If experiments are designed taking these factors into account, the assay gives a simple and rapid method for obtaining information, not about total virus concentration, but data regarding the relative concentrations of infective virus particles in the samples compared.

2. Materials

1. Grinding buffer, for infected plant material and for dilution of standard virus preparations; generally, 0.05*M* phosphate buffer, pH 8.0.
2. Test plants, as uniform as possible. It is advisable to grow more plants than required and to select the most uniform of the group. In addition, arrange the sets of plants in order to allow equal numbers of each size group of plants in each sample replicate. Plants should be trimmed to leave only the leaves to be inoculated according to the experimental design used.
3. Abrasive powder, preferably Carborundum (600 mesh); Celite can also be used.
4. A number of 3-cm squares of Parafilm, enough for one piece per inoculation.

3. Methods

1. Select the test plants and label the leaves to be inoculated by punching out small disks in the leaf tip, using a glass Pasteur pipet, so that each different inoculum can be identified (*see* **Notes 1** and **6**).
2. Dust the leaves to be inoculated with abrasive powder, to give a light, even covering.
3. Prepare the inoculum by homogenizing the leaf tissue to be tested in a pestle and mortar, on ice in ice-cold grinding buffer (1:1 [w/v]). Serially dilute the stock in the grinding buffer (*see* **Note 2**), keeping all the samples on ice until the inoculations are carried out. Sufficient inoculum is required for ~25-µL inoculations of each dilution per test leaf.
4. Pipet the inoculum onto the leaf and smooth onto its surface, from petiole to tip, using a piece of Parafilm, under even pressure. The leaf must be supported from beneath to prevent damage to the plant. To avoid carryover of inoculum, the Parafilm must be changed for each sample (*see* **Notes 3** and **4**).
5. Immediately following inoculation, spray the leaves of the test plants with water to remove the abrasive powder, to remove sap residues that may inhibit infection or adversely affect the appearance of the leaf, and to help prevent wilting.
6. Incubate the plants at 23°C with a 16-h photoperiod, for approx 3–4 d; then count the local lesions present on the leaf surface.
7. The tabulated data can be presented graphically, plotting the log of the local lesion number (average of the replicates) vs the log of the dilution. From data in the linear part of the graph, it is possible to extrapolate to the intercepts on the *x*- and the *y*-axes, to give both a theoretical dilution end point and an undiluted lesion number, respectively; both are arbitrary measurements, but offer a simple method for making relative comparisons of viable virus concentration among a number of samples. (*see* example in **Fig. 2**; **Notes 5** and **7**).