ENHANCEMENT OF THE SENSITIVITY OF THE SOLID PHASE C1q BINDING ASSAY BY IMMOBILIZATION OF C1q VIA C1q-SPECIFIC RECOMBINANT ANTIBODY scFv9(G)

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ABSTRACT
A new and improved ELISA method for immune complex detection [scFv9(G)-C1q ELISA] was developed. The assay is based on the C1q-solid phase binding test. C1q was immobilized on microtiter ELISA plates, coated with the single-chain antibody scFv9(G), specific to the collagen-like region of C1q. The obtained results indicated a significantly reduced nonspecific background, when C1q was immobilized via the scFv9(G)-capture molecule. The comparative analysis between the two wildly used methods for IC detection (C1q-SP-RIA and QUIDEL CIC–C1q ELISA) and the new improved Fv9(G)-C1q ELISA shows a good agreement. The method is simple, reproducible and may be a good alternative for the routine C1q-SP-RIA.

Introduction
Circulating immune complexes (CICc) are detectable in a variety of systemic disorders, such as autoimmune disease (1-4), allergic diseases (5), different kinds of infections (viral, bacterial or parasitic) (1, 6-8). It is well known that the detection of CICs may provide useful clinical information, regarding immunopathology, prognosis, and follow-up for autoimmune and rheumatic disorders.

A variety of tests that can detect CICs have been described (9-12), but only a few of them are sufficiently sensitive and reproducible. The C1q-solid phase RIA discriminated well between sera from healthy persons and those from patients, and was evaluated as the most reliable test for IC-detection (8). There are several ELISA kits, commercially available however their clinical usefulness is not well established (13). The disadvantage of these ELISA methods is the relatively high nonspecific binding.

The purpose of the present study is to describe a simple and effective immobilization procedure for C1q, using a single-chain antibody scFv9(G), specific toward the collagen-like region of C1q.

Materials and Methods
Buffers
Sodium carbonate (SC) buffer: 0.035M NaHCO3, 0.015M Na2CO3, pH 9.6, citrate-phosphate buffer: 0.05M Na2HPO4, 0.025M citric acid; PBS-phosphate buffered saline: 0.01M Na2HPO4, 0.01M NaH2PO4, 0.145M NaCl, pH 7.4; TPBS buffer: 0.05% Tween 20 in PBS.

Purified proteins
C1q was purified from human plasma as described before (14). The purity of C1q was assessed by standard SDS-PAGE (12% w/v) under reducing conditions where it appeared as three bands, corresponding to the A-, B-, and C-chains of 34, 32, and 27 kDa, respectively. The func-
tional activity of C1q was tested by its ability to form a hemolytically active C1 (15). Human aggregated IgG (HAIgG) was used as an artificial IC. ScFv9(G), specific only to the collagen-like region (CLR) of C1q, was obtained by the phage-display method, using the Griffin-1 library, as it was already described (16).

**Solid-phase binding assay for immune complex detection**

ScFv9(G) (1µg/100µl) in SC buffer (pH 9.6) was coated on microtiter ELISA plates, which were blocked with 2% w/v BSA in PBS for 1 h. After three rounds of washing, the wells were incubated with C1q (5µg/100µl) overnight at 4°C. Following washing, the plates were incubated with different concentrations of HAIgG (2-10µg/well) in TPBS, or in normal serum (diluted 1:50) for 2 h at 37°C. The amount of bound HAIgG was detected by incubation with peroxidase-conjugated goat-anti human IgG. The reaction was developed using o-phenylenediamine (OPD) detection system. Control experiments were carried out, where C1q was directly coated on the ELISA plates.

Sera from 61 healthy donors were used as a negative control population. Patients’ sera with different positivity (total number 86 sera) and 20 negative samples (used as negative controls), kindly provided by Dr. Kishore (Weatherall Institute of Molecular Medicine, John Radcliff Hospital, University of Oxford) were tested with the C1q-SP-RIA. All sera were tested in parallel by QUIDEL CIC-C1q Enzyme immunoassay, according to the manufacturer’s instructions (17) and via the ELISA method, described above. All tested sera were analyzed in triplicate.

**Result interpretation**

61 healthy donors were tested by the scFv9(G)-C1q ELISA. Six of them show significant deviations and were excluded. The mean of the remaining 55 sera was equalized to 100%. Results of patient’s sera were then presented as a percentage of this mean and graded negatively when being less than the mean +2SD, equivocal when being between mean +2SD and the mean +4SD, and positive when being more than the mean +4SD. All assays were performed with diluted serum (1:5 and 1:50).

**Results and Discussion**

In order to improve the ELISA method for IC detection two different immobilization procedures of C1q were performed. One included immobilization of C1q directly on the ELISA plates, while in the other scFv9(G) was coated on the plates and C1q was allowed to interact with the immobilized antibody. The obtained results indicated that the nonspecific background is significantly reduced when C1q was immobilized via scFv9(G) (Figure). C1q is a sensitive and fragile molecule, which can undergo a partial denaturation during the traditional immobilization pro-

![Figure](image)

**Figure.** ELISA for the detection of HAIgG by immobilization of C1q directly (a) or via C1q–specific antibody scFv9(G) (b). 1. C1q immobilized directly on the ELISA plates (5µg/100µl), HAIgG in PBS (10µg/well) 2. C1q immobilized via scFv9(G); scFv9(G)-1µg/100µl; C1q (5µg/100µl); HAIgG in PBS (10µg/well). 3. C1q immobilized directly on ELISA plates (3µg/100µl), HAIgG in normal serum, diluted 1:50 (5µg/well). 4. C1q immobilized via scFv9(G); scFv9(G)-1µg/100µl; C1q (3µg/100µl); HAIgG in normal serum, diluted 1:50 (5µg/well). Each value represents the mean ± SD for five repeated measures.
Comparison of the QUIDEL C1q ELISA and scFv9(G)-C1q ELISA with C1q-solid phase RIA test, for the detection of immune complexes

<table>
<thead>
<tr>
<th>Assay</th>
<th>C1q-SP RIA (++) sera (reference) n</th>
<th>C1q–SP RIA (+) sera n</th>
<th>Healthy donors (61/100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUIDEL C1q-ELISA</td>
<td>49/57%</td>
<td>32/37%</td>
<td>9/15%</td>
</tr>
<tr>
<td>scFv9(G)-C1q ELISA</td>
<td>42/49%</td>
<td>44/51%</td>
<td>5/10%</td>
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</table>

In order to compare the above described ELISA assay for IC detection with other methods for CIC measurement 86 patients’ sera with different positivity, tested by C1q-SP RIA were selected. All sera were analyzed in parallel by QUIDEL CIC–C1q ELISA test. The results are presented in Table. A good agreement was found between scFv9(G)-C1q ELISA test, QUIDEL C1q based ELISA and C1q-SP RIA. The percentage of samples that were negative in the RIA and positive in the ELISA assays or positive in the RIA and negative in the ELISA was 6.2% for scFv9(G)-C1q ELISA and 40% for QUIDEL C1q based ELISA.

In conclusion, the results reported here, indicate that the proposed method for IC detection, based on the immobilization of C1q via CLR-specific scFv9(G) is more effective than other ELISA methods and almost as sensitive as C1q-SP RIA. The method is simple, reproducible and may be a good alternative for the routine C1q-SP RIA. Its clinical importance should be further studied.

**Acknowledgements**

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REFERENCES