

4. *Histamine metabolism, determination and receptors*

Clinical evaluation of a new fully automated enzyme immunoassay for basophil histamine release in whole blood

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Introduction

The double blind placebo controlled food challenge (DBP-CFC) is generally accepted as the “gold standard” for immediate food reactions [1]. In the diagnosis of adverse reactions to foods, skin prick testing (SPT) and specific IgE levels lack diagnostic specificity and are therefore poor predictors of clinical reactivity when compared to the gold standard. Sampson and Ho [2] reported that the clinical specificity of SPT was 47.8% (n = 289) and of specific IgE was 49.6% (n = 784) in a study of adverse reaction to egg, milk, peanut, and fish. Both SPT and DBPCFC pose considerable risk of adverse reactions, especially to highly sensitized patients. Food challenges are also cumbersome, very time consuming, and difficult to perform on pediatric patients. For these reasons it is desirable to have an alternative in vitro diagnostic method. The HY•TEC™ BHR (basophil histamine release) assay is a fully automated EIA system for the measurement of histamine release in whole blood samples [3]. The assay system performs histamine release tests with a total incubation time of 150 minutes. We report here a study on the clinical utility of the HY•TEC BHR in the diagnosis of food hypersensitivity in pediatric patients, when compared to other tests and using oral challenge as a reference method.

Materials and methods

Patients were included in the study only after informed consent and after passing the following rules of inclusion: (1) age 1 year or older, (2) previous history of anaphylaxis or symptoms of food allergy to milk, egg white, peanut, codfish, or mustard, (3) no concomitant treatment with antihistamines or beta-2 agonists prior to testing, (4) no treatment with topical glucocorticoids at the skin test site, (5) positive response to SPT

controls with the absence of dermographism, and (6) positive response to histamine release positive control.

Histamine release was performed using HY•TEC BHR (Hycor Biomedical Inc., CA, USA) according to the manufacturer's specifications. Allergens (egg white, milk, codfish, peanut, and mustard seed) were obtained from Hycor Biomedical in ready-to-use micro-tubes. Skin prick testing was performed on the volar surface of the distal forearm using extracts from Stallergenes using lancets from Dome Hollister-Stier. Wheals were read after ten minutes and the reactions were outlined with a marker and transferred to paper. Positive and negative controls were included to enable interpretation of the reactions. Oral challenge was done after the method of Rance et al. [4]. Specific IgE was determined on serum samples using an ELISA assay (DPC-Behring, France). Specific IgG in serum was determined using an automated EIA system (Hycor HY•TEC Specific IgG).

Results and discussion

The purpose of this study was to evaluate the HY•TEC BHR for measurement of basophil histamine release in whole blood for the diagnosis of food allergy. HY•TEC BHR assay is a micro-plate-based enzyme immunoassay utilizing a highly specific monoclonal antibody against histamine. Whole blood in micro-tubes was challenged with individual food allergens to elicit histamine release. The clinical sensitivity and specificity of BHR were compared with that of SPT, specific IgE, and specific IgG tests, using oral challenge as the reference method.

The standard curve is based on USP traceable calibrators and is very reproducible (Fig. 1). Assay precision is within acceptable limits using both buffer controls or whole blood samples. The sensitivity of SPT, specific IgE, and specific IgG were 82%, 82%, and 57%, respectively (Table 1). However, the specificity was 32%, 24%, and 47%. The reference method used was oral food challenge. The specificity of the HY•TEC BHR assay was 82%, significantly higher than that of the other tests when oral food challenge was the

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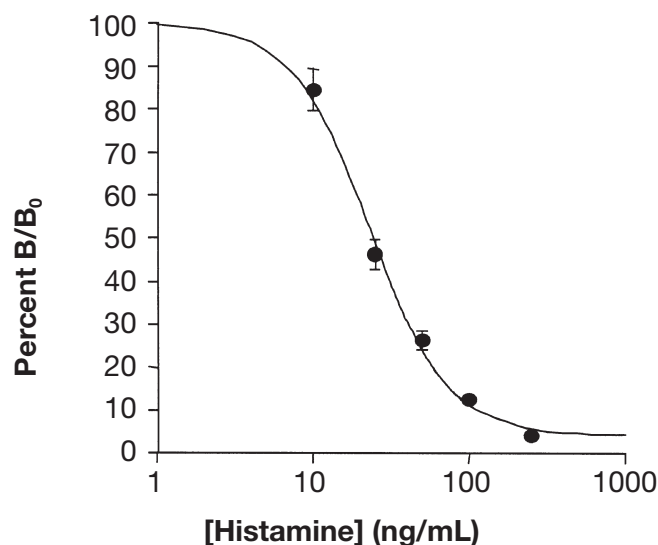


Fig. 1. Standard Curve Reproducibility. Data points represent average percent B/B_0 ($n = 10$) and bars represent one standard deviation. "B" represents the amount of antibody-enzyme conjugate bound at any given histamine concentration (expressed as an absorbance value), and " B_0 " represents antibody bound at zero histamine (therefore it is a maximum signal). The term Percent " B/B_0 " is the ratio of absorbance values.

reference method. Sensitivity was 53%. The HY•TEC BHR assay had the highest concordance, 68%, and was the only method with strong statistical evidence that the concordance did not occur by chance ($p = 0.001$). The differences in concordance between HY•TEC BHR and the specific IgE and specific IgG tests were statistically significant ($p < 0.05$).

Table 1. Clinical Utility of the HY•TEC Basophil Histamine Release Assay (reference method: oral challenge)

	Sensitivity	Specificity	Concordance
BHR	53%	82%	68%
Specific IgE	82%	24%	53%
Skin Prick	82%	32%	57%
Specific IgG	57%	47%	52%

The specificity of BHR was significantly higher than the other 3 tests. Therefore, one may use the superior specificity of BHR to confirm positives that are detected using SPT or specific IgE. We conclude that the HY•TEC BHR may be used as an *in vitro* alternative in oral challenge in cases where this procedure poses a significant risk to the patient or where confirmation of other test results is desired.

References

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