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MONOCLONAL ANTIBODIES FOR THE CONTROL OF INFLUENZA VIRUSVACCINES

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ABSTRACT

Hybridomas producing haemagglutination inhibiting monoclonal antibodies against influenza A/Texas/1/77 H3N2 were developed. One hybridoma producing antibodies reacting with Victoria/3/75, Texas/1/77, Bangkok/1/79 and England/496/80 was selected to determine the potency of influenza virusvaccines.

Tests were performed in a newly developed Enzyme Linked Immunosorbent Assay (ELISA). For this purpose microtiter plates were successively coated with the monoclonal antibody, washed, incubated with vaccine or standard, washed, incubated with the peroxidase conjugated monoclonal antibody, washed and finally incubated with a substrate.

Samples of the vaccine and of a standard containing 25-100 ng of antigen were assayed in the ELISA and the results were compared with those obtained in a rocket electrophoresis method.

Linear regression analysis of the results showed that the correlation coefficients obtained with standards and vaccines for both methods were greater than or equal to 0.96.

The comparison of vaccine potencies determined in the ELISA and the rocket electrophoresis method will be discussed.

INTRODUCTION

One of the main difficulties in both preparing and testing of influenza virusvaccines is formed by the frequently occurring shifts and drifts in the antigenic reactivities of the surface antigens. Each time when one of the three vaccine strains changes, new reference antigen and new antisera have to be prepared to test the potency of the vaccine by either single radial diffusion (SRD) (3) or rocket electrophoresis (RE) (1). This problem may be reduced by using monoclonal antibodies against relatively persistent antigenic determinants. Although a new reference antigen preparation still has to be prepared after each strain switch, the antibody preparations can be used for many years which will improve the standardization of the assay.

MATERIALS AND METHODS

Reference antigens and antisera were obtained from the National Institute for Biological Standards and Control, London.

The RE (1) was performed with a slight modification : the gel used consisted of 1% agarose (Sea plaque, FMC corp., Marine colloids div.) in the Rocket buffer containing 0.0375 M 5.5-diethylbarbituric acid and its sodium salt (Merck), 0.04% calcium lactate (Merck), 0.01% thiomersal (Lilly) and 1% Triton \times -100 (BDH Chemicals) pH = 8.6 and was poured on a levelled plate.

Monoclonal antibodies were produced and screened for as described previously (2). One (broad) monoclonal antibody (6-4-D5 E7) was selected, that reacted with influenza A (H3N2) viruses Victoria/3/75, Texas/1/77, Bangkok/1/79 and England/496/80 in the Haemagglutination Inhibition Test.

Haemagglutinin content was determined with a micro Enzyme Linked Immuno Sorbent Assay (ELISA). The wells of a plate (Serocluster, 96-well E1A plate, Costar) were coated with 0.1 ml of a suspension of monoclonal antibodies containing 2.5 µg protein/ml in PBS (pH = 7.0) overnight at room temperature. Successively the plate was washed thrice with PBS containing 0.5% BSA and 0.01% Tween 80 (PBS-BT) and once with PBS. Then the plate was incubated with 0.15 ml of the hybridoma culture medium for 1 hr at 37°C and washed thrice with PBS-BT and once with PBS. The plate was either incubated with a dilution of the reference or dilutions of influenza virusvaccines in Rocket buffer for 12 hours at 37°C. After washing thrice with PBS-BT and once with PBS the plate was incubated with 0.1 ml 1:8000 conjugate consisting of the monoclonal antibody used for coating (0.65 mg protein/ml) and horse radish peroxidase (Sigma type VI) 0.65 mg/ml for 1 hour at 37°C. Successively the plate was washed thrice with PBS-BT and once with PBS and incubated with 0.1 ml substrate containing tetramethylbenzidine 1.2 mg/ml, 0.003% H₂O₂, 6.8% sodium acetate and 1.4% citric acid for 10 min. at room temperature. The reaction was stopped by adding 0.05 ml 2N sulphuric acid. Extinctions were measured with a Multiscan (Titertek) at 450 nm.

The reproducibility of the ELISA with monoclonal antibodies was investigated in the following manner.

A six fold dilution series of reference antigen of Bangkok/1/79 was tested 20 times in both RE and ELISA. With linear regression analysis (concentration against peak height in the RE and logarithm of concentration against extinction in ELISA) the slope, correlation and regression coefficient (square of the covariance/variance in the dilutions ratio) for each dilution series were calculated.

With trend analysis the slopes of each of the 20 tests were compared with the slope of the mean of the 20 tests and the standard deviation of the first two, three tests etc. were calculated.

The values of the tests should preferably be between + and - two times the standard deviation and at least between + and - three times the standard deviation.

RESULTS

The monoclonal antibody used in RE with antigen Bangkok/1/79 did not produce visible peaks, whereas this monoclonal antibody showed weak precipitation lines in Ouchterlony double diffusion test with antigens Bangkok/1/79 and Texas/1/77.

In the ELISA with the monoclonal antibody as catching antibody there was a clear correlation between the concentration of the antigen and the resulting extinction (Table I). The reproducibility appeared from the 20-fold experiments and trend analysis showed that the results remained between + and - two times the standard deviation (Fig. 1). The same results were found with RE (Fig. 2).

Table I. Linear regression analysis

Number of experiments	20
Number of dilutions per experiment	6
Regression coefficient	≤ 0.10
Correlation coefficient	≥ 0.96

DISCUSSION

Due to their high specificity and low affinity monoclonal antibodies are known to have a limited reaction in precipitation tests. This could explain why in the double diffusion test, with small distance between antigen and monoclonal antibody, a weak reaction was seen, whereas no reaction was found in the RE in which the monoclonal antibody throughout the gel had a uniform concentration that apparently was insufficient for visible peaks.

Interassay quality assurance of references.

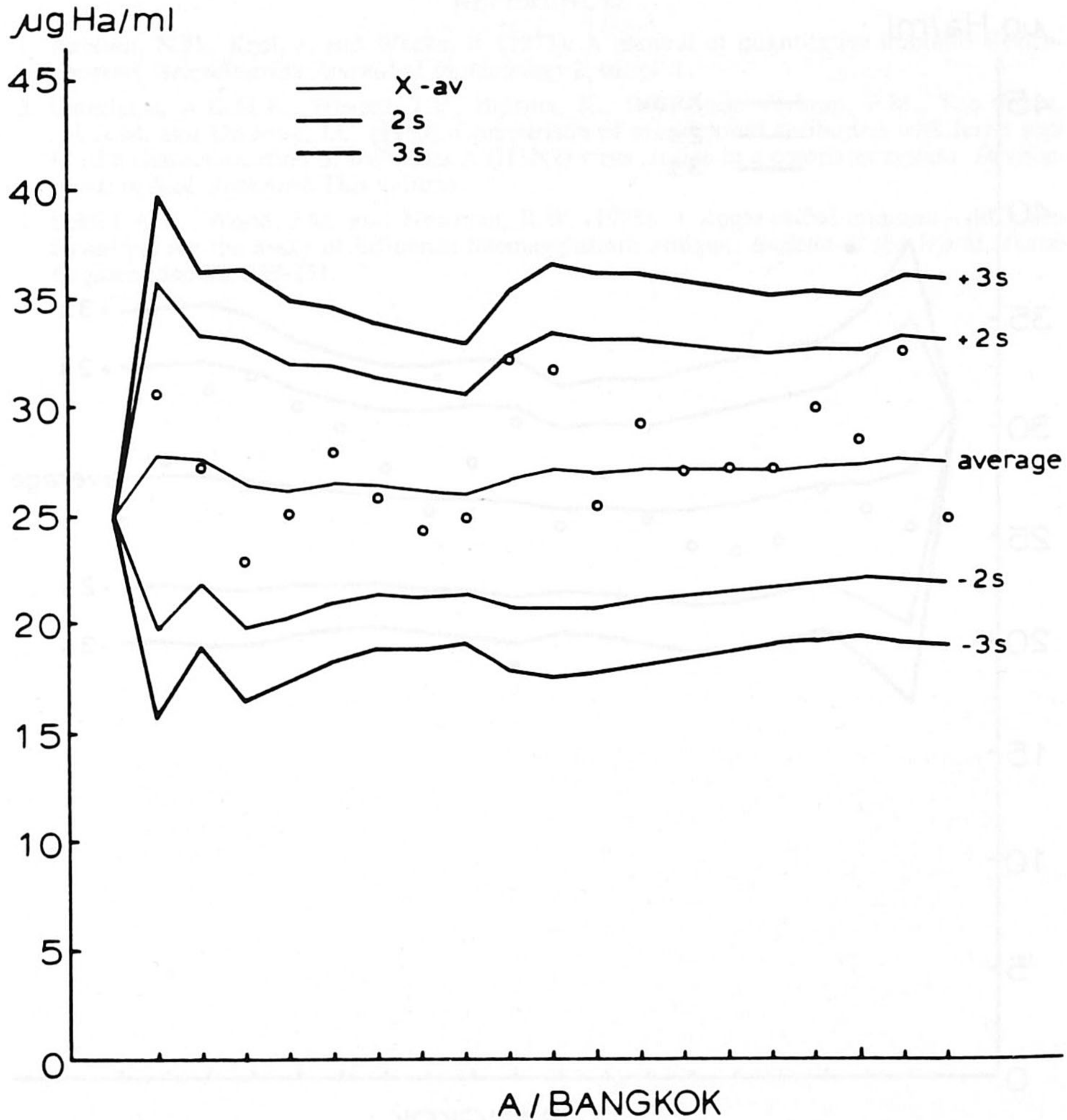


Fig. 1. Trend analysis of 20 ELISAs. The y-axis indicates the amount of haemagglutinin ($\mu\text{g/ml}$) and the x-axis the successive assays. The lines represent from the top downwards respectively the average of the first two, three etc. experiments (a.v.) + three times the standard deviation, a.v. + 2 s.d., a.v., a.v. - 2 s.d. and a.v. - 3 s.d.

The first results of ELISA are promising : there turned out to be a correlation between the logarithms of the concentration and the observed extinction. The correlation coefficient was ≥ 0.96 and the regression coefficient was ≤ 0.10 in 20 experiments. The reproducibility test showed that the variations found, did not exceed 2 times the standard deviation. These results are like those obtained with RE.

A «broad» monoclonal antibody reacting with influenza B/Singapore/222/79 has been raised. After the preparation of a conjugate this antibody will also be tested in the ELISA. Successively, monoclonal antibodies versus Brasil will be

Interassay quality assurance of references

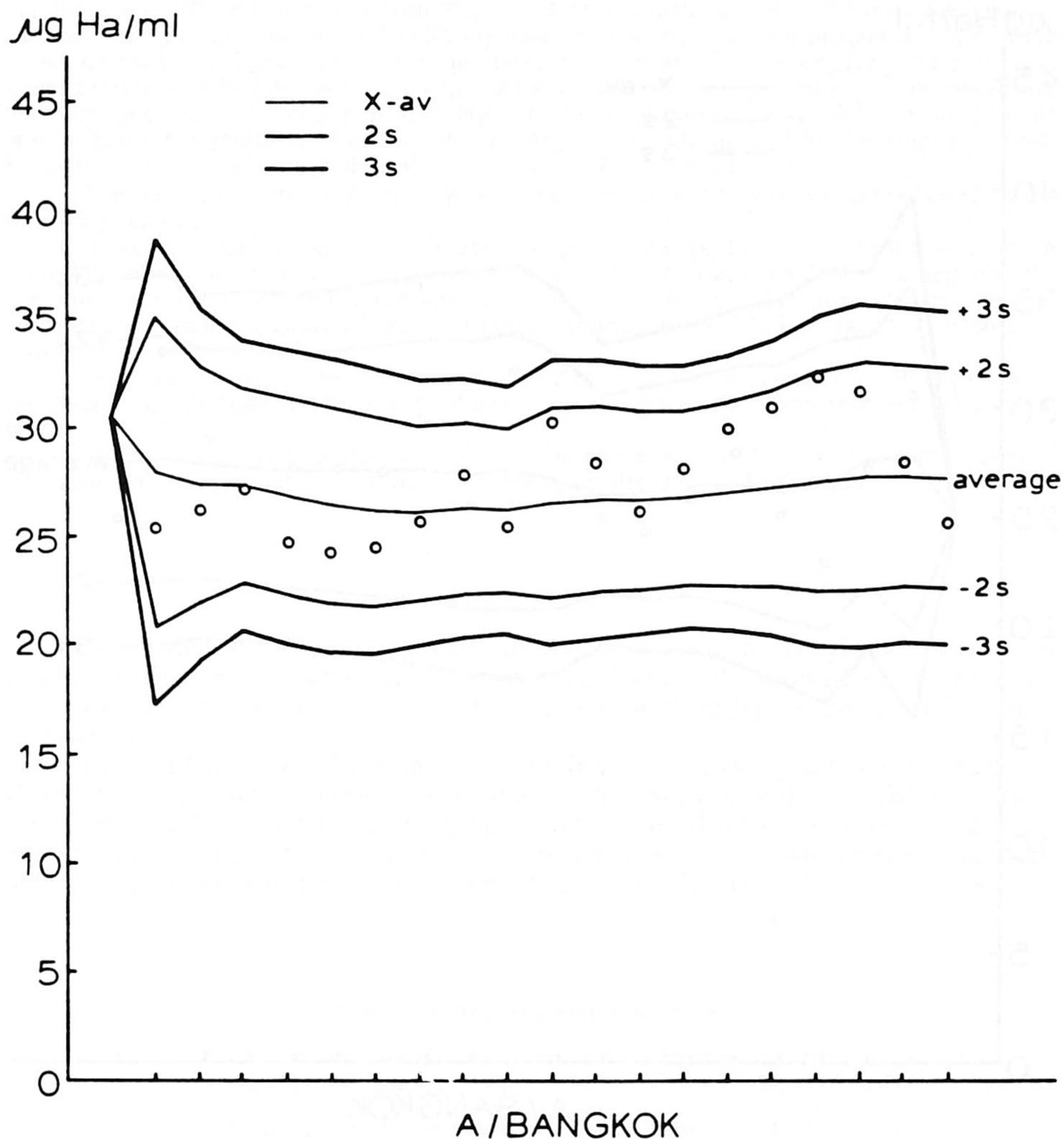


Fig. 2. Trend analysis of 20 REs. The y-axis indicate the amount of haemagglutinin ($\mu\text{g/ml}$) and the x-axis the successive assays. The lines represent from the top downwards respectively the average of the first two, three etc. experiments (a.v.) + three times the standard deviation, a.v. + 2 s.d., a.v., a.v. - 2 s.d. and a.v. - 3 s.d.

raised. Finally we intend to prepare four broad monoclonal antibodies versus each strain.

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