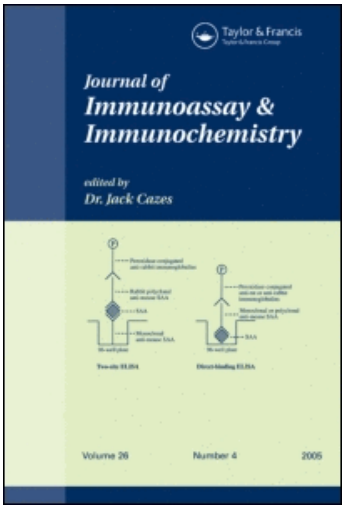

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A HETEROGENEOUS FLUORESCENCE IMMUNOASSAY FOR GENTAMICIN USING A SECOND ANTIBODY SEPARATION

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ABSTRACT

A heterogeneous fluorescence immunoassay (FIA) for gentamicin was developed using a second antibody separation. The separation of bound from free fluorescence label removes a number of endogeneous fluorescent interferences. Correlation with a standard radioimmunoassay (RIA) for gentamicin was acceptable ($r = 0.91$). We conclude that a heterogeneous FIA is a precise, accurate, and convenient alternative to monitoring antibiotic levels. ('KEY WORDS:' Fluorescence Immunoassays, Gentamicin, Heterogeneous Fluorescence Immunoassay, Non-Isotopic Immunoassay, Immunoassay, Fluorescence.)

Nonisotopic immunoassays are currently being developed in order to remove many of the restraints required to perform radioimmunoassays.[1] One such approach is the fluorescence immunoassay (FIA) which substitutes a fluorescence label for the radiolabelled

ligand.[2] The reaction between a ligand (L), a ligand labelled with a fluorescent molecule (L^f), and an antibody [Ab] specific toward both L and L^f may be written:

$$[L] + [L^f] + [Ab] \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} [L - Ab] + [L^f - Ab]$$

where $k_1 >> k_{-1}$.

One difference between an FIA and RIA for a particular ligand is the possibility that the fluorescent labelled ligand (L^f) may undergo significant changes in its fluorescent properties upon binding with an antibody to L^f.

This change may be used as the basis for a homogeneous assay. All homogeneous fluorescence immunoassays (HFIA) are limited by endogenous fluorescent interferences. Such interferences may arise from altered metabolic processes, the presence of drugs such as antibiotics, or any fluorescent material which absorbs light at wavelengths used to excite the fluorescent label and emits fluorescence at wavelengths comparable to the label. Alternately, fluorescence quenching of L^f by endogenous materials may also invalidate a homogeneous FIA.

We decided to investigate the use of a standard RIA separation procedure in a heterogeneous FIA using materials and equipment which did not require a specific manufacturer. The choice of the second antibody approach was made, based on the relative ease and specificity of separation, as well as its acceptance in most laboratories using the RIA technique.

MATERIALS AND METHODS

Reagents and Standards

Antisera. Rabbit anti-gentamicin antisera was prepared according to a previously published method.[3] Goat anti-rabbit antisera was prepared by injection of rabbit IgG into a goat.

Normal Rabbit Serum (NRS). NRS was prepared in our laboratory under sterile conditions.

Phosphate Buffer 0.2 M (pH 7.6) and Glycine Buffer 0.1 M (pH 3.0). Buffers prepared with reagent grade chemicals from Sigma Chemical Company.

Fluoresceinthiocarbamyl gentamicin. Gentamicin was reacted with fluorescein isothiocyanate isomer I (Research Organics, Inc., Cleveland, Ohio 44125) as previously described.[4]

Standards. Gentamicin standards were prepared by adding gentamicin sulfate (Schering Corporation, Kenilworth, N.J. 07033) to tris buffer and making appropriate dilutions with gentamicin free human serum.

Gentamicin radioimmunoassay reagents. Reagents were obtained from American Diagnostics (1598 Monrovia, Newport Beach, CA. 92663).

Apparatus

Spectrofluorometer. A Varian model SF-330 spectrofluorometer (address: Varian Instrument Division, Palo Alto, CA. 94303) was used for all measurements. An anodized aluminum sample holder was constructed in our machine shop to hold the sample tubes used for reading fluorescence. This holder consisted of a 12 mm x 12 mm x

18 mm block of aluminum with a hole drilled in the center and slits 2 mm wide x 8 mm high on two adjacent sides to permit 90^{0} optics. The sample tubes used for reading fluorescence were 6 x 4 mm LP/2 tubes (Luckham LTD., LabroWorks, Victoria Garden, Burgess Hill, Sussex, England).

PROCEDURE

A stock standard (1000 ug/ml) in 0.1 M glycine buffer, pH 3.0 was diluted appropriately with glycine buffer pH 3.0 each time a standard curve was run. Samples and controls were diluted 1:40 in glycine buffer to a final volume of 400 ul. 100 ul of standard, diluted sample or control and 100 ul of label in glycine buffer (10 ng/tube) were added to the appropriate tube. Upon addition of 100 ul of antisera (diluted 1:200 in 0.2 M phosphate buffer), the tubes were incubated for 30 minutes at 37^{0}C.

After this initial incubation, 100 ul of NRS (diluted 1:200 in 0.2 M phosphate buffer) and 100 ul of goat anti-rabbit antisera were added and allowed to incubate for 2 hours at room temperature (a shorter time is acceptable, 2 hours was chosen for our convenience). In order to assure a solid precipitate, 100 ul of saturated sodium sulfate were added along with 400 ul of 0.2 M phosphate buffer, the tubes centrifuged, and the supernatant aspirated. 100 ul of 0.1 M NaOH were added to each precipitate followed by the addition of 400 ul of 0.2 M phosphate buffer to give a final volume of 500 ul. Approximately 300 ul of this volume

were transferred to a 6 mm x 40 mm test tube for measurement of fluorescence.

RESULTS

Precision. The within-run and between-run precision data for three levels of pools are presented in Table 1 and 2. The results

TABLE 1

Within-Run Precision

No. replicate detns.	Mean	S.D.	CV,%
	ug/ml		
10	1.79	0.15	8.4
10	3.89	0.15	4.2
10	8.27	0.37	5.2

TABLE 2

Between-Batch Precision

No. replicate detns.	Mean	S.D.	CV,%
	ug/ml		
10	1.79	0.16	8.8
10	3.89	0.40	10.3
10	8.27	0.86	10.4

TABLE 3

Analytical Recovery Experiment For Gentamicin FIA

Gentamicin Added (μg/ml)	Gentamicin Recovered (μg/ml)	Mean Recovery
2.0	2.1	105
4.0	3.9	97
6.0	5.6	93
8.0	7.8	96
10.0	9.1	91

demonstrate acceptable precision for the homogeneous FIA of gentamicin over the concentration ranges of interest.

Recovery. The stock standard of gentamicin was added to gentamicin free human serum and the results are shown in Table 2. Care was taken not to add a volume of stock standard which would exceed 10% of the final volume.

Method Comparison. Gentamicin values for 127 patient sera were determined by both FIA and RIA. Evaluation of the least squares analysis of the correlation data obtained for both methods yields a correlation coefficient of 0.913 and a scattergram as shown in Figure 1.

DISCUSSION

The results of the second antibody fluorescence immunoassay correlate with the results of a radioimmunoassay for gentamicin. During the course of this study, three batches of labelled gentamicin and first antisera from three different rabbits and seven bleeds, were used over a period of one year. It is evident that this assay is not highly sensitive to a particular lot of reagents.

On comparison of the heterogeneous FIA in this study to the quenching FIA Ref. assay employed by Watson et al (4), we found that the standard curve for the heterogeneous FIA was more sensitive with a drop of 300 relative fluorescence units as compared to a drop of 16 relative fluorescence units over the concentration range of 0 to 16 ug/ml. Another advantage of the HFIA over the quenching assay is that less antisera is required and with our antisera the

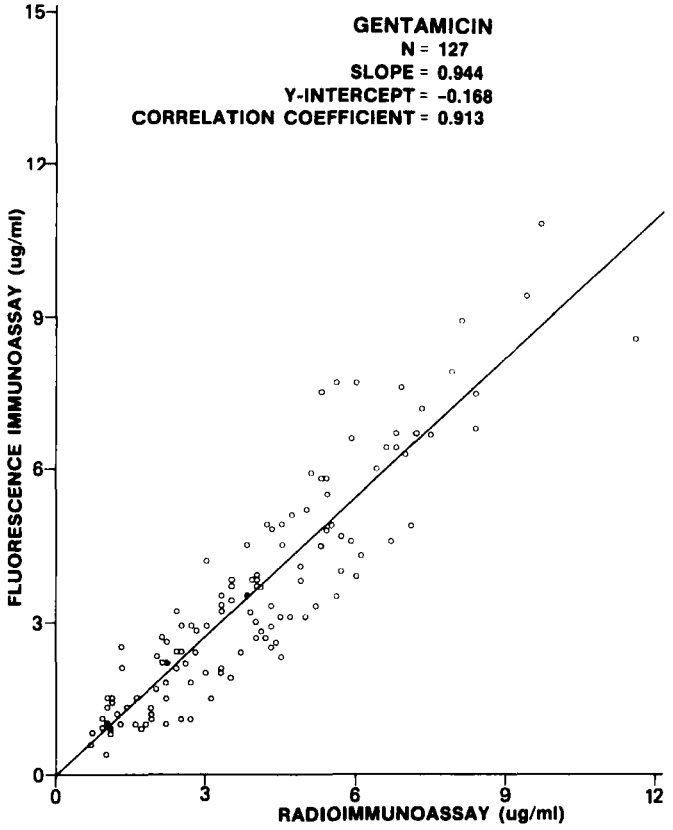

Fig. 1 Correlation between gentamicin levels in patient serum samples determined by fluorescence immunoassay and radioimmunoassay. Closed circles indicate multiple data point.

HFIA used 1/5 of the same antisera per tube required by the quenching assay. In any homogeneous assay, the possibility of an interference must be considered. Theoretically, this objection may be removed by using a blank tube. However, the interference simply raises the background level and reduces the signal to background ratio with a decrease in precision and limit of detection. The patient

population was not selected and included many acutely ill patients on multiple drug therapy. Many specimens were highly lipemic, icteric, and/or hemolyzed. No interference was observed during the course of this study.

In summary, we have demonstrated that it is possible to use the second antibody technique commonly used in many radioimmunoassays in a heterogeneous fluorescence immunoassay. The HFIA presented here is a suitable alternative to the RIA with comparable precision, sensitivity, and freedom from significant interferences. It is anticipated that HFIAs will assume a more important role in the development of non-isotopic immunoassays (NIIAS) to begin to replace RIAs in many applications.

REFERENCES

1. Engvall, E. and Pesce, A.J. Ed., Quantitative Enzyme Immunoassay, Scandinavian Journal of Immunology, 8, Suppl. 7, 1 (1978).

2. O'Donnell, C.M. and Suffin, S.C., Fluorescence Immunoassays, Anal. Chem., 51(1), 33A (1979).

3. Broughton, A. and Strong, J.E., Radioimmunoassay of Iodinated Gentamicin, Clin. Chem. Acta, 66,125 (1976).

4. Watson, R.A., Landon, J., Shaw, E.J. and Smith, D.S., Polarization Fluoroimmunoassay of Gentamicin, Clin. Chem. Acta, 73, 51 (1979).