

## Methods Used to Determine the Virucidal Activities of Disinfectants

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Disinfectants are of great importance in the prevention of nosocomial infections. They are used for a variety of purposes in infection control. Testing of disinfectants for their virucidal activity is complex, so some standard methods to assess the virucidal activity are determined. These methods can be classified as follows: quantitative suspension test methods, carrier test methods, fingerpad methods and whole-hand methods.

Quantitative suspension tests are standardized and have been used for many years. These tests can be used for assess the virucidal activity of disinfectants on animate or inanimate surfaces. This test involves mixing a certain number of viruses with a disinfectant for a certain period of contact time. At the end of the contact time, the virucidal activity of disinfectant is immediately suppressed by transfer of the sample (virus + disinfectant) into 9 volumes of ice-cold cell maintenance medium that contain appropriate disinfectant neutralizer. A dilution series with a factor of ten is prepared in an ice-cold cell maintenance medium immediately and the dilutions are transferred immediately into cell culture units (petri dishes, tubes or wells of microtitre plates) either using monolayer or cell suspension. After incubation, the titre of infectivity is calculated. Reduction of virus infectivity is calculated from differences of log virus titres before and after treatment with the disinfectant. Test virus, test temperature, contact time and interfering substances (clean or dirty) vary according to the required test conditions (instrument disinfection, surface disinfection or hygienic handrub and handwash) [1].

Suspension test methods allow determine the activity of the disinfectants on viruses in a suspension and they are easier to perform. However, viruses are found adsorbed to surfaces and suspension test methods can not represent the nature of the viruses. So that the carrier test methods are more relevant to determine the activity of disinfectants instead of suspension tests [2]. In carrier tests, an object (a carrier such as metal, plastic or glass) is contaminated with virus inoculum. The certain number of viruses applied to carrier and allows dry. The carrier is then exposed to the disinfectant for a certain period of contact time. At the end of the contact time, dried virus - disinfectant mixture is covered with ice-cold cell culture maintenance medium that contain appropriate disinfectant neutralizer and eluted from the surface of the carrier. A 10-fold serial dilution of eluate is performed in ice-cold cell culture medium. Dilutions are transferred into cell culture units for determining the reduction of the virus titre. Performance of the carrier test method is altered by nature of interfering substance, time used for virus drying, contact time between viruses and disinfectant and neutralization of disinfectant [3,4].

In fingerpad method, volunteer's fingerpads are artificially contaminated with viruses and then fingerpads are exposed to the disinfectant. Prior to the test, volunteers' hands are prepared for the experiment. For this purpose, volunteers wash their hands using non-antimicrobial soap and tap water and dry them with paper towel. Then ethanol is dispensed on to the volunteer's hands which are rubbed over the entire surface of the both hands until hands are dry. Test procedure is initiated by dispensing the certain number of virus suspension (approximately 10 $\mu$ L) on a finger pad and the virus suspension is allowed to dry. Inoculum area of finger pad is exposed to disinfectant by using vial. An open vial containing 1mL of disinfectant is placed the inoculum area and by inverting the vial disinfectant is brought in contact with the skin for approximately 20 seconds. The vial is turned upright and the fingerpad is allowed to dry. The virus is eluted using 1mL of cell culture maintenance medium. The eluate is assayed for determining the reduction of the virus titre [4-9].

Whole-hand method is also similar to fingerpad method. Volunteer's entire hands are artificially contaminated with viruses and then hands are exposed to the disinfectant. Volunteers are also washed their hand with non-antimicrobial soap and tap water and dry them. Volunteers' hands are contaminated with the certain number of virus suspension (approximately 1mL) and the hands are rubbed together. The hands are then allowed to dry. 0.5 - 5mL disinfectant is poured on the palm surface of one of the hands, and the hands are rubbed together. At the end of the certain period of the contact time, both hands are placed in a sterile bag (or a sterile glove) that contain elution solution and the viruses are eluted from entire surface of both hand. The eluate is assayed for determining the reduction of the virus titre [4,10-11].

All these methods are standardized by European Committee for Standardization (Comite' Europe'en de Normalisation, CEN) and/or American Society for Testing and Materials (ASTM) [1,5,10].

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