

# A simple and quick method for enhanced detection of specific IgE in serum from lymphatic filariasis patients

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## Abstract

A new simple and quick technique, using a suspension of protein A agarose beads to absorb IgG4 from sera prior to determination of filarial-specific IgE in ELISA, is presented. The optimal ratio between serum and absorbant was determined by absorbing fixed volumes of sera from individuals from a *Wuchereria bancrofti* endemic area with different volumes of the protein A agarose bead suspension and testing supernatants for filaria-specific IgG4 and IgE. The effect of absorption on measured IgG4 and IgE intensities in sera from various categories of individuals from the endemic area was thereafter examined. Overall, absorption resulted in a 96.5% decrease in mean ELISA OD values for IgG4 and a 41.6% increase in mean ELISA OD values for IgE. Higher increases in IgE measurements were seen with sera from circulating filarial antigen (CFA) negative individuals (64.7%), microfilaria (mf) negative individuals (56.1%) and individuals with chronic filarial disease (62.7%) than with sera from individuals who were CFA positive (23.4%), mf positive (10.0%), or without chronic disease (36.5%). These differences indicate that the degree to which IgE detection in unabsorbed serum is blocked by IgG4 varies with infection and disease status. Absorption of IgG4 from serum with a protein A agarose bead suspension prior to measurement of specific IgE is a useful alternative to conventional gel column absorption methods, particularly when processing many samples. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** IgE; IgG4; Blocking antibodies; Protein A agarose beads; *Wuchereria bancrofti*; Lymphatic filariasis

## 1. Introduction

An increased level of specific IgE antibodies is a characteristic feature of the immune response in

helminth infections (Maizels et al., 1993; Allen and Maizels, 1996) including lymphatic filariasis (Hussain et al., 1981; Ottesen et al., 1982; Kurniawan et al., 1993, 1995; Estambale et al., 1994, 1995). Although definitive evidence is still lacking, it seems likely that specific IgE in human lymphatic filariasis plays a role in both protective immunity and pathogenesis (Maizels et al., 1995). For this reason, analysis and characterisation of

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the IgE response in infected individuals and endemic populations may provide important information on the natural history of infection and disease in lymphatic filariasis, which may also be useful for devising new measures for its control.

Elevated levels of IgE are often associated with allergic responses. However, such responses are rarely seen in helminth infections, probably because they are modulated by much higher levels of blocking IgG4 antibodies with affinity for the same antigenic epitopes (Hussain and Ottesen, 1986; Hussain et al., 1992). Although therefore apparently beneficial to the patient, competition between IgE and IgG4 antibodies for similar epitopes interferes with the ELISA quantitation of IgE in serum from infected individuals.

To overcome this obstacle and thereby to maximise IgE detection, IgG is commonly absorbed from test sera using protein A or protein G sepharose columns prior to IgE assays (Estambale et al., 1994; Kurniawan et al., 1995; Steel et al., 1996; Zhang et al., 1999). This technique, however, is quite laborious and time consuming especially when processing many samples. A new, easier and faster technique using a protein A agarose bead suspension in Eppendorph tubes to absorb IgG4 from test sera prior to determination of filarial-specific IgE in ELISA is presented.

## 2. Materials and methods

### 2.1. Serum samples

Sera were obtained from individuals from Ma-saika village (Pangani District) in north-east Tanzania, which is highly endemic for *Wuchereria bancrofti* infection. After informed oral consent to participate, all villagers were examined for signs of chronic filarial disease (hydrocele in males and lymphoedema in both males and females), and blood specimens were collected between 21:00 and 24:00 h. From each individual, samples of 100  $\mu$ l finger-prick blood and 5 ml venous blood were collected. These samples were examined for microfilariae (mf) by the counting chamber method (McMahon et al., 1979) and for circulating filarial antigens (CFA) by the TropBio ELISA kit for

serum specimens (Simonsen and Dunyo, 1999), respectively. Results from the clinical, mf and CFA examinations are presented elsewhere (Simonsen et al., 2001). Based on the findings, sera from 80 individuals aged  $\geq 15$  years were selected for the present study (42 males, 38 females), namely 60 asymptomatic individuals (20 mf and CFA negative, 20 mf negative but CFA positive, 20 mf and CFA positive) and 20 individuals with chronic filarial disease manifestations (10 CFA negative and 10 CFA positive). Five of the CFA positive individuals with chronic disease were mf positive while the other five were mf negative.

### 2.2. Absorption of IgG4 with protein A agarose beads

IgG4 was absorbed from the serum samples using a commercially available 1:1 suspension of protein A agarose beads in phosphate buffered saline (Ken-En-Tec A/S, Denmark; Catalogue No. 1060H). The suspension was gently mixed in the bottle with a stirring pin, and the desired volume was added to 50  $\mu$ l of test serum in Eppendorph tubes. The mixtures were incubated on a rocker at room temperature for 30 mins, and then centrifuged at 1000 rpm for 2 min. The supernatants were transferred to new tubes and further diluted to the required test concentration (see below) before being tested for filarial specific IgG4 and IgE.

### 2.3. Detection of filarial-specific IgG4 and IgE

Detection of filarial-specific IgE and IgG4 antibodies was carried out by ELISA as described previously (Estambale et al., 1994), with an adult *Brugia pahangi* homogenate as antigen. Buffers were prepared according to Voller and Savigny (1981), and optimal dilutions of antigen, sera and conjugates were determined by serial titration using unabsorbed sera.

Wells of ELISA plates (Immuno-plates, Maxisorp 442404; Nunc A/S, Denmark) were coated by overnight incubation at 4 °C with 100  $\mu$ l of antigen diluted in coating buffer to a protein concentration of 1  $\mu$ g/ml for IgG4 and 2  $\mu$ g/ml for IgE. All next steps were carried out at room

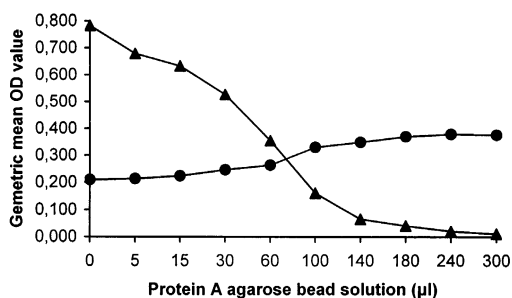


Fig. 1. Geometric mean of filarial-specific IgE and IgG4 intensities (OD values) after absorption with increasing volumes of protein A agarose bead suspension in four pools of sera from individuals from a *Wuchereria bancrofti* endemic area (▲, IgG4; ●, IgE).

temperature. Following three 3-min washes with washing buffer, 200 µl of 0.5% bovine serum albumin in washing buffer was added to each well as a blocking agent. After 1 h, the plates were washed as above and incubated with 100 µl of test serum diluted in washing buffer (1.5 h with 1:1000 serum dilution for IgG4; overnight with 1:20 serum dilution for IgE). Plates were washed as previously, and incubated with 100 µl horse-radish-peroxidase (HRP) conjugated antisera diluted in washing buffer (1 h with 1:2000 dilution of HRP conjugated monoclonal mouse-anti-human IgG4 (CLB, The Netherlands); 2 h with 1:1000 dilution of polyclonal rabbit-anti-human IgE (Dakopatts A/S, Denmark)). After washing as before, 100 µl of orthophenylene-diamine (OPD) substrate solution, prepared from OPD tablets (Dakopatts A/S, Denmark) according to the manufacturer's instructions, was added to each well. The reaction was stopped after 20 min by adding 50 µl of 2.5 M H<sub>2</sub>SO<sub>4</sub> per well. Optical density (OD) values were measured with an ELISA reader (Bio-Rad) at 492 nm. Serum samples were tested in triplicate and the arithmetic mean optical density (OD) value calculated for each specimen. Pre- and post-absorption samples from the same individual were tested for the same antibody type on the same plate. The OD value of a positive control serum included on all plates was used to adjust for minor plate-to-plate variations.

## 2.4. Data analysis

The geometric mean intensity (GMI) of filarial-specific IgE and IgG4 antibodies in OD values was calculated as  $\text{antilog} [(\sum \log x + 1)/n] - 1$ , with  $x$  being the individual mean OD values and  $n$  the number of examined individuals. GMI OD values before and after absorption of sera with protein A agarose bead solution were compared statistically by paired samples  $t$ -test.  $P$ -values  $< 0.05$  were considered statistically significant.

## 3. Results

### 3.1. Optimisation of the protein A agarose bead suspension concentration

To determine the optimal ratio between serum and protein A agarose bead suspension needed for absorption of IgG4 and enhanced detection of IgE, four pools of sera were prepared by mixing 500 µl of serum from each of five different asymptomatic individuals (two pools from mf positive and two pools from mf and CFA negative individuals). Fifty microliter serum samples from each of the four pools were then mixed with increasing volumes of protein A agarose bead suspension (ranging from 0 to 300 µl) in Eppendorph tubes. After incubation and centrifugation, the supernatants were further diluted to give the required test concentration, and tested for IgG4 and IgE in ELISA. The geometric mean intensities of IgE and IgG4 (OD values) at different volumes of protein A agarose bead suspension appear from Fig. 1.

For IgE, OD values increased progressively with the amount of protein A agarose bead suspension added until a volume of ~140–180 µl, after which the increase was minimal. By contrast, OD values for IgG4 progressively decreased with increasing amount of protein A agarose, becoming very minimal at volumes of 140–180 µl and above. Thus, most of the filarial-specific IgG4 in 50 µl of serum was absorbed by 140–180 µl of protein A agarose bead suspension, and this led to considerable enhancement in detection of IgE. A

ratio of 50:140 between serum and absorbant suspension was therefore used in further measurements of filarial-specific IgE.

### 3.2. Pre- and post-absorption IgE and IgG4 levels in relation to infection and disease status

To analyse pre- and post-absorption IgG4 and IgE levels in relation to infection and chronic filarial disease status, all 80 sera were absorbed individually with protein A agarose bead suspension at a volume ratio of 50:140. Filarial-specific IgG4 and IgE levels were measured by ELISA before and after absorption.

Pre- and post-absorption filarial-specific IgE and IgG4 mean antibody intensities in relation to CFA, mf and disease status are shown in Table 1. After absorption, OD values for filarial-specific IgG4 in all groups were reduced to negligible values (overall reduction by 96.5%). It, however, led to a statistically significant increase in mean IgE OD value of 41.6% ( $t$ -test,  $P < 0.001$ ). Higher increases in post-absorption mean IgE intensities were seen in sera from CFA negative (64.7%) than sera from CFA positive (23.4%) individuals; in sera from mf negative (56.1%) than sera from mf positive (10.0%) individuals; and in sera from individuals with chronic filarial disease (62.7%) than sera from asymptomatic individuals (36.5%). Post-absorption increases in mean IgE intensities were statistically highly significant among the individuals who were CFA positive, CFA negative, mf negative, asymptomatic, and with symptomatic chronic filarial disease, but not among mf positive individuals (Table 1).

## 4. Discussion

Several methods have been devised for measuring levels of specific IgE in lymphatic filariasis. Thus, Hamilton et al. (1981) developed an antigen-excess non-competitive solid-phase radioimmunoassay (SPIRA) that permits effective measurement of filarial-specific IgE in sera from individuals infected with *W. bancrofti*, even in the presence of high levels of competing IgG. This technique is highly sensitive and accurate and was

used extensively in early classical work to characterise IgE responses in human lymphatic filariasis (Hussain et al., 1981, 1992; Ottesen et al., 1981, 1982). However, besides the risk posed by using radioactive labelled material, the technique is complicated and requires the use of a gamma counter, a sophisticated and expensive equipment not commonly available in economically disadvantaged lymphatic filariasis endemic areas of the world.

ELISA has now become the most widely utilised serological technique for antibody detection in lymphatic filariasis and in many other infectious diseases. It is relatively inexpensive, easy to use and suitable for field studies with many samples to analyse. ELISA is also a useful technique for semi-quantitation of filarial-specific IgE provided IgG antibodies competing for the same antigenic epitopes are first removed. In this regard, the most commonly used method involves absorption of IgG from test sera using protein A or protein G sepharose gels in columns (Estambale et al., 1994; Kurniawan et al., 1995; Steel et al., 1996; Zhang et al., 1999). The main drawback of this method is that it is laborious, especially when many samples are processed.

The present study examined a simpler method for absorbing IgG4 from sera prior to filarial-specific IgE determination by ELISA. To the best of our knowledge, this is the first time protein A agarose bead suspension, designed for affinity purification of monoclonal antibodies, has been used for this purpose. The product is made from rProtein A (a product of a recombinant *Staphylococcus aureus* gene expressed in *Escherichia coli*) coupled to cross-linked 6% agarose beads. It is available commercially in phosphate buffered saline suspension at a ratio of 1:1. It binds the Fc region of most mammalian IgGs, including human IgG4, with high affinity (information from manufacturer). In the present study, absorption was accomplished by mixing the desired volumes of serum and bead suspension in Eppendorph tubes. Thereafter, the beads were simply sedimented by centrifugation and the supernatant of absorbed diluted serum was examined for antibodies in ELISA. Compared to conventional gel absorption, the method is simple and quick and the cost of materials is of a similar scale.

Table 1  
Pre- and post- absorption filarial-specific IgG4 and IgE intensities (OD values) in relation to CFA, mf and disease status

	No. sera examined	IgG4		IgE		% Increase	<i>P</i> -value (paired <i>t</i> -test)
		Geometric mean OD value before absorption	Geometric mean OD value after absorption	Geometric mean OD value before absorption	Geometric mean OD value after absorption		
CFA positive	50	0.706	0.024	0.158	0.195	23.4	0.001
CFA negative	30	0.505	0.020	0.232	0.382	64.7	<0.001
Mf positive	25	0.666	0.022	0.180	0.198	10.0	>0.05
Mf negative	55	0.610	0.023	0.187	0.292	56.1	<0.001
Disease positive	20	0.672	0.026	0.134	0.218	62.7	<0.001
Disease negative	60	0.613	0.021	0.203	0.277	36.5	<0.001
All individuals	80	0.627	0.022	0.185	0.262	41.6	<0.001

The protein A agarose bead suspension technique was found to be an effective and useful alternative to current conventional methods for absorbing competing IgG4. Thus, when serum and protein A agarose bead suspension were used at the selected volume ratio of 50:140, the OD value for filarial-specific IgG4 was reduced by an overall average of 96.5%, and this was accompanied by an overall mean increase in measured IgE intensity of 41.6%. The enhancement compares favourably with the 41.5% increase observed using protein A sepharose columns by Estambale et al. (1994). Due to the non-linear association between OD values and actual antibody concentrations, whereby a small increase in OD value leads to a large increase in actual antibody concentration (especially at higher values), increases in OD values for IgE in the present study correspond to higher increases in actual antibody concentrations. In contrast to the present and several other studies (Hamilton et al., 1981; Hussain et al., 1981, 1992; Kurniawan et al., 1993; Estambale et al., 1995), a study in Haiti found only marginal evidence that antifilarial IgG4 or other antibodies interfered with parasite-specific IgE detection (Marley et al., 1996). This suggests that there might be regional variation in the extent to which IgG4 is blocking IgE responses, perhaps related to host genetics and/or differences in infection transmission intensity.

The present study indicated that the increase in measured IgE intensity after IgG absorption varied with infection and clinical status. Thus, higher mean increases in IgE were observed in CFA and mf negative individuals and in individuals with chronic pathology (who are often infection negative) than in CFA and mf positive individuals and in individuals without chronic pathology. Differences in IgG4 levels between patient groups did not appear to be large enough to be the major explanation to this, and other factors, such as differences in degree of competition between parasite specific IgG4 and IgE in different patient groups, may play a significant role. The study therefore emphasise the importance of IgG4 absorption in studies comparing IgE levels in different groups of patients, since the level of blocking may be different in different

infection and disease categories. Failure to do so may result in a distorted picture of the relative levels of IgE.

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