APPLICATION OF THE ELISA TECHNIQUE TO PROBLEMS IN THE SEROLOGIC DIAGNOSIS OF PLAGUE[1]

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A new technique, the enzyme-linked immunosorbent assay (ELISA) appears to have advantages in some areas over the standard indirect hemagglutination (IHA) test for detecting antibody to specific F-1 antigen of the plague bacillus, Yersinia pestis. *In this presentation the authors compare the results of ELISA and IHA testing and discuss possible ways the former could contribute to solving certain problems of routine plague serology.*

Introduction

The indirect hemagglutination (IHA) test has been utilized for over 20 years to detect antibody to the specific F-1 antigen of the plague bacillus, *Yersinia pestis*. Although the test is reliable and simple, many problems with it have been encountered concerning standardization of procedures and reagents (*1*). Accordingly, any new serologic technique merits study. This article reports on the evaluation of one such technique (*2*), the enzyme-linked immunosorbent assay (ELISA), in terms of its potential for use in several problem areas of routine plague serology.

Materials and Methods

The methodology of Ruitenberg et al. (*3*) was followed throughout. F-1 antigen was dissolved in carbonate-bicarbonate coating buffer at pH 9.6. One-tenth ml of this solution was then placed in each well of an ELISA plate[7] and dried overnight at 37°C. After that the plates were washed three times in phosphate-buffered saline containing 0.05 per cent Tween 20[8] (PBST) and air-dried.

To decrease possible "background staining," 0.4 ml of a solution of 4 per cent bovine serum albumin (fraction V)[9] in H_2O was added to each well of the ELISA plate. The plates were then incubated 30 minutes at 37°C, washed three times with PBST, and air-dried.

Serial twofold dilutions of inactivated test sera in PBST were then prepared, and 0.1-ml aliquots of appropriate dilutions were transferred to the proper wells of the ELISA plates with an Eppendorf pipette,[10]

[1]Summary in Spanish in the *Boletín de la Oficina Sanitaria Panamericana*, 1979. In conducting the research described in this article the investigators adhered to the *Guide for the Care and Use of Laboratory Animals*, as promulgated by the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council of the United States.

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after which the plates were again incubated for 30 minutes at 37°C, washed three times with PBST, and air-dried.

One-tenth ml of a suitable dilution of horseradish peroxidase conjugated to species-specific anti-immunoglobulins in PBST containing 1 per cent bovine serum albumin was then added to each plate well. Plates were incubated for 1 hour at 37°C, washed three times with PBST, and air-dried.

A substrate solution was prepared by: dissolving 80 mg of 5-aminosalicylic acid in 70°C distilled water, cooling to room temperature, adjusting to pH 6.5, and adding 1 ml of a 0.05 per cent H_2O_2 to each 9 ml of the solution. This was followed by placing 0.1 ml of the substrate solution in each well and incubating the plate for 1 hour at 37°C. After final incubation, enzymatic activity was measured immediately by reading the substrates' optical densities at 455 nm.

To serve as additional "background controls" for individual test sera, control plates were prepared using a procedure similar in every way to that described above except that F-1 antigen was not added. Endpoint titers were derived by first subtracting the greatest optical density obtained with negative control sera from that of the serum under test. If the value obtained was a titer $\geq 1{:}4$, it was considered positive.

Horseradish peroxidase conjugated to anti-human IgG and IgM, prepared in goats, as well as anti-rat IgG prepared in rabbits,[11] were titrated against varying dilutions of F-1 to determine the optimal F-1 concentration. These preliminary checkerboard titrations showed the optimal concentration of F-1 for coating to be 10 μg/ml.

Test sera were obtained from 38 albino *Rattus norvegicus* recovering from experimental infection with *Y. pestis*; from 10 patients with bacteriologically confirmed plague; and from 26 contacts of plague patients. All the sera were tested simultaneously for F-1 antibody by both the IHA and ELISA methods (*4*).

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Results

Comparison of the ELISA and IHA test results showed them to be in good agreement, indicating comparable ability to demonstrate the presence or absence of F-1 antibody. Use of the purified F-1 antigen and the horseradish peroxidase conjugated to immunoglobulins provided a remarkably clean and reproducible test system. This was particularly true when 50 per cent endpoints from the linear portions of the curves were compared. No problems with high levels of background activity were encountered in any of the controls.

F-1 antibody (IgG) was readily detected in the sera of convalescent *R. norvegicus*, an important plague reservoir. Figure 1 shows

Figure 1. Observed levels of antibody (IgG) to the specific F-1 antigen of *Y. pestis* in the sera of plague-infected *R. norvegicus*, as measured by the ELISA technique.

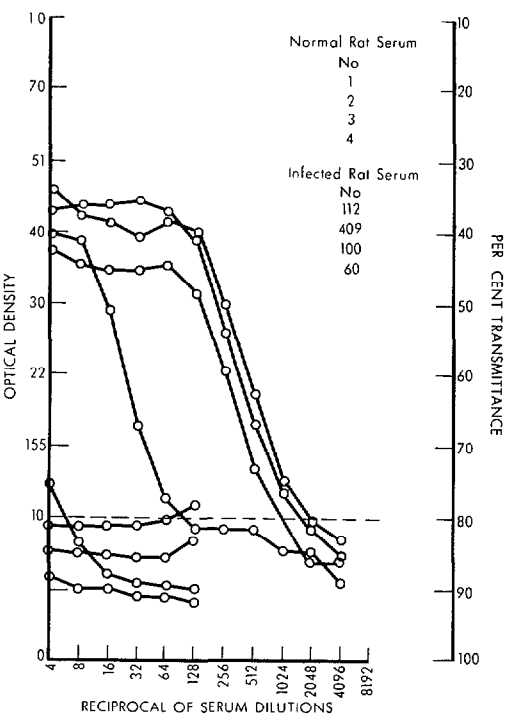

Table 1. Serologic responses to infection or presumed infection observed in bacteriologically confirmed plague patients or contacts, as measured by the ELISA technique.

	Acute sera		Convalescent sera	
	IgG	IgM	IgG	IgM
Patients				
1	1:32	—	1:16,384	1:2,048
2	—	—	1:512	1:128
3	—	—	—	—
4	1:64	—	1:4,096	1:128
5	1:128	1:128	1:16,384	1:2,048
6	1:32	—	1:2,048	1:2,048
7	—	—	1:1,024	1:1,024
8	1:64	—	1:256	1:256
9	1:4,096	1:256	1:32,768	1:4,096
10	1:128	1:256	1:2,048	1:2,048
*Contacts**				
1	N.D.	N.D.	1:4,096	1:1,024
2	N.D.	N.D.	1:256	1:256

— = Less than 1:4.
N.D. = Not done.
*Sera from 2 of 26 contacts exposed to plague in nature. Neither acute sera nor specimens for bacteriologic examination were collected.

data obtained from titrations of convalescent sera collected from four plague-infected rats. Similar results were obtained from tests on another 34 convalescent rat sera not shown in Figure 1. The ELISA technique was also very useful in examining sera collected from bacteriologically confirmed plague patients (Table 1). A classic serologic response to infection with *Y. pestis* was observed in nine cases, with both IgG and IgM antibody to F-1 being detected. Patient number 3 was found to be negative serologically. Unfortunately, the strain of *Y. pestis* recovered from this patient was not available to test the production of F-1 antigen.

As the table also indicates, both IgG and IgM immunoglobulins to F-1 were detected in sera from 2 of 26 contacts tested. Contact number one resided in the home of an individual who died as the result of a bacteriologically confirmed plague infection; she was treated for presumptive plague infection several days following the onset of adenopathy with high fever. Contact number two had a similar history but apparently experienced a milder form of the disease (pestis minor). Unfortunately, specimens for bacteriologic examination were not obtained from these two subjects.

Discussion

The ELISA technique provides a simple and highly reproducible method for demonstrating the presence of F-1 antibody in sera collected from plague-infected *R. norvegicus* or human beings. It has particular value in that both IgG and IgM antibodies to F-1 can be detected and measured directly.

While precise records were not available for the plague patients listed in Table 1, acute sera were drawn on hospital admission and convalescent sera were collected following a 10-day course of treatment with antibiotics. Sera collected from contacts of plague patients were obtained as soon as possible following exposure, but at varying periods of time after they received treat-

ment. The rapid appearance in convalescent sera of both IgG and IgM antibodies to F-1 at rather high titers is of diagnostic value and of great potential use for establishing the time-frame during which infection probably occurred.

The good quality of the results obtained in testing infected *R. norvegicus* sera indicates that the ELISA technique may have some applications in field surveys. The equipment required is relatively simple and should be available even in smaller laboratories; ELISA plates coated with F-1 antigen could be lyophilized in central laboratories and supplied to the field for use; and antibody titers can easily be determined by visual examination.

In no way did the ELISA results conflict with those obtained with the simultaneous IHA tests. It is not suggested that ELISA supplant the IHA test; however, the ELISA technique appears to have broader applications in the area of plague serology, where it offers the possibility of detecting antibody to specific antigens of *Y. pestis* other than F-1 and permitting the serologic diagnosis of disease resulting from infections with the occasionally encountered nonencapsulated *Y. pestis* (*5, 6*). Further development and evaluation of the ELISA technique is in progress.

SUMMARY

The indirect hemagglutination (IHA) test has been used for many years to detect antibody to the specific F-1 antigen of the plague bacillus, *Yersinia pestis*. Though the test is reliable and simple, standardization of procedures and reagents has posed problems. This article reports on evaluation of a new technique, the enzyme-linked immunosorbent assay (ELISA) which appears to have advantages over the IHA test in certain problem areas of routine plague serology.

More specifically, ELISA has been found a simple and highly reproducible technique for demonstrating F-1 antibody in sera from plague-infected rats (*Rattus norvegicus*) and human beings. It has particular value in that both IgG and IgM antibodies to F-1 can be detected and measured directly. The quality of the results obtained to date also indicate that the ELISA technique may have some applications in field surveys.

It is not suggested that this technique should supplant the IHA test. However, the former does appear to have broader serologic applications, where it offers the possibility of detecting antibody to specific plague bacillus antigens other than F-1 and permitting serologic diagnosis of disease resulting from infection with nonencapsulated *Y. pestis*.

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