The Replication and Titration of Iridescent Virus 
Type 22 in Spodoptera frugiperda Cells 

(Accepted 9 August 1977)

SUMMARY

A plaque assay for iridescent virus type 22 (from Simulium sp.) using Spodoptera frugiperda cells has been devised, and the kinetics of growth of the virus in this cell line have been determined. The virus particle/p.f.u. ratio was 75 ± 8, and the p.f.u./TCID₉₀ ratio was 0.56 ± 0.11.

Fundamental research on the cell biology of iridescent viruses has been hindered by the lack of a reliable assay system for these viruses. Bellett (1965) described a fluorescent antibody infectious centre assay for iridescent virus type 2 (from Sericesthis pruinosa) using Antheraea eucalypti cells but this method is both tedious and inconsistent in practice (Kelly, 1972).

Subsequently a tissue culture infectious dose (TCID₉₀) assay was developed for iridescent virus type 22 (from Simulium sp.; Batson et al. 1976) using Aedes albopictus cells (Kelly, 1976a) and though this method is both convenient and economical it lacks the precision, versatility and reliability of a plaque assay. We have recently devised a plaque assay for iridescent virus type 22 and we report the method in this paper. The procedure was originally used in this laboratory for plaque assay of baculoviruses of Spodoptera sp. (D. A. Brown & K. A. Harrap, unpublished observations). Spodoptera frugiperda cells were chosen because small iridescent viruses replicate more rapidly in this cell line than other cell lines used in this laboratory (e.g. Aedes aegypti, Aedes albopictus, Antheraea eucalypti and Drosophila melanogaster; D. C. Kelly and T. Lescott, unpublished observations); because a pronounced c.p.e. occurs; and because this cell line is the only cell line we possess which allows the virus to leave the cells by budding (D. C. Kelly, unpublished observations) which may enhance lateral cell to cell transfer. Iridescent virus type 22 was chosen as a model virus because it is one of the few small iridescent viruses with an accredited ‘pedigree’. This virus is also the only viral pathogen isolated from naturally infected Simulium sp. (blackflies) which are causing considerable nuisance in tropical and subtropical climates, and which in the case of Simulium damnosum act as vectors of the protozoan Onchocerca volvulus (the causative agent of river blindness in West Africa). The virus may eventually prove to be useful in controlling population levels of blackflies.

Iridescent virus type 22 was grown in Galleria mellonella larvae, and the virus used in these experiments had been passaged twice from the original isolate. The virus was purified as previously described (Kelly & Tinsley, 1972; Elliott, Lescott & Kelly, 1977) and then filter sterilized using a 0.22 µm Millipore filter. Virus particle counting was performed as described by Kelly & Tinsley (1974) using 108 nm latex particles. Spodoptera frugiperda cells (J. Vaughn, unpublished observations) were grown and maintained in BML-TC₁₀ medium (Gardiner & Stockdale, 1975) as previously described (Kelly, 1976b). TCID₉₀ titrations were carried out in Spodoptera frugiperda cells as previously described (Kelly, 1976a, b; Elliott et al. 1977) using Falcon plastic microtest plates (3034). Cells (1200) were allowed to grow in 5 µl of BML-TC₁₀ for 24 h at 28 °C. Ten microlitre volumes of
Fig. 1. Plaques of iridescent virus type 22 on Spodoptera frugiperda cells, photographed in a 6 cm Petri dish.

dilutions (normally tenfold) of the virus in BML-TC10 were then added, and the incubation was continued at 21 °C for 96 h when the plates were read for cytopathic effect. Titrations were performed in quintuplicate.

Plaque assays were performed as follows. Cells (1.2 x 10⁷) in 4 ml of BML-TC10 were added to 6 cm plastic Petri dishes and the cells allowed to attach for 3 h at 28 °C. The medium was removed and replaced with 100 μl of inoculum (in BML-TC10) which was allowed to absorb for 1 h at 21 °C. The overlay medium comprised equal volumes of 1.5% (w/v) Sea Plaque agarose (Marine Colloids Inc., P.O. Box 308, Rockland Maine 04841, U.S.A.) in Dulbecco PBS (PBS-2; Stollar & Thomas, 1975) and a solution containing half the concentration of organic salts but twice the concentration of the other constituents of
Fig. 2. (a) Correlation of plaque numbers of iridescent virus type 22 with relative virus concentration (error bars represent standard deviation). (b) Time sequence of iridescent virus type 22 accumulation in Spodoptera frugiperda cells; infection was at a m.o.i. of 30 p.f.u./cell.

Typical iridescent virus type 22 plaques are shown in Fig. 1. They comprise about 20 to 30 lysed cells and have a diam. of 0.1 to 0.3 mm. A linear dose response was observed between relative virus concentration and plaque numbers (Fig. 2a) demonstrating that one plaque is initiated by 1 infectious unit (Fenner et al. 1974). The particle/p.f.u. ratio was found to be 75 ± 8 [5 determinations ± standard deviation, counting 1000 particles of the lower concentration (virus or latex) in each determination]; and the p.f.u./TCID₅₀ ratio was 0.56 ± 0.11 (3 determinations), which approximates to the theoretical ratio of 0.67 (Bryan, 1957), and indicates the sensitivity of the method.

The growth of iridescent virus type 22 is shown in Fig. 2(b). In this experiment cells were infected at a m.o.i. of 30 p.f.u./cell (10⁶ cells in a 25 cm² Falcon flask) and the inoculum was allowed to absorb for 1 h at room temperature; the cells were then rinsed three times with BML-TC₁₀ and 5 ml of BML-TC₁₀ was added to each flask; at various times the cells were scraped off the flasks into the medium, and the cells were disrupted by sonication at 5 amp output for 5 min in a Dawes Soniprobe type 113A sonicating water bath; the virus was then titrated. It can be seen that the virus replicated rapidly in this cell line, with infectious progeny virus appearing 5 h post infection, and that production is virtually complete by 12 h post infection.

The replication of iridescent virus type 22 in Spodoptera frugiperda cells is faster than in Aedes albopictus cells (about 18 h: Kelly, 1976a) and appreciably faster than other small iridescent viruses in a variety of cell lines (Bellett, 1965; Kelly & Tinsley, 1974). The rapid replication of small iridescent viruses in this cell line lends the system to analysis of virus-specific macromolecular synthesis. The ability of this virus (and other small iridescent
viruses (D. A. Brown and K. A. Harrap, unpublished observations) to plaque efficiently means that the virus can now be plaque purified, cloned and used for genetic analysis.

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REFERENCES


(Received 28 June 1977)