

Diagnosis of Occupational Asthma: HMW Allergens

Jack Pepys Workshop 2016

David I. Bernstein MD

Professor of Medicine and Environmental Health

Division of Immunology and Allergy

University of Cincinnati

Questions

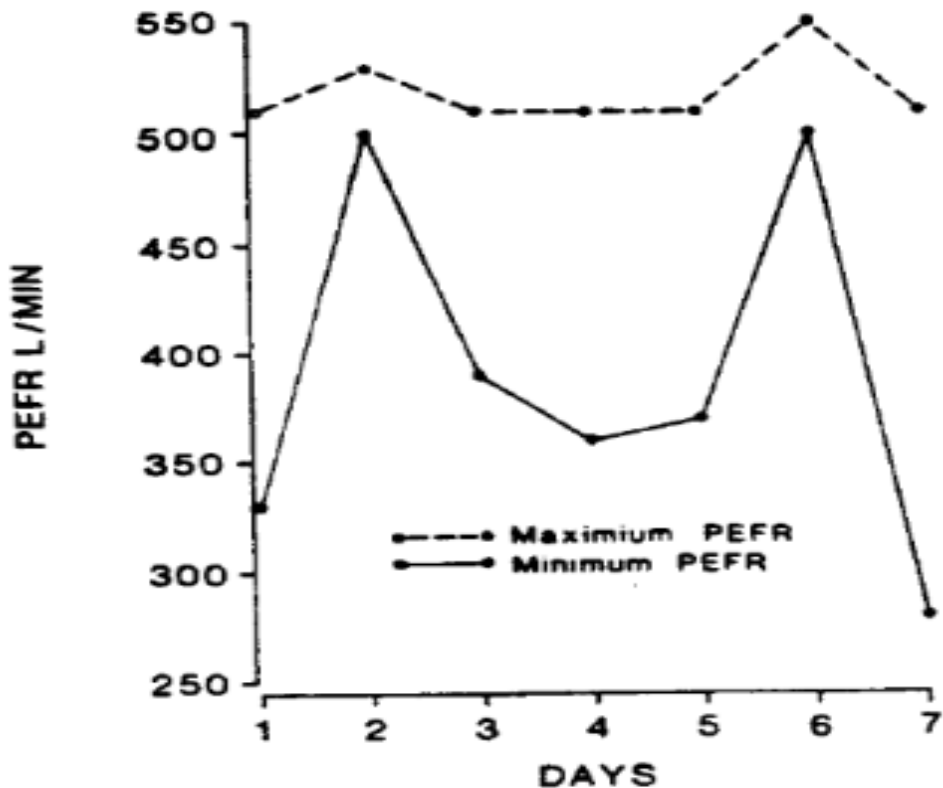
1. Can test performance be enhanced with more specific allergens?
2. What is the relative value of SPT with well defined allergens versus serum specific IgE in predicting clinical sensitivity?
3. Are there other applications for purified allergens, e.g., surveillance of exposed workers at risk?

Evaluation of Egg Processing Workers (NIOSH HHE)

- Egg processing workers – exposed to liquid egg white aerosol, dried powdered egg yolk and whole egg (N=25).
- Evaluation
 - Clinical exam,
 - Skin prick testing (SPT) to factory powdered whole egg, whole egg and egg yolk commercial extracts, ovalbumin, conalbumin, lysozyme and ovomucoid.
 - Serum specific IgE to above Ags (RAST).
 - Serial PEFr x seven days while at work.
- Definite Asthma defined by:
 - Physician diagnosis of asthma or OA
 - $\geq 20\%$ variability in PEFr on at least one day + temporally associated symptoms

Bernstein et al. JACI 1987

Evaluation of Egg Processing Workers – JACI 1987



Egg Exposure	+	-	+	+	+	-	+
Medication	-	-	+	+	-	-	+
% PEFR Variability	35.2	5.7	23.5	29.4	27.4	12.0	49

FIG. 1. Daily maximum and minimum PEFR plotted in worker No. 9. Percent variability in PEFR, concomitant asthma medications, and daily record of egg-processing exposure are illustrated.

Evaluation of Egg Processing Workers – JACI 19

TABLE II. Skin prick test responses, PEFR variability status (+ equals $\geq 20\%$ daily variability), and physician-derived diagnoses in 25 egg-processing workers

Worker	Egg skin tests*	PEFR variability†	Physician diagnosis‡
4	—	—	OA vs A
7	FWE	—	Irr
8	—	—	A vs Br
9†	CON, L	+	OA
10†	FWE, OVA, L, OVO	+	A vs Irr
12†	L, OVO	+	OA
13†	WE, EY, Con, L, OVO	+	OA
25†			OA
28			A vs Br
30			AO vs Irr
14			NS

Prick skin test pos with egg allergen fx in 4/5
with OA dx + PEFR variability but
RAST testing did not correlate

Natural Rubber Latex :
*A prototype occupational
HMW allergen*

Natural rubber latex (NRL) – Prick Skin testing

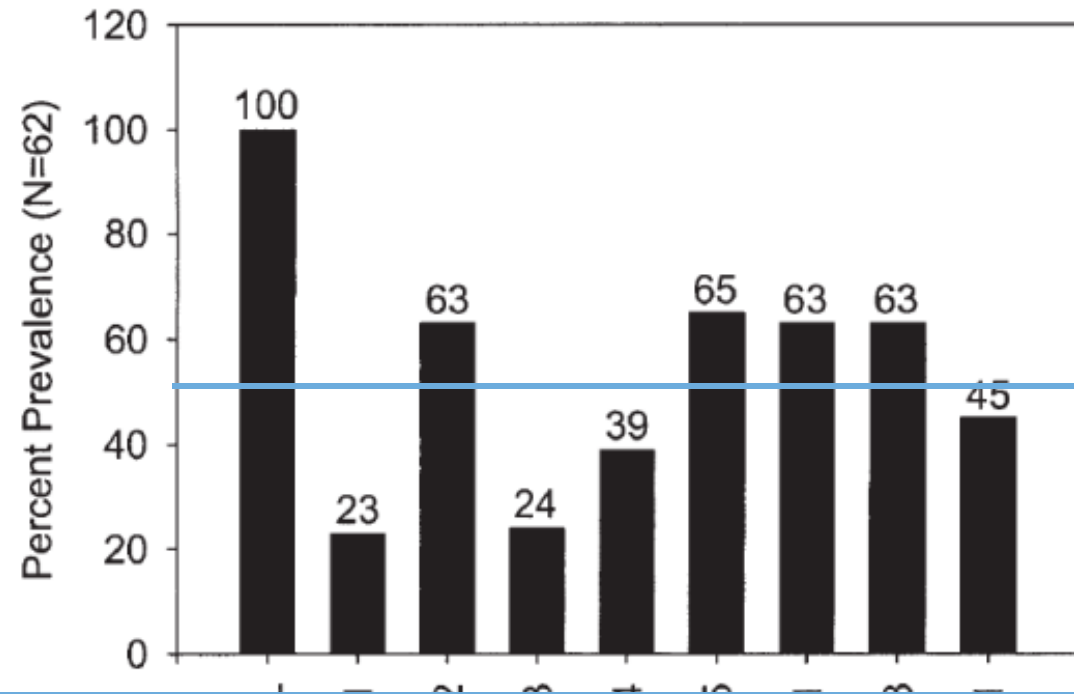
TABLE II. Characteristics of *H brasiliensis* proteins used for skin testing

Name	Trivial name	Predicted physiologic roles	Molecular weight
Hev b 1	Rubber elongation factor	Rubber biosynthesis	14.6 kd
Hev b 2	Beta-1,3-glucanase	Plant defense	34-36 kd
Hev b 3	Small rubber particle protein	Rubber biosynthesis, latex coagulation	22 kd
Hev b 4	Microhelix, cyanogenic glucosidase	Plant defense	50-57 kd
Hev b 5 fusion protein (MBP-Hev b 5)	Acidic latex protein	Undocumented	16 kd
Hev b 6.01	Prohevein	Plant defense	19 kd
Hev b 7.01	Patatin homologue	Rubber biosynthesis inhibitor	44 kd
Hev b 13	Esterase	Undocumented	43 kd
NAL	Nonammoniated latex		

Hev b proteins were freshly made from 50 µg/mL stock solutions in saline fortified PBS (PBS, pH 7.4 containing 2.0% NaCl).

Bernstein et al. In vivo sensitization to purified Hevea brasiliensis proteins in health care workers sensitized to natural rubber latex. JACI 2003;111:610-6.

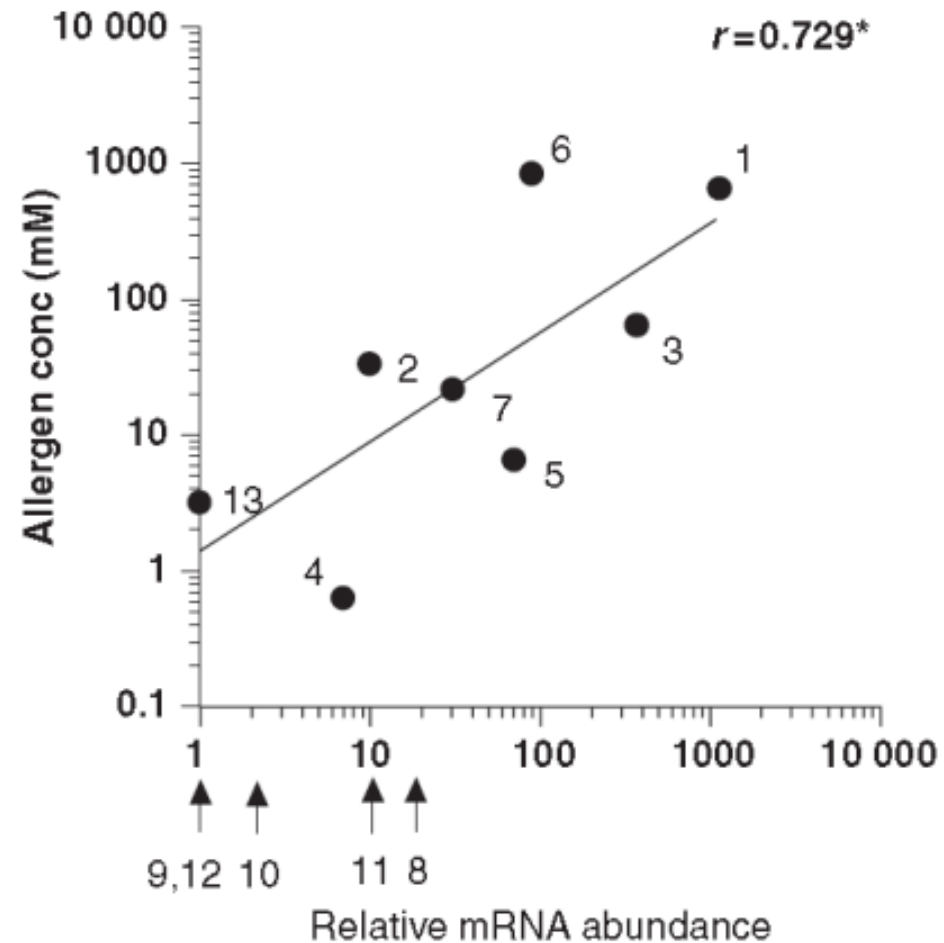
In vivo sensitization to purified Hevea brasiliensis proteins in health care workers sensitized to natural rubber latex. JACI 2003;111:610-6.



Various combinations of 8 Hev b proteins exhibited a maximal 92% concordance with skin test responsivity to NAL

Allergen concentration in natural rubber latex

H.-Y. Yeang, R. G. Hamilton_w, D. I. Bernstein_z, S. A. M. Arif, K. -S. Chow, Y. -H. Loke, M. Raulf-Heimsoth_o, S. Wagner_z, H. Breiteneder_z and R. E. Biagini_k *Clinical and Experimental Allergy*, 36, 1078–1086



Bernstein D, Biagini R et al. In vivo sensitization to purified Hevea brasiliensis proteins in health care workers sensitized to natural rubber latex. JACI 2003;111:610-6.

NRL sensitized (NAL, non-ammoniated extract) health workers (N=62) and non-sensitized atopic HCWs (N=49)

- 15 (22%) - contact urticaria
- 23 (34%) - work-aggravated rhinitis
- 25 (37%) - work-aggravated asthma,
- 4 (6%) - work-aggravated anaphylaxis

SPT titration testing to 8 Hev b purified major allergen proteins

FDA approved sp IgE assays:

- AlaSTAT sp IgE ↑40 of 60 (67%) with + ST to NAL
- CAP immunoassay ↑ 33 of 62 (53%) of NAL-reactive workers

In vivo sensitization to purified Hevea brasiliensis proteins in health care workers sensitized to natural rubber latex. JACI 2003;111:610-6.

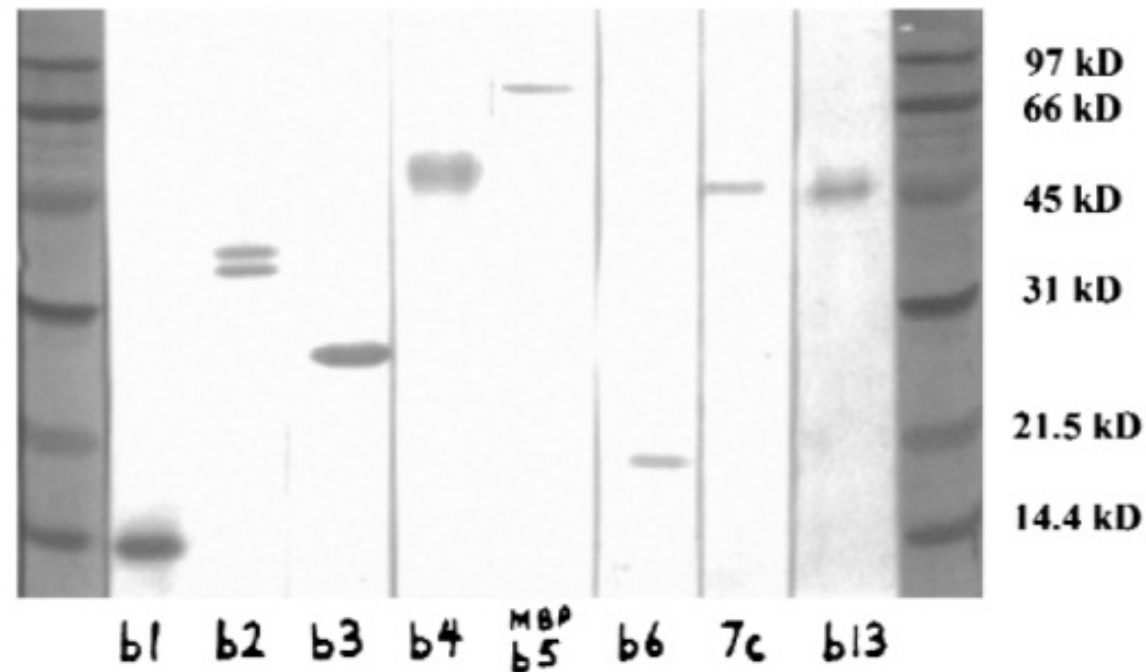


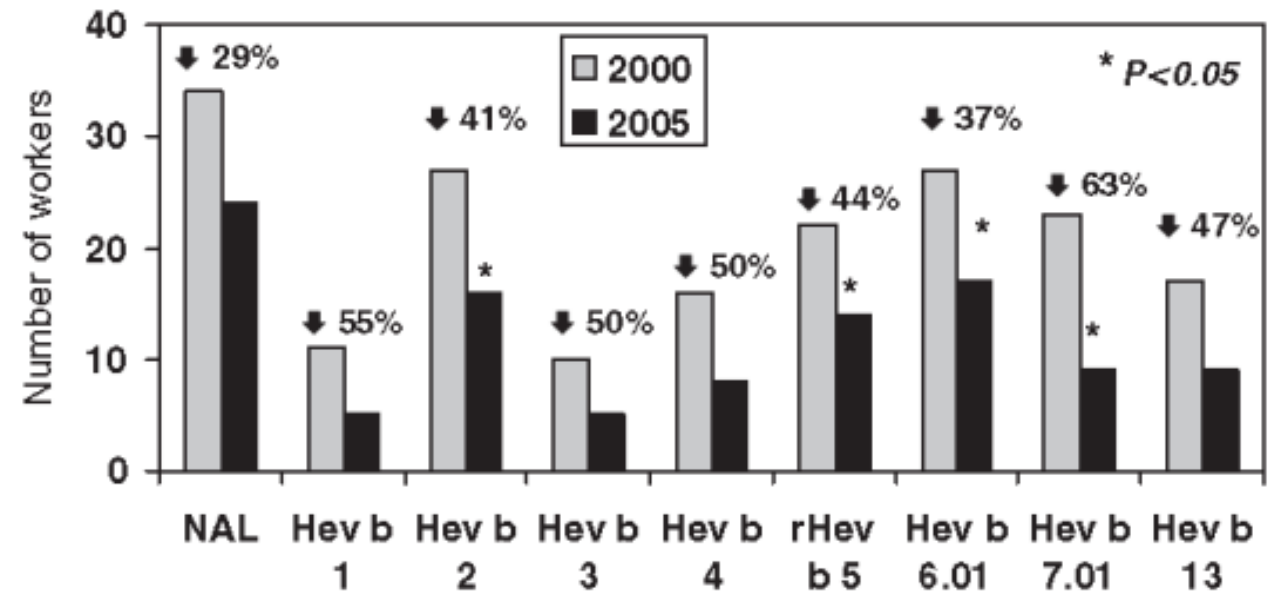
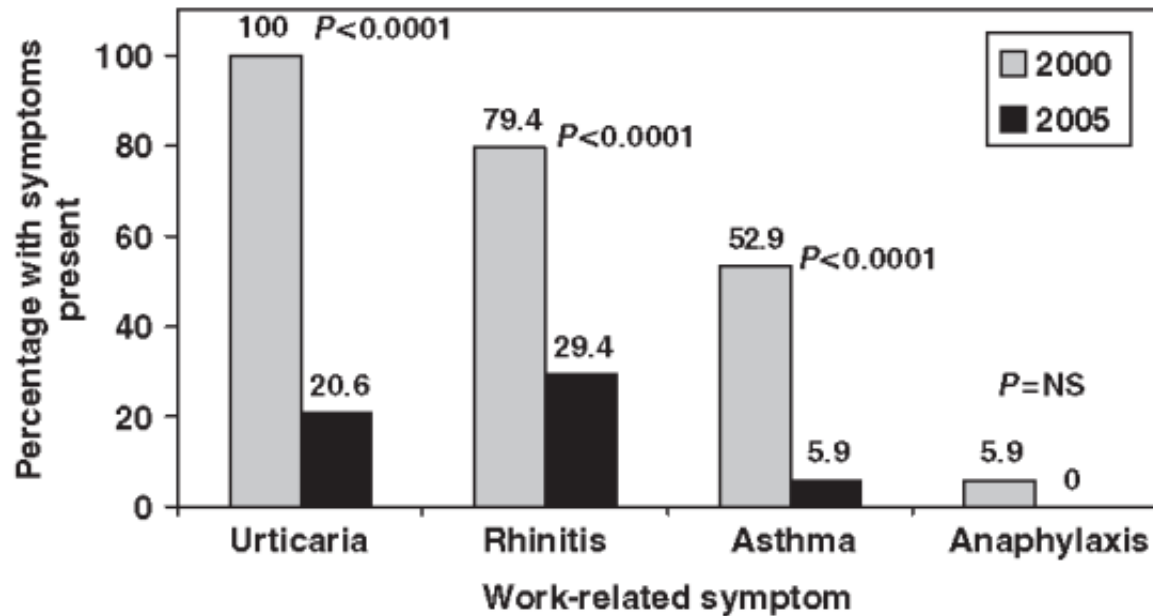
FIG 1. Western blots show IgE binding to the purified allergenic latex proteins Hev b 1, Hev b 2, Hev b 3, Hev b 4, recombinant MBP-Hev b 5, Hev b 6.01, Hev b 7.01, and Hev b 13. Molecular-weight markers are shown.

Percutaneous reactivity to natural rubber latex proteins persists in health-care workers following avoidance of natural rubber latex

A. M. Smith, H. S. Amin, R. E. Biagini^w, R. G. Hamilton^z, S. A. M. Arif[%], H. Y. Yeang[%] and D. I. Bernstein
Clin Exp Allergy 2007

- Five-year followup of 34 HCWs with NRL allergy of original 62
- Skin prick testing performed with NAL, seven NRL allergens (Hev b 1, 2, 3, 4, 6.01, 7.01, and 13), and recombinant Hev b 5 (rHev b 5)
- NRL and enhanced NRL (rHev b 5-enriched allergosorbent)-specific IgE antibodies with ImmunoCAP assay.
- Avoidance recommended in initial study (2000).

Frequencies of NRL associated symptoms and # of health care workers SPT+ to NRL allergens



Results – 5 year follow-up

1. Only 24 of 34 (70.6%) remained SPT positive to NAL
2. Skin test sensitivity decreased after 5 years →
 - **Median endpoint percutaneous concentration to NAL increased 100-fold over 5 years from 0.0001 mg/mL to 0.01 mg/mL (P<0.03)**
3. Specific IgE ImmunoCAP™ 5.25 KUA/L in 2000 → 2.2 KUA/L in 2005
 - **NAL rHev b 5-enhanced ImmunoCAP – mean 8 KUA/L (2000)
3 KUA/L (2005)**

ImmunoCAP specific IgE-diagnostic performance?

rHevb 5 enriched vs. non-enriched allergosorbent?

Smith et al. Clin Exp Allergy 2007
18/34 of NAL SPT + HCWs in 2000 had Pos sp IgE sensitivity: 53% → 60% rHevb 5 sp IgE

- 14/26 NAL SPT + HCWs in 2005 had POS sp IgE sensitivity: 54% → 77% rHevb 5 sp IgE**

What have we learned?

- Skin prick test is useful in the evaluation of occupational allergic disorders caused by HMW agents (thank you Jack Pepys!)
- Specific IgE to date lack necessary sensitivity for general screening
- Use of purified or component allergens may improve diagnostic performance of both sp IgE and skin testing.