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# A New Conjugate for the ELISA Quantitation of Porcine IgA

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The preparation of a new soluble immune complex conjugate (anti-IgA-IgA-peroxidase), suitable for use in the ELISA technique is described. This conjugate requires pure IgA and specific anti-IgA, but the enzyme (peroxidase) was easily conjugated to the IgA.

Key words: *pig – IgA – ELISA*

## Introduction

Secretory IgA is the predominant immunoglobulin in external secretions. The important role of this immunoglobulin for protection against bacterial and viral infections at mucosal surfaces is extensively documented (Tomasi, 1972; Bienenstock et al., 1979, 1981; Tagliabue et al., 1983). The detection and quantitation of IgA antibodies in mucosal secretions is therefore very important. In the case of the pig, the IgA of both milk and intestinal juices have been extensively studied (Curtis and Bourne, 1971; Porter et al., 1978; bourne and Newby, 1981; Butler et al., 1981; Inoue, 1981).

Confronted with neonatal viral diarrhoea in pigs (rotavirus and coronavirus) we have been obliged to perfect techniques to analyse and quantify the mucosal immune response. For this we have chosen the ELISA technique (Scherrer and Bernard, 1977) and employed a new conjugate not covalently linked to the specific antibody. This conjugate is a soluble immune complex made with anti-IgA and IgA-linked to peroxidase. It is easy to prepare and the assay as sensitive as the classical technique.

## Materials and Methods

### *IgA purification*

The IgA was purified from sow milk, by standard chromatographic procedures.

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*ELISA reaction*

*(1) IgA peroxidase conjugate.* The IgA (5 mg) (Lowry et al., 1953) was labelled covalently with 5 mg of peroxidase (grade I, Boehringer) as described by Nakane and Kawari (1974). The conjugate was filtered (0.22 $\mu$m, Millex-GS, Millipore) and then stored at 4°C.

*(2) Non-covalent conjugate.* The ELISA reaction was performed in the classical manner (Engvall and Perlmann, 1972) on 96-well plates (Nunc-Immuno plates II-C-F). The IgA antigens were coated overnight in carbonate buffer (0.2 mg/ml) and the remaining steps carried out in PBS buffer containing 0.05% Tween 20 and 1% gelatine (Difco).

The conjugated immune complex was made with specific anti-IgA antibody and labelled IgA. The specific rabbit anti-IgA was made after several passages through IgG and IgM Sephacryl S-300 immunosorbent columns (Wright and Hunter, 1982). The purity of the antibodies was tested with the ELISA technique until the optical density of the reaction was less than 0.05 against IgG and IgM (plate coated with 0.1 mg/ml). Different dilutions of these 2 substances (antibody and IgA-peroxidase) were mixed for 2 h at 37°C, and gently shaken with a stirring movement (200 rpm, Gyrotary shaker G10). The soluble complex was immediately used for the ELISA reaction (100 $\mu$l/well, 2 h, 37°C).

The reaction was evaluated at 405 nm with a Vernon spectrophotometer after a 2 h contact with ABTS-$H_2O_2$ (Boehringer).

## Results

*Immune complex conjugate (anti-IgA-IgA-peroxidase)*

Three different dilutions of a specific anti-IgA (1/100, 1/200, 1/500) were mixed

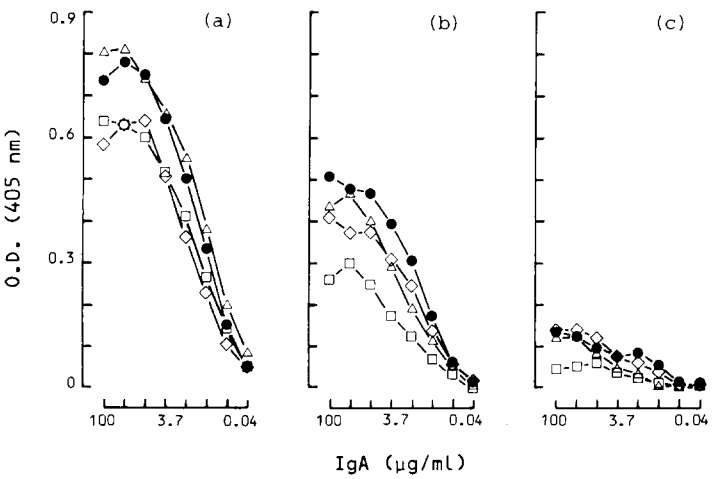


Fig. 1. Optical density of the ELISA reaction (405 nm) with different combinations of the immune complex conjugate in relation to different quantities of IgA coated onto the plate: Anti-IgA antibodies: (a) 1/100, (b) 1/200, (c) 1/300. IgA-peroxidase: □———□, 1/500; △———△, 1/1000; ●———● 1/2000; ◇———◇, 1/4000.

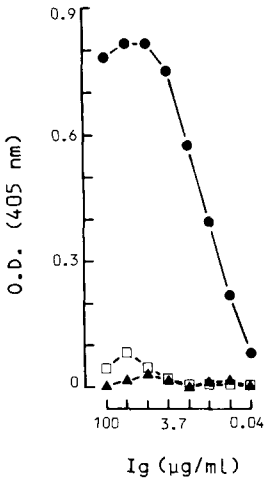


Fig. 2. Optical density of the ELISA reaction (405 nm). Cross reaction between the immune complex-conjugated anti-IgA and different immunoglobulin preparations; ●———●, IgA; □———□, IgG; △———△, IgM.

with 4 different dilutions of the previously prepared and labelled IgA (1/500, 1/1000, 1/2000, 1/4000). Fig. 1 illustrates the results of this reaction.

The only antibody solution which gave a suitable reaction was 1/100. The labelled IgA was used at 1/1000 or 1/2000.

*Specificity of the reaction*

To test the specificity of the reaction against the other classes of pig immunoglobulins, purified IgG and IgM were coated onto the plate. Practically no cross-reaction was found, even against high concentrations of IgG or IgM (0.1 mg/ml) coated onto the plate. (Fig. 2).

*Applications*

With this technique we have detected the kinetics of excretion of IgA-specific rotavirus coproantibodies in experimental infections of fattened pigs (Bernard et al., 1984) and the longitudinal distribution of the IgA rotavirus antibodies in the pig gut. We have also found a heterogeneous rotavirus antibody distribution, with many 'waves' between 9.3 µg and 1.1 µg per intestine segment of 25 cm length (S. Bernard, 1985, submitted).

## Discussion

A major disadvantage of the ELISA test originally described for the measurement of antibodies was the requirement of specifically purified antibodies to be conjugated directly and covalently to the enzyme. The linkage of alkaline phosphatase

to antibodies with glutaraldehyde is relatively difficult and requires the purification of the specific antibodies by affinity chromatography.

The use of peroxidase is very simple but sometimes leads to a loss of specificity (personal observations). To avoid these problems it is possible to use complexes with anti-alkaline phosphatase (Butler et al., 1980) or with peroxidase (Sternberger et al., 1970). It is also possible to make soluble complexes with a labelled immunoglobulin and a specific anti-immunoglobulin. This technique does not require the purification of the antibody immunoglobulins and the linkage of IgA to peroxidase is very easy.

The conjugated IgA is stored, after 22 $\mu$m filtration, at 4°C and the antibody at −70°C. The reaction has the same lower limit of detection as the classical reaction is, 10 ng/ml and the optical reaction obtained with 40 ng/ml of IgA coated on the plate is the same as that given by 30 $\mu$g/ml of IgG. Cross reactions are relatively low. We have shown that this process can be extended to all the immunoglobulin classes, with similar results.

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