Guidelines and testing methods for virucidal activity, requirements for inclusion in the VAH List

In recent years the EN Standards covering the practice-related testing of virucidal activity of disinfectants have been published. This not only impacts the registration procedures for biocides but also the listing of virucidal surface and instrument disinfectants at the VAH (*Verbund für Angewandte Hygiene* / Association for Applied Hygiene).

The testing procedures already known in connection with bactericidal activity which require the performance of quantitative suspension tests (phase 2, step 1) and carrier tests (phase 2, step 2) now also apply to virucides. Classification in the three possible efficacy levels for virucides: "limited virucidal activity", "limited virucidal activity PLUS" and "virucidal activity" remains.

The quantitative suspension test in accordance with EN 14476 applies as phase 2, step 1 testing for all products listed as virucides.

In the case of EN 16777, the carrier test for surface disinfectant **without mechanical action**, statement of virucidal efficacy for this group of preparations is now no longer valid without corresponding practice-related verification. An analogous procedure applies to instrument disinfectants. In this case, in addition to successful suspension testing, phase 2, step 2 testing in accordance with EN 17111 is now also required.

A product is only regarded as having virucidal activity on conclusion of the practice-related testing.

Practice-related testing methods for virucidal activity concerning hand disinfection, surface disinfectants with mechanical action and textile disinfectants still do not have practice-related testing methods available; however these are currently being planned. Only in this case may the results of the quantitative suspension test in accordance with EN 14476 (or the RKI/DVV suspension test, as per 2015) be used solely. Table 1 provides an overview of the current European standards for testing virucidal efficacy in human medicine.

In Germany, testing according to the standard of the *Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten* (DVV, German Registered Association for Combating Viral Diseases) is currently also accepted. These are in part the basis for the listing of virucidal efficacy of preparations in the RKI list (Robert Koch Institute). However this only applies to the current DVV suspension test (DVV, as per 2015), since the carrier tests demanded additionally by RKI represent specific RKI standards and have requirements beyond those of the DVV carrier test (as per 2012). However the DVV has already adopted the carrier test in accordance with EN 17111 for instrument disinfectants without developing its own testing guideline.

Which testing method should be selected?

If product dossiers for the registration and marketing of a preparation as a biocide are being prepared then proof of the virucidal efficacy should preferably be via testing in accordance with European standards. There is little point in this case in testing according to national standards, regardless of type.

If a preparation is also to be offered in Germany for special situations specified by the Infection Protection Act then testing in accordance with DVV/RKI guidelines is unavoidable. Testing has to start with a suspension test in accordance with RKI/DVV guidelines. However is subsequently concluded with a carrier test in accordance with RKI specifications.

The VAH List is still of key significance in human medicine in routine or prophylactic disinfection. Therefore, it currently makes sense to have a product included in the VAH List not only for its microbiocidal but also its virucidal efficacy.

The VAH accepts all valid EN standards or RKI and DVV guidelines. The VAH demands in addition testing with polyomavirus SV 40 to have the product listed as "virucidal".

The VAH insists on successful performance of the entire testing procedure (that is: phase 2, step 1 suspension and associated phase 2, step 2 carrier tests). In the case of existing preparations already listed, any missing test results must be submitted subsequently to continue inclusion in the VAH List. It should also be noted that the VAH insists on double evaluation by 2 independent, accredited testing laboratories.

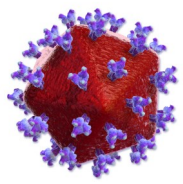
Furthermore, the VAH makes further demands regarding the statements on virucidal activity. The pre-condition for virucidal listing of a disinfectant is an expert assessment concerning the bactericidal/yeasticidal efficacy. The exposure time for effectiveness against viruses which is stated in the VAH List may not be less than that for bactericidal/yeasticidal exposure time. Contact times of more than one hour are generally no longer accepted.

All these amendments have thus led to a considerable improvement in the testing concept for disinfectants and major progress in the safety of patients and medical staff.

What does the spectrum of activity selected mean?

The concept of testing for virucidal activity of disinfectants is based on the assumption that testing certain surrogate viruses can back up statements concerning comparable human pathogenic viruses. Thus making extensive testing against individual pathogens unnecessary. According to the same concept, products are classified and the corresponding test viruses are selected according to the spectrum of activity against viruses in one of the three groups "limited virucidal activity", "limited virucidal activity PLUS" or "virucidal activity".

Limited virucidal activity

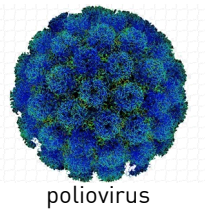


HIV

Products in this category are effective against **enveloped viruses** whereby the virus particle is surrounded by an additional envelope comprising components from the host cell membrane.

Enveloped test viruses are vaccinia virus and bovine viral diarrhea virus (BVDV). These are surrogate viruses for hepatitis B and C virus as well as for HIV. Efficacy against pathogens of significant diseases such as e.g. measles and influenza is also covered by "limited virucidal activity".

Limited virucidal activity PLUS



poliovirus

Disinfectants in this category are effective against enveloped viruses from the group "limited virucidal activity" **and some non enveloped viruses** with **sensitivity to lipophilic** disinfectants. Non-enveloped viruses do not have an envelope around the virus particle. However it would be wrong to assume that they are thus more sensitive to environmental influences and disinfectants. The simpler the outer layer of a virus is, the more stable the virus particle is.

Non-enveloped, slightly lipophilic test viruses are adenovirus and the murine norovirus (MNV). These are deemed as surrogate viruses for e.g. human norovirus and some other pathogens causing diarrhea.

Virucidal activity

Products in this category are effective against enveloped and non-enveloped viruses and thus include all applications with "limited virucidal activity" and "limited virucidal activity PLUS".

Testing for the VAH listing as a virucidal product is somewhat more extensive since adenovirus, murine norovirus, poliovirus and polyomavirus (SV 40) are defined as test viruses.

Poliovirus is a non-enveloped virus highly resistant to disinfectants. The polyomavirus serves as a surrogate virus for human papillomavirus and other oncoviruses. It is also particularly resistant to disinfectants.

The murine parvovirus is exclusively used to test products for chemothermal disinfection. Parvoviruses are non-enveloped viruses and in all probability the most resistant of all vertebrate viruses. In addition to tenacity against environmental and chemical influences, they are also extremely thermostable. They even survive pasteurization procedures (80°C/10 min).

A summary of the efficacy statements, test viruses and practice-related applications can be seen in Table 2.

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Application	Efficacy according to EN specifications				Efficacy according to VAH specifications			
	Phase 2, step 1 (suspension test) EN 14476	Test viruses	Phase 2, step 2 (carrier test)	Test viruses	Phase 2, step 1 (suspension test) EN 14476 or guideline RKI/ DVV (2015)	Test viruses	Phase 2, step 2 (carrier test)	Test viruses
Hand disinfection	**Limited virucidal activity**	*Vaccinia virus*	Currently no valid EN standard adopted	Under discussion	**Limited virucidal activity**	*Vaccinia virus BVDV with oxidative acting products*	Currently no testing procedure available	No test viruses currently stated
	Limited virucidal activity PLUS	*Adenovirus + murine norovirus*			**Limited virucidal activity PLUS**	*Adenovirus, murine norovirus*		
	Virucidal activity	*Poliovirus, adenovirus, murine norovirus*			**Virucidal activity**	*Poliovirus, adenovirus, murine norovirus, polyoma virus SV-40*		
Instrument disinfection Temperatures <40°C	**Limited virucidal activity**	Currently no valid EN standard adopted	EN 17111	*Vaccinia virus*	**Limited virucidal activity**	*Vaccinia virus*	EN 17111	*Vaccinia virus*
	Virucidal activity	*Poliovirus, adenovirus, murine norovirus*		*Adenovirus murines norovirus*	**Virucidal activity**	*Poliovirus, adenovirus, murine norovirus, polyoma virus SV-40*		*Adenovirus, murine norovirus*
Instrument disinfection Temperatures >40°C	**Virucidal activity**	*Murine parvovirus*	EN 17111	*Murine parvovirus*	**Virucidal activity**	*Murine parvovirus*	EN 17111	*Murine parvovirus*
Surface disinfection	**Limited virucidal activity**	*Vaccinia virus*	EN 16777	*Vaccinia virus*	**Limited virucidal activity**	*Vaccinia virus BVDV with oxidative acting products*	EN 16777 or DVV guideline, as per 2012	*Vaccinia virus*
	Limited virucidal activity PLUS	*Adenovirus + murine norovirus*		*Adenovirus murine norovirus*	**Limited virucidal activity PLUS**	*Adenovirus, murine norovirus*		*Adenovirus, murine norovirus*
	Virucidal activity	*Poliovirus, adenovirus, murine norovirus*		*Adenovirus murine norovirus*	**Virucidal activity**	*Poliovirus, adenovirus, murine norovirus, polyoma virus SV-40*		*Adenovirus, murine norovirus, murine parvovirus*
Chemothermal disinfection procedure including textile disinfection ≥30°C	**Virucidal activity**	*Murine parvovirus*	Currently no valid EN standard adopted	No valid EN standard	**Virucidal activity**	*Murine parvovirus*	Currently no testing procedure available	No test viruses currently stated

Tab. 1: Current statements of efficacy concerning virucidal activity corresponding to EN standards and VAH specifications

Efficacy	Test viruses	Practice-relevant for: (examples)
Limited virucidal activity Effective against enveloped viruses	Vaccinia virus bovine viral diarrhea virus (BVDV)	**Bloodborne pathogens** Hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) **Significant pediatric disease pathogens** Mumps virus, measles virus, rubella virus, varicella virus (VZV), *respiratory* syncytial virus (RSV) **Pathogens of other important, in part also notifiable diseases** Influenza viruses, parainfluenza virus, cytomegalovirus, Epstein-Barr virus Hanta viruses, dengue virus, yellow fever virus including FSME, filo virus (Marburg, Ebola virus), Lassa virus, etc.
Limited virucidal activity PLUS Effective against enveloped and some non-enveloped viruses sensitive to lipophilic acting disinfectants	Adenovirus murine norovirus (MNV)	**All pathogens included in "limited virucidal activity".** **Viral pathogens of gastroenteritis, in particular** Adenoviruses, noroviruses, rotaviruses **Other hygiene-relevant pathogens** Coronaviruses
Virucidal activity Effective against enveloped and non-enveloped viruses.	Adenovirus, poliovirus, murine norovirus (MNV), polyomavirus (SV40), murine parvovirus	**All pathogens included in "limited virucidal activity" and "limited virucidal activity PLUS".** ECHO viruses, coxsackieviruses, rhinoviruses, picornaviruses (on particular hepatitis A virus), papilloma viruses, humane parvoviruses

Tab. 2: Key to efficacy statement