Variability in EIA plates used in ELISA tests for the detection of antibodies to *Legionella pneumophila*

We recently reported the development of an ELISA test for the detection of antibodies to *Legionella pneumophila* using Flow EIA plates (76-381-04). In subsequent batches, however, we have noted that there were four different kinds of plate with different properties within each box, which made results of further studies unreproducible. We therefore examined EIA plates from various manufacturers, assessing them by our whole plate assay. In this assay all the wells of an EIA plate were coated with EDTA-extracted *L. pneumophila* serogroup 1 antigen, followed by a positive control serum at the maximum dilution previously found to give a positive reading. Anti-IgM peroxidase conjugate and then enzyme substrate were added as in the assay procedure. The absorbance values were measured and the standard deviation for the whole plate was calculated.

Results from Nunc plates were found to have the lowest standard deviation. When tested with a few sera with anti-*L. pneumophila* IFA titres of <1/16 and a few sera with titres of ≥1/16 the results showed good correlation with previous values obtained using Flow plates.¹

Four hundred and eight sera, collected during 1981 in the Cambridge area from patients with lower respiratory tract symptoms which were considered worth screening for *L. pneumophila* infection, were tested by ELISA using Nunc plates.

When the results with these sera were compared with IFA titres (Figs. 1 and 2), many sera which had IFA titres of <1/16 gave high ELISA anti-*L. pneumophila* titres. The effect was more marked with IgM than with IgG. Two hundred and seventy-eight/384 sera with IFA IgM titres of <1/16 had an ELISA titre of <1/20 but there were some sera with IFA IgM titres of <1/16 with ELISA titres of 1/2560; 294/398 sera with IFA IgG titres of <1/16 had an ELISA titre of <1/20 but one serum with an IFA IgG titre of <1/16 had an ELISA IgG titre of 1/2560. Thus, for both IgM and IgG detection, ELISA assays using Nunc plates did not satisfactorily differentiate between sera with IFA titres of <1/16 and ≥1/16 (see Figs. 1 and 2).

Since these results were at variance with our published results¹ using Flow EIA plates, we felt it imperative to seek a better solid phase, and since Falcon Flexible plates (Becton Dickinson) had just been introduced, these were examined by the whole

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Fig. 1 *Anti-L pneumophila* ELISA IgM titres in sera with IFA titres of <1/16 and ≥1/16 using Nunc EIA plates.

Fig. 2 *Anti-L pneumophila* ELISA IgG titres in sera with IFA titres of <1/16 and ≥1/16 using Nunc EIA plates.

Fig. 3 *Anti-L pneumophila* ELISA IgM titres in sera with IFA titres of <1/16 and ≥1/16 using Falcon Flexible plates.
plate assay and found to be satisfactory.

Results of ELISA assays in Falcon Flexible plates (Figs. 3 and 4) showed much better correlation to IFA titres. Three hundred and five 384 sera with IFA IgM titres of <1/16 had an ELISA IgM titre of <1/20 and the highest ELISA titre in this group was 1/320.

The relatively small number of sera with IFA titres of <1/16 which gave ELISA titres of 1/20 in Figs. 1–3 is an artefact due probably to harsh selection of sera for further titration. However, since this was done in the same manner for all assays, it should not have biased the results.

In our assay employing Falcon Flexible plates, we obtained results which were in good agreement with our previous study and the correlation between IFA and ELISA was good. Relatively few sera with IFA titres of <1/16 gave ELISA titres of >1/80. All sera with IFA titres of ≥1/16 had ELISA titres of ≥1/20, and the ELISA titres (particularly IgG) were generally much higher than the IFA titres.

We have therefore found that using the correct microtitre plate, our modified indirect ELISA test is reliable and specific, and more amenable to large scale routine or survey work than IFA.

This study underlined the value of performing extensive evaluation of the ELISA assay conditions. Our tests with small numbers of sera showed Nunc plates to be satisfactory, and it was only when a large number of sera (by IFA) were tested by ELISA that we realised there was a problem.

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References

Book reviews


The last decade has seen a revolution in our understanding of the processes involved in the inflammatory process. This expansion of knowledge has come about largely through the application of the experimental approaches and the insights derived from cell biology. The picture thus revealed in this volume, inchoate in part though it still remains, makes fascinating reading. Historical perspectives receive their due need of attention in the form of an elegant overview of some of the “historical highlights” of inflammation written by Guido Majno. The rest of this fairly slim volume is divided into seven chapters dealing with various aspects of the pathobiology of acute inflammation and five chapters which consist of “updates” of our knowledge in a number of specific disease areas. Amongst so many excellent contributions perhaps it is invidious to single any out, but the chapters on chemotaxis by Peter Ward and on the role of proteases and oxidants in tissue injury by Aaron Janoff and Harvey Carp are, in my view, of outstanding interest.

The book is well produced and by present day standards moderately priced. I can thoroughly recommend it to all biologists, medical and otherwise, with an interest in this important field.

N WOOLF

Recent Advances in Neuropathology. Ed W Thomas Smith and JB Cavanagh. (Pp 301; illustrated; £23.00.) Churchill Livingstone. 1982

Since the publication of the first volume in this excellent series many new developments in clinical and experimental neuropathology have led to a growing need for a sequel. As with the previous volume this book takes the form of a series of reviews written by neuropathologists and neuroscientists of international reputation. The aim has again not been to survey the whole field of neuropathology, but rather to highlight various subjects of current interest. These range from experimentally orientated topics, including chapters on microglial function and neuronal cytoskeletal proteins through to recent developments in more clinically related areas such as Creutzfeldt-Jakob disease and non-missile head injury. There is also an important chapter on immunohistological techniques which are of increasing relevance in both research and diagnosis. There is a high standard of illustration throughout the book and the texts are clear and informative. This is not only an essential item for all neuropathologists but can be recommended as stimulating reading for any clinician or pathologist with an interest in the nervous system.

DB BROWNELL


This is the first of a series of volumes on immunocytochemistry which are intended to cover all the major techniques and applications in this field. The authors include anatomists, pathologists, micro-