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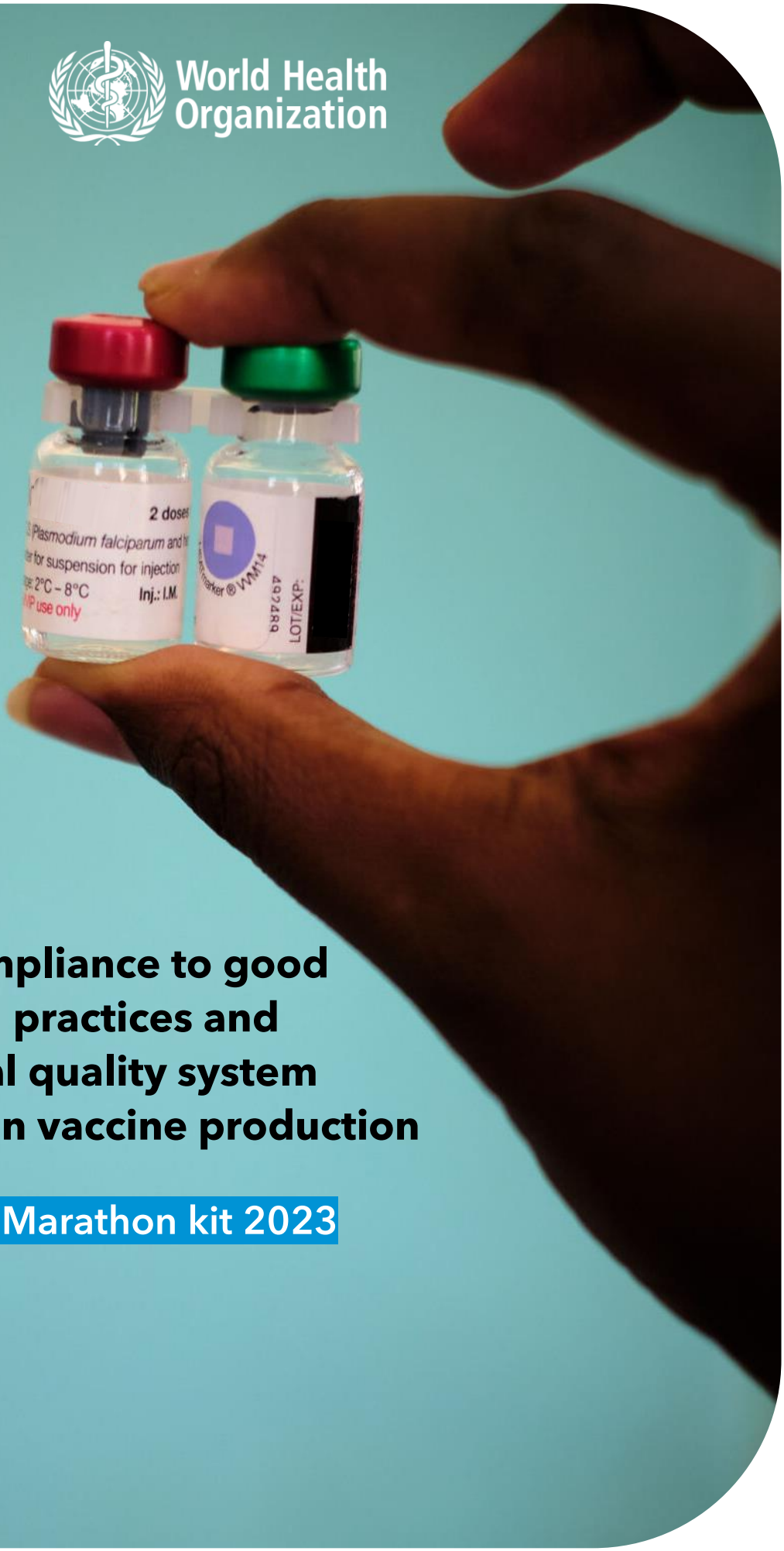

Enhancing compliance to good manufacturing practices and pharmaceutical quality system requirements in vaccine production

Virtual Training Marathon kit 2023

World Health Organization

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Contents

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Abbreviations

µm	Micron
AI	Artificial intelligence
ALCOA+	Attributable, Legible, Contemporaneous, Original, Accurate, Complete, Consistent
API	Active pharmaceutical ingredient
APR	Annual product review
AP	Aseptic process
APS	Aseptic process simulation
APV	Aseptic process validation
AQL	Acceptable quality limit
ASTM	American Society for Testing and Materials
BCG	Bacillus Calmette Guerin
BI	Biological indicator
BP	Batch process
BSL	Biological safety level
BUCU	Blend Uniformity Content Uniformity
CAPA	Corrective action preventive action
CCS	Contamination control strategy
CCTV	Closed-circuit television
CFR	Code of Federal Regulations
CFU	Colony forming unit
CIP	Clean in place
CMA	Critical material attribute
CNC	Controlled non-classified
COTS	Commercial-off-the shelf
CpK	Process capability
CPP	Critical process parameters
CPV	Continued process verification
CQA	Critical quality attribute
cRABS	Closed restricted access barrier system
CSV	Computer system validation
CTD	Common technical document
DI	Data integrity
DIRA	Data integrity risk assessment
DIT	Diffusive integrity test
DMS	Document management system
DoE	Design of experiment
DP	Drug product
DQ	Design qualification
DS	Drug substance
DTP	Diffusive test point
EDMS	Electronic data management system
EM	Environmental monitoring
EMA	European Medical Agency
EMPQ	Environmental monitoring performance qualification
eREC	Electronic record
ERP	Enterprise resource planning
eSIG	Electronic signature
EtO	Ethylene oxide
FDA	Food and Drug Administration
FIT	Filter integrity test
FMEA	Failure mode effects analysis
FRS	Functional requirement specification
GAMP	Good automated manufacturing practices
GCP	Good clinical practices
GDUFA	Generic Drug User Free
GEP	Good engineering practice
GLP	Good laboratory practice
GMP	Good manufacturing practice
GPT	Growth promotion test
GxP	Good practices
HAZOP	Hazard & operability study
HBEL	Health-based exposure limit
HEPA	High efficiency particulate air
HPLC	High-pressure liquid chromatography
HVAC	Heating, ventilation, and air conditioning
IaaS	Infrastructure as a service
ICH	International Council for Harmonisation
IMP	Investigational medicinal product
IQ	Installation qualification
ISO	International Organization for Standardization
ISPE	International Society of Pharmaceutical Engineers
IT	Information technology
KPI	Key performance indicator
LAF	Laminar airflow
LIMS	Laboratory information management system
LMIC	Low- and medium-income countries
MES	Manufacturing execution systems
MHRA	Medicines & Healthcare Regulatory Agency
MLAF	Mobile laminar airflow
mRNA	Messenger ribonucleic acid
MTBF	Mean time between failure
NRA	National regulatory authority
OOS	Out-of-specifications

OPV	Ongoing process verification
OQ	Operational qualification
oRABS	Open restricted access barrier system
PaaS	Platform as a service
PAHO	Pan American Health Organization
PAT	Process analytical technology
PD	Process development
PDA	Parenteral Drug Association
PDUFA	Prescription Drug User Free Act
PHSS	Pharmaceutical Healthcare Sciences Society
PIC/S	Pharmaceutical Inspection Co-operation Scheme
PLC	Programmable logic controller
POU	Point-of-use
PpK	Process performance
PPM	Primary packaging material
PPQ	Process performance qualification
PQ	Performance qualification
PQS	Pharmaceutical quality system
PSA	Process safety analysis
PSF	Product summary file
PUPSIT	Pre-use, post-sterilisation integrity test
PV	Process validation
PW	Purified water
QA	Quality assurance
QbD	Quality by design
QC	Quality control
QMS	Quality management system
QPBR	Quality practices in biomedical research
QRM	Quality risk management
QTPP	Quality target product profile
R&D	Research & development
RABS	Restricted access barrier system
RFID	Radio frequency identification device
RG	Risk group
RMT	Risk management tool
RO	Reverse osmosis
RTP	Rapid transfer port
RTU	Ready to use
RU	Receiving unit
SaaS	Software as a system
SAL	Sterility assurance level
SCDM	Soybean casein digest medium
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SIP	Sterilisation in place
SOP	Standard operating procedure
SST	Structural simulation toolkit
SU	Sending unit
SURF	Single-use redundant filtration
SUS	Single-use systems
TOC	Total organic carbon
TPP	Target product profile
TRS	Technical report series
TSB	Trypticase soy broth
TT	Technology transfer
URS	User requirement specifications
USP	United States Pharmacopeia
VCA	Variance components analysis
VMP	Validation master plan
WFI	Water for injection

Introduction

Ensuring quality of the health product ensures its safety and efficacy. Manufacturers in low- and middle-income countries (LMICs) face challenges to achieve quality in local production, such as the lack of an available manufacturing workforce trained in quality and understanding regulatory quality standards and difficulties in implementing a quality culture in the manufacturing facility.

The Local Production and Assistance (LPA) Unit in the Regulation and Prequalification Department (RPQ), Access to Medicines and Health Products Division (MHP), WHO, supports Member States (MS), particularly low- and middle-income countries (LMICs), to strengthen sustainable local production and technology transfer to improve timely, equitable access to quality, safe and effective essential medical products. The LPA Unit provides assistance and support to MS with an ecosystem-wide and holistic approach, such as conducting ecosystem assessments for sustainable, quality local production, developing and implementing strategies/roadmaps and tools, providing comprehensive capacity building and technical assistance, including for WHO Prequalification (PQ)/Emergency Use Listing (EUL), and facilitating technology transfer (TT).

In response to Member States' requests for capacity building to achieve local production of quality-assured pharmaceuticals and vaccines, the LPA Unit has been organising the Virtual cGMP Training Marathon annually since 2020. A selection of key current good manufacturing practices (cGMP) topics is delivered virtually in a marathon fashion for several consecutive weeks with content based on current WHO GMP guidelines.

The first Virtual cGMP Training Marathon in 2020 strengthened foundational knowledge of WHO cGMP for pharmaceutical manufacturing. The second Virtual cGMP Training Marathon in 2021 continued to build the understanding of the fundamentals of cGMP with a focus on GMP for vaccine manufacturing. The 3rd Virtual cGMP Training Marathon for Vaccine Manufacturing in 2022 delivered training with progressively in-depth content on facility design, technology transfer, and advanced cGMP concepts for vaccine production. The 3rd Virtual cGMP Training Marathon also employed an innovative approach to capacity building with hands-on group work for a small number of participants to solidify their learning and skills based on real-life scenarios and quality risk management tools.

In 2023, the fourth Virtual cGMP Training Marathon for Vaccine Manufacturing: Principles into Practices, organised from 12 September to 10 October, continued to progressively build capacity in critical GMP topics requested by participants in previous training marathons and recent regulatory changes in sterile processing: data integrity, computer systems validation, and aseptic process simulation, to name a few. During each of session of the Virtual cGMP Training Marathon, attendees raised questions that have been selected and assembled in this training material with technical questions and peer-reviewed answers from the GMP experts who delivered the different topics.

This is the 3rd training material released for the Virtual cGMP Training Marathons organised by the LPA Unit; the 2nd training material was released following the Virtual cGMP Training Marathon for Vaccine Manufacturing in 2022.
Its format allows the reader to easily refer to the questions under each specific session and topic.

This is a continuous learning resource for participants and other relevant stakeholders to acquire new capacities to strengthen their local production of safe and quality vaccines and other essential medicines.

Session 1 Facility design

1. What are the main differences between WHO, the United States of America, and the European good manufacturing practices (GMP) in terms of facility design?

There might be minor details, but basically, apart from their definition of hygiene classes, requirements and philosophy are similar. Often WHO tends to offer detailed descriptions, Europe provides somewhat less detail, while the United States of America maintains consistency and relies on state-of-the-art practices, quality risk management (QRM), and interpretation.

2. How is the incorporation of vaccine storage conditions considered in the facility design?

By having intermediate storage capacities between the different production steps, where necessary, and by calculating filling capacities in line with the possibilities of keeping vaccines at room temperature.

3. Is it feasible to manufacture both viral live vaccines and inactivated vaccines within the same multiproduct facility? Would segregation through dedicated airlocks, separate heating, ventilation, and air conditioning (HVAC) systems, entrances, and exits suffice, or is it advisable to handle this within a dedicated facility?

WHO Technical Report Series (TRS) 999, 2016, section 9.1, mentions "In general, preparations containing live microorganisms or live viruses should not be manufactured and containers should not be filled in areas used for the processing of other pharmaceutical products. However, if the manufacturer can demonstrate and validate effective containment and decontamination of the live microorganisms and viruses then the use of multi-product facilities may be justifiable. In such cases, measures such as campaign production, closed systems, and/or disposable systems should be considered and should be based on QRM principles".

This approach should be limited to low biosafety risk groups (RG) and should be pre-approved by the national regulatory authorities (NRA). Upstream processes (e.g., spore-former Clostridium, or Bacillus Calmette Guerin - BCG) would require separate buildings and utilities. Based on risk assessment the situation must be assessed on a case-by-case basis depending on the risk brought by the product.

4. Must Influenza vaccines be manufactured in dedicated facilities?

Given the most common egg-based technology, it would be difficult to produce anything else, but filling can be common for other inactivated products.

5. Could mRNA vaccine facilities be used for many antigens in the same facility?

Yes, on a campaign basis, and with appropriate cleaning validation.

6. What are the main consideration points for a flexible multi-fill facility?

Careful planning of capacity, large portfolio allowing resources to be shifted from one product to the other, flexible use of formulation and filling, reserves from the organisational point of view, as well as an intelligent masterplan encompassing a facility design compliant with flexibility and flexible processes.

7. What design recommendation would be appropriate for emerging countries that plan to build a GMP

facility for biopharmaceuticals or multi-products that have similar characteristics (e.g., modular, ballroom, ordinary facility)?

There can be no standard answer, as it will depend on several factors: type of product, process step(s) envisaged, quantities, indications given by technology providers, local construction possibilities and skills, etc.

8. What is the meaning of direct and indirect impact systems?

An indirect impact system is a system that is not expected to have a direct impact on product quality but typically will support a direct impact system. These systems are designed and commissioned following good engineering practice (GEP) only. It follows therefore that a Direct Impact system is a system that has a direct impact on product quality; such systems must be properly validated.

9. Are GMP facilities moving to a closed system technology as single-use systems (SUS) begin to be introduced and mentioned in the newest GMP Sterile Guidance (e.g., TRS 1044 Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022 / EU Annex 1 Manufacture of Sterile Medicinal Products, 2022)?

Closed system technologies and SUS do not necessarily go together; it is a matter of quantities, as SUS items are comparatively more expensive than reusable items, but yes, in the frame of a contamination control strategy (CCS), there is a definite move towards closed system technologies. SUS systems can add some more flexibility as they are sterilised out of the site, whereas stainless steel equipment needs to be cleaned sterilised, and cooled before starting a new production.

10. Is there a specific facility design requirement for the flow (material, people, waste, etc.)?

Flows should preferably be unidirectional, to minimise transfers between different hygiene zones.

11. Are there specific guidelines to produce adenoviral vector vaccines regarding facilities and workflow, considering the risk of "from clean to dirty, non-infectious to infectious"?

Adenovirus vaccines follow the same general principles.

12. During the planning stage, is it required to prepare user requirement specifications (URS) before conceptual design?

The URS is the starting point of a facility construction project. We need to know from the very beginning what we want to do in our facility. We can start with a short document of a few pages and then the design studies will introduce new requirements in the URS. No need to have a full 500-page description from the very beginning.

13. What is meant by footprint?

The shape and size of the area something occupies.

14. What are the GMP requirements for laminar flow in aseptic filling and vial capping operations?

Grade A unidirectional airflow with a class B background is required for aseptic processing (e.g., filling). "Unidirectional airflow – An airflow moving in a single direction, robustly and uniformly, and at sufficient speed, to reproducibly sweep particles away from the critical processing or testing area" (EU GMP, Annex 1, Manufacture of Sterile Medicinal Products, 2022). For the capping operation, a grade A class air supply may be implemented, with a

class background that could be class B or C refer to the Manufacture of Sterile Medicinal Products, Annex 1 PIC/S Europe, 2022, that gives a guidance value for air velocity 0.45 m/sec for the unidirectional airflow.

15. What is the relationship between the gowning procedures and the design of a vaccine manufacturing facility?

Gowning procedures follow the GMP guidelines and must consider toxicity and hazard issues, by using personal protection equipment where necessary. Separate rules apply to gowning worn in infectious (bio-positive) and non-infectious (bio-negative areas), for example for decontamination.

16. Are there any specific requirements for the material of construction? Does it need to be mentioned in the conceptual design phase?

Not necessarily. An important issue is to have at an early stage an idea of the number of floors and weight of equipment, as the grid used can depend on the use of concrete or metal.

17. Is there a difference between critical quality attributes (CQA) and critical material attributes (CMA)?

"The CQA is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality" (International Council for Harmonisation - ICH Q8(R2) Pharmaceutical Development, 2009). ICH Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances, 1999, further defines CQA specifications as the criteria that a drug substance or drug product must meet to be considered acceptable for its intended use. This includes attributes like identity, strength, purity, and potency. Once CQAs are identified, the systematic approach (e.g., a risk assessment) should continue to process development, understanding the impact of CMAs and critical process parameters (CPPs) on the CQAs.
A CMA is a physical, chemical, biological, or microbiological property or characteristic of an input material that should be within an appropriate limit, range, or distribution to ensure the desired quality of output material.

18. How is it ensured that a GMP-compliant waste management system is in place at a vaccine facility? Carry out an immediate separation and inactivation of waste as early as possible in the process (e.g., heat, chemical treatment). Also, consider the need to comply and coexist with other non-GMP related standards as well, especially when biological or toxic waste is involved.

19. Does the Gantt chart help in project planning and execution? Is there any other software you suggest for project planning?

In many cases, excel can be useful for simple planning matters, but when complexity arises, Microsoft Project or similar may be used.

20. Does WHO require having process safety analysis (PSA) be performed?

For any process, a risk analysis will be performed, but the question here is if we talk about process risk or risk to the people; basically, the methods are the same (Failure mode effects analysis - FMEA, Hazard & operability study - HAZOP, etc.).

21. What does the design space concept refer to?

Design space is a key concept in pharmaceutical quality by design, providing a better understanding of manufacturing

processes and enhancing regulatory flexibility. It is of paramount importance to develop computational techniques for providing quantitative representations of a design space, by the ICH Q8 (R2) Pharmaceutical Development, 2009 guideline. Design space exploration is the process of finding a design solution (unique combinations of the settings of the independent variables) or solutions that best meet the desired design requirements from a space of tentative design points.

22. In a situation where a facility previously produced live attenuated viral vaccines and is transitioning to manufacturing anti-serum products, what would be an optimal change-over protocol?

Consider implementing a comprehensive dismantling approach coupled with thorough decontamination. However, due to the diverse processes involved, it is advisable to conduct a feasibility study before finalising the decision.

23. What are the environmental classification grades needed for quality control (QC) in a vaccine manufacturing facility?

QC is normally not a GMP function and may be done in a non-controlled environment. For sterility testing purposes, either class A/B or an isolator technology can be applied, with precautions to be taken for media preparation. Precious indications can be found in the WHO paper on Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities - Points to Consider for Manufacturers of Human Vaccines (2012).

24. Should an airlock placed before an activity room for biological safety level 2 (BSL2) be a "sink" or "bubble" type?

The question can only be answered if the process or layout is known, as it depends on the status of the material (live, inactivated, etc.).

25. Are isolators required? Or are closed restricted access barrier systems (cRABS), and open restricted access barrier systems (oRABS) acceptable too?

All barrier systems including cRABS and oRABS are perfectly acceptable, but there are more limitations from an environmental point of view (A/B background instead of C background). The selection is based on QRM, but costs must be considered as well.

26. Is it recommended to sample the incoming sterile active pharmaceutical ingredients (API) and sterile primary packaging material in the sterile area in the microbiological laboratory?

Any sampling of sterile materials brings a risk to the materials. The most critical point is to have a procedure to check the integrity of outer packaging to be sure it is not damaged and to make sure that the sampling procedure will not contribute to the contamination of the product. It is very common to receive advance samples to perform a conditional release at the beginning of the production and perform sterility testing within the sterile section of the microbiological laboratory, but the pre-requisite is that the supplying company was thoroughly audited for quality assurance measures.

27. Is it necessary to validate leachable/extractable levels for all components that come into contact with the product, such as fermenter bags, tubes, chromatography resins, and Flexboy® bags?

In principle, yes, but you can approach specialised companies for such tests.

28. What does modular production facility refer to?

Modularisation is a rational way to simplify and streamline the construction of a building or the fabrication of equipment.
Modularisation enables the movement of most of the construction works from the site to a dedicated fabrication workshop where the modules a prefabricated and save time on qualification and validation.

29. Which air grade class must be maintained for closed systems?

Though a D class is theoretically possible, a C class is recommended for aseptic products. EU Annex 1 states that Grade D background is acceptable for closed isolators and Grade C is required for open isolators.

30. Does WHO have guidelines for the stages of conceptual design and basic design?

No, there are no special guidelines. General GMP guidelines, WHO TRS, etc. must simply be observed.

31. Are there specific qualification procedures for vendors who supply pre-treated primary packaging materials?

The main issue is the verification of the quality assurance (QA) procedures and the measures taken to guarantee the sterility of the material (integrity of packaging materials) from production to reception on the user's side.

32. For pre-treated primary packaging, how is the process flow defined into the filling line (i.e., decartoning and introduction into the filling line; need for decontamination; air grade class)?

There are different layers of protection for the glassware (cartons, bags, tubs), and they must be removed successively, in hygiene classes of always higher grade, observing the decontamination methods prevailing.

33. Which is the recommended water type for vaccine production? Distilled or reverse osmosis (RO)?

It depends on the stage of the process, but in the final phases, water for injection (WFI) is required.

34. Is it necessary to establish separate entry and exit points for personnel in a gowning room classified as grade A/B?

As per TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022 / EU Annex 1 Manufacture of Sterile Medicinal Products, 2022, separate airlocks are desirable. Where this is not practical, time-based separation of activities (ingress/egress) by procedure should be considered.

35. If several different processes take place in the same air grade class, is it necessary to separate the rooms or is an open space allowed?

If the same batch is processed, there are normally no issues, but your inspectorate may look at this differently.

36. When is the best time to design a production-scale facility for a research & development (R&D) product?

Given the fact that patents are limited to 20 years and a development period that can last 10 to 12 years, planning should certainly start at the time of phase I of clinical trials. Building times and delivery times of equipment must be considered at an early stage.

37. For legacy equipment, is it needed to redo installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ)?

Although legacy equipment means any equipment that is of such age or condition that it is no longer warrantied or supported by the manufacturer, GMP rules on qualification and validation still apply.

38. How is risk management applied in facility design?

By looking at Target Product Profile and Critical product attributes of the products to be manufactured. Risk management applies in facility design to protect the most critical part of the process. This is also the starting point of the CCS.

39. When designing the filling room for a level 4 high-risk injectable vaccine in a cGMP facility, what equipment (RABS or isolator) and pressure differential (positive or negative) should the manufacturer consider?

For an injectable high-risk vaccine, the facility design based on QRM needs to take into consideration staff protection. This point could lead to an isolator with negative pressure with additional requirements to protect the product as well as pressure control measures to avoid air ingress into the isolator.

40. Are any major disadvantages seen in using mobile laminar airflow (MLAF) for transporting equipment from the autoclave to the air class A supply in the vicinity of the filling machine?

The use of a MLAF does not change anything from the fact that we are dealing with aseptic manipulations that rely on the skill of operators; the MLAF may even give a false sense of security. It would be better to focus efforts on closed systems, to introduce tools, components, and parts inside the restricted access barrier system (RABS).

41. Is it acceptable to exit from the sterile block into a controlled non-classified (CNC) class, not only considering the contamination risks but also the complexity of the gowning process?

It would seem logical not to have to go through the complete cascade of hygiene zones when exiting the sterile block (assuming you mean class A/B), but experience has shown that the procedure can depend a lot on how the NRA sees the issue. Regardless, it is advisable not to omit more than one hygiene class specifically, transitioning from class A/B to class D, rather than from A/B to CNC. This presupposes the presence of an efficiently functioning and well-monitored differential pressure concept, the elimination of airflow reversal, and the completion of a comprehensive contamination risk assessment to prevent compromising the sterility of the block.

42. What is the recommended procedure for handling rejects before reaching the capping machine? Can the vial capping take place as an aseptic process with a barrier that separates the process of closing the vials from the filling which is completely separated by an additional barrier?

Vials with raised caps/missing stoppers are normally sorted out before the capping machine. The capping machine is normally separated from the filling machine (this is done for possible contamination by aluminium particles, though the newer machines have crimp rails and suction devices). The filling/stoppering and the capping machines can be in the same room (A/B if we speak about aseptic processing under RABS), with the transport between the 2 machines under grade A protection or they can be in separate rooms, whereby the background of the capping machine could be lower, as long as the transport to the capping and the capping are done under grade A air.

Session 2 Lyophilisation

1. Is the evaluation of the product temperature required during the freeze-drying routine process? What are the monitoring requirements in the lyophilisation chamber?

Product temperature monitoring is needed during the process development to have a good knowledge of the product and lyophilisation process. This must be defined to make links between the shelves´ temperature and pressure in the chamber. This information will become a critical process parameter (CPP) for the process monitoring and linked to critical quality attributes (CQA) used for the product release. In routine production, the shelves' temperature is considered for process monitoring. Vials incorporating probes are mainly used during process qualification. The lyophilisation process parameters to be monitored are:

- Leak test and filter integrity test before the beginning;
- Shelves temperature during the process;
- Pressure in the chamber;
- Time and duration of each process step.

2. Is a fast freeze-drying process an alternative to conventional freeze-drying?

Fast freeze-drying can be an alternative to conventional freeze-drying. The development of such a process should ensure that the product manufactured with this technology meets its specifications defined during development. If there is a change of technology in freeze drying for an existing product a risk assessment must be carried out to identify where potential variation in product specification and risk to the patient could occur.

3. Should a laminar airflow (LAF) unit be installed surrounding the lyophilised product filling line where operators can move?

Air grade class B is the mandatory background for grade A. However, a grade A air supply may be installed in these areas decreasing the contamination risk during an eventual opening of the restricted access barrier system (RABS) doors. If there is no Grade A air supply surrounding the RABS, there will be a higher contamination risk if the RABS doors are opened. Specific measures should be taken in this case based on quality risk management (QRM) and the possible impact on the aseptic process and product.

4. Should the smoke test be conducted inside the filling machine only, or for the whole filling room? Is there a special recommendation for conducting this study in a lyophilisation process?

Smoke tests are required not only inside the filling machine. Airflow visualisation is required for room classification and part of the qualification for the most critical areas in the aseptic process (e.g., "filling line"). The places and areas to carry out the smoke tests are defined based on QRM. These tests must demonstrate that there is no airflow from less clean areas to cleaner areas (e.g., no flow from B to A). For freeze dryers, it is necessary to demonstrate there is no air coming from the floor and going inside the lyophiliser. Smoke tests, photographs, and videos to demonstrate the airflows are under control and well-oriented are required.

5. Under what circumstances can the lyophiliser cycle be halted manually and subsequently resumed?

This could be acceptable if it is covered by the aseptic process simulation (APS). This

is independent of the impact of these events on the freeze-dryer operation and the quality of the product. This event is expected to be exceptional and should trigger a deviation and investigation to evaluate the impact on the freeze-dryer operation and the quality of the product.

6. How can integrity testing be conducted for lyophilised ampoules, and what are the acceptance criteria for the test?

For integrity testing, the Dye Ingress Test is commonly used, where a sample of lyophilised ampoules or vials is placed in a vacuum chamber with a dye to detect nonintegral units. Other methods are available such as High Voltage and Pressure/Vacuum Decay tests, allowing 100% of units checked, which is required for containers closed by fusion (e.g., glass or plastic ampoules); other containers such as s vials, should be checked for integrity according to appropriate procedures (see WHO TRS 1044, Annex 2, WHO Good Manufacturing Practices for Sterile Pharmaceutical Products (clauses 8.22 and 8.23). Any failure detected during the sample-based or 100% testing should be reported immediately and appropriate actions taken.

7. Does disinfection need to be performed and validated for lyophilisers?

For sterile products, it is mandatory to load the product in a sterile chamber. For that you need to wash and sterilise the equipment and these operations have to be validated. If disinfection is part of your cleaning, it should be validated.

8. Is it mandatory to use clean in place (CIP) and sterilisation in place (SIP) in a lyophiliser? Could we use hydrogen peroxide or formaldehyde to decontaminate the equipment; if not, what is the right method to clean and decontaminate the equipment?

As per TRS 1044, Annex 2, WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022 / EU Annex 1 Manufacture of Sterile Medicinal Products, 2022, the lyophiliser´s cleaning process should be done using validated cleaning procedures. Formaldehyde is not allowed for use in many countries due to its toxicity. Hydrogen peroxide is not considered a sterilising agent but a decontamination one. The only way to sterilise a lyophiliser is by using moist heat sterilisation.

9. Are CIP and SIP mandatory for RABS for aseptic filling including the lyophilisation process?

CIP and SIP are not mandatory. Nevertheless, you must wash, clean, and sterilise your equipment, including parts of the filling line, and stoppers bowls. Isolators and autoclaves are often used.

10. Is it acceptable to transport sterilised vials (in perforated stainless-steel trays) from the dry heat steriliser through a Class B area and subsequently place them under Class A conditions?

This process is not acceptable. Once vials are sterilised in a dry heat steriliser you need to make a connection under grade A from the oven to the filling line. If you must cross a grade B area, you need to keep the vials in A with additional protection.

11. Regarding WHO TRS 1044, Annex 2, WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, section 8.123, for live vaccines, is there an alternative for automatically loading the lyophiliser and for determining the required sterilisation frequency?

As per section 8.123, regardless of the type of product, lyophilisers and associated

product transfer and loading or unloading areas should be designed to minimise operator intervention as far as possible, and the frequency of lyophiliser sterilisation should be determined based on the design and risks related to system contamination during use. Lyophilisers that are manually loaded or unloaded with no barrier technology separation should be sterilised before each load. This clause refers to all lyophilised products including live vaccines. Regarding the frequency of sterilisation, this is based on the defined contamination control strategy (CCS) and cross-contamination risk assessment regarding other vaccines or other batches.

12. The updated EU Annex 1 mentions, "...Airflow may not be fully unidirectional in closed isolators where simple operations are conducted." However, the document doesn't specify examples of "simple operations." What are the instances of such operations and the scientific rationale behind this consideration?

As per clause 4.19.b of EU Annex 1 Manufacture of Sterile Medicinal Products, 2022, certain non-critical operations (e.g., transfer of filling equipment accessories) associated with the aseptic fill in isolators may not require a Class A unidirectional airflow; however, it must be demonstrated that this condition does not represent a significant contamination risk to the aseptic process.

13. What are the lyophiliser qualification critical parameters?

The critical parameters for the qualification of lyophilisers are those needed for:

- Cleaning
- Sterilisation
- Lyophilisation process (shelves temperature at different stages, closing of vials, chamber vacuum, vacuum break, chamber filter integrity).
- De-icing the condenser.

14. In developing countries, there may not be sufficient economic resources to implement the use of automated robotic lyophilisation loading. In that case, what would be an acceptable approach? Would manually loading under class A still be acceptable?

Manual loading must be avoided as much as possible. Semi-automated methods or equipment to load the lyophiliser providing a barrier between the product and the operator in charge of loading the equipment may be used. After the filling, this is the most critical step, and the open-filled vials must remain under grade A conditions.

15. What is the most common and justifiable cycle duration in a lyophiliser?

There is no fixed requirement for this parameter. The duration of the lyophilisation process is based on the process development and linked to a specific product. The process must be as short as possible for economic reasons.

16. Does the recording of the location of failed vials in the lyophiliser tray (e.g., melt, tip over, etc.) need to be done?

Recording the position of failed vials in a lyophiliser is useful for understanding the root cause of the failure. Currently, it is quite difficult to recover this information and trace the defective vial to its position in the lyophiliser.

17. What is the sterilisation process used to sterilise the nitrogen gas before introducing it into the lyophiliser chamber to break the vacuum?

The Nitrogen or air used to break the vacuum of the lyophiliser chamber is sterilised through a hydrophobic sterilising

grade filter for gases that need to be qualified and integrity tested.

18. What are the main points to consider during the qualification of lyophilisers, including scope, frequency, and mapping?

The qualification of the lyophiliser covers the following parameters:
• Shelves temperature with probes distributed uniformly in the chamber.
• Shelves temperature during process monitoring.
• Shelves flatness to avoid containers out of contact with the shelves.
• Chamber Pressure.
• Chamber integrity
• Condenser de-icing performance.
• CIP, SIP
• Lyophilisation process control (e.g., Programmable Logic Controller - PLC)

The frequency will be defined based on risk assessment and experience.

19. During APS, should the entire lyophilisation process, including freezing time, primary drying, and secondary drying, be mimicked? Is it necessary for the media fill growth media to be held in the chamber for the entire lyophilisation process time?

The whole lyophilisation process time is not needed nor recommended for an APS due to, for example, drying out or freezing of the broth media. The duration must be defined based on risk assessment. Most of the operations in the lyophiliser must be performed such as loading on all shelves, creating a vacuum, breaking the vacuum with air, closing the stoppers, and unloading. During the APS for lyophilisation, you must mimic the duration of the filling and loading process to cover the longest holding time of the filled and half-stoppered vials going into the lyophiliser.

20. What is the difference between an air grade class A and a grade A air supply?

Find below the two definitions provided by EU Annex 1. The most important difference is the background air grade classification around the Grade A air supply.
Grade A: The critical zone for high-risk operations (e.g., aseptic processing line, filling zone, stopper bowl, open primary packaging, or for making aseptic connections under the protection of first air). Normally, such conditions are provided by localised airflow protection, such as unidirectional airflow workstations within RABS or isolators. The maintenance of unidirectional airflow should be demonstrated and qualified across the whole of the grade A area. Direct intervention (e.g., without the protection of barrier and glove port technology) into the grade A area by operators should be minimised by premises, equipment, process, and procedural design.

Grade A air supply: Air that is passed through a filter qualified as capable of producing grade A total particle quality air, but where there is no requirement to perform continuous total particle monitoring or meet grade A viable monitoring limits. Specifically used for the protection of fully stoppered vials where the cap has not yet been crimped.

21. Why are two colours of grade A shown in some layout drawings?

In the layout drawings, two grade-A areas have different purposes. Inside the isolator or RABS, it is shown fully red in the drawing as it is the most critical part of the filling where an air grade class A first air is the first air above the filling and pre-stoppering stations. For RABS, it is usually proposed to have a grade A surrounding the machine where operators can open the RABS doors reducing the risk of contamination. The

latter is usually shown with a different or lighter colour in the drawings.

22. In the case of RABS, is it acceptable to cascade from grade A to an unclassified area?

No, RABS (which provides a class A environment) must have a class B background. From class B to controlled non-classified (CNC), there must be class C and D airlocks.

23. For the APS of the lyophilisation process, should the filling quantity be kept consistent with the commercialised batch? Should the simulation of loading and unloading be carried out? Could the filling quantity be reduced based on risk assessment?

The answer to this question is fully addressed in the text of TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022 / EU Annex 1 Manufacture of Sterile Medicinal Products, 2022 WHO, clauses 9.36, 9.40. For lyophilisation, it is not necessary to simulate the full loading and unloading of the lyophiliser. Nevertheless, simulation of the worst case for loading needs to be performed including the holding time of the first vial filled until the end of the filling. Care needs to be taken to identify the filling units as to the time of loading throughout the filling. As per the design of the APS, all these points must be assessed through QRM.

An extract from EU Annex 1, Manufacture of Sterile Medicinal Products, 2022, says “The process simulation should be of sufficient duration to challenge the process, the operators that perform interventions, shift changes, and the capability of the processing environment to provide appropriate conditions for the manufacture of a sterile product. The number of units processed (filled) for APS should be sufficient to effectively simulate all activities that are representative of the aseptic manufacturing process. Justification for the number of units to be filled should be clearly captured in the CCS. Typically, a minimum of 5000 to 10 000 units are filled. For small batches (e.g., those under 5000 units), the number of containers for APS should at least equal the size of the production batch.”

24. During the initial lyophilisation process validation, is it mandatory to validate with the lower and upper in-process control limits?

The process validation at the initial stage is based on the data coming from the development phase and the application submitted to regulatory authorities. The process validation will have to be designed through QRM. The definition of the range for some parameters is linked to the process flexibility defined.

25. If multiple lyophilisers are employed during a single filling session, how should sampling be conducted, and what is the procedure for distinguishing the product units from each piece of equipment?

For a product lyophilised in several lyophilisers, the units from each lyophiliser must be considered as a sub batch and sampling must be traceable to the sub-batches. The sub-batches should be identified to allow proper segregation.

26. Concerning the lyophilisation process, how can justification be provided for not trending some critical quality indicators that do not change or change little over time?

Regarding the lyophilisation process, we cannot justify not trending critical quality indicators, even if they do not change or slightly change during the process. To avoid monitoring such parameters based on QRM we need to reevaluate the criticality of these

quality indicators and make a procedure change.

27. What is the difference between RABS and isolators?

An isolator is an enclosure capable of being subject to reproducible interior bio-decontamination, with an internal work zone meeting grade A condition that provides uncompromised, continuous isolation of its interior from the external environment (e.g., surrounding cleanroom air and personnel). There are two major types of isolators:
i. Closed isolator systems exclude external contamination of the isolator's interior by accomplishing material transfer via an aseptic connection to auxiliary equipment, rather than the use of openings to the surrounding environment. Closed systems remain sealed throughout operations.

ii. Open isolator systems are designed to allow for the continuous or semi-continuous ingress and/or egress of materials during operations through one or more openings. Openings are engineered (e.g., using continuous overpressure) to exclude the entry of external contaminants into the isolator.

RABS is a system that provides an enclosed, but not fully sealed, environment meeting defined air quality conditions (for aseptic processing grade A) and using a rigid-wall enclosure and integrated gloves to separate its interior from the surrounding cleanroom environment. Operators use gloves, half suits, rapid transfer ports (RTP), and other integrated transfer ports to perform manipulations or convey materials to the interior of the RABS. Depending on the design, doors are rarely opened, and only under strictly pre-defined conditions.

Session 3 Investigational products

1. **Is it acceptable to club study phase 1/2 in view of a fast-track program?**

Yes, it is possible to execute clinical trial phase 1/2 concurrently. The clinical trial phase 1/2a was executed for several COVID-19 vaccine candidates to expedite the development.

2. **What constitutes the optimal batch size in the context of clinical trials? Additionally, what percentage of a commercial batch is deemed acceptable for use in a clinical trial?**

For clinical trial phases 1 and 2 where there are tens and hundreds of volunteers, 1000-2000 units will be enough for clinical studies, QC tests, reference samples for testing -if needed- and stability studies. For the phase 3 batch, WHO recommends it to be the same batch size as the commercial scale.

3. **Is there any guideline for quality practices in biomedical research (QPBR)?**

The guideline for QPBR WHO on behalf of the Special Programme for Research and Training in Tropical Diseases, 2010.

4. **Is the International Council for Harmonisation (ICH) Q14 already available?**

The Assembly of the International Council for Harmonisation (ICH) met in person on 31 October and 01 November 2023 in Prague, Czech Republic. During this meeting, the ICH Q2(R2) Revised Guideline on Validation of Analytical Procedures, 2022, and the new ICH Q14 Guideline on "Analytical Procedure Development", 2023, were adopted by the ICH Assembly Regulatory Members.

5. **To produce pre-clinical batches of a biopharmaceutical product on a pilot scale, is it necessary to accomplish all good manufacturing practices (GMP) guidelines?**

Not necessarily. Please follow WHO TRS 1044 Annex 6, 2022: WHO good practices for research and development facilities of pharmaceutical products. However, if the same facilities will be used for clinical batch production, GMP for investigational products (WHO TRS 1044, Annex 7, 2022) should be followed.

6. **Does ICH Q9 Quality Risk Management, Q9(R1), 2023, apply to biologics?**

Yes, manufacturing of biological products requires strong quality risk management (QRM). Pharmaceutical Inspection Co-operation Scheme (PIC/S) GMP Annex 13, EU Guidelines to GMP, 2010, states that "biological processes may display inherent variability, so that the range and nature of by-products may be variable. As a result, QRM principles are particularly important for this class of materials and should be used to develop the control strategy across all stages of manufacture to minimise variability and to reduce the opportunity for contamination and cross-contamination."

7. **If there is a change in the production process during the commercial stage (as compared to the clinical trial phase), are more clinical trials needed? Or is a quality comparability study before and after the change sufficient to guarantee that the change has no impact on efficacy and safety?**

Yes. There could be changes in the production process during the commercial stage. A comparability study will be required depending on the changes. A determination

of comparability can be based on a combination of analytical testing, biological assays, and, in some cases, nonclinical and clinical data. If a manufacturer can assure comparability through analytical studies alone, nonclinical, or clinical studies with the post-change product are not warranted. However, where the relationship between specific quality attributes and safety and efficacy has not been established, and differences between quality attributes of the pre-and post-change product are observed, it might be appropriate to include a combination of quality, nonclinical, and/or clinical studies in the comparability exercise. Please refer to ICH Q5E Comparability of Biotechnological/biological Products subject to Changes in their Manufacturing Process; 2004.

8. How do prescriptive and descriptive documentation differ?

Prescriptive documents are instruction-type documents such as research proposals, study plans, protocols, and standard operating procedures (SOP). Descriptive documents are raw data, records, and reports that describe what, when, where, and how the activities have been done.

9. For compassionate use products, is the manufacturer required to comply with GMP and be certified by an authority?

Compassionate use must be undergoing clinical trials or have entered the marketing authorisation application process and, while early studies will generally have been completed, its safety profile and dosage guidelines may not be fully established. Therefore, manufacturing of the product must comply with GMP for investigational products. GMP certification of clinical material manufacturers depends on each country's requirements.

10. Can biological-origin oral solid products be manufactured in a general oral solid facility (e.g., oral Semaglutid)?

According to the US Food and Drug Administration (FDA), Semaglutid is not considered a biological product because this peptide drug is shorter than 40 amino acids. However, the product may have different risks from other oral dosage forms and hence, QRM must be applied to determine a control strategy to minimise the risk of contamination and cross-contamination in the manufacturing facilities.

11. Can pre-clinical batches be used to establish the target product profile (TPP) and critical quality attributes (CQA)?

TPP and quality target product profile (QTPP) are determined from the start of product design. After that, quality attributes and CQAs will be determined based on QRM which links to patient safety. Then, the process will be designed to produce a product as required. Preclinical batches will be produced to meet the predefined QTPP.

12. What is the average timeline for manufacturing all batches required for preclinical and clinical studies, development, and validation?

The timeframe for the completion of both preclinical and clinical studies, coupled with the regulatory authority's approval process leading to the marketing approval of a vaccine, typically ranges from 5 to 15 years.

13. Can validation batches be released to the market?

Processes must be shown to be robust and ensure consistent product quality before any product is released on the market. Hence, the release of process validation batches for commercial use will require satisfactory completion of the validation study for that

process. Batches of product manufactured before completion of process validation (PV) activities may be released for commercial use following verification of acceptable results for all tests, verification that the acceptance criteria have been satisfied, and the critical process parameters, ranges, and materials used are the same as the proposed commercial manufacturing process and fulfilment of other requirements for product release as required by the national regulatory authority (NRA) of each country.
According to the GMP guideline, PIC/S GMP PE 009-16, 2022, Guide to Good Manufacturing Practice for Medicinal Products, Annex 15 (Qualification and validation), "where validation batches are released to the market this should be pre-defined. The conditions under which they are produced should fully comply with GMP, with the validation acceptance criteria, with any continuous process verification criteria (if used), and with the marketing authorisation or clinical trial authorization."

14. Do research and development (R&D) facilities undergo inspection as per WHO TRS 1044, Annex 6 for certification?

Currently, WHO does not inspect R&D facilities for certification. Some agencies conduct inspections to verify the reliability, integrity, and compliance of clinical and non-clinical research being reviewed in support of pending applications like the US FDA.

15. Before technology transfer (TT), is the receiving unit (RU) required to audit the sending unit (SU) (e.g., R&D)?

The first steps of a TT should include a process of due diligence and gap analysis through visits to the SU and RU. This is not considered as an audit. During an initial discussion, it should be identified whether a RU has any interest in such a project. The suitability and degree of preparedness of the RU should be assessed before the start of the transfer. The SU should make available in relevant documents all the necessary information and knowledge regarding the product, process, or procedure to ensure a successful transfer. See more details of Technology transfer in WHO TRS 1044 Annex 4, 2022.

16. Are suppliers required to be audited before commercial procurement or should they be audited at the R&D stage?

In industrial practice, all starting materials should be fixed from the clinical trial phase 3 batch(es). Not all suppliers will be audited as vendor audits should be based on QRM. Only vendors of critical materials should be audited, as appropriate according to GMP requirements.

17. In clinical trial phases 1 and 2, where the production typically involves only 3 to 5 batches, is it necessary to perform cleaning validation?

Cleaning validation follows the same principle of process validation, i.e., life cycle approach, risk-based, science-based, and quality, and comprises 3 phases of activities: process development (PD), process validation (PV), and continued process verification (CPV). The company should start cleaning procedure development as early as possible in the R&D phase as described in WHO TRS 1044 Annex 6, WHO Good Practices for Research and Development Facilities of Pharmaceutical Products, 2022. Cleaning procedure development may include a cleanability study, solubility of substances including cleaning agents, etc. In the clinical trial phase 1 and 2 batches, the company may not provide a full cleaning validation report in the dossier submitted to the NRA. However, such activities should be done

and data available for the full cleaning validation to be executed in clinical phase 3 batches. Refer to WHO TRS 1019, Annex 3, Good Manufacturing Practices: guidelines on Validation, Appendix 3, Cleaning Validation, 2019, and WHO TRS. 1033, Annex 2, Points to Consider when including Health-Based Exposure Limits (HBELs) in Cleaning Validation, 2021.

18. Regarding cleaning validation, must there be a demonstration of product degradation in alkaline and high-temperature conditions, typically by Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)?

In cleaning validation, the final rinse or finally cleaned equipment should be demonstrated and proved to be “clean”. For vaccines, the cleaning procedures usually comprise sterilisation, also known as decontamination, before cleaning with strong alkaline and/or strong acids. Proteins are usually degraded by this procedure and washed away easily. Therefore, SDS-PAGE may not detect protein degradation in the last rinse or on the surface of equipment due to its limit of detection and quantitation. Total organic carbon (TOC) is commonly used to demonstrate cleanliness.

19. What is the requirement for standardisation of a working standard against a reference standard? Is it required to perform impurity testing, or is it sufficient with what we have already performed for batch release?

Each working standard/reference should be prepared as a large batch, characterised, and calibrated against the international or well-established reference standard (if available) by testing the quality attribute to be used as the reference material such as potency, biological assay, etc. Characterisation of the working standard should be done to gain all necessary information based on knowledge and risk management. The batch release data should be available as a minimum. Other data may be required. It should be tested at regular intervals to ensure that it is fit for its intended use.

20. Could further elaboration on quality control (QC) in an R&D facility be provided?

A QC unit should be available and separated from the production unit, with sufficient space and suitable equipment to fulfil activities required to support research and development activities as defined in WHO TRS 1044 Annex 6, WHO Good Practices for Research and Development Facilities of Pharmaceutical Products, 2022. Such activities may include sampling and testing (e.g., starting materials, packaging materials, intermediate products, bulk products, and finished products), sampling and storing reference/retention samples, qualification and validation, evaluation, maintenance and storage of reference materials, managing stability program and testing, and environmental monitoring. Analytical method development may be initiated and proceeded to establish suitable and reliable analytical procedures.

21. What are the requirements to be met for a life cycle approach to R&D data?

The life cycle of R&D data should be the same as those generated in the following phases of the pharmaceutical life cycle. It includes creating, processing, reviewing, analysing, reporting, transferring, storing, retrieving, and monitoring until retirement or disposal of such data.

22. What is the major difference between GMP of investigational medicinal products (IMP) compared to commercial registered products?

In clinical trials, there may be added risk to the subjects compared to patients treated with authorised medicinal products. GMP for IMP addresses specific issues concerning IMPs which shall be manufactured to ensure the quality of such medicinal products to safeguard the safety of the subjects and the reliability and robustness of clinical data generated in the clinical trial. Procedures need to be flexible to provide for changes as knowledge of the process increases and is appropriate to the stage of development of the products. The production of IMPs involves added complexity in comparison with authorised medicinal products by lack of fixed routines, variety of clinical trial designs, and consequent packaging designs. Randomisation and blinding add to that complexity an increased risk of product cross-contamination and mix-up. This increased complexity requires a highly efficient quality management system (QMS), well-trained personnel for GMP, and relevant good clinical practices (GCP). Cooperation between manufacturers and sponsors of clinical trials is required.

23. In the context of clinical phase 3, is it a requirement for the product to be manufactured in a GMP facility, or is production in a laboratory setting permissible?

According to WHO TRS 1044, Annex 7, WHO Good Manufacturing Practices for Investigational Products, 2022, all clinical materials, IMPs, must be produced in GMP facilities.

24. Which are key aspects of how to control the process in R&D?

Two important tools are knowledge management and QRM. Following the concepts described in ICH Q8 (2009), and ICH Q11 (2012), Pharmaceutical Development, and Development and Manufacture of Drug Substance, (chemical entities and biotechnological/biological entities), respectively, the control strategy should be defined and evolved when more knowledge and experience are gained. CQAs and critical process parameters (CPP) should be defined during this stage.

25. Is it acceptable to use IMP for commercial purposes after successful clinical trial approval?

Normally, IMP batches are not used for commercialisation. If the IMP batches are also process validation batches at a commercial scale, then, they may be released for commercialisation if it is agreed by the authority. Please see point 13 as well (i.e., Can you release validation batches to the market?)

26. What is meant by consistency and comparability? At which stage should it be done?

IMPs or commercial products should be manufactured in a manner that ensures consistency between and within batches of the product in terms of quality, efficacy, and safety. However, when there is a change, such as in the manufacturing process or scale of production, both during development and after approval, comparability studies should be done to provide evidence that the manufacturing process changes will not have an adverse impact on the quality, safety, and efficacy of the drug product (DP).

27. What accounts for the variation in endotoxin limits, with some vaccines having higher thresholds, while others adhere to more stringent standards?

It is known that certain families of vaccines such as toxoids contain much higher levels of endotoxin, whereas others such as purified recombinant subunits and gene vectors may contain very low levels. This is because the manufacturing processes of

different vaccines are different. Vaccine manufacturers must show that the vaccine is safe and the endotoxin limits in the release specification are appropriately justified based on data from R&D, preclinical, and clinical studies. If the endotoxin limits are defined in the compendia such as Pharmacopoeia or specific WHO TRS, they should be followed. A manufacturer may define endotoxin limits for investigational products following the guidance document entitled "Setting Endotoxin Limits During Development of Investigational Oncology Drugs and Biological Products " Guidance for Industry- US FDA, July 2020. After getting more experience from batch manufacturing, more appropriate endotoxins may be justified.

28. Are PIC/S and ICH considered guidelines or regulations from the WHO perspective?

Yes. PIC/S GMP and ICH guidelines are in line with WHO GMP and other TRS guidance.

29. Why is Bioavailability not appropriate for vaccine production?

Vaccines work by stimulating immune responses. Therefore, bioavailability data is not relevant to its function.

30. If the sending unit has already transferred the validated analytical method to the receiving unit's R&D and QC, and there is a method's optimisation, which unit should perform the analytical method validation?

If the method is validated in the sending unit, only method verification is required in the receiving unit. Either R&D or QC or both could be the receiving lab. However, the QC lab of the receiving unit may be better than R&D for analytical method transfer and verification. If R&D is the recipient of the transfer and verification, there must be another transfer from R&D to QC.

31. Is QPBR also applicable within R&D platforms? Or only within production facilities?

QPBR is applied in research laboratories for all activities to assure the quality of the research work. Please refer to the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Quality Practices in basic Biomedical Research (QPBR) training manual (2010).

32. Is there any limitation to achieving a successful R&D trial batch for TT in the case of small companies?

Possible limitations could be a lack of qualified personnel, suitable facilities, and equipment, and an efficient QMS.

33. In the PV of vaccines, which one of the different types (concurrent or prospective) is preferred and why?

Prospective validation is the gold standard. The validation studies will ensure intra-batch and inter-batch homogeneity of the product, thus demonstrating the consistency of the manufacturing process.

34. Can the production process of a drug substance (DS) employed in clinical trial phase 3 be altered? Specifically, is it feasible to adjust the manufacturing process for producing the DS designated for clinical trial phases 1 and 2, making it distinct from the manufacturing process tailored for the DS utilised in clinical trial phase 3 due to the introduced modifications?

Yes, it is possible. The scale and batch size of the phase 3 batch DS could be different from those of phases 1 and 2 batch(es). However, comparability data should be available. Refer to ICH Q5E Comparability

of biotechnological/biological products subject to changes in their manufacturing process; 2004.

35. Should the Integrity of primary data be considered?

Yes. Primary data or original data is the first or originally captured data or information and any data subsequently required to fully reconstruct the performance of the good practices (GxP) activity and hence it is the foundation to assure data integrity.

36. What are the key points to be considered in the analytical method lifecycle through phases 1 to 3?

The analytical method should be developed from the R&D stage and preclinical development. Then, method validation should be done as appropriate to the stage of product development from phase 1 to 2 as there will be more understanding and experience from batch production. In phase 3, the method should be fully validated according to the ICH Q2 (R2) Validation of Analytical Procedures, 2022 guideline. After marketing authorisation, any significant change could result in revalidation. Continued test performance should be monitored (for example, using control charts) to ensure the validated status of the analytical procedure.

37. What parameters are related to contamination, cross-contamination, and biosafety throughout the manufacturing of phases 1 to 3 material?

Contamination may be caused by 3 types of contaminants, i.e., microorganisms, pyrogens, and particulates. Cross-contamination is a contamination caused by foreign chemical, microbial, or physical substances that are inadvertently transferred from different batch(es) or different product(s) to a certain batch with possible harmful effects that might affect the quality and safety of the pharmaceutical products.
Biosafety: Containment principles, technologies, and practices that are implemented to prevent unintentional exposure to biological agents or their inadvertent release. It is fundamental to protecting the personnel involved, the environment, and the wider community against unintentional exposures or releases of pathogenic biological agents (WHO Laboratory Biosafety Manual, 4th ed., 2020). Biosafety considerations are related to the risk group of organisms/ biological agents used together with other risks such as manipulation procedures, quantity/ scale of work, equipment, facilities, primary containment, etc. Risk assessment and control strategy should be evaluated and defined.

The contamination control strategy (CCS) should address the required contamination controls according to the contaminants type, based on current product and process understanding and QRM assuring process performance and product quality. Parameters and attributes related to starting materials including cells and seeds used, active substance (DS), excipients, DP, facilities and equipment, in-process control, DP specifications, monitoring and control method, and frequency of controls should be included in the CCS.

38. What does "blinding" mean?

Please refer to the definition from PIC/S GMP Annex 13 EU Guidelines to GMP, 2010. It mentions that blinding is a procedure in which one or more parties to the trial are kept unaware of the treatment assignment(s). Single-blinding usually refers to the subject(s) being unaware, and double-blinding usually refers to the subject(s), investigator(s), monitor, and, in some cases, data analyst(s) being unaware of the treatment assignment(s). An IMP blinding refers to the deliberate disguising of

the identity of the product by the instructions of the sponsor. Unblinding shall mean the disclosure of the identity of blinded products.

39. Can a supplier be considered qualified because it has already been approved by another major multinational company? i.e., leverage another company's supplier qualification?

It is possible to establish a risk-based approach to qualify suppliers. Risk control measures should be implemented as appropriate. Furthermore, the supplier has performance, and quality certification(s), etc., should be considered.

40. Can PV still be based on 3 consecutive batches or is it now expected to do quality by design (QbD), design of experiment (DOE), design qualification (DQ), and performance qualification (PQ)?

EMA, Guideline on Process Validation for Finished Products - Information and Data to be provided in Regulatory Submissions, (2016), mentions that "the number of batches should be based on the variability of the process, the complexity of the process/product, process knowledge gained during development, supportive data at commercial scale during technology transfer and the overall experience of the manufacturer. Data on a minimum of 3 production scale batches should be submitted unless otherwise justified".
WHO (WHO TRS 981 Annex 3, 2013: WHO guidelines on Variations to a Prequalified Product) still requires a PV report for 3 batches of the proposed batch size.
However, the US FDA does not simply accept "3 batches" but requires that "The approach to process performance qualification (PPQ) should be based on sound science and the manufacturers overall level of product and process understanding and demonstrable control."

41. For blind studies, is it easier to put radio frequency identification devices (RFID) as part of the packaging? (i.e., easier to differentiate between placebo vs. non-placebo)?

For blind studies, we should keep blinding until the time we need to break this blinding. Any blinding mechanism that ensures blinding while maintaining patient safety and clinical trial integrity should be acceptable.

42. What happens if a clinical study gets interrupted? Is there any position by WHO on how to continue clinical studies in cases like that? Does the study need to be restarted, a new protocol obtained, new subjects recruited, etc.? Can some of the data that has already been generated be leveraged?

Protocol modification is possible. Communication and discussion with authorities are encouraged.

43. If an already approved vaccine is being worked on, can the clinical studies be fast-tracked?

Communication and discussion with authorities are encouraged.

44. When a biopharmaceutical product under development moves from R&D to commercial production, what are the major changes in GMP components and why?

The principles of GMP apply to investigational medicinal products and commercial manufacturing are the same. However, some differences exist due to the nature of product stages and risk.

45. Is it possible to do a PV batch and a clinical trial batch simultaneously or from the same batches?

Yes. Most big vaccine companies manufacture clinical trial phase 3 batches, and the same batches are process validation batches. These batches are also put on the stability study program.

46. Is it necessary for the sources of raw material and primary packaging material (PPM) to be qualified before clinical trial batches?

Not necessarily. However, it is better to qualify the materials of biological origin and high-risk materials as early as possible.

47. If a company does not have a small-scale filling machine, can it fill vials manually under a laminar cabinet, and these vials be used for a clinical trial, or just for a stability study?

For phases 1 and 2, it may be possible providing that all necessary validation activities have been executed to ensure aseptic processing and sterility of the product. Aseptic operators must be very well-trained and qualified. The laminar airflow (LAF) cabinet should be qualified (grade A) and located in a grade B environment. All facilities must be fully qualified. The vials can be used for clinical study if they conform to the specification described in the product summary file (PSF) and the clinical trial protocol is approved by the NRA. These vials should also be used for stability studies. For clinical batches to be used for the phase 3 trial, the candidate vaccine should be manufactured on an industrial scale.

48. Are there any graphs available related to the precision of the results in analytical method validation?

For analytical method validation, the relative standard deviation is generally applied and therefore, a graphical presentation is not.

49. Does submission to WHO follow the electronic common technical document (CTD) format?

The document submitted for WHO performance qualification (PQ) is the product summary file (PSF). A manufacturer whose application letter is accepted will prepare and submit one hard copy and five electronic copies (on CD-ROM), in either Microsoft Word or PDF format, of a PSF, which should be completely updated and written entirely in English following the WHO format provided. The WHO format is required; however, the CTD format can be accepted so long as (a) a detailed cross-referencing of contents is presented; and (b) those aspects required by WHO but not included in the CTD requirements are presented. Where the PSF cross-references to the CTD format, the documentation may be in electronic form only. Electronic documents should be in searchable text where possible. (Refer to WHO Technical Report Series No. 978, 2013, Annex 6: Procedure for assessing the acceptability, in principle, of vaccines for purchase by United Nations agencies.)

50. What does the term "scientifically sound" mean?

It means that it adheres to established scientific principles, follows rigorous and well-accepted scientific methods, and produces reliable and trustworthy results based on scientific evidence.

51. What is WHO's procedure for reviewing submissions, including factors such as the order of submission, the timeline for reviewers, and the definition of Prescription Drug User Fee Act (PDUFA) or Generic Drug User Fee (GDUFA) fees?

Refer to WHO TRS No. 978, 2013, Annex 6: Procedure for assessing the acceptability, in

principle, of vaccines for purchase by United Nations agencies.

52. What strategies can low- and medium- income countries (LMICs) employ to expedite the development of vaccines?

There may be 4 major points to be considered:

1. Infrastructure readiness such as R&D, good laboratory practice (GLP) for pre-clinical studies, and GMP of both DS (one or more technology platforms) and DP.

2. Availability and training of qualified personnel for production (DS and DP), quality assurance, QC, and regulatory affairs.

3. Collaboration with well-established technology owners will be very useful for TT, public-private partnerships, etc.,

4. Good and early communication with the NRA to facilitate the regulatory process.

Session 4 Use of statistics and metrics in manufacturing process qualification and monitoring

1. How are the number of batches for process performance qualification (PPQ) (stage 2B) and continued process verification (PV) stage 3-A determined? (e.g., statistically, or risk-based).

Statistical methods are typically not used to determine the number of batches for stages 2B and 3A. It results in an impractical number of batches, and the methods are complex. A risk-based approach is typically applied. The typical minimum number for PPQ is still 3 as in the past, but more can be necessary to assess the risk of key raw materials, multiple equipment trains, multiple dosages, etc. A matrix or bracketing approach can be applied. The number chosen should be justified. The number of stage-3A batches with enhanced sampling is often based on an overall product and process assessment, for instance, it may be performed for the first campaign after PPQ, or for a small number of batches to show that any residual risk that exists post-PPQ has been addressed. The necessity for heightened sampling in stage 3A is contingent upon the specific details and reasons involved.

2. Is extensive sampling and analysis required for a selected batch in a continuous process verification (CPV) program, or is it trending and evaluation of CPPs and critical quality attributes (CQAs) of all batches, or a combination of both?

CPV is trending and evaluation of CQAs and CPPs (potentially a subset) of every batch. In stage 3A, enhanced sampling of multiple batches may be appropriate for a limited number of batches to evaluate batch variability post-PPQ. It is followed by ongoing monitoring of CQAs and the subset of CPPs that is required to display process control (not all CPPs must be trended; it can be limited to those that vary enough to affect a CQA or if the relationship between CPP and CQA is not well established). The choice of the CPPs to monitor is risk-based and should be justified.

3. How many data points are required for a reliable statistical analysis?

It depends on the analysis. A variance component analysis may need only a few samples per component. But if you want a reasonably process estimate of standard deviation, 25-30 is required. Control limits can be computed before that but should be considered tentative. It may require more than 30 if the typical sources of variability have not yet been observed (for instance, in a high-volume product). If you are computing a tolerance interval, the k factor adjusts to the sample size, which may result in unacceptably wide intervals when the sample size is small. However, that is the intent of the k-factor - to incorporate the uncertainty of the estimate of the mean and standard deviation when the sample size is small.

4. Can the PPQ batches be replaced with engineering runs?

PPQ runs must be manufactured consecutively using commercial conditions, and according to an approved protocol including acceptance criteria. Thus, engineering runs cannot be considered PPQ runs. However, for new products with accelerated approval, less than three PPQ runs, or concurrent validation may be acceptable if agreed to with a regulatory agency, and successful engineering runs may be used for that justification.

5. What's the minimum number of batches to calculate the process capability index (CpK)?

CpK and process performance (PpK) are unstable until a large number of samples are incorporated - about 60-90 samples. However, they can be computed after 25-30 if the process is stable and updated as more data are collected.

6. What is the difference between CpK and PpK?

CpK uses a short-term estimate of the standard deviation derived from the average moving range for individual measurements. PpK uses a long-term standard deviation derived from the overall standard sample deviation. Typically, Ppk is used because it incorporates mean shifts and sub-populations typical in pharmaceutical manufacturing.

7. For monitoring and evaluating the consistency of batches, can control charts be replaced by CpK or PpK?

No, they provide different information about the process. Charts display the process control, that is, the process variability. CpK only provides the capability of the process to meet specifications. A process can be in control but not capable, or capable, but not in control.

8. Sometimes the control limits are outside the specification limits or CpK/PpK is less than 1.0, but to reduce this variation it is necessary to make a considerable investment. Is it acceptable if the company accepts that risk because the process has sufficient controls to avoid an out of specifications (OOS)?

Indeed, a CpK higher than 1.33 is desirable. It indicates that the process is capable and meets specification limits. CpK between 1 and 1.33 is common if the specifications are derived from manufacturing performance (which is typical for many biological products). They are considered capable by many manufacturers.

In those cases, a CpK of 1.0 is just not achievable and when CpK is larger than zero but less than one, the process mean is within specification limitations, but a portion of the manufacturing output might have exceeded them. Low process capability is primarily a business risk if there are process controls to prevent OOS products from reaching the patient. Opportunities to reduce the risk of OOS (even for unmeasured units) include, for example, effective sampling of each batch (not just a single result) or robust models relating process parameters to quality attributes.

9. In some cases (for safety or stability reasons), we need to target our process quite close to the specification, which results in a small CpK or PpK value. Should we still use capability analysis for these parameters? Is there any other method to determine the capability of the process?

In any case, CpK and PpK are still useful to indicate the risk of exceeding specifications. There are other methods, such as a Bayesian model, that can provide a direct assessment of the probability of exceeding specification. These approaches are not readily available in one of the standard statistical packages and require coding in R, for example.

10. What is the acceptable CpK value for a vaccine /biological process?

Because the specifications for biological processes are often derived from performance data, the CpK is limited to around 1. However, if specifications are patient-focused - a range not related to the performance, instead based on scientific knowledge of the range acceptable to the

patient - then CpK can be higher, and typically > 1.33 is considered acceptable.

11. For the design of experiment (DoE) to determine the number of batches for the qualification, is the Taguchi matrix approach recommended?

The DoE is not used to determine the number of batches. It is used during process design to understand the relationships of process parameters and process attributes (inputs and outputs). The Taguchi method can be used to design the experiments; however, it tends to be more complicated than necessary and does not have some of the desirable properties of other types of design.

12. What should be the sample size in blend uniformity? What are the acceptance criteria of blend uniformity for an individual unit and the mean as part of the statistical evaluation?

Sample size shall be defined and justified. Generally, a 1-3X dosage unit range is used. Sample quantities larger than 3X can be used with adequate scientific justification. Typically, there are multiple replicate samples at 10 locations within a blender. There are criteria for mean, individual, and standard deviation. Other sampling plans may be used, if justified, including reduced quantities for smaller batches. However, sampling plans should be representative of the entire blender or batch. Refer to the blend uniformity content uniformity (BUCU) pages of the International Society of Pharmaceutical Engineers (ISPE) website.

13. Is it possible to perform any adjustment of CPPs during PV?

During PV, the process can be controlled as necessary according to the batch record. If that allows adjustment of CPPs, then it is acceptable. However, they should not be deliberately adjusted across the batch record range.

14. Statistics are used to determine the number of samples, but how are statistics used to determine which location of the lyophiliser's shelves to be sampled (including shelf number, etc.)?

The selection of shelf locations and the number of samples collected from each location should be based on prior knowledge of variability in shelf surface temperature and moisture. The locations selected for sample collections should include the worst cases in terms of impact on moisture content. That is a scientific assessment. Specifically, sample where variability might be introduced due to the physical/chemical/biological properties of the operation.

15. Should a confidence limit always be included in our PV? How will it help in establishing intra- and inter-batch consistency?

Statistical confidence allows a statement to be made about the quality of the unmeasured units, that is, the entire batch. It incorporates the uncertainty that results from measuring a sample from a total population. Hence, it brackets where the true, unknown population mean, or individual doses may fall.

16. Is the industry moving toward incorporating artificial intelligence (AI) technology to manage statistical data for predictive quality outcomes?

Yes, there are examples of AI for prediction and process control. It is not common. But as digitalisation increases, we will see more modelling in general, some with an AI component.

17. Is a three-batch validation and stability study required for batch size

change in the same equipment train though all CQA is comparable with the previous batch size?

For a change in the batch size, PV followed by a stability study is necessary. The number of batches can be justified based on experience and scientific knowledge. If comparability has been robustly shown with scientific and/or empirical evidence (with experiments), then less than 3 batches may be justifiable.

18. Is it necessary to undergo PV if the batch size will be only temporarily changed?

If a product manufactured using a different batch size is going to be given to patients, then comparability with the validated batch size must be shown. It is possible that less than a full 3-batch validation may be required if comparability can be robustly shown with scientific and/or empirical evidence (with experiments).

19. How is the confidence and coverage required determined and decided?

It is a risk-based decision. Confidence represents the level of risk you are prepared to assume when making statements that may be incorrect, and coverage is the percentage of the population that will be included in an interval. Typically for high criticality attributes 95% confidence and 95% or 99% coverage are used. For less critical attributes, or ongoing assessment during CPV, after PV, the confidence may be decreased to 50-80%.

20. As there could be a statistical shortfall, why is there an emphasis on using it? Can the batch just be released using the CQAs/ Critical process parameters (CPPs), if all my CQAs are met?

A criterion of a single sample passing the batch does not provide any confidence that the next sample would also pass. It provides information on that measurement only. It says nothing about the potential distribution of all samples from the batch. The statistical intervals allow a statement to be made about the unmeasured units.

21. What are the formulas for variance components analysis (VCA)?

There are multiple ways to compute variance components. See the description at the link for the fully nested random effects model computed in Minitab. Refer to the Methods and formulas for Fully Nested ANOVA – Minitab webpage.

22. Is variability in analytical measurements already addressed during analytical method validation, eliminating the need for consideration during the PV?

Correct. Analytical variability should be addressed before PV when the requirements for method validation are met.

23. What does the concept "influence of margin" mean, and how is the equivalent margin determined? Is it always -10 to 10?

The equivalence margin is not always -10 to +10. It depends on the amount that is acceptable from a risk perspective, which is different for every situation. In a method comparison, it may be what we expect from analytical variability. For a process change, it may be based on the needs of a patient (for example, no expected change in efficacy). Some common ranges for specific measurements can be found in various guides.

24. The analytical method for a specific CQA (e.g., protein content), is validated; the assessment of intermediate precision, which reflects reproducibility, involved the analysis of six samples; during the PPQ phase, sampling consisted of three

samples for each evaluation, with two replicates per sample. In terms of comparability, is the above approach deemed acceptable?

Without knowing the specifics of the variability, it is not possible to be sure if that sampling design is appropriate. If the product is an aqueous homogenous solution, then 3 PPQ samples are adequate (I am assuming it is 3 per batch, not a total of 3). Otherwise, more samples may be required to assess the variability within the batch. The number of samples for intermediate precision (minimum of 6) and the number of replicates depends on the variability of the method determined during assay development.

25. If US good manufacturing practices (GMP) in vaccine manufacturing, including US Food and Drug Administration (FDA) guidelines such as Process Validation, 2011, ICH Q9-R1 Quality Risk Management, 2023, and ICH Q10 Pharmaceutical Quality System, 2008, are followed, will it be equivalent to meeting WHO GMP expectations, or are there any additional WHO requirements?

WHO TRS No 1019, Annex 3, Good manufacturing practices: guidelines on validation, 2019, supports the concept of process validation linked to principles of quality risk management and quality by design, as described by WHO and the ICH of Technical Requirements for Registration of Pharmaceuticals for Human Use. If a vaccine is developed according to the US FDA and ICH requirements, it will follow the life-cycle approach that links product and process development, validation of the commercial manufacturing process, and maintaining the process in a state of control during routine commercial production. However, there are specific regulatory details of some agencies that are different. Manufacturers must clearly understand the regulatory details of the global agencies their product is required to follow.

26. In what circumstances a process (product) is returned to PV stage 1 during CPV?

If the process cannot routinely meet specifications, essentially showing that assurance of quality is not met, it should be returned to PV 1 to future understand and reduce PV. It may also be required if significant process changes are made with potential effects that are not fully understood.

27. Does the consideration of as many as 6 tests for a control chart need to be made, especially when working with biological data?

Assuming you are referring to the Nelson rules, the application of multiple tests will likely result in many signals, some being false positives. It is best to choose a subset of 2-3 (typically rules 1, 2, 3, 5, or 6).

28. How important is it to correctly analyse signals in a control chart? How do we avoid misinterpreting these signals? How is this related to trend analysis?

It is critical to evaluate signals correctly. Otherwise, resources can be wasted investigating signals when the process is not truly out of control. Because sources of variability such as raw material lots are not used randomly, there are natural clusters in data that can trigger signals of a mean shift. Indeed, it may be a mean shift, but it is not necessarily unexpected, or an indication that the process is out of control. Signals are expected because of this clustering. They should be investigated based on risk considering proximity to specification and whether the behaviour is truly unexpected. Research the concept of "independent and identically distributed" to further understand why signals must be carefully interpreted (it

is an assumption for typical evaluation of control charts that pharmaceutical manufacturing data do not meet).

29. Could further elaboration on the difference between ongoing process verification (OPV) and CPV be provided?

There is little difference between OPV and CPV. They are both programs to monitor the trends in process attributes and parameters. The WHO TRS No 1019, Annex 3, Good manufacturing practices: guidelines on validation, 2019, and FDA´s CPV, in Guidance for Industry: Process Validation- General Principles and Practices, 2011, put more emphasis on the potential need for enhanced sampling following PPQ. Continuous PV in the European Medical Agency (EMA) PV Guidance refers to the continual monitoring of selected process parameters using process analytical technology (PAT) tools as in continuous manufacturing (not batch).

30. What is the recommended frequency of CPV reporting (stage 3A)? Is it necessary to generate a cumulative report for each batch?

It depends on risk, specifically based on the product volume, process capability, and process understanding. For instance, a well-understood, highly capable process would be reviewed less frequently than a new process with lower capability. The report does not need to be overly detailed by batch. A summary of the assessment of the trends and potential actions to be taken is adequate (there could be none if the process trends exhibit nothing unusual or present a risk to supply). It can be a relatively simple template. Also, trends can be (and in some cases, should be) evaluated more often than a formal report is written.

31. What kind of statistical software is commonly used in the pharma industry?

The most common are JMP and Minitab, followed by Statgraphics. Design-Expert and MODDE are common for DoE.

32. Is using Clement's equation to calculate the capacity index in a non-normal distribution process recommended?

If data are non-normal, the first question should be "Why is the data non-normal?". Specifically, is there a true biological/physical/chemical reason that will predictably result in the same skewed distribution? In those cases, instead of using Clement's equation, it is appropriate to transform the data. For instance, many biological assays are log-normal, so a log transition is often appropriate. Note that non-normality is often due to sub-populations in the data. Neither transformation nor Clement´s equation is appropriate in those cases. The reason for the sub-populations should be evaluated and appropriate responses taken (either accepting the inaccuracy of the capability metric or separating the sub-populations).

33. What are the recommendations to consider when selecting a minimum number of batches to set specifications?

Before final specifications are set, it is important to have enough batches, that the expected sources of variability have been incorporated, and a sample size of 25-30. That of course is not possible at the time of setting initial specifications. That is why the specification derived as mean +/- 3s will be too narrow when only a few batches have been manufactured. Tolerance intervals are appropriate at that time because they incorporate uncertainty in the mean and standard deviation to set specifications when there are few batches. Note, however,

that with sample sizes less than 8 or so, tolerance intervals will be quite wide, so there may be some adjustments based on clinical or scientific data. It is also critical to allow specifications to be updated after additional batches have been made. However, after an acceptable number of batches have been incorporated (essentially capturing the expected variability of the process), they should be re-established. In the case of a low-volume product, 25-30 batches may take many years and multiple updates may be planned/agreed upon with the regulatory agency. Also note, when available, patient-focused specifications that reflect a patient's need and are not derived from process performance, should be utilised.

34. For bacterial endotoxin testing, should the manufacturer be aligned with the calculated limit, or could the clinical limit be used?

Manufacturers are responsible for determining what the endotoxin limit should be. A limit wider than the calculated limits but still within the clinical limits may be accepted by a regulatory agency with adequate justification of patient risk. The use of alert and action limits can be set based on historical data trends to ensure the maintenance of product quality and to justify the limits. It may be decided that tighter limits are desirable.

35. What are some examples of justifications that would permit the use of fewer than three batches for process performance validation studies?

If a process change is expected, based on a scientific justification, to have little to no effect on product quality, less than 3 batches may be acceptable. Examples: 1) the fill volume of a product is minorly changed) minor equipment change and comparability can be scientifically or empirically justified.

Session 5 Data integrity

1. **If there is already recorded data in different electronic systems (e.g., maintenance), is it also needed to record this information in a logbook? Is it an acceptable approach to create electronic logbooks where the personnel check bar codes/ type information?**

Generally, if the GXP activities are documented within the quality system, traceable (attributable), and easily retrievable for inspection, different systems can be used for documentation. Duplication of documentation is to be avoided as it can cause confusion.

2. **What does the concept of double-checking mean, and how many people are needed?**

The concept of double checker can be further broken down into a "witness" and a "reviewer". There are limited instances where 2 witnesses are required. The reviewer does not witness the activity but reviews the documentation. These are very different risk management strategies for review and need to be fully defined within the batch record standard operating procedure, etc.

3. **How can a sincere and honest data integrity (DI) compromise be differentiated from intentional data manipulation?**

It might be difficult to distinguish between these two categories. What may assist with the investigation is to evaluate the motivation, which generally comes back to an inadequate process, but can sometimes be malicious (rare).

4. **Can paper-based records still be used instead of electronic records (eREC) and electronic signatures (eSIG)? And can an old analogue integrator, manual sampling injection, etc., still be used for high-pressure liquid chromatography HPLC?**

It might be difficult to justify using outdated technology such as manual injection, considering the current industry standard and the need for accurate results. It is recommended to upgrade systems to current industry standards as soon as possible.

5. **What steps should be taken if an analyst neglects to make contemporaneous entries? Is it required to document a deviation in a non-contemporaneous record correction?**

It depends on the requirements outlined in the deviation SOP. Also, this decision depends on the criticality of the data that was not recorded contemporaneously. There are no clear guidelines published by the regulators.

6. **Is there any example that describes attributable, legible, contemporaneous, original, accurate, complete, and consistent (ALCOA+++) for paper-based systems instead of electronic systems?**

Refer to the PIC/S Good Practices for Data Management and Integrity in Regulated GMP/GDP Environments, 2021, a guide for data integrity, which gives examples for both paper and electronic-based processes.

7. **What corrective action preventive measures (CAPA) should be implemented for a deficient system review, encompassing errors in documents, especially when the oversight extends to the analyst,**

supervisor, and quality assurance (QA)?

The CAPA should focus on why the employees do not value the integrity of data. Generally, this is a top management issue, and due to bad process design. The quality culture at the site must be evaluated.

8. Is handwritten correction allowed in any approved document?

Yes, if an SOP is directing how to make hand-written corrections to approved documents. If not, an SOP must be created.

9. What should be the retention period for electronic and paper-based data?

That depends on the markets in which the applications are approved, and the types of products marketed.

10. If there is no data printout feature available in the production equipment, then, how could we ensure DI?

Procedural controls such as witnessing could be considered until upgraded equipment can be purchased. Use the risk management principles in ICH Q9 as a guide.

11. When is the right time for periodic data review?

The frequency of periodic data review is an outcome of the data integrity risk assessment (DIRA). Until we complete the DIRA, we cannot speculate on the frequency of review.

12. What is the impact in DI terms in the case of water sampling if the SOP states to open the point-of-use (POU) sampling and let the water flow out for 1 minute but many firms do not time this 1-minute requirement?

The impact could be false positives, which is an indirect risk to patient safety, as it wastes resources investigating water contamination that was due to recovery from the sampling point rather than the water itself.

13. What is the most common root cause for DI violations?

Typically, the most common root causes are due to bad process design, such as unclear SOPs on how to perform an activity.

14. If it is found that the purified water (PW) system has no deviation or excursions for 3 years (e.g., total colony count of 0 and 1, all the time), how is DI checked?

Perform an unannounced visit to the micro laboratory immediately after the counting has been completed, pull the plates out of the garbage before they are autoclaved, and perform a secondary check to see if the data is accurate.

15. Can the absence of an audit trail be accepted in old machines, such as in the old version of autoclaves?

It really depends, but generally speaking that equipment should be replaced whenever possible.

16. Is there a specific WHO guideline for auditing facilities systems on data integrity?

The guidance for inspectors is published by PIC/S. The WHO guidance is written for industry.

17. Regarding, ALCOA´s attributable principle, if an activity is carried out by at least 3 persons, who should complete the documentation? (e.g., during raw material dispensing who should sign the weighing balance ticker tape)

This should be pre-determined and outlined with clear instructions in the SOP. We see these types of SOPs when dealing with

aseptic connections during line setup, where only one person can fill out the documentation, due to the potential for contamination.

18. What is the relationship between continuous process verification (CPV), ongoing process verification (OPV), and annual product quality review?

CPV is a continuous evaluation of process control and validation so that the annual product review (APR) becomes a simple exercise of compiling the CPV data from throughout the year for a holistic overview.

19. Looking at the design of a process to prevent the recurrence of unplanned deviations, is that not another way of performing root cause analysis? Is the design/process mapping a new concept as presented?

Yes, this is correct. The process mapping tool is relatively new in pharma but has been in the PIC/S data quality risk management (QRM) guidance for more than 10 years. Most companies did not incorporate mapping into their QRM toolbox until recently.

20. According to ICH Q9, which risk management tool (RMT) is recommended to ensure alignment of the data governance system across the entire organisation?

Data and process mapping combined with QRM.

21. Is data generated by a non-validated software reliable?

We need to change our perspective of software validation - and replace it with workflow validation. All workflows must be validated, and then software is one component of the workflow. Otherwise, we will never be able to use new and innovative software to improve the production of medicines. The Food and Drug Administration (FDA) guidance for DI provides excellent guidance on this matter (e.g., Data Integrity and Compliance with Drug CGMP, 2018).

22. If an enterprise resource planning (ERP) and a laboratory information management system (LIMS) are installed, what is the impact or non-compliance when the material status in the two systems may not be in synch? (e.g., in LIMS the material status was approved but in ERP the material status is still 'blocked' due to the delay in updating the material status from LIMS to ERP). How long will out-of-synch data be accepted by the national regulatory authority (NRA)?

It depends on the risks posed to patients by this discrepancy. If there is a risk of the release of a product without full evaluation, then that could be serious.

23. Does the need to distinguish QRM in medical devices (21 Code of Federal Regulation - CFR 820) from QRM in other areas exist? Are there specific requirements for QRM in medical devices that differ from the QRM practiced in other disciplines?

Yes, this is true, medical devices have very specific requirements for QRM (design control), while for pharmaceuticals, it is more flexible, but this flexibility does not mean the QRM is optional.

Session 6 Computer system validation

1. If computer system validation (CSV) of a laboratory information management system (LIMS) has already been conducted, does the need arise to perform CSV again for the backup system in the quality control (QC) Laboratory? Or could only the installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ) be undergone?

According to Section 7.2 of EU Annex 11 (EudraLex Good Manufacturing Practice: Medicinal Products for Human and Veterinary Use, Volume 4, Annex 11: Computerised Systems, 2011, regular back-ups of all relevant data should be done. Therefore, all analytical equipment on which electronic data is generated should be included in the data backup. Electronic data backup plays a crucial role in safeguarding critical information, complying with regulations, and supporting the operations and growth of the pharmaceutical industry's QC processes. Backups ensure that critical data is protected against loss or corruption, maintaining the integrity of QC records and test results. Pharmaceutical companies are required to have robust data backup procedures to ensure the validity and traceability of QC data. These processes should be verified when they are established and regularly tested regarding backup and restore capability. The integrity and accuracy of backup data and the ability to restore the data should be checked during validation and monitored periodically. In case electronic data back-up is conducted through LIMS or other software, back-up process verification testing should be part of the software validation. It is an acceptable common practice to combine testing of the backup process with testing of disaster recovery procedures. A possible reference is PDA TR80, 2018: Data Integrity Management for Pharmaceutical Laboratories.

2. Does Minitab statistical software need to be validated?

Refer to the EU Annex 11 (EudraLex Good Manufacturing Practice: Medicinal Products for Human and Veterinary Use, Volume 4, Annex 11: Computerised Systems, 2011, This reference states that "when computers or automated data processing systems are used as part of production or the quality system, the manufacturer shall validate computer software for its intended use according to an established protocol". The built-in functionality of Minitab, like other commercial-off-the-shelf (COTS) (Good automated manufacturing practices - GAMP category 3) software programs, is considered acceptable as far as validation. Risk assessment of the intended use of the software would be highly recommended, and according to the risk associated with the use, the required testing can be determined or skipped in case of negligible risk. Testing typically covers correct installation, tests that demonstrate fitness for intended use, and any further tests related to risks and supplier assessments. COTS software developers provide some testing you can perform to validate the program after installation. Like Excel, if macros are written within Minitab, those added functions should be validated.

3. When should CSV start for new equipment? What is the approach for existing equipment? Does retrospective validation apply?

CSV activities shall begin before machine procurement starts. It starts during the preparation for the software user requirement specifications (URS). An initial risk assessment should also be performed based on an understanding of processes, user requirements, regulatory requirements, and known functional areas. The results of this initial risk assessment should include a decision on whether the system is good practices (GxP) regulated

(i.e., GxP assessment). It also should include an overall assessment of the system's impact. High-impact systems require validation and verification. Typically, the deliverables of the validation exercise would encompass the URS, risk assessment document, design qualification (DQ), installation qualification (IQ), operational qualification (OQ), performance qualification (PQ), and finally the validation summary report (including user requirements traceability). In the case of a currently functioning system that has never been validated, URS shall be established, and a thorough risk assessment should be performed based on an understanding of processes, user requirements, regulatory requirements, and known functional areas. The results of this risk assessment should include a decision on whether the system is GxP-regulated.

In case gaps identified in a legacy system design prove to be of low risk based on the system´s history, stability, number of failures, and vendor risk, then verification (e.g., OQ or functional testing) may not be necessary. It needs to be objectively demonstrated and documented that the system operated as it was designed to do. As such, efforts could be placed on the performance qualification (PQ). The PQ (i.e., user acceptance or intended use testing) always falls upon the end-user organisation to perform. The current expectation is to perform prospective validation; retrospective validation is no longer accepted by authorities.

4. Should a spreadsheet utilised for internal stock card management in the Quality Control (QC) laboratory undergo validation, considering the presence of a manual stock card maintained in a written format?

Regulated companies shall adhere to regulatory guidance in which they should focus on data governance: the design, operation, and monitoring of the workflow in which this spreadsheet is used. Unless the spreadsheet is adequately controlled, it may be advisable to consider a paper printout as the master record. When Excel spreadsheets are used solely to produce paper documents like a word processing document rather than a traditional application to record and manipulate GxP data or just as templates, it is advisable to manage them as documents rather than applications. This includes establishing an appropriate level of security conditions to be maintained, including password protection and secure storage.

5. Is it necessary to validate the backup server system in the QC laboratory?

In general, servers hold good manufacturing practices (GMP)-regulated data and therefore become regulated servers that must be managed and operated according to regulatory guidelines on validation and must therefore be qualified. It is critical to ensure that the server is secured with appropriate access controls that are managed by company policies. A risk assessment should be performed to assess infrastructure including but not limited to consideration for complexities, potential miscommunications, and vulnerabilities. The extent of the qualification and verification testing will be based on the identified risks associated with the IT infrastructure that could directly or indirectly affect product quality, safety, data, and information. The control measures for system-critical components including but not limited to servers should be included, verified, and documented within the installation qualification (IQ) process.

6. Does a spreadsheet used to monitor key performance indicators (KPI) need to be validated? All data uploaded on this spreadsheet comes from a system, however, it needs to be input by an operator.

Regulated companies shall adhere to regulatory guidance in which they should focus on data governance: the design,

operation, and monitoring of the workflow in which this spreadsheet is used. Spreadsheets fall into GAMP category 3, so they do not require full validation but should be held under appropriate security and accuracy controls like password protection, secure storage, access to the sheet and data by users and developers as well as data input methods. When Excel spreadsheets are used for the development of template solutions, where data can be subjected to a standard manipulation and the result saved as a unique document or statistical analysis or data mining applications are used to facilitate the decision-making process, it is critical to validate those Excel spreadsheets for their intended use to ensure the information is accurate, consistent, complete, and true.

7. What could be the period needed for revalidation? And for how long should data from computer system validation (CSV) be archived?

There is no explicit frequency and scope of the system periodic reviews mentioned in guidance documents. It depends on a variety of factors, such as the criticality and complexity of the system, the risk of errors or failures, the frequency and nature of changes, and regulatory expectations. Moreover, according to WHO TRS 1019, 2019, annex 3; Good Manufacturing Practices: Guidelines on Validation, as a principle, ongoing review should take place, to ensure that the qualified or validated state is maintained and opportunities for continuing improvement are identified. Therefore, it is expected that even if the site has defined a timeframe for re-evaluation of the validated state, that does not preclude them from adhering to the constant lifecycle approach to workflow validation outlined in the guidance.
According to WHO TRS, No. 1044, 2022. Annex 5; WHO Good Manufacturing Practices for Medicinal Gases (clause 6.8), records should be made or completed when any action is taken and in such a way that all significant activities concerning the manufacture of pharmaceutical products are traceable. Records should be retained for at least one year after the expiry date of the finished product. Hence, as in process validation reports, equipment IQ, OQ and PQ reports and supporting systems including utilities and computerised systems related directly or indirectly to the finished product lifecycle shall be maintained for the product lifecycle, plus one year.

8. What is the need for audit trail review for corporate and information technology (IT) systems such as document management systems (DMS)?

According to EudraLex Volume 4 Annex 11: Computerized Systems, 2011, consideration should be given, based on a risk assessment, to building into the system the creation of a record of all GMP-relevant changes and deletions (a system-generated "audit trail"). For change or deletion of GMP-relevant data, the reason should be documented. Audit trails need to be available and convertible to a generally intelligible form and regularly reviewed. The audit trail is a crucial component of a document management system. Electronic data management system (EDMS) oversees electronic GXP-related documents, records, and workflows. They are vital for maintaining controlled and organised documentation, including standard operating procedures (SOPs), batch records, and regulatory submissions. CSV ensures that documents are securely stored, accessible, and in compliance with version control. This provides a framework to consistently assess the risk to data integrity and perform standardised reassessments as the systems and processes change and evolve.

9. Do software formulas used for calculation need to be validated

through a manually validated calculator like high-pressure liquid chromatography (HPLC) formulas for resolution, structural simulation toolkit (SST), etc.?

If the software is purchased off-the-shelf and calculation formulas do not require configuration, or where the default configuration is used by the regulated company, the product should be developed and maintained by the supplier in accordance with GxP and the supplier should be involved typically with the provision of documentation, training, support, and maintenance.

10. For production equipment, if software features like access authorization, audit trail, etc., are not present, what actions would be deemed acceptable regarding CSV?

In the case the software controlling production equipment is not supported with controlling software, it is recommended to start by establishing a URS for the system followed by a thorough risk assessment and a criticality assessment of each requirement to understand how the system was informally being kept in control. It is important to identify gaps and their impact on patient safety, PQ, and/or processes. According to the identified risks, actions shall be taken. If the risk is medium or high, actions shall be taken either to upgrade the system - if practical- or replace it. If the risk is neglectable, the company may just verify system functionality.

11. What would be the evidence or raw data of all the tests being performed during the whole validation? Screenshots or photos of all the tests?

During the execution of validation activities, the process of conducting the actual validation processes (VPs) should be outlined in the approved validation documents. This ensures that the system or software meets its intended specifications and functions as expected. This involves the execution of verification testing following approved test scripts. During test script execution, data is collected to document the outcomes of the tests. Data collected includes observations, measurements, screenshots, and any deviations encountered as hardcopy test evidence. Test evidence may also be retained electronically providing adequate security and retention mechanisms are established.

12. How could CSV failures and data loss in case of disasters be handled?

WHO TRS1019, 2019, Annex 3, Good Manufacturing Practice; guideline on Validation, Appendix 5 Validation of Computerized Systems, requires a documented business continuity plan and disaster recovery plan; a documented process or set of procedures to recover and protect a business information technology (IT) infrastructure, in any event, causing the system to be unavailable. It appropriately defines resources and actions to be taken before, during, and after a disaster, to return the system to operational use.
This plan is expected to establish a comprehensive strategy that outlines how an organisation will continue to operate during and after disruptive events, such as natural disasters, power outages, cyberattacks, or other emergencies. It encompasses not only data recovery but also overall business processes, resources, personnel, and communication strategies. This plan includes key components like data classification according to their criticality and accordingly priorities for backup, restoration, and archival efforts. It also involves the description of backup procedures, frequency, locations, protection, data retention, and restoration policies. It is also expected to outline the steps to be taken when a data loss or disruption occurs. It defines the roles and responsibilities of individuals involved in

responding to incidents and how to regularly conduct testing to ensure that all stakeholders are familiar with their roles and responsibilities during a crisis. Regulated companies shall prepare plans collectively contributing to safeguarding critical data and enabling a quick recovery from unforeseen disruptions. In the pharmaceutical industry, data integrity and patient safety are paramount.

13. Is requalification necessary when there is a change in equipment motherboards?

Among the qualification objectives is to demonstrate that an IT component works, or that a software functions as it was designed. Qualification documents the result of direct measurements and observations that prove a piece of hardware or software was installed correctly in compliance with the requirements and that it functions in conformance to a design parameter. Any repair or replacement of defective computerised system components, typically hardware or infrastructure-related, should be managed following a defined process. The extent of documentation and verification is to be scaled based on the nature, risk, impact, and complexity of the change. Generic like-to-like repair and replacement activities at a high level are likely to require documentation.

14. What should be the vendor qualification criteria for laboratory equipment like HPLC? Is the reputation enough or should an audit be conducted?

Supplier qualification follows the risk-based approach dictated by the GMP guidelines that there is a certain obligation to audit other critical suppliers. Regulated companies should ensure that suppliers are made aware of the need for regulatory compliance. This requires understanding the various risks and the related threats and vulnerabilities to identify potential risk and their impact and how to manage and/or mitigate them. The regulated company should verify, before contract placement, that the supplier has adequate expertise and resources to support user requirements and expectations. It should also verify the supplier´s QMS and how it is implemented for a particular product, application, or service.
The decision whether to perform a supplier audit should be documented and based on a risk assessment. During the audit, the team shall engage in cross-functional discussions to identify risks, vulnerabilities, implications, and action plans, and gather evidence. Collect relevant documents and assess the effective implementation of the supplier quality management system (QMS).

15. What should be the criteria for qualification and periodic qualification of small laboratory and production equipment like balances, pH meters, etc.?

Generally, the extent of qualification depends on the instrument's functionality and complexity. Hence, for the least complex, standard instruments that do not have measurement capabilities like vortex mixers, it is acceptable to verify the proper functioning of the instrument by observation. For simple standard measuring instruments like pH meters, calibration, and performance checks without extensive qualification activities would be also acceptable. Unlikely, analytical instrumentation with a significant degree of computerization and complexity like HPLC, requires all phases of qualification activities including but not limited to establishing URS, DQ, IQ, OQ, and PQ in addition to software validation activities.
Additionally, the instrument´s intended use shall be taken into consideration, for example, the use of a pH meter to analyse a critical quality attribute (CQA) will require more effort than the same instrument used to collect a non-critical pH value.

16. Does the laboratory equipment require DQ?

DQ aims to establish a high degree of assurance that the instrument was procured with due consideration of the requirements of GMP, good laboratory practices (GLP), or International Organization for Standardization (ISO) 17025 General Requirements for the Competence of Testing and Calibration Laboratories, 2017, as appropriate. This entails establishing appropriate URS and converting these into functional requirement specifications (FRS) and operational specifications. Design specifications must be documented and approved in a DQ before the purchase or installation of custom analytical instruments and lab equipment.

17. What is the expectation for enterprise resource planning (ERP) system periodic qualification?

The periodic review is explicitly requested by EU GMP Annex 11 (EudraLex Good Manufacturing Practice: Medicinal Products for Human and Veterinary Use, Volume 4, Annex 11: Computerised Systems, 2011), section 11: "Computerised systems should be periodically evaluated to confirm that they remain in a valid state and are compliant with GMP. Such evaluations should include, where appropriate, the current range of functionality, deviation records, incidents, problems, upgrade history, performance, reliability, security, and validation status reports".
Indications on how to carry out the periodic review are given in Appendix O8 of the GAMP5 Guidelines- ISPE GAMP 5, A Risk-Based Approach to Compliant GxP Computerized Systems, 2008. A periodic review must be carried out according to the established procedure that defines the timing and scheduling of reviews according to a documented risk criterion that considers the degree of criticality of the system (GxP impact), its complexity, and its degree of novelty. Furthermore, it includes problems encountered in the operation of the system or significant or several minor changes made to the system.

It is also expected that ongoing monitoring including reviewing system logs, audit trails, and user access records to identify any anomalies or deviations from the expected behaviour, also helps to confirm that the system continues to operate as intended after the change. Regular periodic reviews and monitoring of the system's performance, data integrity, and compliance should be fully documented.

18. What should be the retention time for electronic data of the lab?

WHO guidance of finished products regarding the unified requirement for paper-based and electronic records states: "Records should be made or completed when any action is taken and in such a way that all significant activities concerning the manufacture of pharmaceutical products are traceable. Records should be retained for at least one year after the expiry date of the finished product. Hence, any laboratory documentation relating to batch processing and/or testing should be retained for one year after the expiry date of the batch. Other documents like process validation reports, equipment/instruments IQ, OQ and PQ reports, and supporting systems including utilities and computerized systems related directly or indirectly to the finished product lifecycle shall be maintained for the product lifecycle + 1 year. Any data collected to support a regulatory application must be retained until the product is retired.

19. Is the infrastructure application subject to inspection?

In addition to the CSV and associated documentation that should be ready for regulatory audits and authorities' inspections, IT IQ is also a regulatory requirement according to regulations like FDA 21 CFR. Part 11 Code of Federal

Regulations - Guidance for Industry, Electronic Records; Electronic Signatures — Scope and Application, 2003. EU Good Manufacturing Practice: Medicinal Products for Human and Veterinary Use, Volume 4, Annex 11: Computerised Systems, 2011, requires the same: "The application should be validated; IT infrastructure should be qualified". Hence, it is expected that inspectors may inspect IT infrastructure like cabling, network cabinets and connectors, active network components, peripheral devices, buildings and premises, backup, and archiving, etc. as part of the inspection of computer-based systems to verify system design and maintenance.

20. How is a cloud service provider audited (whether for software as a system - SaaS, platform as a service - PaaS, or infrastructure as a service - IaaS)?

Regulated companies should ensure that suppliers are made aware of the need for regulatory compliance. This requires understanding the various cloud models, cloud computing risks, and the related threats and vulnerabilities to identify potential risk and their impact and how to manage and/or mitigate them. Among important aspects are security, privacy, data integrity, contractual clarity and protections, business continuity, process and system reliability, effectiveness/efficiency of new business processes, configuration management, compliance with cross-jurisdictional regulations, etc. The regulated company should verify, before contract placement, that the supplier has adequate expertise and resources to support user requirements and expectations, the supplier´s QMS, and how it will be implemented for a particular product, application, or service. The decision whether to perform a supplier audit should be documented and based on risk assessment. During the audit, the team shall engage in cross-functional discussions to identify risks, vulnerabilities, implications, and action plans, and gather evidence. Collect relevant documents and assess the effective implementation of the supplier QMS.

21. Is there a reasonable period to complete the validation? For example, is it acceptable if a 2-year timeframe is set to finish the CSV?

It is required that software validation is completed, accepted, and approved before formal handover for live operation. This is essential to ensure that the system will function as intended. There is no explicit requirement or standard for the time duration of the validation exercise. Yet the validation plan shall cover all required activities and their timelines.

Session 7 Contamination control strategy, environmental monitoring, cleanroom qualification

1. Regarding terminally sterilised products, is it mandatory to perform continuous particle monitoring during the filling process especially if the filling process is performed in class A?

For any grade A area used for aseptic fill-finish processing, particle monitoring should be undertaken for the full duration of critical processing, including equipment assembly. The same concept is underlined also in TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clause 9.24. Terminally sterilised products are normally filled in a grade C environment but if unusually at risk, grade A (in a C background) protection should be considered. The decision and justification of the use of a grade A air supply for filling and the monitoring thereof should be justified in the contamination control strategy (CCS). The CCS should consider risks from a non-viable particulate and microbial perspective, and support the sampling frequency, locations, and methods of monitoring and control. Refer to TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022 clauses 9.2; 9.3.

2. Regarding facility design principles for closed processing, is it acceptable to reduce the cleanroom classification principles based on quality risk management (QRM)?

In general, it is not acceptable to reduce the cleanroom classification principles based on QRM but, considering "closed processing" and the design of the system, for example, TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, paragraph 8.10, Table 4, allows for a minimum of grade D for the assembly of closed and sterilised single-use systems (SUS) using intrinsic sterile connection devices. Paragraph 8.130 clearly states that "for aseptic processing (AP) and where there are any risks that system integrity may be compromised, the system should be located in grade A. If the system can be shown to remain integral at every usage (e.g., via pressure testing and/or monitoring) then a lower classified area may be used". According to paragraph 8.137, SUS should be designed to maintain integrity throughout processing under the intended operational conditions.

3. What are the differences in performing ongoing monitoring versus periodic monitoring?

Monitoring must demonstrate that the design and procedures have been correctly implemented and continue to perform in line with expectations (TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clauses 2.1.i.; 2.2). The frequency of monitoring and control must be included and defined in the CCS. Ongoing monitoring should be identified and described as well as periodic review, resulting in updates to the quality system as appropriate. Monitoring methods are based on risk assessment. Any item (instrument or monitoring method) used must be assessed considering potential routes of contamination and must be included in the environmental monitoring program.

4. Is particle monitoring required during sterility tests in an isolator?

Particle monitoring is required inside the isolator even if for sterility testing. The environmental monitoring (EM) results are part of the batch release and fundamental

for an adequate investigation in the case needed.

5. Should active monitoring of viable particles by the volumetric method be carried out continuously during aseptic filling?

The new TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, emphasises the importance of continuous monitoring during processing. Where aseptic operations are performed, microbial monitoring should be frequent using a combination of methods such as settling plates, and volumetric methods (clause 9.22). Monitoring sample volumes should be justified (clause 9.21).

6. Will the routine period decision (risk-based analysis) be questioned or challenged by the inspectors?

Yes. There is a need to have a detailed rationale for that decision because it is the "mandatory requirement" to describe any precaution adopted to minimise the risks of contamination, and this is based on risk assessment and risk management. Inspectors will want to understand that rationale. The principles of quality risk management should be applied to all sections of this document (TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clauses 1.; 2).

7. Regarding personnel disqualification, is a single excursion of gloved hand monitoring grounds for disqualification?

Personnel must be qualified for operational activities. There should be systems in place for the disqualification of personnel from working in or given unsupervised entry into cleanrooms that are based on specified aspects, including ongoing assessment or identification of an adverse trend from the personnel monitoring program or implication in a failed aseptic process simulation (APS) as per TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clause 7.6). The cause of the excursion needs to be assessed and resolved.

8. In the gowning process, is there a specific requirement for the sequence, such as starting from top to bottom, or is the reverse, from bottom to top, also considered acceptable?

The higher risk of contamination must be assessed and considered. Compliance with aseptic gowning procedures should be confirmed by assessment and periodic reassessment at least annually that should involve both visual and microbial assessment using monitoring locations such as gloved fingers, forearms, chest, and hood (face mask and forehead). As per TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clause 7.10, cleanroom gowning should follow a written procedure designed to minimise contamination of cleanroom clothing or the transfer of contaminants to the clean areas.

9. What would be an acceptable sampling duration and rationale for settling plates?

Not more than 4 hours is indicated. However, the sampling duration may depend on the applied media and the environmental conditions. Desiccation could have an impact on the recovery of microorganisms.

10. What part of the operator gowning needs to be swabbed?

All gowning areas must be properly monitored. Referring to gloves, all the uneven parts between the fingers must be swabbed, not only the fingers' top part or just the centre of the palm. All other

locations need to be considered depending upon risk (e.g., neck, zip, etc.), and the sampling rationale should be discussed and should be justified in the CCS.

11. What are the acceptable methods for bringing paper batch records into the clean room area (what sanitation methods are acceptable)?

Decontamination and preferably sterilisation methods must be described in the CCS, and the choice must be supported by a validation study. PDA TR13-2 Fundamentals of EM Program, Annex 1: EM of Facilities Manufacturing Low Bioburden Products, 2020, and TR90 Contamination Control Strategy Development in Pharmaceutical Manufacturing, 2023, could be good references. Normally steam sterilisable paper is used but is increasingly superseded by electronic batch records (e.g., Manufacturing execution systems - MES).

12. At which stage or processing area, should full flora identification be conducted?

During cleanroom qualification, identification of the microbiota isolated during baseline sampling and the environmental monitoring performance qualification (EMPQ) should be conducted. It is critical to obtain accurate species-level identifications during this process and during routine monitoring to fully understand your plant's microbiota and facilitate microbial investigations. Refer to EU Annex 1 Manufacture of Sterile Medicinal Products, 2022, and PDA Technical Report # 13-2 Fundamentals of EM Program, Annex 1: EM of Facilities Manufacturing Low Bioburden Products, 2020 (Revised 2022) for additional information. Risk assessments may justify the need for periodic evaluations of systems and materials. In these cases, culture-based methods may not be suitable for these microbial surveys due to labour and materials costs and the low-throughput nature of culturing microorganisms. Advanced methods such as next-generation sequencing may be used for a cost-effective approach to periodic microbial surveys, with the distinct advantage of being able to detect microorganisms that are unculturable or difficult to culture in a typical pharmaceutical microbiology laboratory.

13. If there is a power failure that impacts the clean room operation, what needs to be done to decide if the clean room is ready or suitable for operation again after the power is restored?

Air pressure differentials identified as critical should be continuously monitored and maintained. All non-conformities (failure or outages) or deviations from established procedures, should be adequately investigated before certification or release of the batch. The investigation should determine the potential impact on process and product quality and whether any other processes or batches are potentially impacted. Root cause analysis of power failures, including procedures, processes, or equipment, is required to correctly identify and understand while ensuring that appropriate corrective and preventive actions are implemented. (TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, 3.1.iii).

14. Is "trending" performed on a product-specific basis, or is it a requirement for all products, such as trending environmental monitoring results?

Trending should be done product-specific in the case of single-product facilities, and as part of an overall trending activity included in your CCS for multi-product facilities. You could refer to PDA TR90 Contamination Control Strategy Development in Pharmaceutical Manufacturing, 2023.

15. Is it required to do a microbial flora study based on seasons (e.g., summer and winter) to establish the microbial flora baseline?

Yes. In the updated TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, it is a minimum requirement to know the typical microbial flora isolated from the environment (clause 9.4). This includes changes in microbial flora type and numbers, and the predominance of specific organisms, such as spore-forming and mould (particularly critical to properly identify). Depending on the location of the manufacturing facility, seasons will have a significant impact on the microbial flora in clean rooms. This impact needs to be assessed.

16. Why the absence of fungi in a class A/B cleanroom is not mentioned in environmental monitoring guidelines considering that it is usually not tolerable in cleanrooms?

In some way, it is specified, because in cleanroom class A, no count of a microorganism is acceptable. That includes fungi. Any detection of a microorganism should result in an investigation. Any organism found must be identified at the species level, as well as for Class B (any of the detected viable microorganisms must be identified at the species level). The type of organism found in these cleanrooms as well as class C and D must be assessed versus the risk to the product and patients (TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clause 9.4). Furthermore paragraph 9.11. states that "changes in microbial flora type and numbers and predominance of specific organisms, paying particular attention to organisms recovered that may indicate a loss of control or deterioration in cleanliness or organisms that may be difficult to control such as spore-forming microorganisms and moulds".

17. Is there any microbial monitoring requirement for interlocks, pass-through boxes, etc.?

The monitoring requirements are not related to a specific location (unless described otherwise) but more to the "classification" of the area/location. For example: if interlocks are defined as clean room class C, they need to be monitored like any other clean room classified as C.

18. Is there a checklist helping to determine the location of monitoring plates in the clean room area?

You can find Points-To-Consider for assessing and determining a suitable risk-based monitoring program in the Pharmaceutical and Health Care Sciences Society (PHSS) guideline; ISO 14644 (2015) series of standards, Cleanrooms and Associated Controlled Environments guideline; and the WHO Annex 8 TR number 1010, 2018.

19. What are the main points to consider in the qualification for a restricted access barrier system (RABS), and what are the routine checks?

Any control and minimum points to consider for a RABS depend on facility design. The minimum requirement is "surrounding B", and any requirement referring to that must be respected. The following sections must be considered: TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clauses 4.4; 4.19-4.23; 4.32; 8.13; 8.18; 8.29.

20. Is there a difference between requalification and revalidation activities of a clean room?

Yes. "Qualification is a method of assessing the level of compliance of a classified cleanroom or clean air equipment with its

intended use", and validation is a series of defined activities that you must follow and perform as indicated in WHO TRS 1019, Annex 3, Annex 3 Good manufacturing practices: guidelines on validation, 2019. Please refer to TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022.

21. Are closed Restricted Access Barrier Systems (cRABs) considered sealed areas (same as isolators)?

cRABS are systems that provide an enclosed environment. But they are not fully sealed.

22. For the isolator gloves, what is the frequency of pinhole checks?

Everything depends on procedure and use. A justification for the frequency must be included in the CCS, based on a worst-case approach to ensure minimising the risk of contamination. Inspectors are very concerned because holes in gloves are one of the major risks to consider for microbial contamination considering that the surrounding area of isolators is usually a C or D environment. Specifically, TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, paragraph 4.21 (i) states that "for isolators, leak testing of the glove system should be performed using a methodology demonstrated to be suitable for the task and criticality. The testing should be performed at defined intervals. Generally, glove integrity testing should be performed at a minimum frequency at the beginning and end of each batch or campaign. Additional glove integrity testing may be necessary, depending on the validated campaign length".

23. What are the main differences between open and closed isolators used in aseptic filling? (e.g., which is the background area classification for an open isolator?)

Isolators and RABS, which are different barrier technologies, should be installed in a suitable surrounding environment. Where an isolator or RABS is used, the background should be in accordance with TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clauses 4.20 and 8.13. The background environment for open isolators should generally correspond to a minimum of grade C. The background for closed isolators should correspond to a minimum of grade D. The decision on the background classification should be based on risk assessment and justified in the CCS (clause 4.20.i.a-i.b).

24. If there's a change in the growth media supplier, does the growth media need to be requalified (although the specifications are the same)?

Any selected nutrient medium either for bioburden or for sterility testing must be validated for the intended use, to demonstrate the capability to support the growth of a designated group of reference microorganisms, as described by the relevant pharmacopeia, and representative local isolates (TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clauses 9.36.4; 9.36.6; 9.43; 10.9; 6.10). Specifications are very similar, if not identical, from different suppliers. However, the end user must validate the media under their conditions versus product and production.

25. For filling machines with RABS in the aseptic filling procedure, can the filling machine doors be opened to add machine parts before starting the filling procedure?

Adding any item or material to the filling line must follow a controlled process. All

activities are put in place to minimise the risk of contamination. If any changes or deviations happen, the impact on potential product contamination must be assessed. The impact should be determined through risk assessment and documented as part of the CCS (TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clause 6.1). The aseptic process should be clearly defined. The risks associated with the aseptic process, and any associated requirements, should be identified, assessed, and appropriately controlled. The site's CCS should clearly define the acceptance criteria for these controls, requirements for monitoring, and the review of their effectiveness (clause 8.7).

26. When is a cleanroom integrity study required?

A cleanroom must be installed based on quality-by-design (QbD) and the effectiveness should be verified and regularly controlled. If integrity refers to a closed system like a closed isolator, for instance, the verification of the validation study (Performance qualification - PQ after installation qualification - IQ and operational qualification - OQ) must be performed following the supplier/system specification. In addition, some cleanrooms where highly pathogenic organisms of biological safety level (BSL) 4 or very high-risk organisms (e.g., Polio type 2 virus), are handled in absolute negative pressure rooms environments to prevent them from escaping the bio-secured zone. In these cases, containment must be proven.

27. How can the testing frequency through risk assessment be established? If the outcome of the risk assessment differs from established regulations or guidelines, such as the recommended frequency for a validation test, is such a deviation considered acceptable?

Unless a requirement is clearly defined, like for instance the requalification period, all other frequencies must be defined based on risk assessment (refer to ICH Q9(R1), Quality Risk Management, 2023, and for some practical examples, and PDA TR # 90 Contamination Control Strategy Development in Pharmaceutical Manufacturing, 2023). Multiple approaches and different statistical tools are available and can be used.

28. What is the rationale for removing specification for 5 µm particles in a grade A environment?

It is removed only for qualification and harmonised with ISO 14644 (2015) series of standards, Cleanrooms and Associated Controlled Environments. But it is kept for monitoring.

29. According to the updated guidelines regarding aseptic filling, are RABS mandatory for old filling machines?

Where possible, the use of equipment such as RABS, isolators, or other systems should be considered to reduce the need for critical interventions in grade A and to minimise the risk of contamination. There should be a program to mitigate any risk by uninstalling barriers to old lines and, where not possible to mitigate the risk, the lines should be scheduled for replacement.
Robotics and automation of processes can also be considered to eliminate direct human critical interventions (TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, clause 8.9). For instance, where human intervention is required at the capping station, appropriate technological and organisational measures should be used to prevent direct contact with the vials and to minimise contamination. RABS and

isolators may be beneficial in assuring the required conditions (clause 8.29).

30. What are the parameters considered for the qualification and periodic review of RABS?

For RABS, the parameters needed for periodic review are related to the barrier system protecting the aseptic process. These points need to be reviewed and checked in addition to six monthly high efficiency particulate air (HEPA) filter integrity tests:

- Airflow visualisation
- Airflow in the RABS
- Air velocity
- Total particles
- Viable particles
- Microbial contamination on the equipment parts after sanitisation.

31. How often should the non-high-risk areas be monitored?

Non-high-risk areas should be monitored periodically, not continuously as in grade A or grade B, based on the risk. It can be once a day for Grade D.

32. What is the difference between clean room classification and clean room qualification?

Room classification is carried out to define and check the system of heating, ventilation, and air conditioning (HVAC) is delivering air with the right number of particles of different sizes. This must be performed when installing the HVAC system. The purpose of classification is to confirm the level of air cleanliness against a specification by measuring the particle concentration. Clean room classification is part of the qualification. Qualification is the overall process of confirming the level of compliance of a classified cleanroom or clean air equipment.

33. Is it acceptable to have different classes at rest and in activity for the same cleanroom?

A cleanroom has one air grade class with 2 different specifications: in operation and at rest as defined in TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022.

34. For EM for aseptic processing, what kind of trending data is needed?

For aseptic processing, the system of particle counts 0,5 µm, 5 µm, bacterial count, room pressure, and room temperature will be monitored. These data are used for batch release and can be trended to have a good understanding of what is happening in the clean area and anticipate some out-of-specifications (OOS) based on trends of compliant information.

35. Does WHO also require CCS to be in place and available for inspections as EU Annex 1 Manufacture of Sterile Medicinal Products, 2022, requires?

TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022 / EU Annex 1 Manufacture of Sterile Medicinal Products, 2022, and PIC/S texts are now harmonised. They differ only in minor editing and phraseology, and all require having in place a CCS.

36. Does CCS documentation have to be detailed or make appropriate referrals to relevant procedures within the quality management system (QMS)?

The answer to this question relates to the organisation of the company. Some are revising 100% of their QMS. It is recommended, based on QRM, to have an umbrella document to define the CCS policy in the company and where are the documents located. This umbrella document shows how the different aspects of CCS interrelate, starting with the facility design,

and process knowledge, up to manufacturing.

37. Is material monitoring integrated into the CCS alongside equipment and personnel?

As identified in TRS 1044, Annex 2 WHO GMP for sterile products, 2022, clause 2.5, all these points need to be covered by the CCS. Material monitoring (e.g., primary packaging), and risk assessment are part of the CCS as well as the personnel involved. The list in the annex is not exhaustive. For example, for the handling of sterile biological drugs additional contamination risks such as host cell proteins, adventitious virus or mycoplasma need to be considered in addition to the items listed.

38. During facility audits, what specific records should be examined in relation to CCS?

In TRS 1044, Annex 2 WHO GMP for sterile products, 2022, clause 2.5, there is a list of all items required to be considered by CCS. The first document that could be audited or reviewed is the umbrella document describing the aseptic process validation (APV) as part of the CCS.

39. What is the expectation for the development of CCS for bioburden-controlled processes such as in drug substance (DS) manufacturing?

CCS is not mandatory for non-sterile products, but it is recommended where the control and reduction of microbial contamination is considered important (see also USP <1115> Bioburden Control of Nonsterile Drug Substances Products). The manufacturer should document which principles have been applied and acknowledge that compliance with those principles should be demonstrated. In the case of low-bioburden biological bulk substance, the CCS is considered mandatory in upstream processing including purification.

40. What is the relationship between process validation (PV) and CCS?

Aseptic process validation (APV) is part of the CCS.

Session 8 Filter integrity – Pre-use, post-sterilisation integrity test – Aseptic process simulation

1. **Which filter should be incorporated in pre-use, post-sterilisation integrity test (PUPSIT)? Is the filter in the filtration room, or the one placed just before filling?**

In the case of having redundant filtration, the main filter (e.g., the one closest to the filling point) should undergo PUPSIT. It is advisable to perform PUPSIT on the second filter as well to have the chance to be able to initiate the filling process after a PUSPSIT failure of the main filter, and a successful PUPSIT of the redundant filter.

2. **How are the limits for the filter integrity test (FIT) determined?**

The FIT limit value (e.g., bubble point) is determined by the vendor for each type of filter if water is used, or by validation studies when the product is used.

3. **In post-use FIT, why is water needed for wetting since the filter is already wetted with the product?**

To perform FIT the filter must be fully wetted either with water or product. This decision should be fully justified in a documented manner, considering the feasibility of doing the test, the nature of the product, and its cost.

4. **For introducing PUPSIT to the filling procedure, which tests should be done to validate the new filling procedure?**

A series of at least three consecutive successful media fills must be performed including the PUPSIT operation before starting commercial manufacturing.

5. **What should be the frequency for the FIT of vent filters?**

Vent filters used as part of the product FIT system should be integrity tested at least at the end of the filtration process. "The integrity of critical sterile gas and air vent filters (that are directly linked to the sterility of the product) should be verified by testing after use, with the filter remaining in the filter assembly or housing" (refer to WHO TRS 1044, Annex 2 Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clause 8.88).

6. **How are PUPSIT setting parameters defined if we use the product as a wetting agent?**

PUPSIT operational parameters must be based on the specific batch process (BP) or downstream process (DTP) of the product used. This determination must be made per product and is usually performed by the filter vendor.

7. **If alarm sensors are placed in the outlet of filters, can the FIT frequency be reduced?**

All sterilising grade filters used during the product filtration process must undergo a FIT in each batch manufactured regardless of the technology used.

8. **Should a sterilisation grade filter be single-use, or can it be reused if it passes the FIT? Can PUPSIT be performed at the beginning and end of the campaign, or does it have to be done for individual batches?**

As mentioned in WHO TRS 1044, Annex 2 Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clause 8.94, "Liquid sterilizing grade filters should be discarded after the processing of a single batch and the same filter should not be used continuously for more than one working day unless such use has been validated".
Typically, for campaign production, filters may be used for an extended period with the same product. This practice should be fully validated as per section 8.95 of the above mentioned TRS.

9. **The manufacturer certificate usually provides the maximum allowed**

temperature for sterilisation of the filter, so how could a filter be damaged?

Despite having a vendor´s recommendation on sterilisation conditions, the actual sterilisation process used may still affect the structure of the filter (e.g., equipment differences, cycle operational parameters such as vacuum pulses, manipulation during the autoclaving process, etc.). Thus, PUPSIT will ensure that this and other factors have not altered the filter.

10. Which are examples of alternative approaches to PUPSIT mentioned in WHO TRS 1044, Annex 2 (clause 8.87)?

The alternative approach mentioned in WHO TRS 1044, Annex 2 Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clause 8.87, refers to not performing PUPSIT if it is clearly justified in a documented manner based on quality risk management (QRM) that performing PUPSIT is not feasible due to the inherent characteristics of the product and the associated risks (refer to WHO 1044, Annex 2, section 4.21.b).

11. Is sterile gas and water required for PUPSIT? What is the applicability of PUPSIT for gas filters?

No sterile gas or water is required to perform PUPSIT upstream of the product sterilising grade filter, however, consideration should be given to using prefilters to reduce the bioburden. It is noted that sterilising grade vent filters are required after the product sterilising grade filter. As per WHO TRS 1044, Annex 2 Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, section 8.88, "The integrity of critical sterile gas and air vent filters (that are directly linked to the sterility of the product) should be verified by testing after use, with the filter remaining in the filter assembly or housing". A typical consideration for using sterile gas and water for PUPSIT is that when PUPSIT is performed during aseptic process simulation (APS), the integrity test execution can lead to contamination growth upstream of the filtration system.

12. How can PUPSIT be conducted without posing a risk of contamination to the already sterilised line?

A comprehensive documented QRM must be conducted to assess the risks involved in the implementation of PUPSIT.

13. What is the relationship between the pressure hold/leak test and FIT? Do these tests need to be done simultaneously or can only one of them be conducted?

The FIT must be performed whenever there is a sterilising grade filter involved. The pressure hold/leak test needs to be performed for closed systems. As per WHO TRS 1044, Annex 2 Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, sections 8.129 and 8.137, "The appropriate measures should be in place to ensure the integrity of components used in aseptic connections. How this is achieved should be determined and captured in the CCS. The appropriate system integrity tests should be considered when there is a risk of compromising product sterility. The supplier assessment should include the collation of data concerning potential failure modes that may lead to a loss of system sterility" and SUS "should be designed to maintain integrity throughout processing under the intended operational conditions".

14. In the case of oily products sterilised by filtration it may be difficult to do a diffusive integrity test (DIT) before filtration, then, will BP be acceptable?

The decision to use DIT or BP test depends on the given vendor´s specification which usually will include one value or the other. If the type of test is not considered in the vendor´s specifications,

the required test specifications need to be determined by the vendor of the filter.

15. What is the risk of filter damage during the FIT?

The risk of damage to the filter must be determined case by case and is related to many factors such as the degree of manipulation during transport, handling, storage conditions, and the selected sterilisation process.

16. With every part and material (e.g., filter, equipment, etc.) there's always the "AGING" factor whereby the material may experience physical changes (e.g., metal fatigue). Can a mean time between failure (MTBF) be established to aid risk analysis and replace the filter before it fails?

A preventive and predictive maintenance approach is encouraged to avoid deviations and product loss during the aseptic process including the filtration system (e.g., filter stainless steel housing, "O" rings, etc.).

17. Can PUPSIT be done offline?

The filtration system should be designed to permit in-place integrity testing of the 0.22 µm final sterilising grade filter, preferably as a closed system, both before and following filtration as necessary; in-place integrity testing methods should be selected to avoid any adverse impact on the quality of the product. (WHO TRS 1044, Annex 2 Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clause 8.82).

18. Is PUPSIT required for sterile products manufactured by terminal sterilisation?

PUPSIT is a requirement when sterilising the product using sterilising grade filters. Therefore, it is not mandatory for terminally sterilised products, where it would be optional to perform. However, bioburden reduction filters (e.g., 0.45 µm) used for terminally sterilised products require a FIT.

19. What would be the approach to FIT being repeated due to failed results?

An integrity test failure does not automatically mean that the filter cartridge has a defect. The failure needs to be handled as per procedure to determine the cause of filter damage or test problem, and a retest is possible. For example, a failure could be a consequence of a non-complete wetting of the filter membrane. If the failure persists, a deviation is opened, and a detailed investigation is launched to find the root cause and propose corrective actions.

20. It is often required to perform an analysis of extractables and leachables. What would be the recommendation for manufacturers who cannot do this?

Usually, extractables and leachables determinations are performed by vendors. The vendor/supplier should have this information, and it should be able to perform additional tests in the presence of the product to prove compatibility.

21. Are extractables and leachables considered in one test? Or would regulators expect two separate tests and separate results?

These are two different tests: extractables are "chemical entities that migrate from the surface of the process equipment, exposed to an appropriate solvent at extreme conditions, into the product or material being processed", whereas leachables are "chemical entities that migrate into a product from the product contact surface of the process equipment or containers under normal condition of use or storage" (WHO TRS, No. 1044, Annex 2, 2022).

22. In the case of pre-sterilised (Gamma radiated) filters, is it required to perform PUPSIT?

Regardless of the sterilisation method used, all filters are required to pass FIT and PUPSIT.

23. Besides *Brevundimonas diminuta*, which other organisms can be used for filter validation?

Sterilising grade filters, as defined by the American Society for Testing and Materials **(**ASTM) F838-15 standard, are validated using 10^7 colony forming units (CFUs) of *Brevundimonas diminuta* organisms per cm^2 of filtration area at a differential pressure of 30 psig, and still be integral after being exposed to the product of interest for a period (e.g., 24 hours).

24. If two 0.22 µm filters are used before filling, will these two filters be regarded as redundant filters or one of them can be claimed as a bioburden reduction filtration?

Redundant 0.22 µm filtration is expected wherever possible. If it is demonstrated that 0.22 µm redundant filtration is not convenient due to the risks, then a prior filter (e.g., 0.45 µm) may be considered for bioburden reduction.

25. Regarding PUPSIT, in the case of large-volume parenteral applications where 3-5 filters are installed in one housing, is it required to remove the filters from the housing to perform the test?

PUPSIT is performed once on all filter units placed in the housing as one filtration assembly. Each filter unit is not tested individually out of their housing.

26. What should be the batch size for a media fill trial?

The batch size for an APS should be sufficient to simulate all activities /interventions and reflect the real batch size. Typically, a minimum of 5 000 to 10 000 units is required, and this number should be justified in a documented manner (refer to WHO TRS 1044, Annex 2 Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, sections 9.37 and 9.39).

27. For APS, is it a prerequisite that growth media is guaranteed as sterile? In the case that media (Trypticase soy broth - TSB) is hydrated and filtrated with a sterilising grade filter (not the same filter as the product), before execution of an aseptic filling simulation, is it an expectation to validate the media filter (microbial retention test, compatibility, integrity test)?

It is not mandatory to use sterile media to perform APS. However, it is advisable to sterilise the APS media (e.g., sterilising the dehydrated media using gamma radiation) to avoid possible contamination with Mycoplasma which may pass through the 0.22 µm filters. In the case that the APS media is pre-sterilised by a sterilising grade filter, the filter integrity test usually is performed with water using the bubble point or diffusion point values given by the vendor. It is not mandatory to perform a full validation study on this filter.

28. What are the requirements to manage major and minor changes when deciding to perform 1 or 3 media fills, and who is responsible for this decision?

All critical changes should be managed through the change control system individually on a case-by-case approach using a multidisciplinary team where quality assurance (QA) has the ultimate responsibility to approve the decisions made (e.g., a refurbishment of the aseptic filling machine would at least require three consecutive successful APS; a change in rubber stoppers supplier may require at least one APS run).

29. Are media fill vials inspected after 14 days keeping vials 7 days at 20 to 25°C and 30 to 35°C, or will they

be checked after 14 days only once?

As per WHO TRS 1044, Annex 2 Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, clause 9.44, for filled APS units, "The selection of the incubation conditions and duration should be scientifically justified and validated to provide an appropriate level of sensitivity of detection of microbial contamination". The standard practice is to incubate for 7 days at 20 to 25°C and an additional 7 days at 30 to 35° C, for a total of 14 days.

30. Are there any specific WHO requirements for APS of pre-filled syringes (PFS)?

In the case of PFS, the plunger rod may not necessarily be attached to facilitate the incubation and inspection process. However, the impact of placing the plunger rod in terms of container closure integrity must be known.

31. Why is it recommended to inspect the APS incubated units at day 3 and 10 intervals, in addition to days 7 and 14?

Early detection of APS-contaminated units is useful to initiate an investigation as soon as possible and to make decisions regarding the batches produced or being produced. Also, an early detection of contamination contributes to a successful microorganism recovery and subculture to speciate it.

32. About the statement "the filled volume used for APS may not be up to nominal fill volume", is the container headspace not a factor in risk for contamination?

During APS, there is no need to use the routine fill volume if it is sufficient to contact all the internal surfaces of the container closure system. If a larger headspace remains, then it would represent a worst-case scenario which is one of the objectives of the APS.

33. Which are examples of methods for visual inspection of media filled in opaque plastic containers?

When opaque containers are routinely used, the APS strategy requires the use of the same container but transparent. If this is not possible, after the 14 days of incubation transfer the contents to clear sterile containers for visual examination.

34. For a 30 or 100 L batch size using manufacturing vessels, double sterile filters, storage vessels, multiple fill volumes (e.g., 2, 3, 5 & 10 ml), different modes of sterilisation processes, and container closure systems, what would be the recommended plan for APS?

In the case of the same container closure type (e.g., glass vials), multiple fill volumes may be bracketed (e.g., 2 ml, 5 ml, and 10 ml) to cover the whole range described. Bracketing would not be recommended for different container closure systems (e.g., vials and PFS) and sterilisation modes (e.g., depyrogenation tunnel and oven). The worst-case scenario should be considered for all possible combinations (e.g., double filtration would be included in the APS rather than single filtration).

35. In case a filling machine has a door in the middle that allows the operator to pass through the machine during filling, would this be acceptable as per the current standards? Is it a good practice to consider this movement an inherent intervention and validate it through APS? Would it be convenient to go around the machine to avoid going through the filling line to the rubber stoppers station?

The current WHO TRS 1044, Annex 2 Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, emphasises the requirement for avoiding human intervention during the aseptic filling process, thus, the practice described is not allowed for new installations where

a RABS or an isolator design is expected. For legacy or poorly designed setups and equipment, a thorough risk assessment and procedures should be in place to implement measures to control the contamination risk created by personnel accessing the filling area to perform certain exceptional activities (e.g., vial jam), until the equipment train is upgraded or changed. Any routine intervention (e.g., rubber stopper periodic feed), should avoid the presence of personnel in the class A filling station. As mentioned during the session, APS should not be used to justify non-compliant design or practice.

36. What is the recommended frequency of personnel requalification to operate in aseptic processes (AP)? Is it required to requalify with a media fill an operator who is working in a clean area in case this person needs to be transferred?

The recommended frequency for personnel requalification accessing grades A and B is based on the APS requirement which is every 6 months. Personnel accessing RABS or isolators are required to participate in one APS per year, at least. The qualification of the operator is based on training for the specific process involved and on participation in the corresponding APS. If aseptic processes are similar, and a case-by-case QRM analysis is carried out, the operator may not need to do additional APS. If the aseptic areas and processes are different (such as the example mentioned in the question), then at least one APS would be required to finalise the personnel qualification.

37. If a fermentation upstream process takes 7 days or more, is it required to perform a media fill after holding the media for 7 days in the fermenter, or can a 24-hour media hold test of keeping media in the fermenter following the APS?

The APS strategy defined should be as representative as possible considering the availability of equipment and the phase of the project. The fermenter's hold test should be as long as possible, include worst-case challenges, and samples should be drawn for sterility.

38. How should the APS be approached for both upstream and downstream process?

Any sterile product hold included in upstream and downstream is expected to be challenged by performing media hold tests.

39. Is there any consideration regarding the optimal location for the incubator for media fill units?

There is no specific requirement for the location of the APS incubation area. However, the chosen location should not create a risk of mixing between APS and commercial units.

40. Considering that sterility testing, environmental monitoring (EM) program, and media fill cannot assure sterility (or sterility assurance level - SAL) by themselves, what does?

The goal is to avoid microbial contamination holistically and address each of the supportive GMP elements to optimise them individually and collectively to provide the highest confidence in the overall aseptic process. The sterilisation process must guarantee a $SAL10^{-6}$. It is not only the sterility testing nor the EM testing that proves the sterility of the batch.

Session 9 Common good manufacturing practice deviations

1. Are there guidelines specifying which types of vaccines can be manufactured together in the same premises and which cannot?

TRS 999 (2016) Annex 2 WHO good manufacturing practices for biological products replacement of Annex 1 of WHO TRS, No. 822, states that in general, preparations containing live microorganisms or live viruses should not be manufactured and containers should not be filled in areas used for the processing of other pharmaceutical products. However, if the manufacturer can demonstrate and validate effective containment and decontamination of the live microorganisms and viruses then the use of multi-product facilities may be justifiable. In such cases, measures such as campaign production, closed systems and/or disposable systems should be considered and should be based on quality risk management (QRM) principles. EU/Pharmaceutical inspection co-operation scheme - PIC/S good manufacturing practice (GMP) states similar conditions but additionally states that Bacillus Calmette Guerin (BCG) vaccines should be manufactured in dedicated facilities. In all cases, the company should also refer to product-specific guidance concerning specific products for any additional measures in the relevant TRS.

2. Can Pentavalent and anti-snake venom be aseptically filled in the same facility and/or the same filling line?

In principle, this is possible, for example, by using a campaign approach. The decision to use a facility or filling line for campaign manufacture should be justified in a documented manner and should be based on a systematic risk approach for each product (or strain) considering the containment requirements and the risk of cross-contamination to the next product. Campaign changeover procedures, including sensitive techniques used for the determination of residues, should be validated. For finishing operations (formulation and filling) the need for dedicated facilities or the use of campaigns in the same facility will depend on the specific characteristics of the biological product, on the characteristics of the other products (including any non-biological products), on the filling technologies used (such as single-use closed systems) and on national regulatory authority (NRA) regulations.

3. In a virus vaccine manufacturing plant, can a mRNA vaccine also be developed in the same facility used for production?

This will depend entirely upon the nature of the other vaccines being manufactured and filled in the facility. Manufacture of the drug substance (DS) will depend upon the expression system used and other products present in the manufacturing facility as well as the possibility of campaign processing. Filling could most likely be performed on lines already used for inactivated or subunit vaccines. In all cases, robust risk assessment and cross-contamination mitigation strategies would need to be established and validated. Depending upon the scale of production a dedicated workshop would probably be preferable for this group of vaccines. See similar questions above.

4. With most NRAs emphasising a risk management approach, is it recommended to implement preventive action corrective action (PACA) instead of corrective action preventive action (CAPA)? CAPA means reacting after an event happens and correcting it, while

PACA means preventing the event from happening.

Yes. In all cases, it is already expected that risk assessment is prospective and continuing throughout any development of a product or facility or change thereof is performed. Preventive actions are always better than corrective actions.

5. In the present scenario, the emphasis is on fostering a culture of "Continuous Quality Improvement and Quality Culture." However, a pertinent question arises: why isn't GMP compliance and adherence to product specifications sufficient? Introducing a continuous quality improvement program is noteworthy, yet it raises concerns about the potential escalation in operational and production costs.

This is a misconception and a misunderstanding of GMP. An effective quality management system (QMS) has always required a good quality culture and a continuous improvement mentality and senior management commitment. In the medium and longer term having a continuous quality improvement program reduces operational and production costs. Quality Culture is understanding not just the price of quality but its holistic value to the business. For example, in EU GMP for Medicinal Products (2013), Part 1, Chapter 1 (Pharmaceutical Quality System), paragraph 1.4 (xi), requires continual improvement.

6. Is there an accepted definition for "Quality Culture"?

Whilst there is no official GMP definition of "Quality Culture" there is a wide consensus that quality culture is an environment where team members genuinely care about the quality of their work and make decisions based on achieving that level of quality. Harvard Business Review defines a "true culture of quality" as an environment in which employees not only follow quality guidelines but also consistently see others taking quality-focused actions, hear others talking about quality, and feel quality all around them. Developing a mature quality culture and measuring progress across the supply chain can be challenging. The Parenteral Drug Association (PDA) Quality Culture initiative is designed to help pharmaceutical quality personnel guide their organisations toward a mature quality culture.

7. How can Quality Culture be measured? How can it be documented and show the evidence to auditors?

Much has been written about quality culture and its measurement by regulators such as the USFDA and UK MHRA, as well as industry organisations such as the Parenteral Drug Association (PDA), and the International Society of Pharmaceutical Engineers (ISPE). For an example of measurement tools refer to the PDA website.

8. In case a facility is experiencing a recurring non-conformance with a specific group of individuals, how many times should a (re)training be performed?

If problems are recurring, the CAPA approach by QA and supervisors is ineffective and they have failed to address the true root cause of the problem. Re-training alone is never a successful CAPA and normally some re-engineering or the design of the task is required.

9. GMP expects companies to regularly update their process design, what are the specific regulations related to this requirement? What will be the trigger to initiate a process design review?

In many territories such as the EU, it is the "GMP principles" that are defined in binding legislation (such as "Regulations" or "Directives") and these regulations also state that the national regulatory

authorities will from time to time, issue "Regulatory Guidelines" as to current good practices for the implementation of these GMP principles. So, the GMP guide is normally a "Regulatory Guideline" and not a "Regulation". The benefit of this approach is that it is normally much quicker to update guidelines rather than legislation. In most countries, the text of the GMP guideline allows for alternative approaches to achieving the GMP principle so the text of the GMP guideline is normally not considered a Regulation, and therefore not legally binding, though applicants and manufacturers need to provide scientific and risk-based justifications for any deviations from the "regulatory guidelines".
Concerning continual improvement and updating processes and their design, these requirements are typically in the GMP regulatory guideline. For example, ICH Q10 Pharmaceutical Quality System (2008), which is reproduced in Part III of EU GMP, states that improvements may be triggered by new technologies or emerging concerns regarding existing technologies. EU GMP and PIC/S GMP also have a specific section on Continual improvement of process performance and product quality. Furthermore, the new Sterile annex (e.g., WHO TRS 1044, Annex 2 Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022), has paragraphs requiring processes and premises to be upgraded to improve product and process protection.

10. What is the expectation of trending deviations? Is it necessary to look at each product's deviations or also look at all deviations and do trending for all products?

Deviations should be documented and recorded in a manner and according to a variety of criteria to allow for the identification and trending and analysis for common causes (e.g., facility, department product groups, processes, and equipment). The intent is to allow for the identification, trending, and analysis of any common factors leading to improved process and product consistency. From a yield standpoint of view alert limits should be established from historical trend data to determine when further investigation may be appropriate.

11. How is batch-to-batch consistency defined in terms of biological substances, as biological products show variability in their yield?

Due to the differences in their nature and how they are produced, biological therapeutics are regulated, tested, and controlled differently than other medicines. To help ensure their quality, safety, and efficacy, each batch of a biological therapeutic product must be tested extensively at each stage of production to ensure consistency with prior batches. The use of WHO International Reference Standards helps to further ensure the consistency of a product across many batches as well as to allow the comparability of biologicals between manufacturers and/or countries. The establishment of general requirements applicable across a diverse range of product classes governing starting materials, manufacturing, and regulatory oversight is an essential aspect of this process. Whereas WHO guidelines have been established for some specific biologicals to guide implementation.

12. Are ICH documents considered guidelines or regulations?

ICH documents are guidelines but may be implemented in some but not all countries as national regulations in those countries. For example, ICH Q7 Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients (2000), has been incorporated into some jurisdictions as GMP regulations whilst in others they may be binding guidance.

13. When are video recordings considered as official records?

Videos become electronic quality records when used as evidence in a QA or quality control (QC) investigation or validation. For example, air visualisation studies (smoke studies) or videos of media simulations are considered official electronic records, and the data integrity (DI) principles of Attributable, Legible, Contemporaneous, Original, Accurate, Complete, Consistent (ALCOA+) apply. Closed circuit television (CCTV) records are not at the time of recording GMP electronic records but may become so if they are used in the investigation of a deviation or some other GMP incident.

14. Why is the process for gowning and gloving usually done alone instead of having two people help each other?

It is generally considered good practice to limit the number of operators in any area at any one time. The design of gowning required for class A/B operations also does not lend itself to the use of a helper unlike the gowning for example in a hospital operating theatre.

15. May the single-use system (SUS) be used more than once (e.g., a bioreactor bag)?

The trend toward single-use systems has been matched by the trend to question whether some of these devices might be reprocessed to allow reuse. In part, this is the result of organisations seeking cost savings by reprocessing instead of using a SUS device once and then discarding it. Some claim environmental advantages, arguing that reusing a single-use device is greener, resulting in less regulated waste. The reuse of single-use devices involves regulatory, ethical, medical, legal, and economic issues and is extremely controversial. Depending upon the type of SUS, this practice carries significant risk to the process and possibly the patient. These devices and supplies are often complex in design, and cleaning efforts, either by users or third-party reprocessors, may be inadequate. Reprocessing and reuse may compromise the product's performance, and the SUS manufacturer will have no liability when an SUS is not being used according to the manufacturer's instructions.

16. What should be the proposed frequency of controlled non-classified (CNC) area monitoring and how to identify critical locations for monitoring?

The frequency and locations of any monitoring location should be based on science and risk and the activities performed in the CNC and adjoining areas. It is not possible to make any universal recommendation.

17. Is the use of ethylene oxide (EtO) for decontamination or sterilisation acceptable?

EtO is an effective sterilant when used in accordance with ISO 11135:2014 Sterilization of health-care products – Ethylene Oxide: Requirements for the Development, Validation, and Routine Control of a Sterilization Process for Medical Devices. It is widely used for the sterilisation of single-use medical devices. EtO is explosive and requires significant safety precautions in use as well as needing aeration post-processing to allow residues to disperse. It was once widely used for spice decontamination in the food industry. It is very rare to see the use today of EtO in pharmaceutical plants except for those manufacturing certain implants. where used the process is normally performed by specialist contractors.

18. Is fumigation in a clean room mandatory or a risk-based requirement?

In many parts of the world, including the United States of America and Europe, periodic fumigation does not form part of routine environmental control of manufacturing facilities for sterile products. Fumigation is always a

supplemental microbiological control procedure in addition to robust cleaning and disinfection. In these facilities, fumigation is used exceptionally typically after major maintenance and multiple cleaning cycles before the requalification of an area. If a company finds that routine fumigation is necessary, then the robustness of routine cleaning and the materials being used should be investigated. The reason for this is that fumigation is not without product and personnel safety risks and local occupational health and safety regulations should always be followed. In the past formaldehyde fogging was frequently used but due to serious safety concerns the use of formaldehyde has ceased in many territories and today the use of vapor phase or fogging with hydrogen peroxide is usually the method of choice. The use of formaldehyde is still relatively common in live viral vaccine facilities or other biological product facilities where the virucidal properties of formaldehyde are needed. For this reason, fumigation is a risk-based process not a mandated process.

19. How is fumigation process validation (PV) performed?

Fumigation PV is performed by placing chemical and biological indicators (BI) in those locations shown in smoke studies to be relatively poorly swept. It must be remembered that fumigation is only effective on clean surfaces. It must be shown that the fumigant reaches all parts of the facility being fumigated in adequate concentrations but not so high that residues are a problem. The PV must also show that any residues are at a satisfactorily low level.

20. What should be the medical health checkup frequency for working in sterile area class A and B?

High standards of personal hygiene and cleanliness are essential to prevent excessive shedding or increased risk of introduction of microbial contamination. Personnel involved in the manufacture of sterile products should be instructed to report any specific health conditions or ailments that may cause the shedding of abnormal numbers or types of contaminants and therefore preclude cleanroom access. The new sterile annex (e.g., WHO TRS 1044, Annex 2 Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022) does not specify a frequency for health checks though staff are required to notify their supervisors of any health problem that could impact the product such as infections. The Guideline prepared by PAHO for GMP Inspections, suggests that health examinations ask for a check that should be at least annual.

21. Is it possible to have a clean room having a grade A restricted access barrier system (RABS) filling line with a grade C background?

It is possible to install a RABs line for terminally sterilised products in Class C, but this line is not suitable for aseptic operations for products such as vaccines.

22. How often should the integrity test for the isolator gloves/sleeves be done?

WHO TRS 1044, Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, Annex 2, paragraph 4,.21 (i), requires that "For isolators, leak testing of the glove system should be performed using a methodology demonstrated to be suitable for the task and criticality. The testing should be performed at defined intervals. Generally, glove integrity testing should be performed at a minimum frequency at the beginning and end of each batch or campaign. Additional glove integrity testing may be necessary depending on the validated campaign length. Glove integrity monitoring should include a visual inspection associated with each use and following any manipulation that may affect the integrity of the system. For manual aseptic processing activities where single unit or small batch sizes are

produced, the frequency of integrity verification may be based on other criteria, such as the beginning and end of each manufacturing session."

23. What should be the compliant alternative of a perforated table in case of a dispensing booth in a grade C area/ clean room?

The primary purpose of a downflow booth for dispensing is to protect the operator from dust. Vertical downflow booths offer limited protection to the materials being dispensed especially when dispensing from larger containers or drums. The most important feature of the downflow booth is, therefore, the provision of dust-free air to the operator and good dust extraction. Perforated tables may be useful if they aid dust extraction but will nonetheless be more difficult to clean than a solid table surface with a rear-facing extract duct.

24. What is the appropriate procedure for cleaning RABS?

RABS should be cleaned and disinfected thoroughly both before and after use following a written program. Initial cleaning should be with water, or a solution validated to remove product residues. For disinfection to be effective, prior cleaning to remove surface contamination should be performed. Cleaning programs should also effectively remove disinfectant residues. More than one type of disinfecting agent should be employed to ensure that where they have different modes of action, their combined usage is effective against bacteria and fungi. Disinfection should include the periodic use of a sporicidal agent. Period fumigation of the RABS may be part of the decontamination program especially if campaign processing is in place. Gloves should be periodically removed and sterilised before replacement. Between batches, they should be cleaned and decontaminated with the rest of the machine. This should include the use of a sporicidal agent. Special consideration should be given to additional cleaning after aseptic process simulation (APS) runs due to the use of nutritive media. The ability to remove media should be validated.

25. What should be the routine checks for RABS during production?

During routine production, gloves should be regularly wiped using disinfectant (most likely alcohol due to the risks of over-spraying onto product contact parts. Door opening should be minimised and when doors are opened, they should be wiped clean using the approved disinfection agents. Wiping should include the gloves and arms that have been exposed to the B environment with special attention to the fingers of the glove. Where contact plate monitoring is performed on machine surfaces these are best performed at the end of the fill run due to the possibility of leaving media residues on machine surfaces. The cleaning and decontamination procedures should be comprehensively discussed and justified in the contamination control strategy (CCS).

26. For optical inspection of sterile products, what should be the criteria defined for the acceptable quality limit (AQL)?

All containers must be inspected. This is irrespective of the product being aseptically filled or terminally sterilised. After 100% inspection, an AQL sample should be taken, and this AQL sample re-inspected. This AQL inspection is normally performed by QA staff but in the production area. Sample sizes will depend upon the batch size and the ISO 2859 (2020) on Sampling by Attributes should be referred to. There should be at least two product-specific defect classes defined. Defining more defect classes may be appropriate (e.g., critical defects: may cause a lack of sterility, container integrity or cause harm to patients). Major defects: may alter the content or the function of the product. Further guidance on AQL sampling can be found in USP <1790> Visual Inspection of Injections, and in FDA

Inspection of Injectable Products for Visible Particulates - December 2021 Guidance for Industry. There are special defects (e.g., turbidity) for which it is inappropriate to set an AQL-based limit, and in these cases, the acceptance limit of 0 is set.

27. Is it required to change gown from class D to class C if we put an extra head cover and extra hand gloves before entering?

No external clothing should enter a changing room leading to class C or B areas. This includes socks. Wearing an additional gown over grade D gowns might be acceptable if changing into grade D gowns required the removal of all personal clothing except underwear. Extra head cover and extra hand gloves alone would not be acceptable.

28. What is the air cleanliness class expected under an extended laminar airflow (LAF) area in an aseptic filling line with RABS?

This is an often-debated question and one that has still not found consensus. An extended LAF contiguous with the class B area without any physical barrier should be considered a grade A air supply rather than a class A area. The distance that a unidirectional flow will be maintained will depend upon flow rates and the width of the extended LAF. It can be argued that the space is either class A or B, but the important consideration is that microbial alert and action limits should be based on actual data. In practice class A microbiological performance would be expected to be routinely found. If secondary barriers were in place e.g., partial fixed panes or even curtains then the zone could be reasonably considered to be grade A unmanned and grade B when occupied.

29. If the International Organization for Standardization (ISO) requirement is 40 air changes per hour and 70 air changes per hour is kept, does it still need to be validated?

Yes. Validation is required irrespective of set conditions.

30. Regarding terminal sterilisation at F0 > 8 (e.g. 15), is the microbiological performance qualification requirement and challenge (e.g., using BIs) necessary, or a physical performance qualification (PQ) (e.g., reaching 121.1°C in the hardest-to-reach point) sufficient?

For the terminal sterilisation of aqueous solutions, initial validation should include BI unless otherwise justified. Routine re-validations of overkill cycles (F0 > 15) would not normally utilise BIs, however regional regulatory expectations should be respected. Much depends upon the size of the container being sterilised. Large (>10L bottles of media for example) will heat slowly and if a time/temperature requirement in the bottle contents is set then F0 could easily exceed 40-50 if 121.1°C is a validation criterion. In the case of the steam sterilisation of stoppers or hard equipment loads, empty vessels, etc., BIs should always be used in the validation of the vacuum/air removal sterilisation cycles used for this type of load.

If the container is not a sealed container but for example a bottle with vent filters, then it would be appropriate to insert BIs in the vent filter as liquid load cycles do not normally have a vacuum phase. Any container with a complex closure should have BIs placed in the closure system to demonstrate adequate penetration of steam.

31. Is the use of plastic curtains no longer acceptable in any situation, especially in low- and medium-income countries (LMIC) where there are budget or supply chain constraints? For example, when using sterile rapid transfer port

(RTU) vials or stoppers which need to be fed into the filler?

The new annex states a company to use RABS or isolators for filling drug products as the default position for any new facility and any alternative approaches must be robustly justified in the CCS. In all cases, the physical separation of the process from the operator is recommended or where technically not feasible the risks should be mitigated, and the mitigations discussed and justified in the CCS. Plastic curtains are outdated technology for filling operations and alternatives should be sought when upgrading legacy facilities. Curtains may be useful in other lower-risk areas of operation but are always best avoided due to their difficulty in cleaning. WHO GMP does not provide exemptions for low- and middle-income countries (LMICs).

32. How could a legacy aseptic fill line be upgraded?

It is sometimes possible to mitigate legacy fill lines by the installation of secondary fixed barriers and glove ports to replace direct access and curtains and if well designed this may extend the operational life of a legacy line. Unfortunately, it is often not easy to design such protection effectively, and effective containment may require the line to be re-engineered by the machine manufacturer, and this may be a very long process to execute the changes and completely re-qualify the line. For this reason, early replacement of the legacy line should also be considered as an alternative, albeit a more expensive option, before proceeding with the line modification of an aging machine.

33. Is it recommended that the autoclave be equipped with an air detector even though a regular Bowie Dick test is performed?

Yes, it is better for an air detector to be present and confirm every cycle is effective than solely relying on daily Bowie Dick test pack tests.

34. In case silicone hoses for bulk product are more than 3 m long, and the bulk is a suspension, is it mandatory use SUS for the aseptic connections, and laminar flow, or is it possible to make the connections in a class C?

Traditional aseptic conditions are only acceptable under class A in a B background when there is no further filtration in the system (e.g. formulated adjuvanted vaccines). If a lower-grade environment is to be used, then intrinsically safe single-use aseptic connectors should be used in either a grade C or D background. If tube welders are to be used and there is no further filtration in the product stream, then these are best used in a localised grade A background due to the relative weakness of the tube welding process compared to intrinsic aseptic connector technology.

35. Why does EU Annex 1 Manufacture of Sterile Medicinal Products, 2022, no longer state that LAF protection above the unloading door of the autoclave, where the load is taken out, is mandatory?

The old EU annex did not explicitly state that LAF protection above the unloading door of the autoclave, where the load is taken out, was mandatory. It was however the usual design for aseptic processing facilities as a protection measure especially when items were sterilised for example in s/s tins. Today most steam sterilised items are sterilised in several layers of Tyvek® sealed wraps. The new annex focuses on risk management and robust CCS but also in TRS 1044, Annex 2 WHO Good Manufacturing Practices for Sterile Pharmaceutical Products, 2022, paragraph 8.10, requires grade A protection for the “Removal and cooling of unprotected (e.g., with no packaging) items from sterilizers" and "staging and conveying of sterile primary packaging components in the aseptic filling line while not wrapped".

36. In the case of moving a vessel containing bulk product to a class B for filling, is it acceptable to disinfect its outer surface with a sporicidal agent?

In general, the movement of tanks, especially those on wheels between grade B and lesser classified areas should be avoided due to the difficulty in adequately manually decontaminating the exterior of the tanks and especially their wheels. In new facilities, alternative designs should be used to eliminate the need to move the tanks, and if so then a tank lower cart which stays in the class B area should be used. This should be regularly subjected to a high-performance sterilisation process (e.g., autoclaving). Where unavoidable, in legacy facilities, then the transfer should be effectively decontaminated preferably through several stages of repeated treatment using a sporicidal treatment and robustly monitored with an ongoing program of assessment of decontamination effectiveness. The possibility of using a class B captive vessel or the implementation of SUS should be considered. The mitigation strategy and its monitoring should be discussed and scientifically justified in the relevant CCS documentation.

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