

Short communication

Elimination of viral infection risk from blood samples for allergy testing

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The worldwide dissemination of acquired immunodeficiency syndrome (AIDS) and various forms of hepatic viruses increases the risk of virus transmission in connection with the handling of blood samples for allergy testing. In order to reduce the risk of viral infection in laboratory staff and others handling blood samples, we have implemented a method (2) for destruction of lipid-encapsulated viruses, comprising hepatitis B (HBV), non-A, non-B hepatitis (NANB), and HTLV-III, in serum and plasma samples.

In comparison with other immunoglobulins, IgE is very labile. Temperatures around 56°C drastically reduce its biologic activity, as do reducing agents such as dithiothreitol (1). For this reason, treatments involving high-energy radiation or strong oxidants were not considered. Instead we applied the more gentle *solvent-detergent treatment* (SD method), by which the number of live virus particles in plasma has been shown to decline by a factor greater than 10000 (3).

The SD method has been developed and validated for intravenous blood products (4). Originally, a mixture of 20% diethyl ether and 1% Tween 80 was employed, but later it was shown that 0.1% tri(*n*-butyl)phosphate (TNBP) could replace the ether (2). Comparison of different detergents showed that Triton X-100, sodium cholate, and sodium deoxy-

cholate could be used interchangeably with Tween 80. The killing of viruses was strongly dependent on time and temperature, and, at ambient temperature, more than 4.5 and 5.5 logs of vesicular stomatitis virus and of sindbis virus, respectively, were inactivated within 3 h (2). These *in vitro* data have been confirmed in animal experiments for hepatitis B and Hutchinson non-A, non-B virus, and in *in vitro* systems for HTLV-III virus (3). It should be emphasized that the SD method can be applied only to lipid-coated virus particles. This was demonstrated in the non-lipid-enveloped encephalomyocarditis virus (EMC), which did not lose its infectiousness after SD treatment (2).

In the experimental setup, we tested whether determinations of IgE protein or specific IgE changed when serum samples from allergic patients had undergone a treatment which should eliminate viral infectivity. To control for the stability of IgE protein, we SD-treated three serum samples from patients with inhalant allergy (0.3% TNBP and 1.0% Tween 80 for 6 h at room temperature) in combination with 0.15 mmolar of the bactericide sodium azide. The sera were tested by the Pharmacia PRIST system (Pharmacia, Uppsala, Sweden). As shown in Table 1, the recovery of IgE was satisfactory for both treatments alone or in combination.

In order to demonstrate the stability of specific

Table 1. Recovery of IgE protein as determined by the PRIST method

Serum	Treatment							
	None		SD		NaN ₃		SD+NaN ₃	
	Units	Units	Recovery		Units	Recovery	Units	Recovery
A	173	194	121%		127	73%	177	102%
B	400	408	102%		414	104%	407	102%
C	128	114	89%		124	97%	121	95%
Geom. mean			103%			90%		100%

ND=not done.
1 unit of total IgE corresponds to 2.4 ng/ml.

IgE and the allergens used for preparation of two commercial tests for specific IgE, we SD-treated 13 serum samples from patients attending the outpatient clinic of the Allergy Unit, National University Hospital, Copenhagen, in combination with sodium azide. The samples were tested for specific IgE by two different assays employing dissimilar principles: the Magic Lite system (ALK, Horsholm, Denmark) makes use of paramagnetic beads onto which allergen is covalently coated, whereas the CAP system (Pharmacia, Uppsala, Sweden) uses a sponge-like solid phase with covalently linked allergen. The two assays are differently calibrated (Magic Lite: 1.43 to 300 units; CAP: 0.35 to 100 units), but for both assays a translation of the quantitative results into semiquantitative allergy classes is made. Sera were chosen to provide a spectrum of positive and negative samples, and nine inhalation allergens were employed: timothy pollen (g6), birch pollen (t3), mugwort (w6), cat dander (e1), dog dander (e2 for Magic Lite and e5 for CAP), horse dander (e3), house-dust mites – *Dermatophagoides pteronyssinus* and *D. farinae* (d1 and d2) – and *Cladosporium herbarum* (m6). At least three serum samples (of which at least two were positive) were tested for each allergen.

As shown in Table 2, the recoveries of specific IgE, i.e. the ratio of treated to untreated sample, varied for both assays, but the overall recovery was within the interassay variation (i.e. coefficients of variation of 20–25%) of the tests. None of the samples changed more than one allergy class, from one

Table 2. Recoveries (geometric means, in %) of specific IgE after various treatments as determined by the CAP and Magic Lite methods

Allergen	Treatment					
	SD		NaN ₃		SD+NaN ₃	
	ML	CAP	ML	CAP	ML	CAP
Timothy pollen, g6	114	131	100	117	144	102
Birch pollen, t3	122	113	131	115	123	129
Mugwort, w6	109	111	94	85	105	112
Cat dander, e1	104	102	151	104	117	127
Dog dander, e2/e5	99	105	105	117	111	87
Horse dander, e3	188	108	116	106	133	96
<i>D. pteronyssinus</i> , d1	92	140	89	108	85	87
<i>D. farinae</i> , d2	75	123	73	136	80	102
<i>C. herbarum</i> , m6	100	98	107	114	107	105
Geom. mean	107	113	102	111	109	100

At least three sera (two of which were positive) were tested for each allergen.

treatment to another. The change in specific IgE did not depend on the level of specific IgE, and no trends toward systematic variations were found either in positive or in negative samples. These data confirm the nondenaturing properties of the treatment. The SD method should ensure the sterility of serum samples from hepatitis B, NANB, and HTLV-III vira, but not from non-lipid-encapsulated vira. Moreover, if blood drawing and centrifugation take place in environments with high bacterial load, the sterility of serum may be ensured by means of the addition of sodium azide.

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