Disinfectant spraying
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Virucidal Testing of Cleanroom Disinfectants

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In “normal” times, with the exception of live vaccine manufacturers, control of viruses in cleanrooms is not usually the highest priority within contamination control. This is because viruses are not considered to be independent living organisms, they need a host organism to survive. They are obligate parasites, just nucleic acid surrounded by a protein that can only replicate within a host cell, so their ability to survive on hard, dry surfaces is minimal.

What is a virus?

Viruses are very, very small (20–200 nm Ø), this is much smaller than bacteria. On average, the size of bacteria ranges from 0.5 to 5 µm, think mouse versus elephant. Viruses do not contain the components of a normal organism like plants, animals or bacteria so they cannot reproduce without a host. To reproduce, they use their genes (encoded in DNA or RNA) to trick the host cell to use its own machinery to make more copies of the virus. For viruses to trick the host, they must enter the proper host cells. Viruses have two components that they all share, but the structure of which varies, their nucleic acid and their capsid, the combination of which is called a nucleocapsid. Some viruses have additional structures, such as an envelope or a tegument.

The classification of viruses is based on the collection and comparison of these various characters that describe the virus. These can then be used to distinguish one virus from another[1]. Characters can consist of any property or feature of the virus, its genome (DNA or RNA), its symmetry (Helical, Icosahedral or Complex), whether it has a lipid envelope (Enveloped or Non-enveloped), the diameter of the capsid etc.

Enveloped viruses

Enveloped viruses have an additional layer that covers the capsid. This membrane is composed of lipids and proteins it “stole” from the host cells and viral glycoproteins. The bumps, knobs and spikes that artists use in images of enveloped viruses like SARS-CoV-2 depict structures on the viral envelope.

Enveloped viruses are the least resistant to inactivation by disinfection, this is because the lipid envelope is easily compromised by most disinfectants and detergents. Once the lipid envelope is damaged, the integrity of the virus is compromised, thereby neutralizing its infectivity. The following are viral families in this subgroup: Arenaviridae, Coronaviridae (i.e. SARS-CoV-2), Filoviridae, Flaviviridae, Herpesviridae, Paramyxoviridae, Poxviridae and Retroviridae.

Non-enveloped viruses

In terms of resistance to disinfectants it helps to separate these again by size. Small non-enveloped viruses less than 50 nm can be highly resistant to inactivation by disinfection, as despite the lack of a lipid envelope, these organisms have a very resistant protein capsid. Families in this subgroup include: Picornaviridae, Parvoviridae, Caliciviridae, Astroviridae and Polyomaviridae. Large non-enveloped viruses, from 50–100 nm in size are less resistant to inactivation by disinfection, as although they have a resistant protein capsid, their larger size makes them more vulnerable than their smaller viral counterparts. The following are viral families in this subgroup: Adenoviridae, Reoviridae and Papillomaviridae.

This difference in resistance to disinfectants can be seen in Fig.3. This demonstrates Gerald McDonnell and A Denver Russell’s hierarchy of microbial susceptibility to antiseptics and disinfectants[2]. This clearly shows that we cannot group viruses together when discussing the effec-

tiveness of a disinfectant. This is acknowledged within the virus efficacy tests that are available.

Disinfectant efficacy tests against viruses

BS EN 14885:2018 Chemical disinfectants and antiseptics — Application of European Standards for chemical disinfectants and antiseptics lists three EN standards for the testing of the virucidal efficacy of chemical disinfectants; EN 14476, EN 14675 and EN 13610 which are all suspension tests. The EN efficacy tests normally used by manufacturers to make claims for cleanroom disinfectants are the tests for evaluation in food, industrial, domestic and institutional areas, however there is no virucidal test specific for this area, probably because viruses do not survive long outside the host organism. However, EN 14885 does state that if a standard doesn't exist for a particular area, a standard from another area can be used, so EN 14476 the test for evalulation in the medical area is recommended for use in the pharmaceutical industry.

The Biocidal Product Regulation[3] which authorises disinfectant products for sale in the European Union also states that a hard surface disinfectant for industrial use (not healthcare) needs to meet the test requirements of EN 14776 and a phase 2, step 2 test from the medical area, using Adenovirus and Murine Norovirus to be classed as a virucide.

There is a recent surface test which is not included in BS EN 14885 or yet noted by the BPR, BS EN 16777:2018[4] is a quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area.

Virus testing is unique within the laboratory because the presence of viruses before and after product treatment is not determined by observing growth of virus but rather by observing the damage caused by infection to mammalian host cells. After a study, individual sets of cells are analyzed microscopically to identify where healthy cell layers become damaged. This damage is known as the cytopathic effects (CPE) of infection. The quantity and quality of CPE is used to calculate the amount of virus present.

There are two ASTM International methods for testing hard surface disinfectants against viruses. ASTM- E1052 is a test method intended to demontrate the virucidal activity in suspension[5] and ASTM-E1053 is the assessment of virucidal activity of chemicals on inanimate, non porous environmental surfaces[6]. A wide range of viruses are suggested for testing but to demonstrate that the test substance has broad virucidal activity, it should be shown to be effective against at least one non-enveloped virus.

For a disinfectant manufacturer to claim virucidal activity in the EU, the European Chemicals Agency (ECHA) strongly advocates the use of EN testing[7]. If there is no appropriate test, then an existing EN test should be modified if possible. Only if that isn't possible can a manufacturer use the ASTM methods. As there is a European test for viruses on a surface you will not see many (probably no) EU disinfectants tested to the ASTM methods unless they were carried out before EN 16777 was available. Table 1 shows a summary of the virus efficacy tests available.

BS EN 14476

BS EN 14476 was first published in 2005, needing a log 4 reduction against two non-enveloped viruses to show virucidal activty. An RNA virus Picornavirus group (Poliovirus) and a DNA virus Adenovirus group (Adenovirus 5). Poliovirus is selected as a test virus because it has a high resistance to chemicals, is acid-stable and is unaffected by lipid solvents such as ether, and most detergents or quaternary products[8]. In 2013, Mu-

Table 1: Virucidal Efficacy Test Methods for Cleanroom Disinfectants © Contec

Test	Type	Organisms	Log Red'n	Time (mins)	Conditions
EN 14476:2005	Suspension	**Two non-enveloped viruses** (RNA Virus Poliovirus / DNA Virus Adenovirus 5)	4	60	Clean / Dirty
Pr EN 14476:2011	Suspension	As above plus human norovirus surrogate (**Non-enveloped RNA Virus** Murine Norovirus)	4	60	Clean / Dirty
EN 14476:2013+A1:2015	Handwash/rub	As above plus **one enveloped virus** (Vaccina virus)	4	60	Clean / Dirty
EN 14476:2013+A2:2019	Suspension	As above plus **one enveloped virus** (Vaccina virus)	4	5**– 60	Clean / Dirty
EN 16777:2018	Surface	**Two non-enveloped viruses** (RNA Virus Murine Norovirus / DNA Virus Adenovirus 5 *) **One enveloped virus** (Vaccina virus)	4	5**– 60	Clean / Dirty
ASTM - E1052	Suspension	Must include **one non-enveloped virus** for broad virucidal claim	4	Not specified	Clean / Dirty
ASTM - E1053	Surface	Must include **one non-enveloped virus** for broad virucidal claim	4	Not specified	Clean / Dirty

*Poliovirus is not included due to drying problems

**High touch surfaces

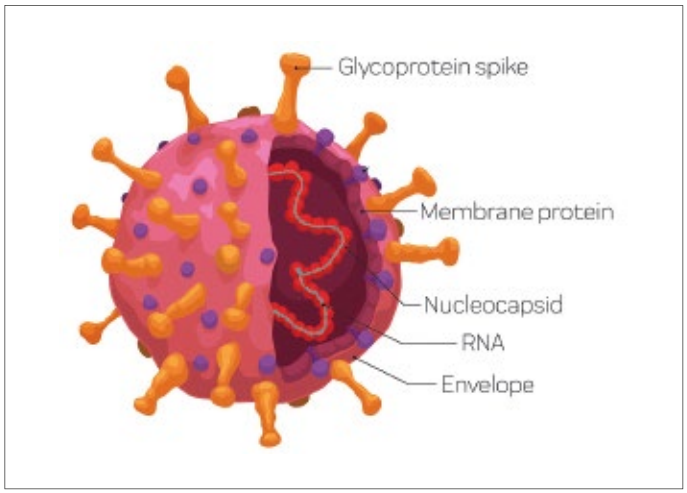

Fig. 1: Enveloped virus © CDC

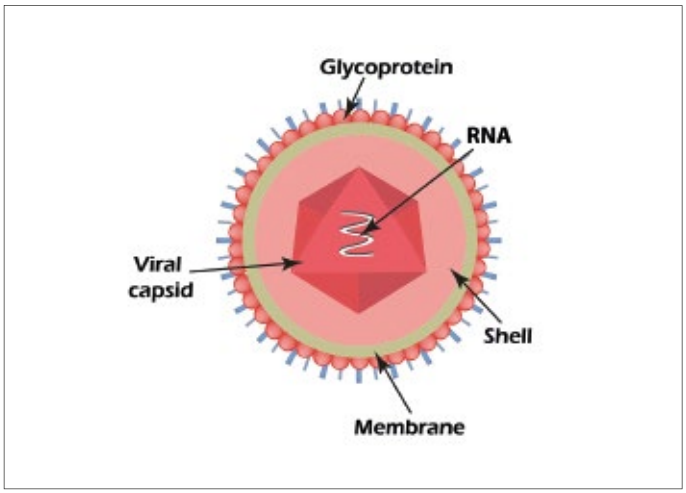

Fig. 2: Non-enveloped virus © CDC

rine Norovirus (MNV) was included as test virus as surrogate for human Norovirus. Further addendums 2015 and 2019 added Vaccinia virus as an enveloped virus for hand wash testing and surface disinfectants. However, the results from previous versions of the test are still valid.

Different levels of virucidal claim can be made using the current suspension test, BS EN 14476:2013+A2:2019[9]. For full virucidal activity a log 4 reduction is needed against Poliovirus, Adenovirus and Murine Norovirus. This would also cover a claim against all enveloped viruses, such as SARS-CoV-2. For limited spectrum virucidal activity: a log 4 reduction is needed against Adenovirus and Murine Norovirus. This would cover a claim against all enveloped viruses and Norovirus, Rotavirus and Adenovirus. If only a claim is needed against enveloped viruses a log 4 reduction is needed against Vaccinia virus.

More recently launched is a surface test BS EN 16777:2018[10] which stipulates as standard organisms, two non-enveloped viruses, Adenovirus and Murine Norovirus, Poliovirus isn't used because of drying problems and one enveloped virus, Vaccinia virus. A test suspension of the virus is inoculated onto a test surface and dried, the solution under test is then applied to cover the dried virus film. A log 4 reduction is needed to pass, there is no reduction in criteria versus the suspension test unlike other EN disinfectant test methods. For full virucidal activity a log 4 reduction is needed against Adenovirus and Murine Norovirus, however the product also needs to have passed EN 14476 for Poliovirus. This would also give a claim against all enveloped viruses. The other pass criteria are the same as EN 14476:2013+A2:2019.

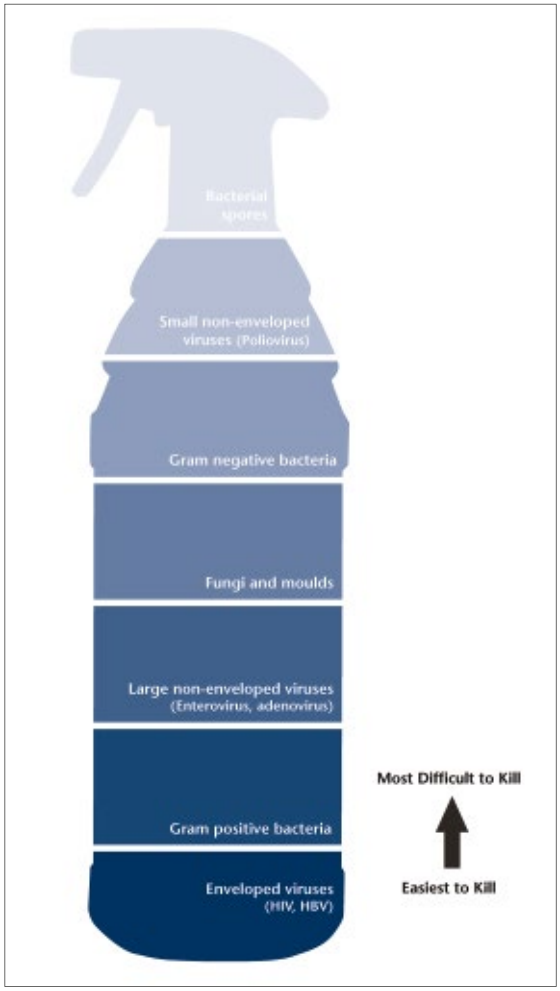

Fig. 3: Hierarchy of microbial susceptibility to antiseptics and disinfectants[2]

Virucidal disinfectants

Most disinfectants are not effective against all micro-organisms. Cleanroom disinfectants are broken down into two main groups, broad spectrum disinfectants and sporicidal disinfectants. As viruses cannot survive outside a host for long periods of time, they are not usually a key organism for cleanroom testing, so cleanroom disinfectants will not always have viruses as part of their standard test portfolio. More testing has been carried out recently to show that a particular disinfectant has efficacy against the current Corona virus. Always check whether a manufacturer does have any virus testing data available for their cleanroom disinfectants, as they may have carried it out for a specific customer but not routinely publish it.

As the resistance to disinfectants is so different between enveloped viruses and non-enveloped viruses we always need to consider these separately, as reinforced by the efficacy tests. As enveloped viruses are easy to kill, most broad spectrum biocides will kill them. As small non-enveloped viruses are so difficult to kill, a sporicide is probably needed for these organisms.

60 – 80 % alcohol solutions will have efficiacy against enveloped viruses, including SARS-CoV-2[11]. Most disinfectants based on quaternary ammonium compounds, amphoteric surfactants and biguanides will have efficacy against enveloped viruses, but contact times will vary. These are unlikely to have any affect on non-enveloped viruses. To kill non-enveloped viruses a sporicide such as hypochlorous acid (>1,000 ppm), hypochlorites (>5,000 ppm), peracetic acid, hydrogen peroxide/peracetic acid blends would be needed, again contact times will vary.

So, although there are no specific virucidal efficacy tests for the evaluation of disinfectants in the food, industrial, domestic and institutional areas, as usually used by cleanroom disinfectant manufacturers, there are EN tests for virucidal effiacacy which can and are used by manufacturers. If a company is looking for a validated disinfectant for SARS-CoV-2 then any disinfectant which passes the EN tests for non-enveloped viruses or enveloped viruses will be acceptable.

References

[1] Virus Taxonomy: Classification and Nomenclature of Viruses: International Committee on Taxonomy of Viruses

[2] Antiseptics and Disinfectants: Activity, Action, and Resistance, Gerald McDonnell and A Denver Russell, Clinical Microbiology Review, Jan. 1999, p. 147–179

[3] EU Biocidal Product Regulation 528/2012

[4] BS EN 16777:2018 Chemical disinfectants and antiseptics. Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area - Test method and requirements (phase 2/step 2).

[5] ASTM E1052 Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension

[6] ASTM E1053 Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces

[7] ECHA Guidance on the Biocidal Products Regulation Volume II Efficacy — Assessment and Evaluation (Parts B+C) Version 3.0 April 2018

[8] BS EN 14476:2005 Chemical disinfectant and antiseptics — Quantitative suspension test for the evaluation of virucidal activity in the medical are — test methods and requirements (Phase 2/Step 1)

[9] BS EN 14476:2013 +A2:2019 Chemical disinfectant and antiseptics — Quantitative suspension test for the evaluation of virucidal activity in the medical are — test methods and requirements (Phase 2/Step 1)

[10] BS EN 16777:2018 Chemical disinfectants and antiseptics. Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area - Test method and requirements (phase 2/step 2).

[11] Stability and inactivation of SARS coronavirus. H.F. Rabenau, J Cinati et al. Medical Microbiology and Immunology 2005 (194:1-6).

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