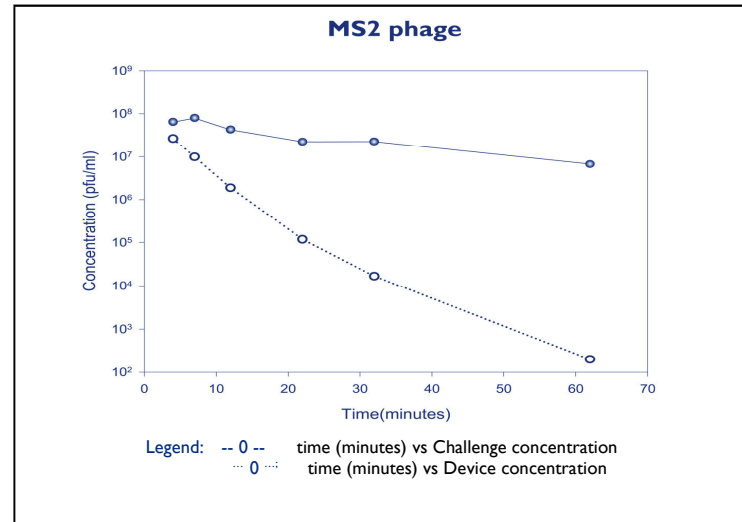


The chart on the right is a clear demonstration of the fast effects of Hydroxyl Radicals in clearing viruses in a contained area.
Source: HPA Tests 2007.

A surrogate is used because producing viruses in large enough quantities to do meaningful tests is extremely difficult and expensive; viruses only reproduce in living tissue so their production is slow and difficult. The surrogate that is used is the MS2 coliphage which is in all respects similar, but tougher than the virus but can be grown as rapidly as bacteria. MS2 is also used as a challenge organism for ANSI/NSF Standard 55 "ultraviolet Water Treatment Systems" (Bourget S et al (2007), Walker C. M. et al (2007), Fitzgibbon J. E., (2008)).

The tests conducted at Porton Down can be found on the Inov8 website: www.inov8.com.



References

Balasubramanian B., Pogozelski W. K., Tullius T. D., 1998, "DNA strand breaking by the hydroxyl radical is governed by the accessible surface areas of the hydrogen atoms of the DNA backbone", *Proc. Natl. Acad. Sci. USA*, Vol. 95, pp. 9738-9743, August 1998, Chemistry.

Bourget S., G. Schumer, A. Staffa, M.-E. S. Cartier, P.J. Messier, 2007, "Improved Respirator for Protection Against Exposure to Airborne Viruses", *American Journal of Infection Control*, Volume 35, Issue 5, pp E36-E37.

Choi, Jae-Mun, Anne M. Hutson, Mary K. Estes, and B. V. Venkataram Prasad. "Atomic resolution structural characterization of recognition of histo-blood group antigens by Norwalk virus", 2008, Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston. *The National Academy of Sciences of the USA*. 2008.

Fitzgibbon J.E. and J.-L. Sagripanti, 2008, "Analysis of the survival of Venezuelan equine encephalomyelitis virus and possible viral simulants in liquid suspensions", *Journal of Applied Microbiology*, Volume 105, Issue 5, Nov. 2008, pp: 1477-1483.

Garrison W. M., "Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins", (1987), *Chem Rev* 87:381-398 -9920

Hennessy, E. P., A. D. Green, M. P. Connor, R. Darby, and P. MacDonald, "Norwalk virus infection and disease is associated with ABO histo-blood group type", 2003, *J. Infect. Dis.* 188:176-177.

Ming Tan and Xi Jiang, "The P Domain of Norovirus Capsid Protein Forms a Subviral Particle that Binds to Histo-Blood Group Antigen Receptors", 2005, *J. VIROLOGY*, Vol. 79, No. 22 p. 14017-14030.

Ming Tan and Xi Jiang, "Norovirus and its histo-blood group antigen receptors: an answer to a historical puzzle", 2005, *Trends in Microbiology*, Volume 13, Issue 6, 285-293, 1 June 2005.

Patrick R. Harrington, Jan Vinje, Christine L. Moe, and Ralph S. Baric, "Norovirus Capture with Histo-Blood Group Antigens Reveals Novel Virus-Ligand Interactions", 2003, *J. Virology*, Vol.78, No.6 3035-3045.

Singh J & Thornton J M (1992). *Atlas of Protein Side-Chain Interactions*, Vols. I & II, IRL press, Oxford.

Walker C. M., and K. GwangPyo, 2007, "Effect of Ultraviolet Germicidal Irradiation on Viral Aerosols", *Environ. Sci. Technol.*, 2007, 41 (15), pp 5460-5465.

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Why Use the Inov8 Solution?

- Effective solution against Norovirus.
- Changes the virus structure through a known chemical oxidation process.
- Effective against airborne and surface Norovirus.
- Solution for hospitals, cruise ships, care homes, etc.
- Low energy consumption.
- Discrete, silent and easy to use.



"In the absence of any opportunity to prevent Norovirus outbreaks in hospitals, the AD units have become the only means by which we can minimise the threat."

Gill Hill,
Matron in Infection Prevention and Control,
Hereford Hospitals NHS Trust 2009.

Inov8 Air Disinfection Combats Norovirus.

Hydroxyl Radicals Provide a Solution to Norovirus Infection—the Highly Infectious Gastro-intestinal Infectious Microorganism

As one of the main causes of non-bacterial gastro-intestinal infections, Norovirus is increasingly prevalent in Western Europe's healthcare system and throughout the world where the number of cases reported yearly is growing rapidly "WHO, CDC, rki".

The virus measures around 25 to 35 nm and is transferred either via the faecal-oral route or via droplets originating from infected vomit. The virus starts to propagate after an incubation period of 1 to 5 days, following which the body reacts with diarrhoea and vomiting. These are usually the first signs of symptoms which may continue up to two weeks following the initial infection.

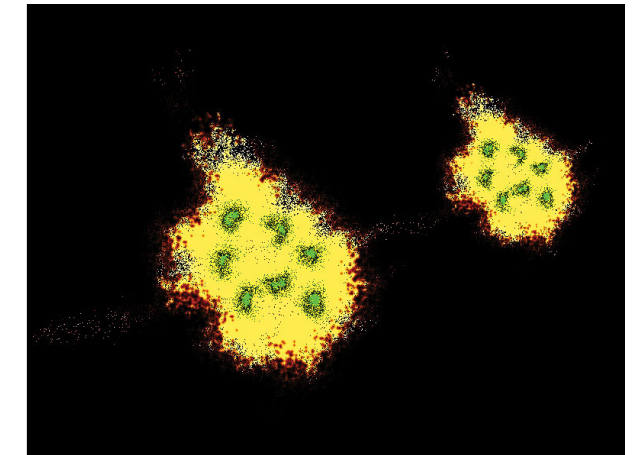


Figure 1. Norovirus under the microscope

According to the Centre for Disease Control (CDC), people may become infected with Norovirus through eating food or drinking liquids infected with this virus. Other routes to infection include touching one's mouth, nose or eyes after contact with infected surfaces or objects or through person to person contact. There is no known effective treatment against Norovirus, therefore once a patient is infected, rehydration the main treatment recourse.

Recent studies (Hennessy et al, 2003 and Ming and Xi, 2005) show that the mechanism of infection with Norovirus is via attachment to human histo-blood group antigens, HBGAs, which serve as receptors for the virus. ABH histo-blood group antigens are carbohydrate epitopes present at high concentrations on mucosal cell surfaces of the gut. The interaction of human HBGAs and Noroviruses is a typical protein-carbohydrate linkage, in which the protruding domain of the viral capsid protein forms an interface with the oligosaccharide side-chains of the antigens.



The Inov8 AD in use in a ward.

Figure 3 shows the form of Norovirus viral capsid, a single major structural protein forming an icosahedral particle which is composed of two major domains (the (S) and the (P) domains). It has been shown (Ming and Xi, 2005) that the isolated P domains, with the hinge form dimers in vitro, are responsible for the binding function to HBGA receptors.

Mechanism of Norovirus Deactivation

Since Norovirus can propagate both via air (microdroplets) or surfaces, oxidation systems can be particularly effective at disinfecting the environment from the virus. The most important oxidizing agent in atmospheric chemical reactions is the hydroxyl radical (OH•) which plays a central role in the oxidation of many organic compounds through a series of cyclic chain reactions. Hydroxyl Radicals are highly reactive and lead to a cascade of reactions that may target all biomolecules. Such reactions will result in damage to the structural and functional properties in capsid proteins of Norovirus.

Inov8 AD air disinfection technology produces hydroxyl radicals, and instantly denatures Norovirus in the air and on surfaces by oxidising the capsid protein structure enveloping the virus particle. For example protein *backbone* damage (Garrison WM (1987)) due primarily to a hydrogen atom abstraction at the alpha carbon; this process leads to backbone fragmentation.

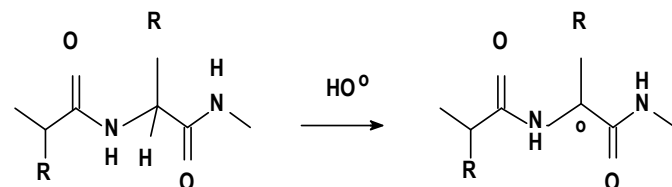


Figure 3. Mechanism of protein backbone oxidation by abstraction of a hydrogen atom.



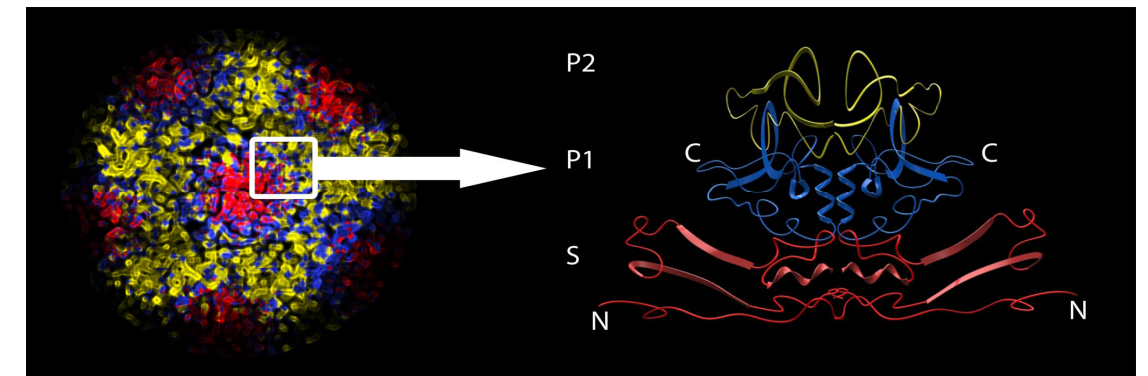
The Inov8 AD combats Norovirus in restaurants, reception rooms, hotels, and cruise liners.

Side-chain damage is another possible protein oxidation mechanism (Singh J & Thornton J M (1992)) and can occur through hydrogen abstraction or oxygen addition.

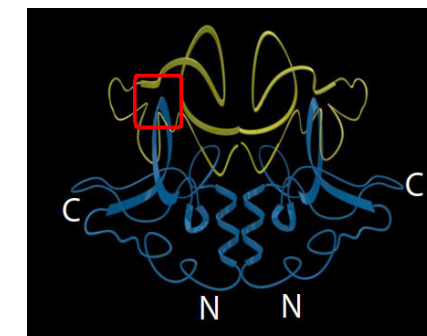
Within a Norovirus capsid protein, the reaction with hydroxyl radical will destruct the main (P) domain structure. This will lead to a dysfunction in the binding ability of Norovirus to HBGA receptors and therefore disable its ability to infect the host cells. The probability of infection is thus reduced.

In summary, the AD air disinfection technology is effective at denaturing the virus's protein structure through a well known chemical oxidation process. This oxidation capability has also been extensively proven against many bacteria, viruses and fungi.

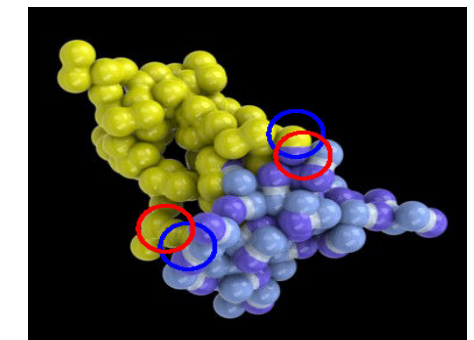
Figure 3, Detail of Norovirus protein and human histo-blood group antigen interaction sites. (Choi et al 2008)



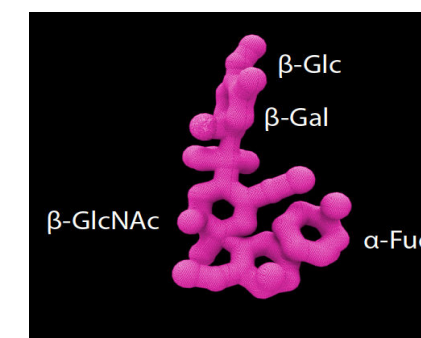
Norovirus capsid protein forms an icosahedral capsid with a T=3 symmetry. The shell domain (S domain) contains element essential for the formation of the icosahedron. The Protruding domain (P domain) is divided into sub-domains P1 and P2.



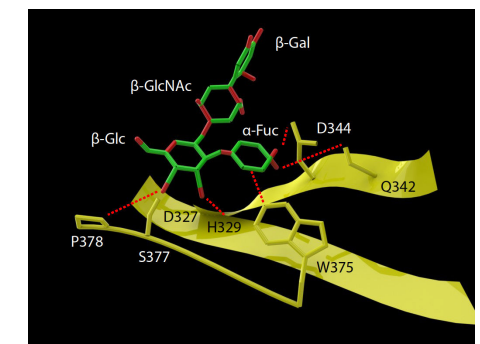
P domain interacts in dimeric contacts that increase the stability of the capsid and form the protrusions on the virion. An hypervariable region in P2 plays an important role in receptor binding and immune reactivity.



Circles indicate the location of histo-blood group antigen binding sites to the virus P domain dimer protein.



HBGA Receptor H-type 1 pentasaccharide.



Example of HBGA receptor interactions with NV capsid protein (sub-domain P2).