A new weapon for decontamination of surfaces and premises from African swine fever virus: accelerated hydrogen peroxide

Jose A. Ramirez, MS., PhD, EVP & Lead Science Advisor
Virox Animal Health, Oakville, ON L6H 6R1 Canada

Introduction

The resurgence of African swine fever over the past 18 months in commercial swine herds in Asia and Eastern Europe, and more recently in European wild boar, has resulted in renewed interest in practical and effective options for eradication of the virus from environmental reservoirs. Given an effective vaccine is currently unavailable, effective disinfection of contaminated surfaces remains a key weapon for prevention and control. Although there are a number of research studies documenting the efficacy of diverse disinfection options against the virus, differences in methodology and criteria make it difficult to select a particular disinfection option with certainty. The fact that the virus is fairly unique - it is the only member of the only genus (Asfivirus) in the Asfarviridae family - also makes application of the usual criteria for disinfectant susceptibility based on virus morphology difficult. This has resulted in a variety of different recommended options for disinfection around the world.

In the US, the Department of Agriculture (USDA) has issued a guidance on which US-EPA registered disinfectants "...may be used against ASFV in/on farm premises and related structures and equipment."[1] The USDA guidance advices that the first recourse in response to the virus should be to use a disinfectant which has been subject to the full evaluation prescribed for disinfectants in the federal pesticides law Section 3.[1] It also allows for several other choices that can be used if a Section 3 registered disinfectant is not available - a provision of the federal pesticides law for emergencies known as Section 18. The latest addition to the Section 3-approved disinfectants for use against ASFV is the accelerated hydrogen peroxide (AHP®) technology branded Intervention® and Prevail™ for the swine production sector.[1]

In this paper, we review the results of recent in-vivo studies evidencing the biocidal efficacy of AHP® against ASFV using the two most widely accepted testing methodologies. We discuss the practical implications of this work, with special focus on a comparative assessment of the different options now available to US producers and veterinarians, and how factors other than biocidal efficacy can affect the outcome of the ASFV-disinfection effort.

Methods for evaluation of virucidal efficacy

Traditionally, in-vitro methods for the evaluation of biocidal activity of chemicals have relied on two types of methods, suspension assays and surface carrier tests. In both cases, the criteria for efficacy can be qualitative or quantitative in nature, depending on whether the criteria is a growth/no growth observation on culture media or a specific count for colonies (in the case of bacteria or fungi) or infected cells (in the case of viruses).

In a suspension method, the organism inoculum (sometimes in the presence of an organic load to simulate use under "dirty" conditions) is added to a volume of disinfectant. After the specified contact time, the mixture is neutralized by dilution or chemical neutralization and any survivors are recovered in culture media. In a carrier method, a specified volume of inoculum and added soil is dried on the surface of a coupon or petri dish. After the appropriate drying period, a specified volume of disinfectant at the required concentration is added to cover the dried inoculum. After the specified contact time, the inoculum-disinfectant mixture is diluted sufficiently to arrest the biocidal activity of the disinfectant. The eluate is then transferred to the appropriate culture media or, in the case of viruses, to monolayers of suitable host cells at the appropriate 10-fold dilutions. In a quantitative test, after incubation, the viability titer of the organisms is compared to the titer in the control carriers and a logarithmic reduction is calculated and compared to a pre-established reduction criterion for efficacy.

In general, suspension tests are simpler and easier to perform; however, it is widely accepted that they may not represent use of the disinfectant under real-world conditions.[2] Additionally, it has been noted that they may not present a sufficient challenge to the disinfectant product.[2,3] In contrast, since in a carrier test the test virus is dried on the inanimate surface; it is widely considered to be a better representation of actual in-use conditions. In most practical situations, the infectious agents will be mixed with organic matter (feces or bodily fluids) and in the worst case will have dried unto an environmental surface. Thus, the disinfectant has to be able to penetrate or dissolve the dried contaminants and suspend the organism-soil mixture so that the disinfectant active can be transported through the membrane or capsid walls of the target organisms. Clearly, test products that do not possess a high degree of detergency or emulsive power will generally be less effective in both the carrier test and in field conditions.[4] This is also the reason why many researchers have found that many disinfectants will pass a suspension test at a given concentration but will require a much higher concentration to pass a carrier test.[2-4]

For many of the so-called transboundary animal disease viruses (e.g. foot-and-mouth disease, African swine fever, classical swine fever, etc.); most of the published data for disinfectant efficacy against these viruses has been based on suspension testing.[5,6] The reason for this is that historically, many of these viruses have been more economically relevant in Europe; where traditionally, the prevalent methodology for virucidal testing has been based on suspension methods - a virucidal carrier method has just been recently published less than two years ago.[7] Furthermore, the first study reporting disinfectant action vs. ASFV using a carrier methodology was the relatively recent paper of Krug et al.[5] As a consequence, many of the disinfectants recommended by the USDA as effective against ASFV most likely will not have been

evaluated under the more demanding and realistic conditions of the carrier test methodology, but rather under the less stringent suspension testing assay.[5]

A key problem with surface carrier testing is that many viruses lose significant infectivity during the drying step on the test carrier.[2,3] This results in a very small difference between the virus that remains intact after drying and the overall limit of virus detection, which makes measuring the reduction in virus titre due to disinfectant action very difficult. One solution to this problem was described by Krug et al.[5] The technique involves using large inoculum volumes in regards to the volumes of disinfectant and neutralizer in order to minimize the loss of detectable virus due to dilution. This technique enabled the successful performance of the carrier testing described below.

Evaluation of efficacy of AHP® disinfectant technology against ASFV

The test was carried out by Dr. Esther Blanco at the Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA) in Madrid, Spain.[8] The INIA laboratory is the European Community Reference Laboratory for African swine fever for the European Union.[9] Both a suspension test and a carrier test were carried out with the AHP®-based products Intervention® and Prevail™, at the recommended dilution of 2 oz. per gallon (1:64) in hard water (1:40 was used for Prevail™ per label instructions), and specified contact time of 5 min. The suspension testing followed the methodology specified in the European standard test method EN14675 for the evaluation of virucidal activity of disinfectants for use in veterinary applications.[10] The carrier testing methodology was in accordance to the standard test method for virucidal activity of disinfectants published by the American Society for Testing of Materials (ASTM);[11] one of the methods accepted by the USEPA as evidence of virucidal efficacy for a commercial disinfectant product.[12]

The results of the testing are shown on Table 1. With a logarithmic reduction of more than 4-logs, Intervention® meets the required efficacy performance for virucidal activity against the African swine fever virus in both the US and in Europe.[10,12] The same type of efficacy was observed for Prevail™, with a log10 reduction of ≥ 4.1.[8]

A further evaluation was carried out with real time PCR (RT-PCR). In this technique, total DNA is extracted from the VERO cells incubated with both control and neutralized disinfectant solution. The results of the RT-PCR assay for the tested samples under the conditions described above showed a cycle threshold of 36.08; below the threshold of positivity of 35.00 for this particular assay. This result further supports the notion that the virus was fully inactivated by Intervention® at a dilution of 2 oz./gal and 5 min contact time.[8]

Practical considerations

The influence of factors other than intrinsic biocidal efficacy of the chemical disinfectant has been discussed previously.[13] Hygiene outcomes in the field are heavily influenced by the quality of the cleaning and disinfection process and the ability (or willingness) of working crews to comply with it. For example, a disinfectant that cannot be easily used with standard pressurized power washing systems prevalent in farms and trailer washing station runs a higher chance of resulting in a failed hygiene outcome. In particular, in our experience in the field, the following factors can determine whether premises will be effectively disinfected or not:

- erroneous concentration due to manual preparation or dilution,
- missed spots due to lack of visual cues for coverage like foam,
- incomplete or sub-optimal treatment of vertical surfaces due to rapid disinfectant run-off,
- less-than thorough application due to worker discomfort from a respiratory and/or eye/skin irritant.

While these potential failure modes are critical in the day-to-day biosecurity program of a healthy farm looking to keep environmental infectious pressure low, they are crucial in the one-time terminal decontamination of an ASFV-positive facility before restocking, or in farms with heightened biosecurity measures due to proximity to an affected area. It is thus instructive to review the different choices recommended by USDA and approved by USEPA based on biocidal efficacy criteria in a practical context, with particular attention to the factors outlined above. A summary of the disinfection options from USDA is presented in Table 2.[1]

Aside from the newly approved Intervention®,[14] the table includes four other formulated products that have gone through a full EPA review and approval for the specific claims against ASFV. It also includes three products that have received an exemption from the EPA for use in case none of the EPA-approval products are available.[1] We will not focus here on the exemption products (Sodium hypochlorite, Citric acid, Thymol) given these are fallback solutions in case others are unavailable and the user has no other choices at their disposal. We focus our attention on the first-choice, commercial products.

All of the EPA-approved products recommended by USDA are chlorine precursors, that is; they generate chlorine species in solution when dissolved in water. Three of the products are based on the substance sodium dichloro-isocyanurate (DCC), the sodium salt of a chlorinated hydroxytriazine that in the presence of water ultimately decomposes into hypochlorous acid and isocyanurates.[15] The fourth product is a powdered mixture of potassium monopersulfate (PMPS) and sodium chloride that when dissolved in water react to form hypochlorous acid.[16,17]

Because these products are highly concentrated powders (or compacted powder tablets) special care must be taken in their

Table 1: Summary of results of testing of Intervention™ accelerated hydrogen peroxide against African swine fever virus

Test method	Recovered virus log-titre (control)	Recovered log-titre after 5 min exposure to disinfectant	log reduction
Suspension Test	5.5	< 0.5	≥ 4.6
Carrier Test	5.5	0	5.5

handling, particularly in avoiding exposure to skin and eyes during preparation of in-use solutions, as well as avoiding inhalation of their dust/fine powder. It is also critical to avoid contaminating the original packaging with moisture, as this could cause the generation of highly poisonous chlorine gas and compromise the shelf life of the powdered stock-product.

An important operational difficulty with powder/tableted products is the need to prepare a diluted liquid stock of an intermediate concentration that can be used as the source to feed a chemical injection system. With DCC powders or tablets, since the solubility limit in water is quite high (approximately 227 gr/L[18]), it is possible to prepare a liquid stock solution of say 10% concentration by weight (100 gr of DCC powder per Liter of water) which is then diluted through an injector with a dilution tip for 4 oz/gal (1:32). In this way, the resulting solution out of the injector nozzle will be diluted by a factor of 300 with respect to the packaged powdered product (1:300); this delivers the roughly 1,000 ppm chlorine required (see Table 2). Having said this, in our experience, this calculation for a two-step dilution just described is not easily understood by most individuals with operational responsibility, thus the potential for incorrect end-dilution is very high.

The same preparation protocol is typically required with the other chlorine-precursor product based on PMPS.[19] In this case however, the choices of intermediate stock dilutions/volume are more limited due to the low water solubility of the product of 62 gr/L (roughly 9 oz. per gallon).[19] At the limit of solubility, the strongest stock solution that can be prepared is at about 6% by weight. Adding more powder product than this will result in precipitation and settling - the liquid in the stock solution will not "hold" more than 62 gr per Liter (or 9 oz per gallon). Because the required in-use concentration is 1% (10 gr per Liter of water), this means that the dilution occurring at the injector drawing from the 6% stock solution should be 1:6. In other words, the injector should be drawing 21 oz. of stock solution for every gallon of water dispensed by the power washer. In practice, this means that to treat a farrowing room that could require, say 50 gal of diluted disinfectant, the operator would have to prepare slightly more than 8 gal of stock solution. This makes it impossible for crews to use hand-held bottle-foamers (as the bottle in these foamers is typically designed to hold between 48 to 96 oz. of liquid) and this would require repeated refilling or multiple units. Furthermore, with a standard power washer delivering 4.5 gallon per minute of water, a 96 oz. bottle-foamer would be emptied in about 1 minute, clearly impractical. For farms with overhead quick-connects affixed to the ceiling that are spread-out through the barn (where the injector-foaming wand assembly is moved throughout each connection point with the chemical concentrate as the crew progress through the barn), it requires using two 5-gal pails of the stock solution and moving them throughout the different connection drop-points in the barn.

Aside from the operational difficulty and risk for calculation errors intrinsic to the preparation of intermediate stock solutions, there is the risk of blockage of the small orifice in the chemical injector from residual debris from the tablet or powder. The orifices in these injectors range in the order of thousands of an inch and can easily be blocked by small particles remaining from incomplete dissolution of the powder in the stock solution. Furthermore, because of the low flow of stock chemical into the injector (in relation to the diluent water jet) blockages are rarely noticed by the operator until they realize that the liquid level in the stock solution is not changing. Furthermore, DCC or PMPS chlorine precursors do not typically exhibit a clear visible cue for application (e.g. highly visible foam), which further contributes to the operator not realizing chemical is no longer flowing properly due to injector orifice blockage.

The lack of visible cues on application also contribute to the misapplication (both under- and over-application) of the chemical, resulting in missed spots or excess use/waste. A more critical issue, however, is the immediate runoff of solution when applied to vertical surfaces. This is particularly critical in the successful terminal decontamination of a farm due to be restocked after a bout with ASFV. In order for the disinfectant to be effective, the surfaces have to remain wet for a period of 10 minutes. With water-thin solutions lacking a foam phase, runoff of excess liquid from vertical surfaces occurs very quickly. This accelerates drying on these surfaces possibly limiting the extent of the contact time of the disinfectants.

In contrast, Intervention® is a liquid concentrated disinfectant that can be easily dispensed through existing chemical injection/power washing equipment increasingly adopted in the day-to-day cleaning & disinfection programs in swine farms and livestock trailer facilities. The ASFV cleaning & disinfection protocol would be no different than that employed in the day-to-day biosecurity program. This reduces any need for additional training and therefore the potential for errors due to changes in protocol. Crews simply connect the 1-gallon bottle of concentrate to their power-washing system injector or fill the handheld bottle-sprayer foaming wands. Crews using larger volumes (such as trailer washing stations) can simply insert the injector dip-tube into a pail or drum of Intervention® concentrate. The risk of miscalculating or mis-preparing an intermediate stock solution is eliminated. The heightened risk of chemical exposure to a concentrated powder or stock solution is unnecessary. The potential for clogging of the chemical injector from undissolved particles is nonexistent.

When dispensed through a standard foaming nozzle, operators can easily judge application by the presence of the highly visible foam. This reduces product waste and minimizes the risk of missed spots. Intervention® has been formulated to produce a durable, high-cling foam on application. Therefore, gravity-induced run-off from vertical surfaces is reduced (due to the foam's lower specific weight); while at the same time, the foam phase impairs the evaporative diffusion process that drives surface drying. Furthermore, the reduced required contact time of 5 minutes (vs. 10 minutes for the other chemical options) further ensures that vertical surfaces are properly decontaminated.

Conclusion

The suitability of a chemical disinfectant to deliver expected hygiene outcomes goes beyond the biocidal properties of the chemical. Many additional factors that arise from the chemical and physical properties of the disinfectant need to be considered as well. We have discussed some of these elements, with particular emphasis on issues related to preparation and application in field conditions as well as the importance of potential health hazards and chemical exposure. The recent validation of Intervention® efficacy versus the ASFV, and subsequent US EPA approval provide producers and veterinarians with a superior alternative to fighting environmental contamination by ASFV.

Table 2: Summary of FIFRA Section 3 approval products recommended by USDA APHIS for mitigating African swine fever virus. Health hazards information has been taken from the safety data sheets published by the manufacturers. The products marked with a **§** are based on an exemption approval, only to be used in emergencies where the recommended US EPA approved products are not available.[1]

Packaged active	In-use active	Health hazards	Potential issues
48.21% NaDCC (29-31% free available chlorine)	Varies with commercial product but in the range of 958-1076 ppm free available chlorine @ 10 min contact time.	• Causes serious eye irritation • May cause respiratory irritation • Contact with small amounts of moisture release poisonous chlorine gas	• Needs to be freshly prepared and used within 1 day • Stock solution needs to be manually prepared • Potential for insoluble particles to clog chemical injectors • Corrosive to equipment and surfaces
21.41% PMPS, 1.5% NaCl (9.75% FAC)	1% (975 ppm free available chlorine - hypochlorous acid), 10-minute contact time	• Corrosive, irreversible eye damage and skin burns	• Stock solution needs to be manually prepared - Insoluble @ higher concentrations than 62 gr/L (1:16). • Potential for insoluble particles to clog chemical injectors • Corrosive to equipment and surfaces
Sodium Hypochlorite**§** (exemption approval 5.25%, 8.25%, 12.0%, 12.5%)	3%, 15 min or 30 min	• Causes serious eye irritation • May cause respiratory irritation	• Stock solution needs to be manually prepared • Needs to be freshly prepared if diluted with hard water
Citric acid**§** (powder close to 100% active).	3%, 30 minutes	• Corrosive, irreversible eye damage and skin burns	• Stock solution needs to be manually prepared • Potential for insoluble particles to clog chemical injectors
Thymol**§** (exemption approval, only for aircraft and aircraft loading ramps)	0.05% Thymol, 15 minutes	• None of note	• Pressurized can, ready to use spray, impractical for farm and livestock trailers

References

1. APHIS ASF virus disinfectants **https://www.aphis.usda.gov/animal_health/emergency_management/downloads/asf-virus-disinfectants.pdf**. Accessed October 30th 2020.

2. Bellamy, K. A review of the test methods used to establish virucidal activity, J. Hospital Infec., 30(S), 1995 389:396.

3. Sattar, S. Springthorpe, S., Adegbunrin, O., M. AbuZafer A. disc-based quantitative carrier test method to assess the virucidal activity of chemical germicides, Journal of Virological Methods, 112(1/2), Sept. 2003, 3:12.

4. Quinn, P. J. Evaluation of veterinary disinfectants and disinfection processes, in Disinfection in Veterinary and Farm Animal Practice, Blackwell Scientific Publications, Oxford, UK, 1987, 66:116.

5. Krug, P.W., Lee, L.J., Eslami, A.C., Larson, C. R. and L. Rodriguez. Chemical disinfection of high-consequence transboundary animal disease viruses on nonporous surfaces, Biologicals, 39 (2011) 231:235.]

6. De Lorenzi, G., Borella, L., Alborali, G.L., Prodanov-Radulovic, Stukelj, M. and S. Bellini. African swine fever: A review of cleaning and disinfection procedures in commercial pig holdings, Research in Veterinary Science, 132 (2020) 262:267.

7. 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). Comité Européen de Normalization, Brussels, EU.

8. Blanco, E. Virucidal efficacy of a disinfectant for use on inanimate environmental surfaces. Virus: African swine fever Virus (ASFV), Project Number 18CISA093.2, 12.26.2019., INIA, Madrid, Spain.

9. EEC Council Directive 2002/60/EC of June 27 2002 laying down specific provisions for the control of African swine fever and amending Directive 92/119/EEC as regards Teschen disease and African swine fever, Official Journal of the European Communities, L 192/27.

10. EN14675:2003 Chemical disinfectants and antiseptics - quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in veterinary field - Test method and requirements (phase 2, step 1). Comité Européen de Normalization, Brussels, EU.

11. Standard Test Method to assess virucidal activity of chemicals intended for disinfection of inanimate, nonporous environmental surfaces, Designation:E1053-11, ASTM, West Conshohocken, PA, 2013.

12. Product performance test guidelines OCSPP 810.2200 Disinfectants for use on hard surfaces- efficacy data recommendations, EPA 712-C-07-074, USEPA, Washington D.C., March 12, 2012.

13. Ramirez, J.A. Theory and practice of cleaning and disinfection in swine biosecurity, Proceedings of the 51st annual meeting of the AASV, Atlanta, March 7-10, 2020.

14. News Release, Virox Technologies Corp., **https://www.viroxfarmanimal.com/wp-content/uploads/2019/08/News-Release-ASFV-Intervention-and-Prevail-02082019.pdf**, accessed Oct. 30, 2020.

15. Falk, N.A., Macnaughtan, M., Taheri, A. and W. McCormick. Chlorine and Chlorine Compounds, in Block's Disinfection, Sterilization and Preservation, McDonnel, G. and J. Haysen (Eds), Wolters Kluwer, Philadelphia, 2021, 280:305.

16. Auchincloss, T.R. Biocidal, particularly virucidal compositions, US Patent No. 4,822,512, granted. Apr. 18 1989.

17. Ramirez, J. New Technologies in Disinfection and Infection Control, in Disinfection & Decontamination, Principles, applications and related issues, G. Manivannan (ed.), CRC Press, Boca Raton, FL.

18. Ulmann's Encyclopedia of Industrial Chemistry, 5th Edition, W. Gerhartz, G. Schultz (eds), 1996. WIley-VCH, Weinheim, Germany.

19. Virkon™ Protecting against African swine fever virus breaching our US borders, Marketing brochure, Lanxess Corp., Pittsburgh, PA, 2019.