247 Receiver Operating Characteristic (ROC) and Reproducibility Analyses of FDA-cleared Latex-specific IgE Assays *Raymond Biagini*, Edward Krieg*, Robert Hamilton†* *DHHS, PHS, CDC, NIOSH, Division of Biomedical and Behavioral Science, Cincinnati, OH †Division of Allergy and Clinical Immunology, Johns Hopkins University School of Medicine, Baltimore, MD

The diagnostic performance of FDA-cleared latex specific IgE immunoassays has been reported using manufacturers' derived cut-offs (JACI, 103:925-930, 1999). We extend these findings using receiver operating characteristic (ROC) curves and evaluate the repeatability of the assays at low specific IgE concentrations for latex and other antigens. Sera from 311 subjects (131 latex puncture skin test [PST] positive and 180 PST negative) were pair-analyzed for latex-specific IgE antibodies in the AlaSTAT® microplate (Ala), Hycor HY-TECH® RAST (HY) and Pharmacia-UpJohn CAP® Systems (CAP). Accuracy was evaluated by ROC analysis. Reproducibility was evaluated by repeatedly testing (3 times, 58 sera; Ala, latex antigen, sera from above) or 86 different sera, 2 or 3 times (CAP, varied antigens). The areas under the ROC curves (AUCs)± standard error (SEM) were: 0.858 ± 0.024, 0.869 ± 0.024 and 0.924 ± 0.017, respectively for Ala, CAP and HY. The HY system had a greater (P <0.05) AUC based on PST than that observed for Ala and CAP. Cut-offs yielding maximal diagnostic efficiencies were <0.35 kU(A)/L for CAP (87.1%) and Ala (88.1%) and 0.11 kU/L for HY (88.7%) (P=NS). Ala repeatability studies showed 66% of re-runs (38/58) yielded equivalent Ala positive (pos) or negative (neg) status, while 20/58 (34%, P <0.0001) yielded one or more repeated results where pos/neg status were discordant. The mean value of Ala pos results where replicates showed discordant neg results was 0.44 ± 0.02 kU/L. The Ala inter-assay coefficient of variation (CV) was 4.0% ± 0.51%. For the CAP system, 61.6% (53/86) of re-runs yielded equivalent CAP pos or neg results; 38.4% of sera (33/86, P <0.0001) yielded one or more repeated result(s) where pos/neg status were discordant. The mean value of CAP pos results where replicates showed discordant neg results was 0.41 0.06 kU(A)/L. The CAP inter-assay CV was 13.1% ± 0.4%. These data indicate that ROC analysis is beneficial in increasing the accuracy of commercial assays for anti-latex IgE antibody by defining a more optimal positive threshold cutoff for some tests. Caution should be exercised when modifying cutoff levels which are different from those suggested by the manufacturers' and the basis of their FDA-clearance. Some commercial tests suffer from significant imprecision at levels close to their recommended positive cutoffs, yielding 35-38% of samples with neg or pos discrepancies upon repeated testing. This may explain the wide disparity in reported seroprevalence rates for latex allergy and discordant data for individual sera between commercial assays, as well as the low sensitivity of these assays compared to PST. These data underscore the need for a well-characterized PST reagent for the diagnosis of latex allergy.

248 Quantification of Hev-b 1/Hev-b 6 Levels in Prospective Latex Allergen Reference Preparations by Immunoenzymetric Assays (IEMAs) *RG Hamilton*, SAM Arija†, HY Yeang†* *Johns Hopkins University School of Medicine, Baltimore, MD †Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia

The modified Lowry, LEAP assay and competitive RAST inhibition assay are analytical methods that are currently used to assess the relative total protein, antigenic content and allergen potency of extracts prepared from natural rubber latex products. These assays suffer from limited sensitivity and specificity and poor reagent reproducibility. Moreover, no consensus latex reference preparation exists that is immunochemically-characterized and available to researchers throughout the world for use in latex allergen assays. The objectives of our study were two fold. First, we developed and validated non-competitive, solid phase, two-site immunoenzymetric assays for quantitation of a soluble and latex particle bound allergen. These so-called "indicator allergens" were Hevea brasiliensis (Hev-b) group 1 allergen (rubber elongation factor, 14.6 kD)] and Hev-b group 6 allergen (prohevein, 20 kD). Second, we used these IEMAs to quantify the levels of Hev-b 1 and Hev-b 6 in latex preparations that are presently used as calibrators in latex allergen assays.

Murine monoclonal (M) and rabbit polyclonal (P) antibodies were prepared to chromatographically purified native Hev-b 1 and Hev-b 6.01 proteins. Antibodies prepared to Hev-b 6.01 were shown to also bind Hev-b 6.02 (Hevein, 4.7 kD). Microtiter plates (Immulon IV) were coated individually with capture M-anti-Hev-b-1 (clone JHU9221-6H3-H3-A1), P-anti-Hev-b-1 (JHU2268) or P-anti-Hev-b-6.01 (JHU3200) at 10 mcg/ml in PBS (0.1 ml/well, 23C-16 hr). Following blocking with PBS-1%BSA, buffer washes (PBS-0.05% Tween 20) were used to remove unbound protein, and purified native Hev-b 1 and Hev-b 6.01 calibrators (1000-1 ng/ml) or latex extracts (normalized to 2 ug/ml of total protein and then diluted 4 fold) were incubated in duplicate at three dilutions (2 hr, 37C, 0.1 ml/well). Following buffer washes, bound allergen was detected with sequential additions of biotinylated P-anti-Hev-b 1 (J2406) or P anti-Hev-b 6.01 (J3202)(1 mcg/ml, 0.1 ml/well,2 hr, 37°C); peroxidase-conjugated strepavidin (1 mcg/ml, 0.1 ml/well, 1 hr, 37°C) and ABTS substrate. Quantitative allergen levels were interpolated in ng/ml from dose-response curves constructed with purified Hev-b 1 or Hev-b 6 following optical density data collection.

Analytical sensitivity of the IEMAs ranged from 2(P)-10(M) ng/ml (Hev-b 1) and 1(P) ng/ml (Hev-b 6). Specificity for the rabbit polyclonal capture/detection antibodies was assessed by direct binding experiments to purified Hev b allergens:

Two non-ammoniated latex preparations (NAL Greer Puncture Skin Test [PST] reagent and Food and Drug Administration [FDA]-E8), ammoniated latex (AL-ASTM) and a Baxter Triflex powdered latex examination glove extract (JHU-011094) were each assessed as candidate latex reference preparations in the IEMAs for Hev-b 1 and Hev-b 6 content. When all extracts were normalized to 1 mg/ml, the measured content of Hevb1/6 allergen in micrograms/ml was as follows:

CROSSREACTIVITY OF CAPTURE/DETECTION ANTIBODIES

	HEVB1	HEVB2	HEVB3	HEVB4	HEVB5	HEVB6.01	HEVB6.03	HEVB7B	HEVB7C
RA-A-HEV-B 1	100%	<0.1%	0.9%	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%
RA-A-HEV-B 6.01	<0.1%	0.8%	<0.1%	0.4%	<0.1%	100%	94%	<0.1%	<0.1%

REFERENCE PREPARATION ALLERGEN CONTENT (MCG/ML)

PREPARATION	HEV-B-1(M)	HEV-B-1(P)	HEV-B-6(P)
FDA-E8-NAL	509.8	114.8	54.4
GREER-PST-NAL	414.1	109.1	161.1
AL-ASTM	534.4	982.3	0.1
GLOVE TRIFLEX	162.1	286.7	0.6

We conclude that the Hev-b 1/6 IEMAs are sensitive and specific. NAL from both sources possesses a higher Hev-b 6/Hev-b 1 ratio than the AL and glove extracts. While further testing is needed to document the presence of other known latex allergens in these preparations, we propose these NAL sources as the most balanced candidate materials available for selection as consensus reference latex preparations.