amb a 1	Symptom scores and nasal challenge	11	0.6, 6 and 12 µg	0.6 subtherapeutic; 6 and 12 equivalent and effective.	8 Creticos et al. 1989	Aqueous product prepared by investigators from ragweed pollen, and compared with CBER reference standard by RID and crossed immunoelectrophoresis. No placebo or untreated control.
Amb a 1	Nasal challenge and antibody responses	40	Up to 0.11 µg	Measurable decreases in Amb a 1-induced nasal histamine and TAME release; decrease in skin test reactivity; and increase in ragweed-specific IgE after a cumulative Amb a 1 dose of only 0.22 µg.	9 Hedlin et al. 1989	Commercial aqueous extract, defined Amb a 1 content. No placebo or untreated controls.
Amb a 1	Symptom scores and systemic reactions	129	0.003, 0.3, 1.8, 2.25 and 4.2 µg	0.003 dose ineffective; all other doses effective. Systemic reaction rate (reactions/injection) using standard protocol: 2.1% at 0.8 µg and 5.6% at 4.2 µg. Rush protocol: 2.3% at 0.003 µg, 2.8% at 0.3 µg, 22% at 2.7 µg, 11% at 4.3 µg. Percent of patients requiring epinephrine: 7.5% when the maximum dose was 0.3 µg, 15% at 0.82 µg, 23% at 2.7 µg, 30% at 4.2 µg, and 25% at 4.3 µg.	10 Turkeltaub et al. 1990	Aqueous products analyzed by RID, RAST inhibition and parallel-line bioassay, and standardized by comparison with CBER reference standard. Untreated control, no placebo control.
Amb a 1	Seasonal and post-challenge nasal eosinophilia	89	2 and 24 µg	High and low doses effective in the challenge phase of study. In the seasonal phase, only the higher dose was effective.	11 Furin et al. 1991	Source and standardization of ragweed extract uncertain. Untreated control, no placebo control.
Der p 1	Symptom scores and systemic reactions	81	0.7, 7 and 21 µg	All three doses therapeutically equivalent. Systemic reaction rate (reactions/injection) 0.56% at 0.7 µg, 3.30% at 7 µg, and 7.10% at 21 µg.	12 Haugaard et al. 1993	Commercial aqueous (skin testing) and alum adsorbed (IT) extracts. Compared to an internal standard by RAST inhibition, immunoelectrophoresis, and bioassay (HEP method). Untreated control, no placebo control.

Summary of clinical data (therapeutic only)

Allergen	Dose range	Observations	Factor
Amb a 1	Up to 93.5 μg	Threshold doses for antibody responses varied 1000-fold.	1000
Amb a 1	0.6, 6 and 12 μg	0.6 subtherapeutic; 6 and 12 equivalent and effective.	2
Amb a 1	0.003, 0.3, 1.8, 2.25 and 4.2 μg	0.003 dose ineffective; all other doses effective.	14
Amb a 1	2 and 24 μg	High and low doses effective in the challenge phase of study. In the seasonal phase, only the higher dose was effective.	12
Der p 1	0.7, 7 and 21 μg	All three doses therapeutically equivalent.	30



- Safety (*anaphylaxis upon bottle change following expiration or change of manufacturer*)

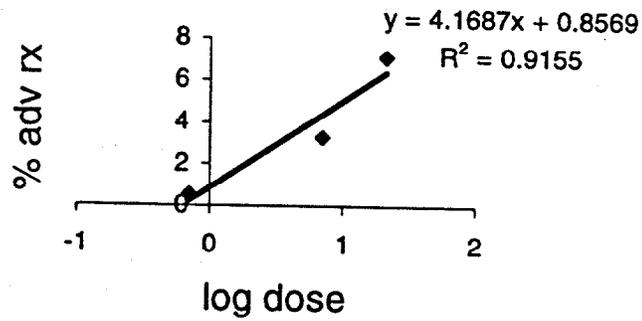


Summary of clinical data (safety only)

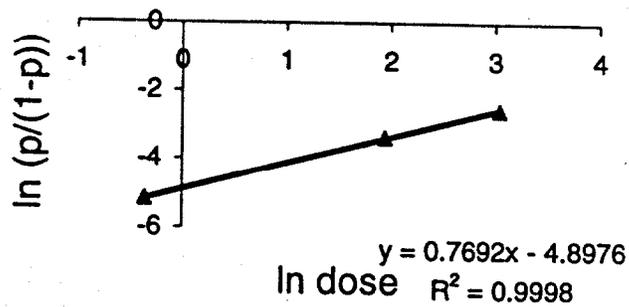
Allergen	Dose range	Observations
Amb a 1	Up to 18.7 µg	7/15 patients undergoing the weekly regimen, and 10/18 patients undergoing the cluster regimen, experienced systemic reactions at doses ranging from 0.13 to 13.1 µg.
Amb a 1	0.003, 0.3, 1.8, 2.25 and 4.2 µg	Systemic reaction rate (reactions/injection) using standard protocol: 2.1% at 0.8 µg and 5.6% at 4.2 µg. Rush protocol: 2.3% at 0.003 µg, 2.8% at 0.3 µg, 22% at 2.7 µg, 11% at 4.3 µg. Percent of patients requiring epinephrine: 7.5% when the maximum dose was 0.3 µg, 15% at 0.82 µg, 23% at 2.7 µg, 30% at 4.2 µg, and 25% at 4.3 µg.
Der p 1	0.7, 7 and 21 µg	Systemic reaction rate (reactions/injection) 0.56% at 0.7 µg, 3.30% at 7 µg, and 7.10% at 21 µg.

Example (Haugarrd et al. (1993))

Linear regression:



Logistic regression:



Summary of fitting adverse reaction rate vs. dose

Source <i>(per injection studies)</i>	Percent increase in adverse reactions associated with ten-fold dose increase <i>(linear regression)</i>	Fold dose increase associated with a 5% increase in adverse reactions <i>(logistic regression)</i>
Haugaard et al. (1993)	4.2	4.6
Haugaard et al. (1993) (maintenance)	9.1	2.4
Turkeltaub et al.(1990)	11.1	5.0

→ tentative safety range: a factor of 4 in potency

So far:

1. Analyze clinical data for efficacy, safety
→ factor of 4 (based on safety) reasonable

2. **Validate ELISA at extremes (RP=0.5, 2.0)**
original validation only at RP=1 (reference to reference)

$$SD [\log RP] = 0.1375; \sigma_{assay} = SD/\sqrt{N}$$

Extract	RP(exact)	N	RP(observed)	SD[log RP]
meadow fescue	0.5	24	0.516	0.097
	1	24	1.104	0.107
	2	24	2.085	0.152
D. farinae	0.5	23	0.499	0.109
	1	23	1.067	0.105
	2	23	2.19	0.113
Bermuda	0.5	23	0.464	0.116
	1	23	0.9914	0.102
	2	23	1.94	0.125

(N is the number of independent determinations)

3. Estimate variability of manufactured lots

Grasses: 412 lots submitted in support of PLA

51 of 412 (12.4%) failed lot release (29 high; 22 low)

Assume normal distribution (in $x = \log RP$)

→ half would have failed high, or implying that 93.8% were below the upper limit,

$$\int_{-\infty}^{x'} f_{obs}(x) dx = 0.938$$

→ $\sigma_{obs} = 0.120$.

Recall,

$$\sigma_{assay} = 0.1375 / \sqrt{3} = 0.0794.$$

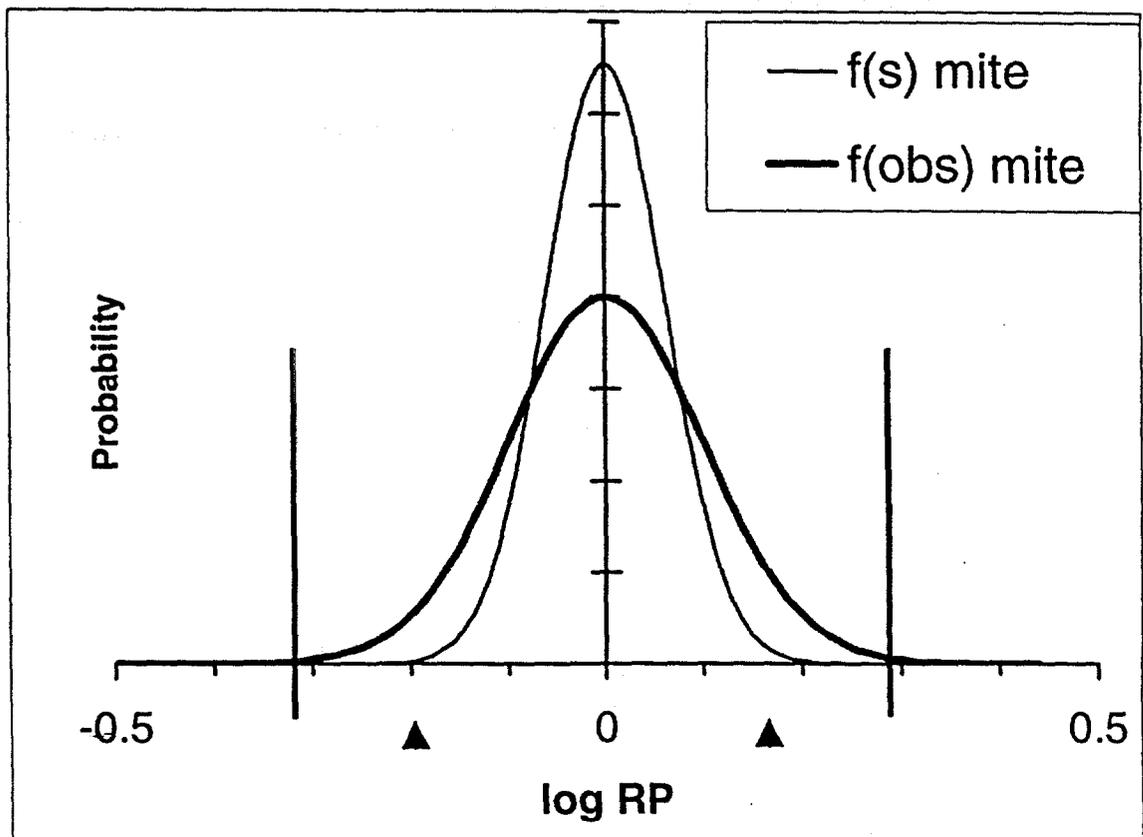
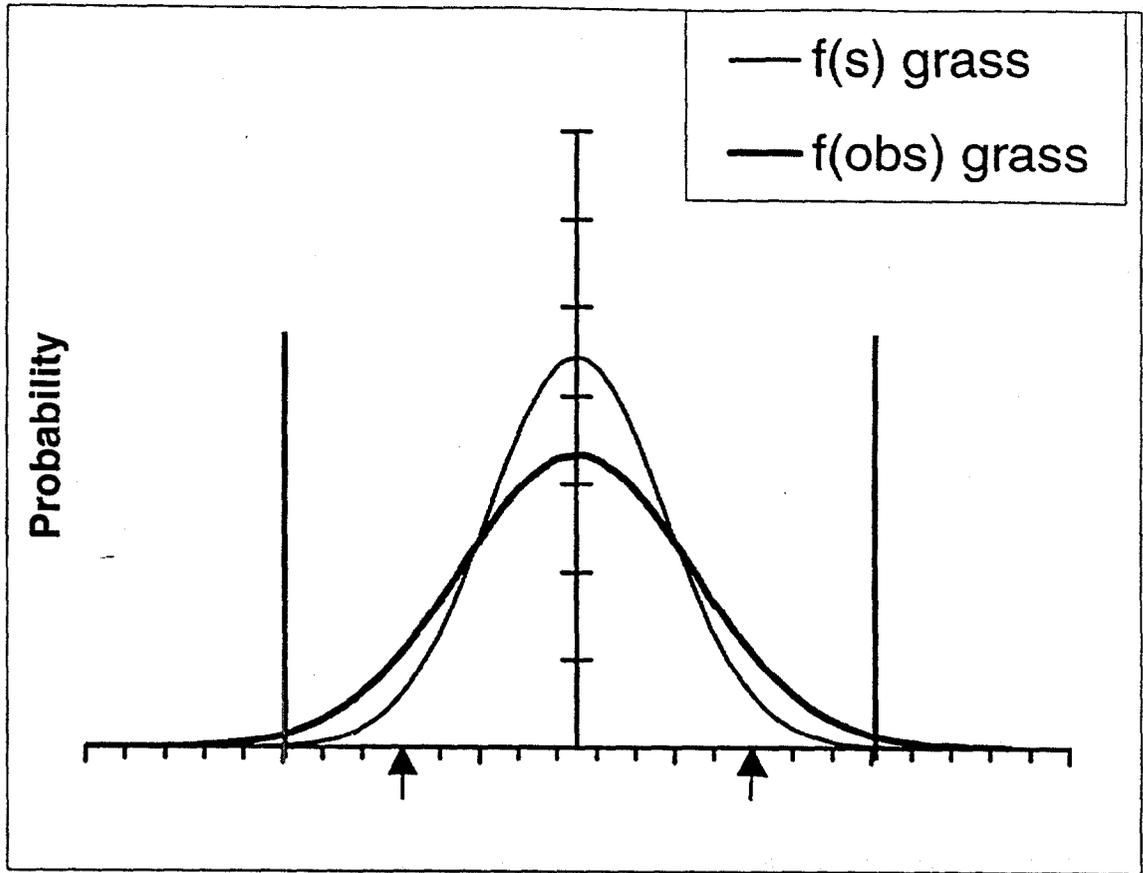
$$\sigma_{obs}^2 = \sigma_{assay}^2 + \sigma_{sample}^2$$

→ $\sigma_{sample} = 0.090$.

Mites: 92 lots submitted for release

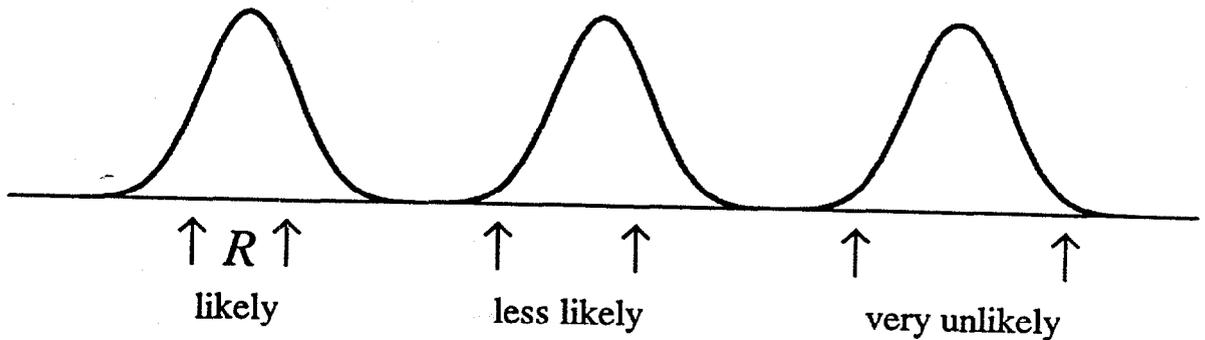
6 of 91 failed (3 high and 3 low)

→ $\sigma_{sample} = 0.061$.



4. Calculate change in RP with bottle change

Statistics Problem: *What is the range, R , of 2 samples picked from a distribution?*



First derive the density:

$$f_R(r) = \begin{cases} 2 \int_{-\infty}^{\infty} f(x) f(r+x) dx & r > 0 \\ 0 & r < 0 \end{cases}$$

$$\langle r \rangle = \int_0^{\infty} r f_R(r) dr$$

$$\int_0^{r'} f_R(r) dr = 0.95$$

95% of the values of the range are less than r'

For a normal (or Gaussian) density with variance σ^2 ,

$$\langle r \rangle = \sqrt{(2/\pi)} \sigma \approx 0.8 \sigma$$

$$- \quad r' = \sqrt{2} \times 1.96 \sigma \approx 2.8 \sigma$$

What are ranges with current (standardized) extracts?

Extract	<u>In terms of log RP</u>			<u>In terms of Δ RP</u>	
	σ_{sample}	$\langle r \rangle$	r'	$\langle r \rangle$	r'
Grass pollens	0.090	0.072	0.25	18%	80%
Mite	0.061	0.049	0.17	12%	48%

Δ RP significantly < factor of 4

Back to Problem:

- RP of lot is “really” 0.7 (*many test result*)
- Manufacturer tests lot as 0.8 (N=3), and passes it
- CBER tests lot as 0.6, and fails it

Is this OK?

Answer: No, too much manufacture's risk

Solution:

- 1. Widen CBER release limits to 0.5-2.0**
- 2. Maintain manufacture's limits**

Advantages:

1. Enforces manufacturing consistency
(e.g., eliminates occasional outliers)
2. Ensures product safety
3. Reduces number of lots rejected
4. Tighter (internal) manufacturing limits rewarded

Probability that CBER will pass or fail an allergen vaccine with a submitted RP of 0.5 to 2.0. Manufacturers will continue to test at 95% CI, indicated in bold typeface, for $N_{manu} = 3$, or for $N_{manu} = 6$.

$N_{manu} = 3$		$N_{manu} = 6$	
RP	P(pass)	RP	P(pass)
0.5	0.500	0.5	0.500
0.6	0.760	0.6	0.792
0.699	0.902	0.7	0.934
0.7	0.903	0.776	0.975
0.8	0.965	0.8	0.982
0.9	0.988	0.9	0.995
1	0.993	1	0.998
1.1	0.988	1.1	0.996
1.2	0.976	1.2	0.989
1.3	0.952	1.288	0.975
1.4	0.916	1.3	0.973
1.431	0.902	1.4	0.944
1.5	0.867	1.5	0.901
1.6	0.806	1.6	0.841
1.7	0.735	1.7	0.766
1.8	0.658	1.8	0.681
1.9	0.579	1.9	0.591
2	0.500	2	0.500

$$P(\text{pass}) = \int_{\log 0.5}^{\log 2.0} f(x) dx$$

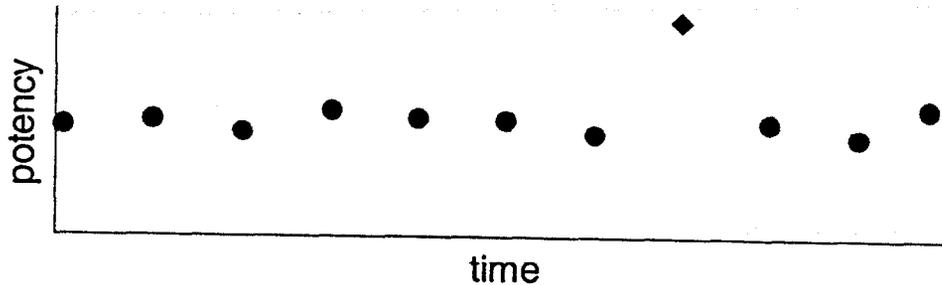
where x is the log of the RP calculated by the manufacturer (with N_{manu} replicates) and subsequently by CBER (with 3 replicates). $f(x)$ is a normal distribution in log RP with variance,

$$\sigma^2 = \sigma_{CBER}^2 + \sigma_{manu}^2 = \frac{(0.1375)^2}{3} + \frac{(0.1375)^2}{N_{manu}}$$

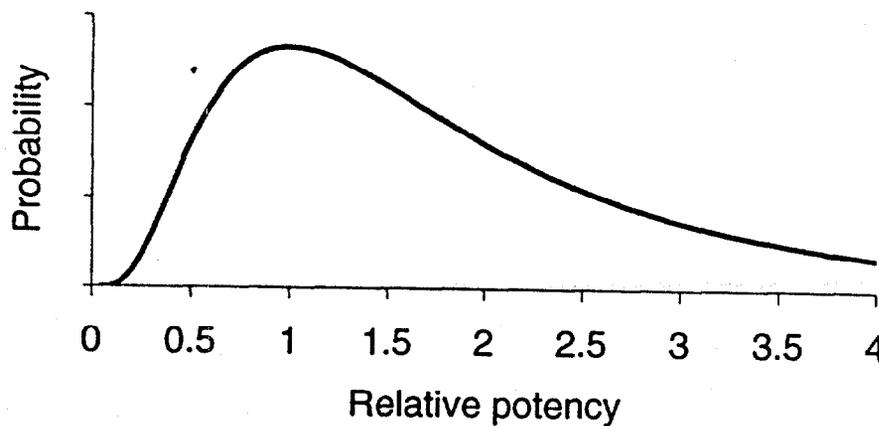
and 0.1375 is the standard deviation in log RP of the current CBER ELISA.

Comments:

1. Essentially, enforces manufacturing consistency



2. Would not widen limits if sample distributions were broad (with respect to safety limit)



(impose equivalence instead)

3. Did not know sample distributions of standardized material until *after* standardization (hence, need for tight initial limits)
4. Range of unstandardized grass extracts was high

Ranges for relative potency as determined by ELISA (compared to a 100,000 BAU/mL reference) for unstandardized grass pollen extracts labeled as aqueous 1:10 w/v (aq) and glycerinated 1:20 w/v (gly), over all manufacturers (from package inserts). N is the number of lots tested.

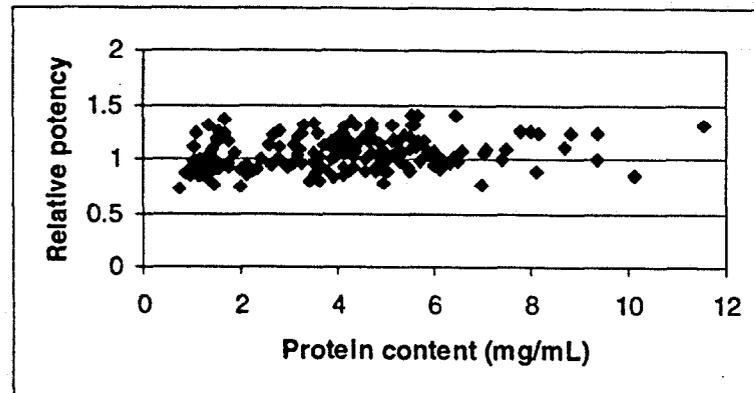
Grass	prep	N	Range of RP	Ratio of highest to lowest RP
Kentucky Bluegrass	aq	27	0.51- 4.49	9
	gly	27	0.32- 1.50	5
Meadow Fescue	aq	21	1.28-11.32	9
	gly	25	1.29- 3.78	3
Orchard	aq	23	0.24- 2.42	10
	gly	25	0.66- 1.32	2
Redtop	aq	22	0.18-15.02	83
	gly	26	0.13- 2.19	17
Perennial Rye	aq	21	0.25- 2.13	9
	gly	23	0.53- 1.95	4
Timothy	aq	29	0.46- 4.49	10
	gly	23	0.43- 1.49	3
Sweet Vernal	aq	14	0.75- 2.56	3
	gly	20	0.64- 2.01	3
Bermuda*	aq	19	0.08- 0.40	5
	gly	28	0.04- 0.16	4

*As a result of standardization, Bermuda is only distributed at 10,000 BAU/mL

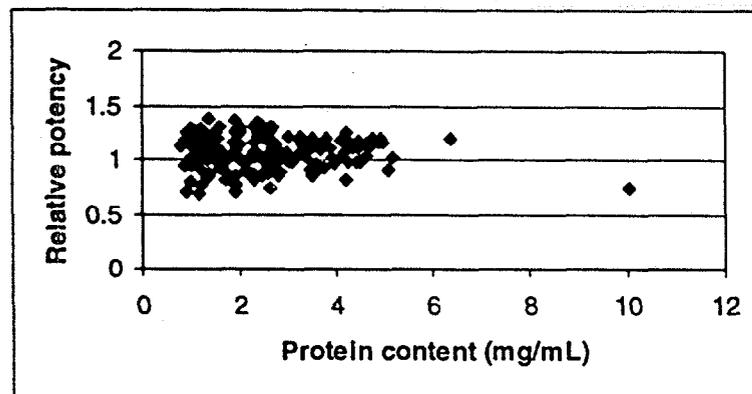
Comments (cont)

4. Could we have just used protein content (easy) instead of ELISA (less precise, more work)?

Protein content for 172 lots of grass, all with RP = 1.0



188 lots of mite (RP = 1.0)



Little correlation → need immunological assay (at least for grass and mites)

Conclusions:

- New ELISA limits and dropping protein content for lot release:
 1. Reduce manufacturer's risk
 2. Negligible increase in consumer's risk
- Variability of potencies for unstandardized grasses unacceptable
- Similar analyses will be carried out for new standardization initiatives