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Short Report

Enzyme Immunoassay for Total Immunoglobulin E in Dried Blood Spots

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ABSTRACT Elevated circulating levels of total immunoglobulin E (IgE) are associated with both allergic disease and repeated macro-parasitic infections. Population-based research on IgE has been limited by the logistical constraints associated with obtaining and processing venipuncture blood samples. In this short report, we present an enzyme immunoassay protocol for quantifying circulating total IgE levels in capillary whole blood, collected from a finger prick and dried on filter paper. The assay demonstrated acceptable levels of accuracy, precision, and reliability. IgE remained stable at room temperature for only 2–4 days and degraded rapidly at higher temperatures suggesting that samples should be refrigerated or frozen within 1–2 days of collection. It is hoped that the relative ease of blood spot collection will expand opportunities for population-based research on IgE. Am. J. Hum. Biol. 19:440–442, 2007. © 2007 Wiley-Liss, Inc.

Immunoglobulin E (IgE), a component of the humoral immune response, is most commonly associated with atopic diseases such as asthma, allergy, and eczema, but also forms an important component of the immunological response against parasitic infections including the gastrointestinal worms (Faulkner et al., 2002; McSharry et al., 1999; Sutton and Gould, 1993). In clinical studies conducted primarily on European and American groups, circulating levels of IgE are much lower than those found among populations frequently exposed to parasitic infections (Buckley et al., 1985; Chan and McKenzie, 2003). It is likely that westernized populations––with low levels of exposure to parasitic infections––do not represent a straightforward model for understanding the biological importance of IgE.

Both total and specific IgE levels are often measured in serum, a method which requires blood collection through venipuncture by a trained phlebotomist. Samples must be immediately processed and stored under controlled conditions to maintain sample integrity. This may prove to be a serious impediment to research in settings where clinical or laboratory facilities are not readily available. Dried blood spots (drops of capillary whole blood collected on filter paper following a simple finger stick) are a reliable and convenient alternative to venipuncture for a growing number of analytes (Mei et al., 2001; Worthman and Stallings, 1997). Stapel et al. (2004) demonstrated the feasibility of using dried blood spots to determine total and allergen specific IgE levels using a radioallergosorbent test (RAST). In this report, we present the results of our validation of an enzyme immunoassay designed to quantify total IgE in dried blood spots.

PROTOCOL

Capillary blood samples are collected by cleaning the participant's finger with isopropyl alcohol and then pricking with a sterile, disposable lancet. Up to 5 drops of whole blood ($\sim$50 μl per drop) are applied to filter paper (Whatman #903). Blood collection procedures require that the drops are drawn onto the filter by capillary action; blotting or smearing onto the paper will lead to uneven absorption and inaccurate assay results. Blood spots are then dried for a minimum of 4 h, placed into an air tight plastic bag with desiccant (Cat. # 61161-319, VWR), and refrigerated (4°C) until they

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can be transported to be stored frozen at −30°C until analysis.

Our method is a modification of a commercially available protocol (Bethyl Laboratories #E80-108). Coating buffer (Sigma Chemical #C3041; 0.05 M carbonate-bicarbonate; pH 9.6), wash solution (Sigma Chemical #T9039; 50 mM Tris buffered saline, 0.05% Tween 20; pH 8.0), postcoat solution (Sigma Chemical #T6789; 50 mM Tris buffered saline, 1% BSA; pH 8.0), and stop solution (2 M H_2SO_4) were made according to kit specifications. Assay/sample diluent was made by adding 0.5 ml 10% Tween 20 to 100 ml of the postcoat solution.

To maximize the comparability between calibrators and unknowns, dried blood spot calibrators were made by serially diluting purified Human IgE calibrator (Bethyl; #RC80-108) 1:2 with assay diluent from 18,000 to 281.25 ng/ml. Each diluted calibrator was then combined with an equal volume of washed erythrocytes (McDade et al. 2004a) resulting in final calibrator concentrations of 9,000; 4,500; 2,250; 1,125; 562.5; 281.25; and 140.6 ng/ml. Assay diluent and washed erythrocytes were combined for a 0 standard. The final diluted standards were then applied to labeled filter paper cards in 50 μl drops using a manual pipette.

The day before the assay was to be preformed, microtiter plates (Nunc MaxiSorp, #445101) were coated with 100 μl/well goat anti-Human IgE antibody (Bethyl; #A80-108A) diluted 1:101 μl in coating buffer. Plates were covered with parafilm and incubated overnight at 4°C. One 3.2-mm disk was removed from each blood spot sample, control, and calibrator using a hole punch, placed into a 12 × 75 mm glass test tube with 250 μl of sample diluent and eluted overnight at 4°C. Samples with high concentrations of IgE can be eluted in higher volumes of diluent (e.g. 500 μl).

On the day of the assay, samples were removed from the refrigerator and rotated at 300 rpm for 60 min at room temperature. The coated microtiter plate was allowed to come to room temperature, washed 4 times with wash solution using an automated plate washer, blocked for 30 min with postcoat solution, and washed an additional 4 times with wash solution. Eluate (100 μl) from each tube was pipetted in duplicate into microtiter plate wells and allowed to incubate for 60 min at room temperature. After this incubation, the plate was washed 4 times using the wash solution. Detection antibody (goat anti-Human IgE-HRP conjugate, 1 mg/ml; Bethyl, #A80-108P) was diluted 1:25,000 in assay diluent, added to the wells (100 μl), and incubated at room temperature for 60 min. After a 4th wash, 100 μl of chromogenic substance (TMB peroxidase substrate and peroxidase solution B; Kirkegaard & Perry) was added to each well for color development. After a 30 min incubation at room temperature, 100 μl stop solution (2 M H_2SO_4) was added to each well. The absorbance was read at 450 nm (BioTek Elx808) and unknown sample concentrations were determined from calibrators using the best-fit four-parameter logistic curve (KC Junior; BioTek).

EVALUATION OF ASSAY PERFORMANCE

We assessed assay accuracy by using commercial laboratory quality control sera with known concentrations of total IgE (Bio-Rad; Liquichek Immunology Control; #590X) to produce two blood spot controls. Sera with high and low concentrations of total IgE were diluted 1:2 in washed erythrocytes and spotted onto filter paper to produce dried blood spots. After correction for dilution with washed erythrocytes, values for both controls were within the expected reference ranges as reported by the sera manufacturer.

Assay linearity was evaluated by eluting and then serially diluting three controls in assay diluent (1:2; 1:4; 1:8). Observed values for the serial dilutions averaged 97.9% of expected, and ranged from 91.0 to 106.4%. The lower detection limit of the assay was obtained by determining the IgE concentration which corresponded to the absorbance value 2 SD above the mean value of 10 replicates of the 0.00 ng/ml calibrator. This value signifies the lowest value of IgE that the assay can reasonably distinguish from 0. The lower detection limit of the assay corresponded with 55 ng/ml total IgE.

We evaluated the precision of the assay by calculating the percent coefficient of variation (%CV; SD/Mean × 100) from eight replicates of three control samples analyzed within one assay. Estimates of within-assay imprecision (CV) across the assay range were 4.62, 4.83, and 4.51%, from low to high concentrations of IgE. Reliability, or between-assay imprecision, was assessed by measuring four laboratory control samples in 15 assays conducted on different days over an 8 week period. CV values of between-assay imprecision across the assay range were 6.48, 8.54, 9.35, and 9.04% from low to high.

We measured the stability of total IgE in dried blood spots by exposing two samples to a

range of environmental conditions for up to 14 days. Blood spots were placed in a plastic ziplock bag with a desiccant pack to control for the effects of humidity and stored at 4, 37°C, room temperature (21–23°C), or an oscillating condition (12 h at 22°C, 12 h at 32°C). On days 1–7 and 14, one blood spot was removed from each condition and frozen at −30°C until analysis. In addition, two blood spots of each sample were exposed to a freeze/thaw procedure in which the blood spots were allowed to thaw at room temperature for 1 h and were then refrozen. This cycle was repeated up to 5 times. These conditions were chosen to approximate various environmental conditions that a sample may be exposed to in fieldwork situations and during laboratory analyses. The use of a laboratory grade freezer (−30°C) inhibits sample degradation during storage. A sample was considered degraded when values fell below 90% of the baseline mean obtained with 5 replicates of the sample which had been frozen at −30°C immediately following collection and drying.

Total IgE remained stable in dried blood spots at 4°C for at least 14 days. At room temperature, total IgE remained stable for 2–4 days, after which IgE concentrations began to decline. Total IgE concentrations degraded rapidly at higher temperatures (both 37°C and 22°C/32°C), falling below 90% of baseline levels within 24 h after sample collection. Total IgE showed no evidence of degradation in response to repeated freeze/thaw cycles. Although we found that refrigerated samples (4°C) showed no degradation after 14 days, we did not asses the impact of longer storage at temperatures between 4 and −30°C.

DISCUSSION

IgE plays an important role in mediating immunological responses to environmental allergens and gastrointestinal parasite infections. IgE levels may also be modified by nutritional and environmental conditions throughout gestation, early infancy, (McDade et al. 2004b) and childhood (Long and Nanthakumar, 2004). Additional research among populations frequently exposed to infectious disease, especially gastrointestinal parasites, is needed to understand the role of IgE in shaping human health and disease.

In this report, we have described and validated a method to detect total circulating IgE levels in dried blood spots based on modification of a commercially available ELISA kit. The protocol demonstrated acceptable levels of assay accuracy, precision, reliability, and linearity. Levels of total IgE remained stable at 4°C for at least 14 days but degraded relatively quickly at warmer temperatures (2–4 days at room temperature and within 24 h at 37°C). This suggests that it is critical that dried blood spots be refrigerated within 1–2 days of field collection to avoid IgE degradation. It is also important to note that this protocol was optimized to measure relatively high levels of total IgE, such as those found in a population frequently exposed to intestinal parasites. The assay detection range may be modified to detect lower total IgE levels such as those found among nonatopic, nonparasitized adults. Because of the relative ease of blood spot sample collection, it is hoped that this assay will expanded the current scope of population-based research on the IgE system.

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