

Simple Procedure for Large-Scale Assays for Chick Interferon

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Details are given of a simple and rapid method for the determination of chick interferon concentrations, using the Cooke microtiter system. The reliability of the result of this assay was examined by multiple determinations and by comparison with the results of another known assay procedure. A comparison of the relative time and cost of performing the two assays is also given.

Assays for chick interferon are usually carried out on monolayers of primary chicken embryo fibroblasts (CEF), using poxvirus as the challenge virus. A very convenient procedure is the plaque inhibition assay of Lindenmann and Gifford (4), used in our laboratory in the modified form of Bodo (1). This assay is based upon a sigmoidal relationship between percentage of plaque inhibition and the logarithm of interferon concentration. The amount of interferon that effects a 50% reduction of the number of plaques produced by a given amount of virus is defined as 1 unit of interferon.

To adapt the above assay to large-scale tests, the introduction of some modifications to the procedure was attempted. In addition to avoiding the tedious counting of plaques, the aim was to minimize manual operations and to scale down tissue culture media volumes in order to minimize cost. This led to the use of the Cooke microtiter system. Microtiter techniques have been used extensively for antibody assays and other virological studies. (For an extensive review, see reference 2). With this system, sterile styrene plates (ca. 12 by 8 cm) are provided which contain small wells (12 times 8) with flat bottoms (Fig. 1) in which cells can conveniently be grown. The most important advantage of the microtiter system lies in the use of the fully automated dilution and pipetting devices. To use these devices, I had to modify the principle of the above assay. Instead of first preparing confluent CEF monolayers and then adding virus plus the interferon dilution, I prepared the serial interferon dilutions first and then added virus plus cells.

The following procedure proved to be useful:

(i) Add 50 μ l of Eagle minimum essential medium (MEM) with 5% calf serum to wells B through H (or B through G as in Fig. 1). This

can be done in one step by using an automatic multidispensing device.

(ii) Add 75 μ l of sample (prediluted in Eagle MEM with 5% calf serum if necessary) to the wells in row A. Usually three wells were used per sample.

(iii) Prepare serial 1:3 dilutions from rows A through H by automatically pipetting 25 μ l from well to well.

(iv) Add 100 μ l (per well) of a suspension containing 2×10^6 CEF and 10^3 plaque-forming units of cowpoxvirus per ml in Eagle MEM supplemented with 5% calf serum. This step can also be automated. Use freshly prepared CEFs. Cowpoxvirus strain Brighton was purified by the method of Joklik (3) and stored frozen at -60°C in Hanks salts containing 0.5% gelatin. It was thawed, sonically treated, and mixed with the cells immediately before the assay.

(v) Incubate in a CO_2 incubator for 3 days.

(vi) Discard medium and stain with gentian violet.

After 3 days of incubation, cells protected by interferon form perfect monolayers, which are evenly stained dark blue, as in Fig. 1 row B, samples 1 to 9, row C, samples 1 to 8, and rows D and E, samples 1 to 3. Cells not protected by interferon show severe cytopathic effects (CPE) and are stained poorly, as is the case with all wells from samples 10 to 12, which did not contain interferon at all, and also with the lower rows of all other samples, in which, according to the serial dilution from rows A through G, the interferon concentration is too small to provide adequate protection of the cells.

The CPE detectable in row A of samples 1 to 9 is unexpected but can be explained readily by the fact that the added samples had not been diluted in Eagle MEM supplemented with 5% calf serum but, rather, were original samples contained in phosphate-buffered saline alone,

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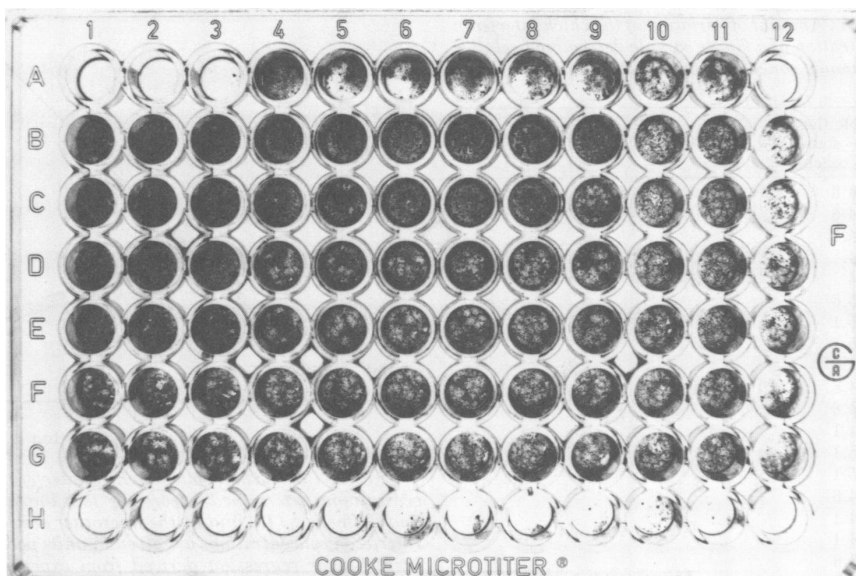


FIG. 1. Microtiter assay for chick interferon after staining with gentian violet. Serial dilutions (1:3) of interferon-containing samples were from rows A to G. (Row H was not used in this test.) Positions 1 to 3, Standard containing 1,500 units/ml. Positions 4 to 9, Standard containing 150 units/ml. Positions 10 to 12, No interferon added. For the unexpected CPE in row A, positions 1 to 9, see text.

which after being mixed with the cell-virus suspension in MEM rendered the resulting medium ineffective in promoting the attachment to the surface and the growth of the cells. This effect is already diluted out in row B.

In evaluating the results of the assay an estimate is made of the point in the serial dilution where the amount of the CPE is about half the amount of the CPE on completely unprotected monolayers. This point can either be the position of a well itself or located in between two wells. This method seems to be arbitrary but, nevertheless, it proved to be highly reliable.

A technique was adopted to determine interferon concentration relative to the concentration of a standard sample. The latter concentration was known from an assay according to Lindenmann and Gifford (4). To determine, for instance, the concentration of the sample contained in positions 1 to 3 (Fig. 1) relative to that of the sample contained in positions 4 to 9, I would first estimate the dilution of the individual samples contained in row A, which leads to the occurrence of a 50% CPE. In the case of the sample contained in positions 1 to 3, this is estimated to lie between rows E and F in all three cases. Therefore, the $\log_3$ dilution of the sample contained in row A up to this point

is 4.5. In the case of the sample contained in positions 3 to 9, the corresponding $\log_3$ is estimated to be 2.5 in four cases (positions 4, 6, 7, and 8), 3 in one case (position 5), and 1.5 in one case (position 9). This gives a mean of 2.42. The $\log_3$ of the relative concentration of the two samples is given by $4.5 - 2.42 = 2.08$, which means that the concentration ratio is $3^{2.08} = 9.86$. This is in good agreement with the actual concentration ratio of 10.

To test the reliability of the microtiter assay, I carried out multiple determinations of a single sample and compared the results of these assays with the results of assays done following the procedure, of Lindenmann and Gifford (4).

Table 1 shows the results of multiple assays of the interferon concentration of a single sample. It can be seen that there is good agreement among the individual values. The standard deviation of about 0.5 $\log_3$ indicates that the point resembling 50% CPE in two out of three cases will be found in the same row of wells or, at most, between that row and the next one. Only in 2% of the cases will this point be off by more than one row in either direction.

Table 2 shows a comparison of interferon concentrations determined by the microtiter assay and by the assay of Lindenmann and Gifford (4). There is good agreement between the re-

TABLE 1. Amount of variation of the chick interferon concentration of a single sample determined relative to the known concentration of a standard in multiple assays^a

Sample (log ₁₀ concn) - standard (log ₁₀ concn)	Mean	Standard deviation
4.6	4.43	0.49
4.6		
4.1		
4.1		
4.1		
3.6		
5.1		
5.1		
4.6		
4.6		
4.6		
4.1		
5.1		
5.1		
4.6		
4.1		
4.1		
3.6		

^a Concentration of standard, 1,500 units/ml. Predilution of standard, 1:10. Predilution of sample, 1:500.

TABLE 2. Comparison of chick interferon concentrations determined according to Lindenmann and Gifford (4) and by the microtiter assay^a

Sample	Interferon concn (log ₁₀ units/ml)	
	Lindenmann and Gifford	Microtiter assay
1	2.49	2.38
2	4.95	5.09
3	3.29	3.13
4	2.51	2.34
5	2.60	2.45
6	2.51	2.65
7	5.18	5.37
8	2.32	2.34
9	4.40	4.43
10	1.48	1.70
11	2.34	2.63
12	3.42	3.32

^a Linear regression: log concentration (microtiter assay) = 1.01 log concentration (Lindenmann and Gifford) - 0.01. Correlation coefficient = 0.99.

sults of both methods. Statistical analysis of the values in Table 1 reveals a high degree of correlation. If the values obtained by either method are connected by linear regression (Fig. 2), it becomes evident that within the limits of statistical errors both methods yield the same results.

To demonstrate the advantages of the described procedure, an estimate of the relative

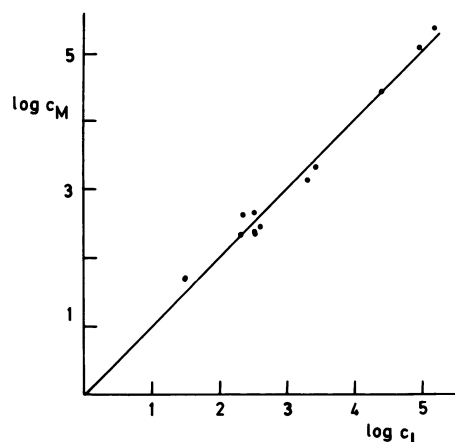


FIG. 2. Correlation between chick interferon concentrations determined according to Lindenmann and Gifford (c_L) (4) and by the microtiter assay (c_M). Interferon concentrations are given in units per milliliter. Linear regression derived from experimental data marked by individual dots. This virtually coincides with the function $\log c_M = \log c_L$.

cost and time of the latter as compared to the method of Lindenmann and Gifford (4) (modified by Bodo [1]) was determined. The fact that each culture contained in a single microwell of the microtiter plate corresponds to a culture grown on a 6-cm petri dish under standard assay conditions (1, 4) results in a drastic reduction of plastic material, tissue culture medium, and CEFs needed. The cost of the described microtiter assay thus amounts to about 10% of that of the standard assay (1, 4).

In comparing the relative time needed to perform the two tests one has to consider that the use of the automated dilution and pipetting devices of the Cooke microtiter system cuts down the time needed to prepare the samples for incubation to about 5 to 10% of the time needed to perform the standard procedure (1, 4). The most striking reduction of time, however, is due to the fact that the microtiter assay avoids the counting of individual plaques. Whereas the inspection of a single microtiter plate for 12 titration end points (50% CPE) takes 1 min at most, counting of plaques on an equivalent number of petri dishes could not be done in less than 1 h.

In conclusion, the described microtiter assay of chick interferon is very reliable and, because of its simple automated performance, may be of great help, especially if a large number of samples is to be tested at a relatively low cost.

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