CEN WS ABF

Date: 2025 -07

Draft CWA ABF: 2025

Secretariat: ASRO

Procedures for assessment of biosecurity in Farm to Fork chain

ICS:

CCMC will prepare and attach the official title page.

Content

European foreword

This CEN Workshop Agreement (CWA XXXX:2025) has been developed in accordance with the CEN-CENELEC Guide 29 "CEN/CENELEC Workshop Agreements – A rapid way to standardization" and with the relevant provisions of CEN/CENELEC Internal Regulations - Part 2. It was approved by the CEN Workshop "Procedures for assessment of biosecurity in Farm to Fork chain", the secretariat of which is held by ASRO, consisting of representatives of interested parties on YYYY-MM-DD, the constitution of which was supported by CEN following the public call for participation made on 2024-10-17. However, this CEN Workshop Agreement does not necessarily include all relevant stakeholders.

The final text of this CEN Workshop Agreement was provided to CEN for publication on YYYY-MM-DD.

Results incorporated in this CWA received funding from the European Commission Horizon Europe research and innovation programme under Grant Agreement No 101084097: Healthy Environmental-Friendly and Resilient Farm to Fork.

The following organizations and individuals developed and approved this CEN Workshop Agreement:

— Chairperson: Mr. Jose Andres CALDERON, San Jorge Tecnológicas SL, *Spain*;

— Secretariat: Mrs. Diana Iorga, ASRO - Romanian Standards Association, *Romania*;

— UAH – Universidad de Alcala: Mr. José Luis PÉREZ-DIAZ, Workshop proposer & Ms. Cristina DEL ALAMO TORANO, *Spain*;

— Institut Pasteur: Mr. Richard PAUL, *France*;

— University of Veterinary Medicine: Mr. Jóźwiak ÁKOS, Ms. Engelhardt TEKLA & Ms. Orsolya STRANG, *Hungary*;

— Counterfog SL: Mr. Francisco Javier PÉREZ DEL ALAMO & Ms. Sonia PEIRO PAREDES, *Spain*;

— San Jorge Tecnológicas SL Mr. Sánchez García-Casarrubios JUAN, *Spain*;

— IRD - Institut de Recherche pour le Développement: Ms. Sylvie MANGUIN, *France*;

— CIOP - Central Institute for Labour Protection: Mr. Marcin CYPROWSKI, Mr. Rafal GORNY, Ms. Agata STOBNICKA-KUPIEC, Ms. Malgorzata GOLOFIT-SZYMCZAK, Ms. Anna LAWNICZEK-WALCZYK, *Poland*;

— ICPOR Castilla La Mancha SL: Mr. Pedro MORALES TIREZ, *Spain*;

— INCARLOPSA: Mr. Luis CALVO ADIEGO, *Spain*.

Attention is drawn to the possibility that some elements of this document may be subject to patent rights. CEN-CENELEC policy on patent rights is described in CEN-CENELEC Guide 8 "Guidelines for Implementation of the Common IPR Policy on Patent". CEN shall not be held responsible for identifying any or all such patent rights.

Although the Workshop parties have made every effort to ensure the reliability and accuracy of technical and non-technical descriptions, the Workshop is not able to guarantee, explicitly or implicitly, the correctness of this document. Anyone who applies this CEN Workshop Agreement shall be aware that neither the Workshop, nor CEN, can be held liable for damages or losses of any kind whatsoever. The use of this CEN Workshop Agreement does not relieve users of their responsibility for their own actions, and they apply this document at their own risk. The CEN Workshop Agreement should not be construed as legal advice authoritatively endorsed by CEN/CENELEC.

Introduction

The current food chain biosecurity assessment paradigm is usually a semiqualitative technique based on binary questionnaires providing typically just a three-level classification 'high, medium or low' for the risk for a particular disease. This simple classification does not allow a precise and optimal decision to take actions and their associated costs. It contrasts with the refined risk assessment methodologies that are well-established in other fields like fire protection engineering. These engineering methods are based on experimental data and computer aided modelling developed through the last decades.

The ambition of this CEN Workshop Agreement is to establish a first step towards a more refined 'transport channel' resolved approach for assessment of food chain biosecurity. This experimental channel-resolved methodology should provide logarithmic 'transport factors' of microorganisms resolved by channels. Such a channel resolved methodology enables a rational optimization of resources as they can be invested on improvements on the actual most critical biosecurity need and not in other channels that may be enough.

According to the 'transport channel concept' of biosecurity of animal farms, all microorganisms need to be transported from one host or reservoir to another host to infect, survive and/or multiply, resulting in subclinical or clinical animal diseases. Effectiveness of these transport channels, the speed of these routes is critical for transmission of diseases. The number of channels is indeed limited to just a few: the project had identified 9 channels. Therefore, a strategy oriented to suppress transport effectiveness for all these 9 routes would have an enormous impact preventing transmission of all diseases. Moreover, most pathogens usually prefer or use effectively just one or a few transmission channels. This means that even the suppression of a single channel would clearly prevent transmission of many diseases associated to that particular channel. In cases, where microorganisms use a combination of several channels, a channel-resolved prevention approach is still useful if applied to one of the critical channels or a set of them simultaneously, utilising also the 'hurdle concept' in food microbiology.

1 Scope

This CWA proposes a methodology to assess biosecurity in the F2F sector, centred on a detailed examination of 'transport channels', in order to initiate the development of a more refined approach, The methodology involves an experimental and quantitative assessment of the risk associated with the transportation of microorganisms, placing particular emphasis on pathogens while considering any other microorganisms present.

This methodology is applicable for two different cases:

— to experimentally assess and monitor channel-resolved biosecurity in a particular farm, or any other element in the F2F chain like transport or industries;

— to evaluate effectiveness and cost-efficiency of a particular biosecurity measure or technology. These experimental data will provide a basis for all stakeholders (like policy makers, veterinarians, or business operators) to estimate or forecast the real effect as well as the cost of the application of a particular measure.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 12353, *Chemical disinfectants and antiseptics — Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity*

EN 13697, *Chemical disinfectants and antiseptics — Quantitative non-porous surface test for the evaluation of bactericidal and yeasticidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas without mechanical action. Test method and requirements without mechanical action (phase 2, step 2)*

EN 17122, *Chemical disinfectants and antiseptics— Quantitative non-porous surface test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area. Test method and requirements. Phase2, step2*

EN 17272, *Chemical disinfectants and antiseptics— Methods of airborne room disinfection by automated process. Determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities.*

3 Terms and definitions

No terms and definitions are listed in this document.

4 Specific objectives

4.1 Risk assessment

Assessing biosecurity risks traditionally has the following four steps:

— hazard identification,

— hazard characterization,

— exposure assessment

— risk characterization.

The risk-based assessment needs to identify and describe the characteristics of the agent (hazard), the extent and mechanism of the harmful effect. In the next step, the dose-response-like relationship needs to be defined, quantifying the effect of the quantity and/or concentration of the agent on the health outcomes. After that, the probabilities need to be weighed in: the exposure to the agent (the probabilities of infection) needs to be quantified. In the last step, the synthesis of the evidence and the characterisation of the risk need to be performed.

4.2 Risk-based interventions

Based on biosecurity risk assessment, also risk-based interventions need to be implemented to mitigate the risks; need to know what the effect and actual cost and benefit of would be implementing a particular measure. The following must be taken into account:

— the severity of the harmful effects of the agents and the probability of these agents to be transported over one or more channels (altogether: the risk),

— the effectiveness of the planned interventions (a quantitative evaluation of the mitigation power of various interventions through various channels),

— the costs of the interventions,

— the quantitative assessment of the benefits (outcomes) of the interventions, in terms of avoided losses (including direct monetary losses, and indirect, animal, and human health related impacts).

4.3 Risk communication

Coordination of risk assessments and risk communication strategies requires information sharing and establishing networks of working relationships between industry groups and regulatory organizations/ agencies. Establishing these relationships necessitates overcoming institutional, cultural, and political boundaries. In the food industry, the need for coordination has been enhanced by industry integration and globalization of both markets and production. Overcoming institutional and cultural barriers, and mistrust is necessary to create consistency in risk messages. Open communication and information sharing can help clarify where risk perceptions diverge and identify points of convergence. The outcome may not be universal agreement about risks, but convergence around the general parameters of risk.

The fundamental goal of risk communication is to provide meaningful, relevant and accurate information, in clear and understandable terms targeted to a specific audience. It may not resolve all differences between parties, but may lead to a better understanding of those differences. It may also lead to more widely understood and accepted risk management decisions. Effective risk communication should have goals that build and maintain trust and confidence. It should facilitate a higher degree of consensus and support by all interested parties for the risk management option(s) being proposed. Risk communication should be: clear, correct, complete, concrete, concise, considered and courteous.

Effective communication of information and opinion on risks associated with real or perceived hazards in food is an essential and integral component of the risk analysis process. The management of hazards enables organizations to effectively identify the hazards and assess the risks inherent in their activities, and to develop prevention and mitigation strategies to eliminate, substitute, control, or reduce the risk so far as is reasonably practicable. Where there are extremely serious risks, all practicable measures

should be employed to eliminate or, where this is not possible, to minimize the risk. The risk management system should be built upon the concept of continual improvement through a cycle of planning, implementing, reviewing and improving the processes and actions that an organization undertakes to meet its goals. Exposure to biological hazards can occur in any work activities involving contact with humans or human-related products, animals or animal products and biological waste, plants and food. The effective, data-centred risk communication should be preceded by precise hazard identification considering the pathological mechanisms, modes of transmission (direct or indirect contact, aerosols, droplet spread, fomites, water, vectors, food, zoonotic) and routes of exposure (for example, inhalation, ingestion, dermal, percutaneous, mucous membranes, parenteral).

Risk communication may originate from official sources at international, national or local levels. It may also be from other sources such as industry, trade, consumers and other interested parties (including: government agencies, industry representatives, the media, scientists, professional societies, consumer organizations and other public interest groups and concerned individuals).

Risk characterization is the primary means by which food safety risk assessment findings are communicated to interested parties. Numerical estimates in the characterization, therefore, should be supported by qualitative information about the nature of the risk and about the weight of evidence that defines and supports it. There are inherent difficulties in communicating the quantitative aspects of a risk assessment. They include ensuring that the scientific uncertainties inherent in the risk characterization are clearly explained and that scientific terminology and technical jargon do not render the presentation of risk less understandable to the target audience. Communications among risk assessors and other interested parties should use language and concepts that are suitable for the intended audience.

Risk communication should be carried out in an open and transparent manner in order to convey credible information in ways that avoid misinterpretation. The information to be communicated should be understandable by the interested parties, including employers, workers and subcontractors. Risk communication should use appropriate means and language at the literacy level of relevant workers to ensure good comprehension, and should be conducive to implementing an effective system for the management of risks, in consultation with workers and their representatives and with their fully-informed participation.

Best practices for risk communication should:

a) infuse risk communication into policy decisions,

b) treat risk communication as a process,

c) account for the uncertainty inherent in risk,

d) design risk messages to be culturally sensitive,

e) acknowledge diverse levels of risk tolerance,

f) involve the public in dialogue about risk,

g) present risk messages with honesty,

h) meet risk perception needs by remaining open and accessible to the public,

i) collaborate and coordinate about risk with credible information sources.

Governments have a fundamental responsibility for risk communication when managing public health risks, regardless of the management methods used. With the responsibility for managing risks comes the responsibility to communicate information about risks to all interested parties to an acceptable level of understanding. Decision-makers within governments have the obligation to ensure effective communication with interested parties when developing scientific and technical analyses and to appropriately involve the public and other interested parties in the risk analysis process.

Industry is responsible for the quality and safety of the food it produces. It also has a corporate responsibility to communicate information regarding risks to affected consumers. Industry participation in all aspects of risk analysis is essential for effective decision making and can serve as a major source of information for risk assessment and risk management.

Early participation in the risk analysis process by the public or consumer organizations can help to ensure that consumer concerns are addressed and will generally result in a better public understanding of the risk assessment process and how risk-based decisions are made. It can further provide support for the risk management decisions that result from the assessment.

Members of the academic and research community may play an important role in risk analysis by contributing scientific expertise on health and food safety matters and assisting in the identification of hazards. They often have a high level of credibility with the public and the media, and may serve as independent sources of information.

The media play a critical role in risk communication. Much of the information that the public receives on food-related health risks comes to them through the media. The media may merely transmit a message, or they may create or interpret a message. They are not limited to official sources of information and their messages often reflect the concerns of the public and other sectors of society.

5 Biosecurity risk assessment

5.1 Principles

The conventional risk assessment paradigm described in 4.1 is insufficient for operating (near-) real-time decision making or prediction systems. The conventional approach focuses on individual agents and provides insight which could be rather used as a pre-requisite input for decision-focused risk evaluation methods.

There are two important principles to be considered when elaborating a risk prediction and mitigation approach at a F2F facility:

— Prevention is better than mitigation and

— Monitor proxies instead of the pathogens themselves at the F2F facility.

5.1.1 Prevention is better than mitigation

At F2F facilities there are more than >300 microbiological and few thousand chemical hazards, so it is nearly impossible to test for everything. Sampling and testing have inherent uncertainties and biases, and it is even theoretically impossible to find hazards/non-compliances with 100% probability with sampling and testing. When a monitoring (surveillance) system detects a pathogen, from a process management perspective, it is already too late: the pathogen is in, and mitigation interventions are needed, which are more costly and time consuming.

Instead of curing the diseases, preventative approach is more cost-effective: preventing pathogens from reaching the animals is a key here. Using a Failure Mode and Effects Analysis (FMEA), preventive, process-based approach would ensure a timely and cost-effective prevention.

The main approach of FMEA emphasises the importance of identifying critical control points across F2F facilities, where zoonotic pathogens such as *Salmonella*, *Campylobacter*, *E. coli*, *S. aureus* and *Listeria* may pose a significant risk to public health. A detailed process flowchart has to be developed to represent the processes of the F2F facility, with risks related of each step assessed using data from the scientific literature or other evidence sources. Hazards have to be scored and evaluated to determine their associated risks (i.e. associated severity and exposure), enabling the identification of key control points

requiring targeted interventions. Complementing FMEA with quantification of the efficacy of various interventions, the methodology demonstrates a structured risk assessment approach for prioritising hazards and supporting evidence-based preventive strategies.

5.1.2 Monitor proxies instead of the pathogens themselves

The prevention paradigm focuses not on detecting pathogens directly but on utilizing proxy information for timely detection and prevention. These proxies include:

- Indicator microbes that signal an elevated probability of specific pathogen presence,
- Environmental conditions (temperature, humidity, pH, etc.) that influence microbial survival and spread,
- Other detectable signs of pathogen presence, such as microbial ATP on surfaces or the metagenomic composition of the environmental microbiome,
- Broader environmental data from the facility's surroundings or connected F2F facilities (e.g., suppliers, buyers).

This specific procedure emphasizes indicator microbes. Tracking the transport of a single or a few microorganisms via a particular channel provides valuable insight into the channel itself and, by extension, its effectiveness in transporting various microorganisms. This approach enables the use of harmless microorganisms as faithful models for testing and experimentally measuring transmission pathways.

When designing a risk-based process control system, direct sampling, testing, and control of pathogens are neither feasible nor effective. Instead, monitoring environmental conditions that minimize pathogen survival, spread, and growth is crucial. In certain cases, abundant microbes occupying the same ecological niche as specific pathogens can serve as proxy indicators. Their presence and levels can be extrapolated and interpreted to assess the risk levels associated with the corresponding pathogens.

5.2 Microbiological ecosystems in animal farms

Microbiological ecosystems in animal farms consist of different communities of bacteria, fungi, viruses, and other microorganisms that reside in the animals, their environment, and farm infrastructure. These ecosystems play crucial roles in animal health, productivity, and disease dynamics by affecting feed efficiency, productive traits, immune responses, and pathogen transmission. Factors such as farm management practices, hygiene, antibiotic use, diet, and environmental conditions shape the composition and function of these microbial communities. A balanced microbiome can promote animal welfare and reduce the need for medical interventions, while disruptions, which are known as dysbiosis, can lead to disease outbreaks, reduce productivity, and increase antibiotic resistance concerns.

5.3 Transport of agents

5.3.1 Aerosols

The transfer of a harmful biological agent in the environment from source to organism can be by direct contact or by indirect routes via carriers. Indirect routes are systems/situations in the environment where transferring a harmful biological agent via carriers is possible. Harmful biological agents pose the most common threat in the (agricultural) environment by being transported via the air-dust or air-droplet route as components of bioaerosols.

Aerosols containing biological particles are called bioaerosols. According to the definition, “bioaerosol” or “biological aerosol” refers to a collection of biological particles dispersed in air or another gaseous phase. Bioaerosol contain live or dead cells of microorganisms, as well as their fragments and products of their metabolism (e.g., endotoxins, β-glucans, peptidoglycans, mycotoxins and VOCs), spores of microorganisms, pollen, fragments of epidermis and its products, plant and animal allergens, as well as viruses. The aerodynamic diameter range of bioaerosol particles ranges in size from nanometre-sized (e.g., bacterial endotoxins: 30-50 nm), through submicron-sized (e.g., fragments of bacterial or fungal cells), to particles that can reach tens to over 100 μm in diameter (e.g., pollen It is proved that single bioaerosol particles may exist in the air, but they tend to aggregate rapidly. When agents are thrown into the air through natural generating processes directly from the respiratory system (e.g., coughing, sneezing, breathing) or by re-aerosolization of fomites from inanimate surfaces, they are carried on particles that are negligibly affected by their size. Due to their small particle size, bioaerosols can be dispersed or transported over long distances and thus can become another source of bioaerosols and cause disease in humans, animals, and plants. Biological agents inhaled with dust or liquid spray can enter the respiratory tract, causing dangerous infectious (Q fever, ornithosis), allergic (bronchial asthma, allergic alveolitis (AZPP), and immunotoxic (organic dust toxic syndrome, ODTS) diseases. Harmful airborne biological agents can also enter the human system through the conjunctiva, the epithelium of the nasopharyngeal cavity, and through the skin.

It should be noted that bioaerosols are ubiquitous in the natural and working environment. Microorganisms transported by air are not subject to growth due to the lack of nutrients in the air and, depending on their characteristics and environmental conditions, can only survive for a certain period of time. Hence, the concentration and composition of the microbial community in the air depend primarily on the sources of their emissions.

Water bodies, soil, plants, and anthropogenic activities are considered the main sources of bacteria, fungi, viruses, and compounds of biological origin in the air. The primary source of biological particles in the indoor environment is living organisms, such as humans, animals, and plants, as well as stored materials (e.g., paper documents). In non-industrial indoor environments, humans are the dominant source of biological particles. The main processes generating bioaerosol particles include the shedding of skin cells and the direct emission of microorganisms during breathing, speaking, coughing, or sneezing. Bioaerosols can be emitted from various work environments, such as farms (poultry houses, pig farms, and cattle farms), landfills, or wastewater treatment plants.

The metabolic activity and associated survival and spread of microorganisms in the environment depends not only on their structure and resulting functions (e.g., size or ability to produce surviving forms), but also on numerous environmental factors, such as temperature, relative humidity, oxygen content, presence of organic and inorganic sources of nutrients, electrostatic and ionic interactions. The structure, concentration and species diversity of microorganisms in the air have distinct geographic variations and influenced by a wide range of climatic and geomorphic conditions.

Understanding bioaerosols' sources and transport mechanisms is crucial to better understanding the role of microorganisms in the atmosphere and to more effectively control the spread of bioaerosol-related epidemic diseases. Over the past two decades, significant progress has been made in source characterization, identification, and diffusion and transport modelling of bioaerosol. Nevertheless, many important scientific questions about bioaerosols still require further research.

5.3.2 Arthropods

Arthropods such as ticks, mosquitoes, and stable flies are potential vectors of pathogens that can impact both animal and human health. These vectors may carry viruses, bacteria, or parasites capable of surviving across different life stages and environmental conditions. Improper handling or containment during transit could result in the escape of live vectors with potential pathogens, posing risks to local

ecosystems, food safety, or public health. Along the Farm-to-Fork chain, their transport – from farm collection to laboratory storage – requires strict biosecurity measures to prevent any accidental spread of infectious agents.

To preserve sample integrity and prevent degradation of genetic material, collected mosquitoes are immersed in RNAlater™ Stabilization solution (Thermo Fisher Scientific Baltics UAB) shortly after identification and before it for ticks. Concerning stable flies, they are preserved in 70% ethanol. RNAlater™ solution penetrates tissues to stabilize nucleic acids, allowing safe transport at room temperature or under refrigeration for several days without compromising molecular quality.

A secure and traceable workflow for the sampling and transport of arthropods ensures that all specimens are properly contained, preserved (with RNAlater solution or ethanol), and transported under controlled conditions. From on-farm collection sites to final -80°C storage in the laboratory, each step is designed to maintain sample integrity while minimizing biological risk during cross-regional transportation.

5.3.3 Animals

Animals can transport biological agents through several mechanisms, acting as reservoirs, carriers, or mechanical vectors. Some examples of transport by animals include:

- Direct Contact. Animals can transfer biological agents through skin-to-skin contact, bites, or body fluids such as saliva, urine, and faeces.
- Aerosol Transmission. Sneezing, coughing, or exhaling can spread biological agents via respiratory droplets or airborne particles.
- Faecal-Oral Transmission. Contaminated faeces can introduce agents into the environment, where they may be ingested through food, water, or contact with surfaces.
- Vector-Borne Transmission. Animals can host ectoparasites like ticks and fleas, or be bitten by mosquitoes, which act as vectors in transmitting pathogenic agents.
- Consumption and Excretion. The agents can be excreted in waste products, contaminating soil, water, and feed.
- Transport. Livestock animals can spread agents through movement in transport vehicles to other farms.

5.3.4 Staff

Farm staff play a crucial role in the transport of biological agents, acting as vectors in microorganisms' transmission. Their daily activities, such as handling animals, equipment, and material, can spread microorganisms across different areas of a farm or even between farms.

- Clothing and Footwear. Farmers, veterinarians, or visitors can unknowingly carry agents on their clothes, boots, or gloves from one location to another.
- Hands and Skin Contact. Direct handling of animals, feed, or infected materials can transfer different types of microorganisms. Inadequate hand hygiene increases the risk.
- Breathing and Aerosols. The staff can inhale and exhale airborne, especially in enclosed farm environments.
- Tools and Equipment Handling. Shared instruments like syringes, feeders, and cleaning tools can spread diseases if they are not properly sterilized.

— Movement Between Areas. Staff moving between different animal groups, barns, or farms without biosecurity measures can facilitate microorganism spread.

— Personal Items – Phones, bags, and other personal belongings can pick up and transfer biological agents.

5.3.5 Fomites

Fomites are inanimate objects or surfaces that can carry and transfer biological agents, including bacteria, viruses, fungi, and parasites. They act as indirect vectors in transmission by allowing microorganisms to persist outside a host and be transferred to animals, humans, or other environments.

— Contaminated Surfaces. Equipment, tools, feeding devices, water containers, and farm structures can harbour microorganisms, which are later transferred to animals through contact.

— Vehicles and Transport Equipment. Trucks, cages, and handling tools can spread microorganisms between farms, markets, or slaughterhouses.

— Bedding and Feed. Straw, hay, or feed contaminated with faeces, saliva, or urine can introduce microorganisms to new hosts.

— Medical Instruments. Reused needles, syringes, and surgical tools can transmit microorganisms if they are not properly sterilized.

5.3.6 Vehicles

Slaughterhouses cannot be considered as a “cull de sac” while assessing biosecurity. There have been reports associated with dirty trucks as important as the classical swine fever outbreak from 1997 in the Netherlands, as well as other important animal diseases as PRRS, PCV2, oocysts of different parasites and Swine Dysentery among others.

Zooming up in the process, specialized lorries transport the animals from the farm to the slaughterhouse and back to different farms after a process of cleaning and disinfecting. This transport can carry all the different diseases and vectors present in the farm through the route and to the slaughterhouse and back to a different farm. Then, depending on the cleaning and disinfection, the fate of the agent is set. If the cleaning and disinfecting process has been successful – ending the path, if has been unsuccessful – leaving the path open for new cycles.

There are several problems when dealing with the biosecurity, starting from the variability of the construction (truck, cages and cleaning facilities), design and materials of the vehicles, the cost (€/time/immobilization), the frequency (every load), the required facilities and staff, and finally the fact that in the end, most operations will require supervision and verification. Furthermore, there are many “Trojan” areas that can harbour pathogens, like the cabin. Cabins are usually left uncleaned or disinfected. All removable things should be cleaned, the floor mats, debris in different areas, the steering wheel, pedals… with products that can cause deterioration over the long run. Boots and clothing are also important and should be cleaned and disinfected. The wheels must also be cleaned, through properly maintained wheel baths and proper cleaning.

The current EU legislation requires slaughterhouses to have approved centres for cleaning and disinfecting of trucks within their facilities (Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin).

Different pathogens have different ecology, as it is clear respiratory diseases might be more prone to be transmitted through the air, and food born ones through manure. Some zoonotic agents as Salmonella

can reach infectivity within very short notice and an animal can turn from negative to infectious through the span of a normal transport (below 4 h). This highlights a potential case of zoonoses where an infected cage can lead to infected animals and disease dissemination after transport or slaughter. We have seen a significant number of cages positive after cleaning (around 30%), reducing this to around 5% after proper disinfection.

PRRS and Classical Swine Fever and ASF are enveloped virus, which are not particularly resistant, but that can be spread through unproper cleaning or management of clothing and trucks. They have been reported to last over 1 week in faeces/water/clothing, stating the case that unproperly cleaned and disinfected means of transport facilitate the propagation of important illnesses.

6 Methods to assess biosecurity

6.1 Experimental methods

6.1.1 Stress tests

Stress tests refer to a testing method that utilizes model and surrogate organisms for the assessment of biosecurity measures or technologies. The objective of stress tests is to determine the protective, mitigating, or disinfectant effect of a procedure or technology using a known microorganism and a defined quantity.

Stress tests serve as an initial assessment, enabling the evaluation, quantification, and determination of the effect to be measured. These tests allow for the characterization of the biosafety measure or technology, as well as the definition of its usage and application procedure to achieve optimal results. Likewise, this method should be used to assess the effectiveness of currently existing measures.

6.1.2 Step tests

Step Tests constitute the next phase in the evaluation of a biosafety measure or technology, whether preventive or mitigation-based. These tests involve the assessment of such measures within a relevant environmental context. Unlike Stress Tests, Step Tests do not use model microorganisms; instead, they rely on the natural microbiota present in the environment.

The objective of Step Tests is to validate and verify the effectiveness demonstrated in Stress Tests under real-world conditions. This approach provides insights into the actual impact of different measures and technologies and enables the study of microorganism transport through various transmission pathways.

Step Tests involve the initial elimination of microorganisms in a specific environment, followed by periodic sampling over time to observe the reestablishment of the microbial community. This allows for an assessment of the rate at which microorganisms return via different transmission routes and the level of protection provided by the biosafety measure under evaluation.

6.2 Theoretical/computational methods

6.2.1 Fluid dynamics

Air-borne micro and nano-sized particulate matter dynamics is governed by viscosity of air. Typically, the Reynolds number for the fall of a water droplet of a radius of 10 μm in air is around 10^{-2}. For smaller radii this number is even lower. In such a case, according to Lamb H. (Lamb, 1994), Navier-Stokes equation for a spherical particle is:

$$\vec{\nabla} p = \mu \nabla^2 \vec{u} = -\mu \vec{\nabla} \times \vec{\omega} \quad (1)$$

where

p is the pressure;

μ is the dynamic viscosity of air;

$\vec{u}$ is the air velocity;

$\vec{\omega} = \vec{\nabla} \times \vec{u}$ is the vorticity.

Assuming that the air exchange with the liquid is negligible, then the continuity equation will stand:

$$\vec{\nabla} \cdot \vec{u} = 0 \tag{2}$$

As the problem is axil symmetric, using cylindrical coordinates *r*, φ and *z* and taking *z* axis as the direction of the air flow, the relations can be written:

$$v_r = \frac{1}{r} \frac{\varphi\psi}{\varphi r}$$

$$v_z = \frac{1}{r} \frac{\varphi\psi}{\varphi r} \tag{3a}$$

where the Stokes Flow Function is:

$$\psi = -\frac{1}{2} V r^2 \left[1 - \frac{3}{2} \frac{R}{\sqrt{r^2+z^2}} + \frac{1}{2}\left(\frac{R}{\sqrt{r^2+z^2}}\right)^3\right] \tag{3b}$$

where

V is the velocity of the air at an infinite distance of the sphere;

R is the radius of the sphere.

The expression for the viscous stress (Force per unit surface) exerted by the air on the surface of the sphere is becoming:

$$\frac{d\vec{F}}{ds} = \frac{3\mu V}{2R} \overrightarrow{e_z} = \frac{3\mu \vec{V}}{2R} \tag{4}$$

where

$\overrightarrow{e_z}$ is the unitary vector in the *z* axis;

s is the unit surface of the droplet.

Air-borne particles will therefore suffer a drag of the air flow due to viscous forces. If the air flow is deflected (for instance in a curved duct) the particles will tend to go ahead along a straight line due to its inertia so that a differential velocity with respect to the air appears. Viscosity will therefore create a centripetal force to make the particles follow the airflow path. This can be seen in the frame of reference of the particle and talk about inertia forces so that the expressions above are still valid substituting the gravity acceleration by minus the acceleration of the frame of reference (or equivalently that of the particle). In fact, there is no way to distinguish inertia forces from gravity (Einstein & Infeld, 1942).

This translates to a practical rule: the smaller the air-borne particle is, the better and faster it follows the air flow. In this sense, a fog can be considered as a whole "fluid" itself and no longer air plus particles. This is similar to the raisin cake that would move integrally. The raisins will not move independently to

the sponge of the cake. Multiphysics computer models can be used to analyse the dynamics of the particles. However, this can be made evident in a simple case that can be treated analytically, i.e. the case of a droplet injected into a lateral air flow.

In absence of gravity or any other external field, if a spherical droplet with initial speed V_0 is suddenly exposed to a uniform air flow with velocity V then the speed of the droplet at time t will be:

$$\overrightarrow{V_p}(t) = \vec{V} + (\overrightarrow{V_0} - \vec{V})e^{\frac{-t}{\tau}} \quad (5)$$

where τ is the damping time.

The damping time τ is the time it takes the speed of the particle or droplet to become the velocity V.

$$\tau = \frac{m}{6\pi R\mu} \quad (6)$$

where

m is the mass of the particle

R is the particle radius

μ is the dynamic viscosity of air.

Therefore, the damping time can be formally defined as the time it takes the difference of the particle speed with respect to the fluid velocity to be 1/e times its initial value. This can be interpreted it as the time the particle takes to "adapt" to the fluid velocity.

EXAMPLE For droplets having a diameter of 2 μm floating in air at room temperature, the damping time is about $5 \cdot 10^{-5}$ s, while for water droplets having a diameter of 20 μm, the damping time is $5 \cdot 10^{-3}$ s. These are the times required for the droplets to reach the velocity of the gas flow around it.

By integration, it is easy to find the depth that the water droplet penetrates for instance in a flow when it impinges into it with velocity $\vec{V_0}$:

$$S = \int_0^\infty (\overrightarrow{V_0} - \vec{V})\, dt \; = \tau(\overrightarrow{V_0} - \vec{V}) \quad (7)$$

These fundamental equations can be used to create computational models to estimate the fraction of microparticles sized equal that or bigger than a selected pathogen that may reach a particular facility. Fluid dynamics computational models can provide accurate description of velocity for air flows and consequently probability for a particle emitted in a point to reach a second point.

6.2.2 Brownian diffusion

In addition to viscous effects due to fluid dynamics, microscopic particles in a fluid experience Brownian motion. This becomes apparent especially in still air or in still water. Computational models of Brownian diffusion can be used for the computation of probability of diffusion under such conditions.

An example of still air condition is the experiment of Pasteur's who used a capillary for equalizing pressure while preventing the arrival of spores and other air-borne microorganisms.

6.3 Module combination

A modular approach to risk assessment is based on probabilistic estimations of "failure". In this case "failure" means that a particular pathogen reaches the assessed facility (farm, industry…) and eventually

is able to be transmitted and infect a new host propagating the disease. It is straightforward mathematical exercise to estimate the probability for a particular microorganism to physically reach a location if we know the probabilities for the several possible channels or means of transporting. Indeed, it is also straightforward to transpose the effect of biosecurity measures on a particular channel if experimental or first-principles model data are available.

7 Experimental test procedures for measuring microorganisms' transport

7.1 Stress tests procedure for surfaces

7.1.1 Principle

A test inoculum suspension containing bacteria, fungi, or viruses in a solution of interfering substances is applied to a stainless-steel test surface and allowed to dry. The test surfaces are then strategically placed throughout the designated area for biosafety assessment, including rooms, objects, or body parts such as footwear. The test is conducted under both conditions: with and without the biosafety measure being evaluated.

Following exposure, the test surfaces are collected and transferred into a previously validated neutralization medium to immediately deactivate any disinfectant present. The number of surviving microorganisms recovered from the surface is then quantitatively determined.

The reduction in viable microbial counts attributed to the tested product or technology is calculated by comparing the results obtained with and without the evaluated biosafety measure.

If necessary, variations in surface materials and textures should be considered, as different substrates may influence microbial adhesion, survival, and the effectiveness of biosafety interventions.

7.1.2 Test organisms

The bactericidal activity shall be evaluated using the following four strains:

- *Pseudomonas aeruginosa* ATCC® 15442™[1];
- *Staphylococcus aureus* ATCC® 6538™;
- *Enterococcus hirae* ATCC® 10541™;
- *Escherichia coli* ATCC® 10536™.

The fungicidal or yeasticidal activity shall be evaluated using the following two strains:

- *Candida albicans* ATCC® 10231™;
- *Aspergillus brasiliensis (ex A. niger)* ATCC® 16404™.

If required for specific applications, additional strains may be chosen from, e.g:

- *Salmonella typhimurium* ATCC® 13311™;
- *Lactobacillus brevis* DSM 6235;
- *Enterobacter cloacae* DSM 6234;
- *Listeria monocytogenes* ATCC® 15313™ or ATCC® 19117™
- *Saccharomyces cerevisiae* (for breweries) or ATCC® 9763™ or DSM 1333;
- *Saccharomyces cerevisiae* var. *diastaticus* (for breweries) DSM 70487.

If additional strains are used, they shall be incubated under optimum growth conditions (temperature, time, and atmosphere) and noted in the test report.

For tests conducted with viruses, the selected organisms may include bacteriophages, such as the Phi29 bacteriophage, due to their well-characterized properties and suitability as viral surrogates in biosafety assessments. Additionally, the use of viruses specified in the EN 17122 is recommended to ensure compliance with established testing methodologies and regulatory requirements. The selection of the appropriate viral model should consider factors such as stability, replication characteristics, and relevance to the specific biosafety measure being evaluated.

7.1.3 Culture media

Tryptone Soya Agar (TSA)

For maintenance of bacterial strains and performance of viable counts.

Tryptone, pancreatic digest of casein	15,0 g
Soya peptone, papaic digest of Soybean meal	5,0 g
NaCl	5,0 g
Agar	15,0 g
Water	1 000,0 ml

Sterilize in the autoclave. After sterilization, the pH of the medium shall be 7,2 ± 0,2 when measured at 20 ° C.

Malt extract agar (MEA)

For maintenance of fungal strains, sporulation and performance of viable counts.

Malt extract (food grade)	30,0 g
Agar	15,0 g
Water	1 000,0 ml

The water shall be free from substances that are toxic or inhibiting to bacteria and fungi. It shall be freshly glass distilled or of equal quality/purity and not demineralized water.

Sterilize in the autoclave.

NOTE 1 If distilled water of adequate quality is not available, water for injectable preparation (see European Pharmacopoeia) can be used.

7.1.4 Test procedure

The Stress Test procedure for surfaces requires the use of controlled surfaces that have been pre-contaminated with a known quantity of a specified microorganism, as previously indicated.

First, the test suspension should be prepared. Adjust the number of cells in the suspension to $1,5 \times 10^8$ cfu/ml to $5,0 \times 10^8$ cfu/ml. The procedure for preparing the test suspension shall be in accordance with EN 13697.

The numbers of units shall be estimated by means of spectrophotometer or any other suitable means. Maintain this suspension in the water bath at 20 °C ± 1 °C and use within 2 h.

The stress test procedure may vary significantly depending on the biosafety measure being evaluated, whether preventive or mitigative. The responsible researcher shall define the test conditions while considering the following aspects:

- The placement or distribution of test surfaces should be homogeneously arranged throughout the testing area to ensure representative sampling and accurate assessment of the biosafety measure's effectiveness.

- The test procedure should have an appropriate duration, considering factors such as the action time of any disinfectants (if applicable), environmental conditions (e.g., temperature, humidity, ventilation), and the expected exposure time of microorganisms to real-world conditions.

- The methodology should aim to replicate typical operational scenarios encountered in the facility, including human movement, equipment usage, and other factors that may influence the spread and persistence of microorganisms.

- If applicable, variations in surface materials and textures should be considered, as different substrates may influence microbial adhesion, survival, and the effectiveness of biosafety interventions.

- The test should incorporate appropriate controls, including positive and negative controls, to validate the reliability and reproducibility of the results.

- The test should be performed multiple times to assess the consistency of the results and to take into account possible variations due to external factors.

7.2 Stress tests procedure for aerosols

7.2.1 Principle

A test inoculum suspension containing a known concentration of bacteria, fungi, or viruses in a solution is aerosolized to create a known aerosol. The procedure should be performed in a controlled environment using harmless microorganisms, such as bacteriophages or other biosafety level 1 organisms that do not pose a risk to human health. If higher biosafety level microorganisms are used, the test must be conducted in specifically designated and equipped facilities.

Once the aerosolized microorganism has been introduced into the environment, the biosafety measure under evaluation — whether preventive or mitigative — is applied. Following the application of the biosafety measure, air and/or surface samples are collected depending on the specific objective of the test and the biosecurity measure. Sampling methods must be validated to ensure accurate and reproducible quantification of viable microorganisms.

This test also allows for the study of microbial transport through the air, making it useful not only for assessing biosafety interventions but also for analysing the dispersion and persistence of airborne microorganisms under different environmental conditions.

Variations in environmental factors such as airflow, humidity, temperature, and ventilation should be considered, as they may influence microbial survival and the effectiveness of the biosafety measure.

The reduction in viable microbial counts attributed to the tested biosafety measure is determined by comparing results obtained with and without its application. Statistical analysis should be performed to assess the significance of microbial reduction and evaluate the efficacy of the intervention under real-world conditions.

7.2.2 Test organisms

Depending on the type of activity targeted, tests shall use all or some of the following test organisms, obtained from culture collections.

For bactericidal activity tests:

— *Pseudomonas aeruginosa*	ATCC 15442
— *Staphylococcus aureus*	ATCC 6538
— *Enterococcus hirae*	ATCC 10541
— *Escherichia coli*	ATCC 10536
— *Acinetobacter baumanii*	ATCC 19606
— *Proteus hauseri*	ATCC 13315

For sporicidal activity tests:

— *Bacillus subtilis* spores	ATCC 6633

For yeasticidal activity tests:

— *Candida albicans*	ATCC 10231

For fungicidal activity tests:

— *Candida albicans*	ATCC 10231
— *Aspergillus brasiliensis*	ATCC 1640

For virucidal activity tests:

- Murine Norovirus souche S99, Friedrich Loefler Institut, Berlin. MNV cultured on RAW 264.7 (ATCC TIB-71) cells line
- Adenovirus type 5, adenoid strain, ATCC VR-5. Adenovirus cultured on HeLa cells or other lines of suitable susceptibility
- Porcine Parvovirus NADL2 strain cultured on ST cells or other appropriate cells line

For phagocidal activity:

- Bacteriophage for Lactococcus lactis subspecies lactis P001 (DSM 4262)
- Bacteriophage for Lactococcus lactis subspecies lactis P008 (DSM 10567)

The multiplication of these two bacteriophages shall be obtained from the host strain: *Lactococcus lactis* subspecies *lactis* F7/2 (DSM 4366).

7.2.3 Preparation and counting of tests suspensions

The test organisms and their stock cultures shall be prepared and kept in accordance with EN 12353.

The preparation of tests suspension of bacteria, spores, fungal spores, yeasts and viruses including bacteriophages, as well as the counting of suspensions shall be in accordance with EN 17272.

7.2.4 Test procedure

The Stress Test procedure for aerosols requires the generation of an aerosol containing the test microorganism selected for the test from the previously mentioned list (see 7.1.). Aerosol generation shall be performed using a nebulizer or any other validated system designed for this purpose. The nebulization process should ensure a homogeneous distribution of the aerosol throughout the test space while adhering to all necessary safety precautions to prevent unintended exposure or contamination. The selected aerosolization system shall be capable of generating particles of a controlled and reproducible size range, ensuring consistency across test conditions.

The number of cells spores or viruses should be adjusted, estimating the number of colony/plaque forming units (cfu/pfu) either spectrophotometrically or by any other suitable means, between 5×10^7 cfu/ml and 2×10^9 cfu/ml.

Following aerosol generation, a stabilization period of at least two minutes should allow the aerosol to disperse evenly and reach equilibrium within the test environment. The duration of this stabilization period may vary depending on factors such as the size of the test area, airflow dynamics, and environmental conditions. Once stabilization is complete, the biosafety measure under evaluation shall be applied according to the predefined test protocol.

The specific test conditions shall be adapted based on the intended function of the biosafety measure being assessed. For example, if the intervention involves disinfection technology or mitigation of airborne transmission, air samples shall be collected before and after the application of the measure. Sampling shall be performed at multiple locations within the test space to assess the spatial distribution of microbial reduction. A control test must be conducted under identical conditions but without the application of the biosafety measure, ensuring a reliable baseline for comparison.

Additional factors such as air exchange rates, humidity levels, and potential interactions between the aerosolized microorganisms and the biosafety measure shall be considered to ensure the accuracy and reproducibility of results.

7.3 Step tests procedure

7.3.1 Principle

Unlike Stress Tests, Step Tests do not involve artificial contamination of air or surfaces. Instead, this method assesses the naturally occurring microbiota present in the environment. This approach offers several advantages, including the avoidance of introducing foreign contaminants that may impact the test area, the ability to conduct assessments in relevant environments, and the opportunity to work under real-world conditions with a diverse range of microorganisms present at lower concentrations. This allows for the validation of the sensitivity and specificity of the biosafety measure under evaluation. However, a key limitation of this method is its inherent variability and lower reproducibility due to natural environmental fluctuations. For this reason, while Step Tests may serve as an alternative to Stress Tests, they provide greater reliability when used as a complementary method.

This test primarily investigates the transport of microorganisms through different transmission channels and the rate at which the microbial environment returns to its baseline state. The method consists of conducting a comprehensive disinfection of a designated space, eliminating microorganisms present in both air and surfaces. Following disinfection, samples are collected at various time intervals to monitor how the microbiota gradually returns to its original state.

To ensure an accurate assessment, the facility shall resume normal operations, allowing for the natural reintroduction of contamination through human and animal movement, air transport, and other transmission channels.

By applying biosafety measures, this method enables the evaluation of how these measures influence, modify, or prevent the transport of microorganisms within the environment.

7.3.2 Test procedure

The comprehensive disinfection should be performed using Counterfog rapid disinfection system. Prior to application, it is essential to determine the dimensions of the test area, as these factors influence key parameters such as the selection of the Counterfog equipment, application duration, and required exposure times.

The Counterfog system is a rapid disinfection technology based on the generation of a nanometric droplet mist, forming a fog cone capable of collapsing with even the smallest microorganisms and penetrating hard-to-reach areas. This system is compatible with any aqueous-based disinfectant, allowing for flexibility in compliance with local regulations regarding the use of chemical agents. Consequently, the system can be adapted to any testing environment. It is recommended to use environmentally friendly biocides, such as hypochlorous acid, hydrogen peroxide, or commercially available biocidal agents that meet regulatory documents.

The application process shall be/is conducted slowly and evenly, ensuring that the disinfectant reaches the entire area. Once the predetermined disinfection time has been completed—based on the dimensions and characteristics of the test space—and the entire area has been covered, the disinfectant is allowed to act for a specified exposure period.

The required exposure time depends on several factors, including the type of disinfectant used, its effectiveness, potential residues left behind, and the size of the test area. As a general guideline, disinfectants should remain active for at least 10–15 min before further procedures are carried out.

After the designated exposure period has elapsed, normal operations within the facility may resume, and periodic sample collection shall begin. The sampling strategy may vary depending on the objectives of the test. Samples are collected from both air and surfaces, ensuring a homogeneous distribution throughout the test area, to obtain representative data.

The following sampling scheme is recommended for assessing microbial dynamics and the effectiveness of the biosafety measure under evaluation:

- **Immediately after** the exposure period.
- **1 hour** after the exposure period.
- **2 hours** after the exposure period.
- **4 hours** after the exposure period.
- **8 hours** after the exposure period.
- **24 hours** after the exposure period.
- **48 hours** after the exposure period.
- **96 hours** after the exposure period.

The methodology shall be adapted based on the data obtained during testing, ensuring optimal assessment of microbial persistence and transmission patterns. It is recommended to conduct this type of test at least three times to accurately evaluate population dynamics and, in cases where a biosafety measure is being assessed, to verify its effectiveness under real-world conditions.

7.3.3 Air sampling methodology

A key aspect of both Stress Tests and Step Tests is the collection and analysis of bioaerosols. To ensure accurate and efficient sampling, it is recommended to use the Bioaerosol Fast Sampler, a device that employs Counterfog technology to effectively capture microorganisms and other airborne particles present in the environment.

The Bioaerosol Fast Sampler provides a liquid sample in approximately 2 min, enabling rapid and repeated sampling at different time intervals throughout the test. This allows for a detailed temporal analysis of airborne microbial concentrations and their variations over time.

The collected liquid sample shall be analysed using appropriate microbiological or molecular techniques, depending on the specific objectives of the test. These techniques may include:

- Culture-based methods, for the quantification and viability assessment of recoverable microorganisms.

- Molecular techniques (e.g., PCR or qPCR), for the detection and identification of specific microbial species, including non-culturable microorganisms.

The analytical methods shall be selected based on the intended outcomes of the study, the type of microorganisms being investigated, and the required sensitivity and specificity of the test.