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# PROPERTIES AND USE OF MUMPS VIRAL ANTIGEN FOR DETECTION OF SPECIFIC IgG AND IgM ANTIBODIES IN EZYME-LINKED IMMUNOSORBENT ASSAY

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*Summary.* — Specific mumps virus antigens of three purification degrees have been prepared and their quality was evaluated in the enzyme-linked immunosorbent assay (ELISA) with specific human sera. Two enzyme immunoassays were elaborated, namely a simple sandwich method for IgG and the IgM-capture technique for IgM detection. Four different lots of mumps antigen were tested in these two ELISA systems. All antigens of corresponding purification degrees showed practically the same properties.

*Key words*: *mumps virus antigen*; *IgG and IgM antibodies*; *enzyme immunoassay*

## *Introduction*

Determination of specific antibodies to mumps virus is of importance for diagnosis of diseases caused by mumps virus, for epidemiological studies, and for determination of immunological efficiency of mumps vaccines (Sakata *et al.*, 1985; Nigro *et al.*, 1986; Fedová *et al.*, 1987; Linde *et al.*, 1987). In the case of parallel detection of IgG and IgM antibodies ELISA is very adventageous, because it enables to reach the results in a relatively fast and easy way. We devel- oped a new method in our laboratory for the preparation of the specific mumps virus antigen suitable in ELISA (Grubhoffer, 1986). For the determination of IgG antibodies a simple sandwich method was used. For the determination of IgM antibodies the IgM-capture technique was applied, because the indirect method of IgM detection often leads to mistakes. Mumps virus antigens were tested with human specific sera using "sandwich" (IgG) as well as "capture" (IgM) techniques.

## *Materials and Methods*

*Antigens.* The Enders strain of mumps virus was used for preparation of viral antigens (Pa-Ag). The virus (reproduced in cells of chicken embryo allantois) was concentrated by precipitation with polyethylene glycol 6 000 and further purified by high speed centrifugation at 100 000 × g through a 20% (w/w) sucrose cushion and on a linear sucrose gradient of 20—55% (w/w) (Grubhoffer and Novotný, 1986).

*Human sera.* For the evaluation of the quality of all antigens tested in ELISA acute (Pa-PS/IgM) and convalescent (Pa-PS/IgG) patient sera were used. The presence of specific anti-

bodies was first determined in haemagglutination-inhibition and immunofluorescence tests. The selected negative sera (Pa-NS) contained no antibodies to mumps virus by these tests.

*Hyperimmune horse serum and peroxidase labelled conjugate of specific horse gamma-globulin.* A serum obtained after immunization of a horse with purified mumps virus antigen was used for isolation of gamma-globulin by the combined precipitation procedure with caprylic acid and ammonium sulphate (McKinney and Parkinson, 1987). Specific horse gamma-globulin was then coupled with horseradish peroxidase (RZ = 3, Boehringer Mannheim) by the oxidation method in the presence of iodine (Nakane and Kawaoi, 1974).

*Protein determination.* Protein content in antigen preparations was determined colorimetrically (Lowry *et al.*, 1951).

*ELISA system for detection of IgG antibodies.* Specific antigens were attached onto the surface of polystyrene microtitre flat-bottom plates (Novogen, ÚMG ČSAV, Czechoslovakia) by passive adsorption in the concentrations from 0.2 to 20.0 μg prot./ml. Then negative and positive control sera were applied in dilutions from 1 : 100 to 1 : 10 000. The conjugate, swine anti-human IgG antibodies labelled with horseradish peroxidase (SwAHu/IgG/Px, SEVAC, Czechoslovakia), was examined in dilutions from 1 : 500 to 1 : 30 000. Ortho-phenylendiamine was used as a substrate. After stopping the enzyme reaction, absorbances of all samples were read at 492 nm using a spectrophotometer Uniscan II (Flow Laboratories).

*ELISA system for the detection of IgM antibodies.* Swine anti-human IgM antibodies (Q-SwAHu/IgM, SEVAC, Czechoslovakia) were attached onto the surface of microtitre flat-bottom plates by passive adsorption in the concentrations from 1.0 to 32.0 μg prot./ml. Control human negative and positive sera were than applied in the dilutions from 1 : 100 to 1 : 10 000. In the further step, the mumps antigen was added in the concentrations from 0.2 to 20.0 μg protein/ml. In the third layer, there was applied the conjugate, horse anti-mumps antibodies labelled with horseradish peroxidase (HoAPa/Px), in dilutions from 1 : 500 to 1 : 20 000. Ortho-phenylendiamine was used as substrate. The evaluation of the reaction was performed in the same way as described above for IgG antibodies determination.

**Table 1. Specificity of the tested mumps antigens in ELISA**

| Mumps virus antigen | Ratio of $\frac{^{A}\text{Pa-PS/IgG}^{**}}{^{A}\text{Pa-NS}}$ | Ratio of $\frac{^{A}\text{Pa-PS/IgM}^{**}}{^{A}\text{Pa-NS}}$ |
|---|---|---|
| Pa-Ag1/I | 3.91 | 2.08 |
| Pa-Ag1/II | 4.58 | 2.65 |
| Pa-Ag1/III | 6.15 | 3.12 |
| Pa-Ag2/I | 4.55 | 2.11 |
| Pa-Ag2/II | 5.30 | 2.45 |
| PaAg2/III | 7.02 | 3.28 |
| Pa-Ag3/I | 3.75 | 1.90 |
| Pa-Ag3/II | 4.72 | 2.32 |
| Pa-Ag3/III | 6.51 | 2.96 |
| Pa-Ag4/I | 3.68 | 1.88 |
| Pa-Ag4/II | 4.66 | 2.53 |
| Pa-Ag4/III | 6.28 | 3.05 |

* Pa-Ag1-4/I — mumps virus antigens after direct ultracentrifugation.
Pa-Ag1-4/II — Pa-Ag1-4/I further purified through a sucrose cushion.
Pa-Ag1-4/III — Pa-Ag1-1-4/II further purified on a linear sucrose gradient.

** $^{A}$Pa-PS/IgG, $^{A}$Pa-PS/IgM, $^{A}$Pa-NS — the absorbances measured in the relevant ELISA tests by the detection of specific anti-mumps antibodies in control human Pa-PS/IgG (1 : 10 000), Pa-PS/IgM (1 : 2 000), and Pa-NS (1 : 500).

*Results*

Four different lots of mumps antigens (Pa-Ag) have been evaluated. All antigens were gradually purified by direct ultracentrifugation (Pa-Ag/I), by ultracentrifugation of Pa-Ag/I through a sucrose cushion (Pa-Ag/II) and by ultracentrifugation of Pa-Ag/II on a linear sucrose gradient (Pa-Ag/III). As a criterion of the quality of the tested antigens their specificity was considered, which was expressed numerically as a ratio of the absorbances related to the control human positive and negative sera in the detection of specific antibodies under optimum conditions. These conditions were found by means of "chequer board" titrations of all components used in ELISA systems. It was found out that the optimum protein concentration for all types of Pa-Ag, to attain the maximum discrimination between positive and negative sera, was in the range from 2.0 to 3.0 μg protein/ml. The data on the antigen specificity are summarized in Table 1. For the final evaluation of the tested Pa-Ag in ELISA the control human positive and negative sera were used in the following dilutions: Pa-PS/IgG (1 : 10 000), Pa-PS/IgM (1 : 2 000), and Pa-NS (1 : 500). These dilutions of control sera were selected so that the absorbances measured after stopping the reaction were in the range from 0.10 to 1.50, which ensured sufficient reliability of the results obtained.

*Discussion*

Four different lots of mumps antigens prepared in the same way were tested under identical conditions in ELISA systems developed for the detection of human anti-mumps IgG and IgM antibodies. The properties of the antigens of various purity (Pa-Ag/I, Pa-Ag/II, and Pa-Ag/III) were compared. The optimum concentrations of all three types of the tested antigens were always in the range from 2.0 to 3.0 μg protein/ml. These results are in a good correlation with the values stated by other authors in similar systems (Sakata *et al.*, 1985; Julkunen *et al.*, 1984; Kristensson *et al.*, 1984). The degree of purification affected the specificity of the tested Pa-Ag strongly. Maximally purified antigens allowed much more better discrimination between control human positive and negative sera in ELISA than the antigens of lower degree of purification. The preparation of more purified Pa-Ag requires larger amounts of initial viral material, but it is worth to ensure the better quality (specificity) of these antigens. The checking of four different lots of mumps antigens confirmed the reliability of the respective preparation procedure, which is of importance with regard to the commercial production of these antigens for the detection of human IgG and IgM antibodies to mumps virus in ELISA.

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