Quantitative Assay of Interferon by the Immunofluorescent Cell-counting Technique

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Although numerous methods have been devised for the assessment of interferon activity, the majority are adaptations of established virus assay techniques based on the reduction of cytopathic effects. The various interferon assay methods that are currently employed have been described by Finter (1966) along with comments on their advantages and limitations. Recently, immunofluorescent cell-counting assays have been developed for a number of viruses that are highly sensitive, precise, reproducible and rapid (cf. Hahon & Cooke, 1967). This report describes an extension of the technique for the quantitative assay of interferon within 24 hr after virus challenge of treated cell monolayers.

Virus strains employed in the study were vaccinia (IHD), yellow fever (ASIM), psittacosis agent (BOLOG) and Venezuelan equine encephalomyelitis (TRINIDAD). The established McCoy cell line was used for the assay of virus infectivity. Nutrient medium for cells consisted of medium 199 containing 0.5% lactalbumin hydrolysate, 10% calf serum and 50 μg. of streptomycin and 75 μg. kanamycin/ml. Cells were maintained in medium 199 with 5% calf serum. For interferon induction, McCoy cell monolayers in plastic tissue culture flasks (75 cm.2) were exposed to 3 ml. of undiluted irradiated vaccinia virus at 35°C for 4 hr. Cells were then washed twice and incubated at 35°C for 24 hr with 5 ml. maintenance medium. Supernatant fluids were harvested, centrifuged at 105,000g for 90 min. to sediment virus and cell debris, then dialysed against HCl-KCl buffer, pH 2.0, at 4°C for 24 hr. Dialysis was again carried out against two changes of Earle's BSS, pH 7.1, at 4°C for 24 hr. Fluids were then stored at −60°C until assayed for interferon activity. Preparations possessed the biological and physical properties ascribed to viral interferons (Lockart, 1966).

To assay interferon, serial twofold dilutions of interferon prepared in maintenance medium were introduced in 0.5 ml. volume directly into vials containing cover slip (15 mm. diam.) cell monolayers and incubated at 35°C for 24 hr. Assays were carried out in triplicate. Virus and cell controls consisted of medium from non-infected McCoy cell cultures that had been processed in the same manner as the interferon preparations. After incubation, cell monolayers were washed twice with maintenance medium and then challenged with approximately 7 × 10⁶ cell infective units of virus in 0.2 ml. volume. Attachment of challenge virus was made with the aid of centrifugal force. The psittacosis agent and vaccinia virus were attached to cells at centrifugation speeds of 500 g for 15 min.; yellow fever and VEE viruses were attached at centrifugation speeds of 19,642 to 29,432g for 15 min. Following attachment, one ml. of maintenance medium was added to vials which were then incubated at 35°C in accord with prescribed periods for each virus assay (18 to 24 hr). Details of the assay procedures have been recorded elsewhere (Hahon & Nakamura, 1964; Hahon, 1965, 1966; Hahon & Cooke, 1967). Coverslip cell cultures were then rinsed twice with cold phosphate-buffered saline, pH 7.1, fixed with acetone (−60°C) and either prepared immediately for immunofluorescent staining and counting, or stored at −60°C.
The direct fluorescent antibody procedure was employed to demonstrate immunofluorescence in infected cells. The preparation of globulin conjugate, the staining procedure and fluorescence microscopy equipment have been described in detail elsewhere (Hahon & Cooke, 1967). For each coverslip monolayer, 50 microscopic fields were examined for infected cells. The reciprocal of the interferon dilution that reduced the number of fluorescent cells to 50% of the controls served as the measure of interferon activity. The 50% reduction value was derived by plotting probit transformations of reduction percentages against corresponding interferon dilutions.

![Graph](image)

Fig. 1. A comparison of the sensitivity of the psittacosis agent, vaccinia, VEE and yellow fever viruses to a preparation of McCoy cell interferon. Psittacosis agent ▲—▲, vaccinia virus ●—●, VEE virus ■—■, yellow fever virus ○—○.

Of four viruses employed to challenge interferon-treated cells, yellow fever virus was the most sensitive to the protective activity of interferon (Fig. 1). The comparison attains greater validity because the assessment procedure was carried out with standardized immunofluorescence assays for each agent and with the same cell line. This challenge agent was subsequently employed to detect and estimate the induction of interferon by Coxiella burnetii (Hahon & Kozikowski, 1968) and of adenovirus-4 from guinea-pig leucocytes (E. H. Kozikowski, unpublished).

When yellow fever virus challenge doses, differing by tenfold in concentration, were employed there was no significant change in interferon titres. That the titre of an interferon preparation is independent of the virus challenge dose agrees with previous findings (Lindenmann & Gifford, 1963; Finter, 1964; Hallum & Youngner, 1966).

To estimate the precision of the assay, seven replicate determinations of the same preparation were made. Interferon titres ranged from 100 to 126 units/0.5 ml. with a mean titre of 111 units. The standard deviation was ±10.6; the coefficient of variation was 9.5%. The precision of this interferon assay was superior to the variety of assessment procedures used by others (Finter, 1966).

Four determinations were performed over a period of three months with another
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preparation of interferon stored at −60°C to determine the reproducibility of the assay. Interferon titres, i.e. 275, 305, 270, 275, showed less than twofold differences (Table 1).

The immunofluorescent assay of interferon adequately fulfils most, if not all, of the proposed criteria for interferon assessment (Finter, 1966). This assay is based on a standardized and highly quantitative virus assay system that provides for efficient and effective virus-cell interactions which result in maximum sensitivity and precision. The precision obtained with the interferon assay is identical to that cited for the assessment of yellow fever virus by fluorescent cell-counting (Hahon, 1966). This emphasizes the view that the ability to detect and to measure interferon potency accurately and rapidly is a reflection of the system used for virus assay.

Table 1. Replicate titrations of the same McCoy interferon pool with yellow fever virus as challenge agent

<table>
<thead>
<tr>
<th>Date</th>
<th>Interferon dilutions</th>
<th>Interferon titre, units/0.5 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/4</td>
<td>1/8</td>
</tr>
<tr>
<td>16. v. 68</td>
<td>90.3*</td>
<td>86.7</td>
</tr>
<tr>
<td>11. vi. 68</td>
<td>93.4</td>
<td>88.7</td>
</tr>
<tr>
<td>25. vii. 68</td>
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</tr>
<tr>
<td>29. vii. 68</td>
<td>96.1</td>
<td>93.0</td>
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</tbody>
</table>

* Percentage reduction of infected cell counts.

REFERENCES


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