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Assaying Levels of Virus with Local Lesion Hosts

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1. Introduction

A number of methods have been used for the quantitation of virus within infected plants. Early ones used physical techniques such as dry-wt and particle counts; more recently, serological and nucleic acid-based methods have been developed. These methods are all based on a physical aspect of the virus particle, and all measure total amounts of virus (or viral component), regardless of infectivity. Holmes ***(1)***, however, was the first to utilize the observation that mechanical inoculation of tobacco mosaic virus (TMV) onto the leaves of *Nicotiana glutinosa* led to the formation of local lesions, and that the number of local lesions was inversely correlated to the dilution of the inoculum. The local lesion assay remains the simplest method to quantitatively measure the most important biological property of a sample of virus, that of the presence of viable virus particles.

A number of points should, however, be borne in mind when using an assay of this kind. First, there is the obvious requirement for the availability of an appropriate host, one which responds to mechanical inoculation with the formation of clear necrotic lesions or ringspots, or chlorotic spots (*see* **Fig. 1** and **Table 1**). Second, there is almost always a significant variation in response between plants, and between leaves on the same plant. The experimental design must take this into account. However, care should be taken not to overcomplicate the layout used, since the length of time required to inoculate and the risk of error in such an experimental design may outweigh the advantages gained from the randomization. In addition, although half-leaf comparisons give least variation in lesion number, the difficulty in carrying out many half-leaf comparisons makes the use of whole opposite leaves more practical. Finally, the nature of the curve (*see* **Subheading 3., step 7**;

From: *Methods in Molecular Biology, Vol. 81: Plant Virology Protocols: From Virus Isolation to Transgenic Resistance*
Edited by: G. D. Foster and S. C. Taylor