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## Measurement of the concentration of murine IgG monoclonal antibody in hybridoma supernatants and ascites in absolute units by sensitive and reliable enzyme-linked immunosorbent assays (ELISA)

J.O. Fleming and L.B. Pen

*Department of Neurology, USC School of Medicine, 2025 Zonal Avenue, Los Angeles, CA 90033, U.S.A.*

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We have investigated an enzyme-linked immunosorbent assay (ELISA) for mouse IgG using affinity-purified goat anti-mouse antibodies for capture and detection. This assay was used to measure the absolute or weight/volume concentration of murine monoclonal antibody in hybridoma supernatants. Bovine or horse serum did not interfere with the assay, which permitted reliable measurement of all murine IgG subclasses except IgG3 in the 1–20 ng/ml range. Antibody capture was essentially complete in the optimized assay. In combination with an antigen-dependent ELISA, the assay allowed estimation of the absolute concentration of specific monoclonal antibody in ascites. These rapid and relatively simple assays may be applicable in many situations in which a practical means of measuring murine monoclonal antibodies in weight/volume units is needed.

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*Key words:* Monoclonal antibody; IgG, murine; Ascites; Protein determination

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### Introduction

Monoclonal antibodies have proven to be very useful reagents in many research and clinical applications. Often, it is helpful or essential to know the concentration of a given monoclonal antibody

in absolute, or weight/volume, units. In this regard, one method which has been used to rigorously determine absolute Ig concentration is to purify the antibody and subsequently perform a protein determination. Although precision may be achieved in this way, purification methods themselves vary as to yield, relative purity, and convenience (Manil et al., 1986). Furthermore, Ig extraction from most monoclonal antibody preparations may co-purify substantial amounts of non-specific antibody molecules. For example, even when tissue culture media containing serum is pre-treated with protein A, substantial amounts of non-specific Ig remain (Underwood, 1986).

Alternatively, Ig is frequently measured by radioimmunoassay or ELISA using a specific antigen bound to a solid-phase support. It is not clear,

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*Correspondence to:* J.O. Fleming, Department of Neurology, 142 McKibben Annex, USC School of Medicine, 2025 Zonal Avenue, Los Angeles, CA 90033, U.S.A.

*Abbreviations:* Ig, immunoglobulin; IgG, immunoglobulin G; ELISA, enzyme-linked immunosorbent assay; viral-ELISA, ELISA measuring antibodies to different coronaviruses; C-ELISA, capture ELISA for measuring murine IgG concentration; GAM-HRP, goat anti-mouse IgG conjugated to horseradish peroxidase; GAM-CAP, goat anti-mouse IgG, unconjugated, used in the capture assay; EM, ELISA medium; PBS, phosphate-buffered saline; SPA, Sepharose-protein A.

however, that such assays provide an unequivocal measure of antibody concentration. While these immunoassays do in part reflect antibody concentration, it has also been established that they depend heavily upon the affinities of particular antibodies, the properties of other reactants, such as conjugated secondary antibodies, and the density of antigen in each assay (reviewed by Steward and Lew, 1985). Most importantly, it has been shown that the binding of an antibody to its specific antigen is quantitatively quite different from the binding of the same antibody to a solid-phase support such as plastic (Mattila, 1985; Dierks et al., 1986). Thus, the common practice of using myeloma proteins bound to plastic as standards in assaying Ig bound to specific antigen rests on a flawed premise. Mattila (1985) has estimated that the magnitude of error when plastic-bound myeloma standards are used to measure antigen-bound Ig may be in the range of 10–70-fold.

A useful alternative strategy in this regard has been to use anti-Ig antibodies to capture the Ig to be quantified in a solid-phase assay. This method, which has been used in many laboratories, potentially provides a reliable means of determining the concentration of specific antibodies in weight/volume units. Nevertheless, the underlying assumptions, methodological details, and practical applications of capture assays for Ig have not always been fully considered. In this report, we describe a solid-phase capture assay for murine IgG. We explicitly address several critical aspects of this assay such as the importance of capture antibody concentration and quality, the lack of interference by exogenous, non-murine antibody, and the influence of IgG subclass on the sensitivity of the assay. In addition, the validity and reliability of the assay are demonstrated, and the application of the assay to common problems is presented.

## Materials and methods

### *Antibodies*

Monoclonal antibodies to coronaviruses JHM (Fleming et al., 1983), A59 (Gilmore et al., 1987), OC43, and 229E (Fleming et al., in preparation)

were produced in this laboratory. The heavy chain isotypes of these antibodies are as follows: J.2.2 (IgG2b), J.7.2 (IgG2b), A.3.10 (IgG2a), 0.3.12 (IgG2b), 0.4.3 (IgG2a), E.1.1 (IgG2a), and E.1.10 (IgG2a). The following myeloma proteins were purchased from Litton Bionetics (Charleston, SC): MOPC 21 (IgG1), RPC5 (IgG2a), MOPC 195 (IgG2b), and FLOPC 21 (IgG3). These reference immunoglobulins are referred to below by heavy chain isotype; for example, 'myeloma IgG2a' indicates RPC5. All monoclonal and myeloma antibodies contained  $\kappa$  light chains. Mouse IgG standard (I-5381) was obtained from Sigma Chemical Company (St. Louis, MO). Anti-coronavirus monoclonal antibodies were purified after growth in serum-free conditions by affinity chromatography using Sepharose-protein A (SPA) (Pharmacia, Piscataway, NJ) as described by Ey et al. (1978). In some instances, serum-free growth of hybridomas was facilitated by the use of HL-1 media (Ventrix, Portland, ME). Hybridoma-induced ascites were produced in BALB/c or C57BL/6 mice by the method of Galfrè and Milstein (1981).

### *ELISA for antibody to coronaviruses (viral-ELISA)*

This was performed as previously described (Gilmore et al., 1987), with minor modifications. Coronaviruses were produced during infection of tissue cultures under serum-free conditions. Supernatants were clarified by centrifugation at  $400 \times g$  for 10 min and diluted in phosphate-buffered saline (PBS), pH 7.2, to approximately  $10^5$  plaque forming units/ml. 100  $\mu$ l of virus suspension were added to each well of a 96-well polystyrene microtiter plate (Immulon II, Dynatech, Alexandria, VA) and allowed to adsorb overnight at 4°C. It was necessary to avoid any detergents at this step, in order to assure adequate binding of antigen to the plate. Plates were emptied and then post-coated with ELISA medium (EM) (PBS containing 0.2% Tween 20 and 0.1% bovine serum albumin) for 1 h. This and all subsequent steps were performed at room temperature. The plates were then washed twice in EM, and 25  $\mu$ l of the primary antibody to be assayed, diluted in EM, were incubated in each well for 1 h. The plates were washed four times in EM, and 25  $\mu$ l of affinity-purified goat anti-mouse IgG antibody conjugated to horseradish peroxidase (GAM-HRP) (Kier-

kegaard and Perry, Gaithersburg, MD, catalog no. 14-18-06, reconstituted to 0.1 mg/ml in reagent quality distilled water, stored at  $-70^{\circ}\text{C}$ , and diluted 1:500 in EM prior to use) added to each well. After an incubation of 1 h, the plates were washed four times in EM, and 150  $\mu\text{l}$ /well of substrate-chromogen solution (50 ml of 0.1 M citrate buffer, pH 5.0, containing 20 mg *o*-phenylenediamine hydrochloride (Sigma, St. Louis, MO) and 10  $\mu\text{l}$  30%  $\text{H}_2\text{O}_2$ ) were added. Plates were then incubated for 30 min in the dark. The reaction was terminated with 50  $\mu\text{l}$ /well of 2 M  $\text{H}_2\text{SO}_4$ , and the absorbance of each sample was read at 490 nm (Minireader II, Dynatech, Alexandria, VA).

#### *Capture ELISA for quantitation of murine IgG (C-ELISA)*

Reagents and conditions were those used above in the Viral-ELISA, with modifications as noted. Immulon II plates were coated overnight at  $4^{\circ}\text{C}$  with 100  $\mu\text{l}$ /well of affinity-purified goat anti-mouse IgG (GAM-CAP) (Kierkegaard and Perry, Gaithersburg, MD, catalog no. 01-18-06, reconstituted to 0.1 mg/ml in PBS according to procedure C of the manufacturer, stored at  $-70^{\circ}\text{C}$ , and diluted in PBS prior to use as noted in the results section). It was essential to eliminate preservatives such as sodium azide in this step in order to avoid subsequent inactivation of GAM-HRP. Plates prepared in this fashion were stable for at least a month. Subsequently, plates were emptied and then postcoated with EM for 1 h. Plates were washed twice with EM, and 25  $\mu\text{l}$ /well of the IgG to be assayed, diluted in EM, were added for 1 h. Plates were washed four times with EM and then incubated in succession with GAM-HRP, chromogen solution, and  $\text{H}_2\text{SO}_4$  as in the Viral-ELISA.

#### *Optimization of GAM-CAP reagent*

To assess the degree of antibody capture by the C-ELISA, a constant amount of the IgG2a myeloma (500 pg/well) was adsorbed to individual wells of a plate (plate 1) prepared with varying amounts (0–1000 ng/well) of GAM-CAP. After an overnight incubation, the unadsorbed IgG in each well was removed and transferred to another plate (plate 2) coated with 40 ng/well, or 100  $\mu\text{l}$

of a 1:250 dilution of 0.1 mg/ml stock solution, of GAM-CAP, and the C-ELISA was performed as noted above. Preliminary studies had indicated that 40 ng/well of GAM-CAP might be near an optimum value. Reference to myeloma standards added to plate 2 and treated identically in the ELISA permitted estimation of the amount of IgG not adsorbed on plate 1.

A second consideration relating to optimization of the GAM-CAP step is the sensitivity of the assay when the concentration of this reagent is varied. Accordingly, different concentrations of GAM-CAP were employed and the relative sensitivities of each assay were compared to that achieved with plates prepared with 40 ng/well of GAM-CAP. In each case, 500 pg/well of myeloma IgG2a was assayed, and resultant absorbances were compared.

#### *Interference by serum in C-ELISA*

Under usual tissue culture conditions, hybridomas are grown with 10% serum supplementation and secrete in the order of several  $\mu\text{g}/\text{ml}$  of Ig. To test the interference of serum proteins in the C-ELISA under stringent conditions, myeloma proteins were diluted to a final concentration of 4  $\mu\text{g}/\text{ml}$  in the presence of 10% serum; this mixture was then further diluted in PBS in order to be in the optimal range for the C-ELISA.

#### *Estimation of specific antibody concentration in ascites*

Our strategy involved two steps: (1) Viral-ELISA (antigen-specific) determination of the relative titers of reference and ascitic preparations of a monoclonal antibody, and (2) C-ELISA determination of the weight/volume concentration of the same monoclonal antibody in the reference preparation. This strategy would not be valid if interfering proteins in ascites prevented a true estimate of the relative reference:ascites titer of the monoclonal antibody. In order to assess this possibility, experiments were performed with mixtures of monoclonal antibodies J.2.2 and J.7.2 (specific for JHM virus, essentially no specific binding to A59 virus) and monoclonal antibody A.3.10 (specific for A59 virus, essentially no specific binding to JHM virus). Each mixture or

'artificial ascites' was produced by mixing 30  $\mu$ l of a SPA-purified antibody with 60  $\mu$ l of ascites from another, heterologous antibody, for example, J.2.2 SPA with A.3.10 ascites. Thus, the known or expected concentration of a SPA antibody (for example, J.2.2 SPA) was three times the concentration of the same antibody in a SPA-ascites mixture (for example, J.2.2 SPA and A.3.10 ascites). Experimentally, relative antibody titers in the SPA, ascites, and SPA-ascites mixtures were determined by Viral-ELISA against the relevant viruses, for example, JHM virus in the case of J.2.2 SPA added to A.3.10 ascites. The Viral-ELISA titers were compared by a modification of the multiple of normal activity method (Tijssen, 1985), utilizing semilog paper in which absorbances were displayed on the ordinate and serial  $\log_{10}$  dilutions of the reactants were displayed on the abscissa. In each experiment, the absorbance of the SPA-ascites mixture was corrected for the small amount of non-specific binding of heterologous ascites to the virus corresponding to the specific antibody (for example, A.3.10 ascites binding to JHM virus, absorbance usually 0.10–0.20). The relative titers were then calculated by comparing the extent of horizontal separation of the curves in their linear ranges.

## Results

### Optimization of the C-ELISA

Initial experiments indicated that when IgG was directly adsorbed onto plastic the assay was relatively insensitive and was also subject to marked interference by horse or fetal bovine serum (data not shown). Having shown that GAM-CAP was necessary for a practical assay, we next sought to determine the optimal amount of this capture antibody. Ideally, capturing antibody should bind all of the IgG to be assayed, since capture that is partial or inconsistent may yield misleading results. As shown in Fig. 1, capture of 500 pg/well of myeloma IgG2a was increasingly efficient as more GAM-CAP was used until 40 ng/well of GAM-CAP was reached; beyond this value, essentially complete capture of all added IgG was achieved. Similar results were obtained with the IgG1, IgG2b, and IgG3 myelomas.

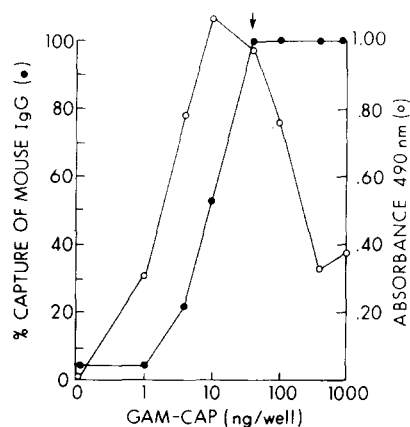


Fig. 1. Optimization of GAM-CAP reagent in C-ELISA. The C-ELISA was performed with varying amounts of GAM-CAP as indicated on the abscissa. In each case, 500 pg/well of myeloma IgG2a was the standard antibody to be captured and detected. The performance of the assay as a function of GAM-CAP added was measured by percentage of myeloma IgG2a captured (●) and absorbance achieved (○). The optimum is indicated by an arrow.

The relative sensitivity of the C-ELISA as a function of amount of GAM-CAP was also investigated. As also shown in Fig. 1, there was a drastic decrease in sensitivity of the assay outside of the range of 10–40 ng/well of GAM-CAP. This unexpected result indicates that the amount of capture antibody employed is a critical determinant of assay efficiency, and, further, that the optimum amount of capture antibody falls within a very narrow range of acceptable values.

Although the experiments shown in Fig. 1 were performed with an overnight capture step, other assays with a 1 h capture step gave essentially the same result. In subsequent assays, therefore, this step was shortened to only 1 h.

### Serum interference and sensitivity of C-ELISA to different IgG subclasses

After optimizing assay conditions, we next sought to determine the degree to which serum components might interfere with the detection of murine IgG. A representative experiment is shown in Fig. 2A, in which myeloma IgG2a was assayed with and without added serum components. There was no interference by either horse serum or fetal bovine serum, in contrast to the marked interference noted in assays in which GAM-CAP was

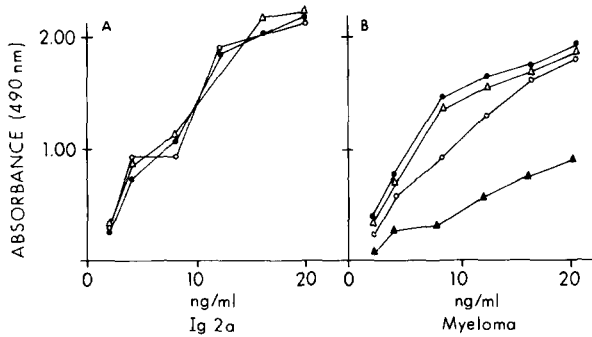


Fig. 2. Representative C-ELISAs under different experimental conditions. *A*: Assay of myeloma IgG2a (diluted in PBS (●), mixed with media containing 10% horse serum (○), and mixed with media containing 10% fetal bovine serum (Δ), as noted in text). *B*: Assay of myelomas of different IgG subclasses diluted in PBS by the optimized C-ELISA (IgG2a (●), IgG2b (Δ), IgG1 (○), IgG3 (▲)).

omitted. To judge the sensitivity of the C-ELISA for all murine IgG subclasses, the study shown in Fig. 2*B* was performed. This indicated reasonably consistent detection of each subclass, with the exception of myeloma IgG3, to which the assay showed significant diminution of sensitivity.

TABLE I  
VALIDITY OF C-ELISA

| Antibodies <sup>a</sup>      | Different methods of protein determination |                                   |                   |
|------------------------------|--|-----------------------------------|-------------------|
|                              | Supplier's specification (mg/ml)           | UV absorbance (OD 280 nm) (mg/ml) | C-ELISA (mg/ml)   |
| <i>Standards</i>             |  |                                   |                   |
| Myeloma IgG2b                | 1.00 <sup>b</sup>                          | 1.12                              | 1.00 <sup>c</sup> |
| Sigma IgG                    | 1.00 <sup>b</sup>                          | 1.42                              | 1.12              |
| <i>Monoclonal antibodies</i> |  |                                   |                   |
| J.2.2                        | —  | 0.75                              | 0.72              |
| J.7.2                        | —  | 1.41                              | 1.56              |

<sup>a</sup> See materials and methods section. Monoclonal antibodies J.2.2 and J.7.2 were obtained from hybridoma supernatants after growth in serum-free conditions and purification by SPA. Purity was greater than 95% as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

<sup>b</sup> Both mouse Ig standards were stated by the suppliers to be 1 mg/ml by the Folin protein assay.

<sup>c</sup> For the C-ELISA, myeloma IgG2b was used to calibrate the assay.

TABLE II  
PRECISION OF C-ELISA<sup>a</sup>

|                           | Monoclonal antibody | Number of determinations | Concentration <sup>b</sup> (μg/ml) | CV <sup>c</sup> (%) |
|---------------------------|---------------------|--------------------------|------------------------------------|---------------------|
| Within-assay variability  | 0.3.12              | 12                       | 68 ± 4.8                           | 7.1                 |
|                           | 0.4.3               | 12                       | 92 ± 4.7                           | 5.0                 |
|                           | E.1.1               | 12                       | 62 ± 4.7                           | 7.6                 |
|                           | E.1.10              | 12                       | 123 ± 8.2                          | 6.2                 |
| Between-assay variability | 0.3.12              | 4                        | 68 ± 2.5                           | 3.7                 |
|                           | 0.4.3               | 4                        | 84 ± 7.3                           | 8.7                 |
|                           | E.1.1               | 4                        | 51 ± 7.9                           | 15.6                |
|                           | E.1.10              | 4                        | 123 ± 8.6                          | 7.0                 |

<sup>a</sup> Four assays were done on successive days. On the fourth assay, 12 determinations were done on each sample to evaluate within-assay variation. In the prior assays, determinations were done in triplicate. Between-assay variation was measured by comparing the mean value for each antibody on the four successive assays.

<sup>b</sup> Mean ± standard deviation.

<sup>c</sup> Coefficient of variation.

#### Validity and precision of C-ELISA

The protein concentrations of commercial murine IgG standards and purified monoclonal antibodies were determined by C-ELISA and UV absorbance (assuming an absorbance at 280 nm of 1.38 for a 1.0 mg/ml solution at 1 cm). In addition, protein concentrations of the commercial standards had been determined by the Folin method by the manufacturers. As can be seen in Table I, there was good agreement among the three methods. Table II demonstrates assessments of the precision of the C-ELISA. The coefficients of variation were from 5 to 7.6% for within-assay variation and from 3.7 to 15.6% for between-assay variation.

#### Representative applications of the C-ELISA

It is often of interest to monitor the production of antibody by hybridomas under different conditions. For example, Fig. 3 demonstrates the growth and monoclonal antibody secretion of hybridoma J.2.2. In Fig. 3*A* these parameters are displayed for J.2.2 after adaptation to serum-free culture in HL-1 media, and in Fig. 3*B* similar data is displayed for growth in media supplemented with 10% horse serum. Although cell growth was comparable in both instances, the yield of antibody

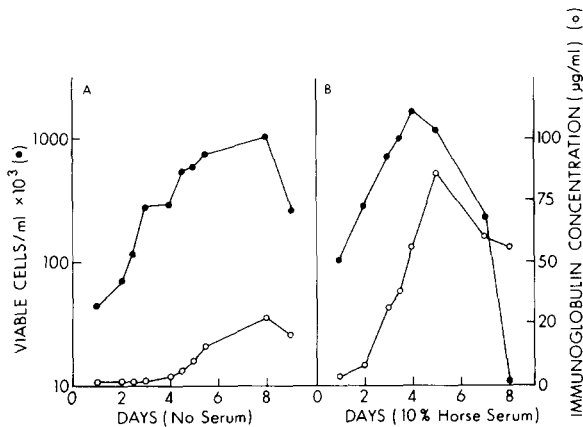


Fig. 3. Application of C-ELISA to the measurement of IgG production by hybridoma tissue cultures. Hybridoma J.2.2 was adapted to serum-free growth in HL-1 media (A) or in media containing 10% horse serum (B). Small samples were removed from each culture for analysis at the times indicated. The numbers of viable cells (●) were determined by hemocytometer counts of cells stained with trypan blue, and the concentrations of murine IgG (○) were determined by C-ELISA.

per cell was approximately three times higher when serum was included in the medium. With regard to the C-ELISA, the low background at day 1 indicates that neither HL-1 nor serum-supplemented media contain detectable protein cross-reacting with murine IgG in the assay. Fig. 3 also demonstrates that the C-ELISA reliably distinguished among closely related determinations, for example, between the concentration of J.2.2 in the morning and afternoon samples.

A vexing problem in hybridoma methodology is to measure the weight/volume concentration of

a specific monoclonal antibody in ascites (Partridge et al., 1985). To address this problem, SPA-ascites mixing experiments were performed as noted in the materials and methods section. Table III summarizes these experiments and shows that there is close agreement between the known and observed concentrations of specific monoclonal antibody in SPA-ascites mixtures. This result indicates that the relative concentration of a specific monoclonal antibody in SPA-preparations and the same antibody in ascites can be reliably determined by the Viral-ELISA, despite the presence of a large excess of other proteins and irrelevant murine IgG in ascites. Since it was demonstrated above that the absolute concentration of a monoclonal antibody may be determined either in purified or tissue culture preparations of the antibody by C-ELISA, it follows that the concentration of specific monoclonal antibody in ascites may reliably be estimated by the combined use of the Viral-ELISA and the C-ELISA. When these methods were applied to a series of ascitic fluids induced by our hybridomas, specific monoclonal antibody was found to be at a concentration of 0.24 to 10.3 mg/ml and constituted approximately 20–90% of the total IgG.

## Discussion

One method to rigorously determine absolute Ig concentration is to employ solid-phase immunoassays using species-specific capture anti-

TABLE III

### DETERMINATION OF SPECIFIC ANTIBODY TITER IN MIXTURES WITH NON-SPECIFIC ASCITES

| Experiment no. | Monoclonal antibody mixtures |                        | Relative concentration of specific SPA antibody in mixture |                       |
|----------------|------------------------------|------------------------|--|-----------------------|
|                | SPA purified (specific) +    | Ascites (non-specific) | Known <sup>a</sup>   | Observed <sup>b</sup> |
| 1              | J.2.2 +                      | A.3.10                 | 3.0  | 2.5                   |
| 2              | J.7.2 +                      | A.3.10                 | 3.0  | 2.6                   |
| 3              | A.3.10 +                     | J.2.2                  | 3.0  | 3.1                   |
| 4              | A.3.10 +                     | J.7.2                  | 3.0  | 4.5                   |

<sup>a</sup> In each experiment, 30  $\mu$ l of specific, SPA-specified monoclonal antibody were added to 60  $\mu$ l of heterologous, irrelevant ascites (see text); thus, the concentration of specific monoclonal antibody in the SPA-purified preparation is known to be three times that in the mixture.

<sup>b</sup> Observed relative (SPA: mixture) titers of specific antibody, measured by Viral-ELISA.

bodies (Panush et al., 1983; Herlyn et al., 1985; Ziai et al., 1985; Ferrante et al., 1986; Sancho et al., 1986; Butler et al., 1986; and others). Building upon this experience, we have examined methodological requirements, underlying assumptions, and several useful applications of such assays. We have shown that this type of assay reliably determines mouse IgG concentration in hybridoma supernatants and in ascitic fluids. To our knowledge, the estimation of specific monoclonal IgG concentration in ascities by these methods has not been reported previously. Specifically, the following characteristics of the C-ELISA we have optimized were demonstrated: (1) essentially complete murine IgG capture, (2) sensitive and comparable detection of all murine IgG subclasses, except IgG3, (3) lack of interference by contaminating, non-mouse Ig from added serum, (4) close correspondence to murine IgG standards measured by the Folin (Lowry) method and UV absorbance at 280 nm, and (5) good precision and reliability (approximately 5–15% coefficient of variation).

With regard to alternative methods of murine IgG measurement, it has been suggested that competitive inhibition enzyme immunoassays (CIEIA) have superior precision to immunoglobulin capture assays (Bosworth et al., 1983). However, the use of affinity-purified capture antibodies, as in the C-ELISA we have studied, results in precision (Table II) which is comparable to that of reported CIEIAs. Another consideration when comparing CIEIAs and C-ELISAs is that inhibition assays may lead to artifactually low estimates of monoclonal Ig concentration (Springer, 1983). This may have been the case in the CIEIA determinations reported by Hunter and Bosworth (1986), where the average concentration of monoclonal antibodies in hybridoma cultures was approximately 1  $\mu\text{g}/\text{ml}$ . By contrast, the range of monoclonal antibodies we have measured in hybridoma supernatants by C-ELISA is more in keeping with the values usually reported in the literature (10–100  $\mu\text{g}/\text{ml}$  of Ig) (Galfrè and Milstein, 1981; Underwood, 1986). Finally, the C-ELISA requires only one set of plates, rather than the two sets of plates in the CIEIA to accommodate liquid-phase and solid-phase reactions. The simplicity of the C-ELISA, both with regard to the reagents and

procedures used, should make it easy to set up in any laboratory with immunoassay experience.

In conclusion, several methodological aspects of the assays we have used require emphasis. First, as Shields and Turner (1986) have pointed out, the use of highest quality affinity-purified antibodies in both the capture and indicator steps (GAM-CAP, GAM-HRP) is very important in maximizing assay sensitivity and reliability. Second, the concentration of capture antibody is an especially critical determinant of the C-ELISA; as we have shown, the optimum value of capture antibody may lie in a very narrow range. The paradoxical decrease in sensitivity of the C-ELISA when excess capture antibody is used may be similar to the 'hook' or prozone phenomenon in solid-phase assays discussed by Pesce et al. (1981). Third, the diminished sensitivity of the assay to IgG3 probably relates to the relatively low concentration of this subclass in murine serum and to the corresponding bias of our anti-IgG reagents against this subclass. This problem could be obviated by isotyping all monoclonal antibodies to be tested and using an IgG3 myeloma reference protein if necessary. Similar considerations may apply in the small minority of monoclonal antibodies with lambda light chains (data not shown). Fourth, a major source of error and imprecision in the C-ELISA related to the marked sensitivity of the assay and the corresponding need for serial dilutions of reactants, standards, and samples to be tested. For reliable determinations, it is essential to dilute samples so that their concentrations fall within the relatively narrow range (1–20  $\text{ng}/\text{ml}$ ) of the C-ELISA; outside of this range, measurements will be misleading and imprecise. Fifth, in the present study data were plotted manually and interpreted by visual inspection. Although this sort of analysis is adequate for most investigational purposes, increased assay sophistication and performance may be achieved by the application of rigorous mathematical methods (Tijssen, 1985; Kay et al., 1986) or computer-based techniques (Butler et al., 1987). Finally, the assays presented have been validated for murine IgG only. While it is likely that similar assays would be useful in measuring other Ig classes of mice and Ig of other species, this must be determined empirically in each case.



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