Autoclave Validation

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Introduction

- Who Am I?
- What's in it for you?
- If we did not have autoclaves (or steam in place), life would be different for the pharmaceutical industry. Sterilisation would involve fire (or heat), chemicals, filtration or radiation. Microbiological media would have a shorter shelf life and there would be a greater risk of contamination.
- Autoclave means "auto lock".
- Interesting info: The man credited with initiating the project which lead to the invention of the autoclave was Charles Chamberland. He worked with Louis Pasteur and also invented a vaccine for chicken cholera by accident.

Presentation Aims/Outcomes

- What is an autoclave
- How an autoclave works
- Why use autoclaves
- Identify the two main types of autoclaves (in pharma)
- Detail how to validate an autoclave (including loading patterns)
- What to look for in an audit

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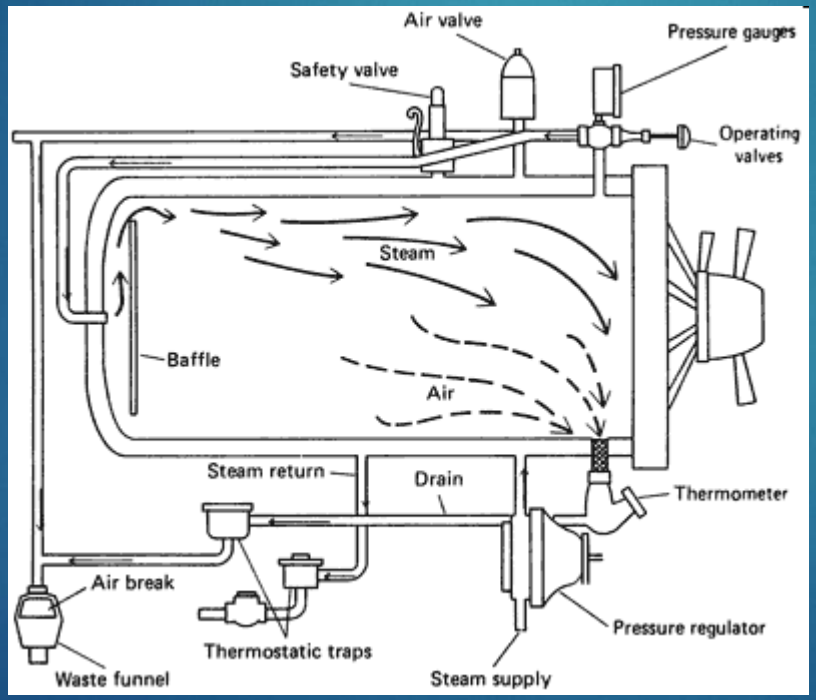
What is an Autoclave?

- An autoclave is a device that uses elevated temperature and pressure in combination with moist heat to accomplish a task
- In biological sciences, this task is primarily to kill anything living, as well as viruses
- In chemical sciences, autoclaves are used to cure coatings, vulcanise rubber and for hydrothermal synthesis.

Hows Does An Autoclave Work?

- Steam enters the chamber jacket, passes through an operating valve and enters the rear of the chamber behind a baffle plate. It flows forward and down through the chamber and the load, exiting at the front bottom. A pressure regulator maintains jacket and chamber pressure at a minimum of 15 psi, the pressure required for steam to reach 121ºC (250ºF). Overpressure protection is provided by a safety valve.



•*(from S.S. Block, Disinfection, Sterilization and Preservation, 2nd ed., Philadelphia, lea & Febiger, 1977)*

Hows Does An Autoclave Sterilise?

- Steam held at elevated temperature and pressure over time is used to transfer moist heat.
- Steam has much greater heat transfer than boiling water. – 80 calories to boil water, 540 calories to produce steam. At 1 ATM, 2257kJ/kg is needed to boil water * so over 15 000kJ/kg for steam.
- Heat acts to denature proteins, effectively killing all cells present.

- *Ref https://www.ck12.org/physics/heat-temperature-and-thermal-energy-transfer/rwa/Boiling-Water/

Common Industry Types

- Production autoclave
  - Usually large
  - Loads one side, unloads the other
  - Used to sterilise production equipment
  - May be used to sterilise filled product (can have one opening)
  - If faulty, potential impact on sterile core or batch disposition
- Microbiology Laboratory Autoclave
  - May be large or small
  - Usually loads and unloads from same side - Sterilised items do not unload directly into production environment
  - Used to sterilise equipment as well as media. Also used to decontaminate materials before disposal - the “decon cycle’

Why Use Autoclaves?

- Easiest way to sterilise large volumes of heat tolerant materials.
  - More effective than dry heat
  - Not as messy as chemicals
  - No need for radiation shielding
- Once validated, simple indicators are used to tell autoclaved and non autoclaved material apart – the temp/time/pressure trace is used to confirm sterilisation occurred.

What may affect Sterilisation

- Sterilisation is dependant on:
  - initial bioload
  - microbe sensitivity to heat
  - time A/C held at sterilising temperature
  - ability of steam to penetrate items being sterilised
- Steam Penetration:
  - As steam is used to transfer heat, tightly wrapped items, or long tubing may not be properly penetrated. Would represent worse case for validation.

What is Validation?

- Validation shows that a process (or item of equipment) does what it is claimed to do.
- Why validate?
  - PIC/S PE009-14 Section 83 requires it
  - Need to show that sterile equipment is being taken into sterile core - do not want to contaminate production (which could lead to adulterated product)
  - For micro testing (both bacteriological and Viable Environmental Monitoring), using sterile equipment helps reduce false positives.
  - Need to show that decontamination cycles are effective

Validation Documentation

- URS. IQ, OQ, PQ.
- URS: Written with no particular brand/model in mind.
- IQ: does equipment meet the URS requirements? Is everything that was on the box, in the box? Is the unit installed properly.
- OQ: does the item operate properly? Eg. Does the unit hold temp and pressure correctly?
- PQ: Does everything confirmed in OQ stand up when the equipment is actually in use?
- Validation Protocol: sets out the procedures for IQ, OQ and PQ and assigns responsibilities
- Validation Report: detail the results, make valid conclusions, document failures and successes.

PQ of Autoclave

- PQ: validation of autoclave cycles and loading patterns.
- What SAL do you need?
  - Need to show a 10^{-6} reduction of microbes
  - What is your starting bioload?
  - Spore strips have >10^{6} CFU.
- What is the microbe's D value?
  - For Geobacillus stearothermophilus, this is around 1-1.5
- Can use physical, chemical or biological indicators.
- How does this all fit together?

- More reading:
  - https://www.sciencedirect.com/topics/immunology-and-microbiology/sterility-assurance-level
  - ISO 17665-1(2006) Sterilization of Healthcare Products – Moist Heat
  - https://www.fda.gov/media/71026/download - FDA Guidance for Industry

D-Value, Z-Value and Fo

- What is D value?
  - refers to **decimal reduction time** - The time required at a certain temperature to kill 90% (eg reduce population by log 1) of the organisms being studied. Thus after an organism is reduced by 1 D, only 10% of the original organisms remain. Dependant on microbe and initial numbers. Eg D value of 1.5 = 1CFU left after 10.5 mins.
- What is Z value?
  - Refers to the temperature change required to produce a 1 log reduction in D value.
- What is F_0?
  - The number of minutes to kill a specified number of microbes with a Z value of 10°C at a temp of 121.5°C.
  - Often confused with the time the chamber is held at elevated temperature and pressure and in practice is the same thing.
- Overkill
  - Use many more microbes than would find on items typically autoclaved. Negates the need to test sample for bioload before running the cycle.
  - Use a sterilisation time exceeding what is necessary to kill a large number of microbes. Negates the need to determine D value of microbe.

Loading Patterns

- Why important?
  - Sterilisation relies on steam penetration. This may not occur in all cases
  - Very important to show what you put in an autoclave comes out sterile
  - When to use spore strips and when to use solutions
- How to validate?
  - 3x successful runs each loading pattern
  - Place BI with each item in worse case spot. Place thermocouple next to BI, but not touching item.
- How often re qualify?
  - This should be stated in your relevant SOP. 6, 12, 24 months are typical. A risk assessment will help determine your schedule
  - For Micro A/C's depends on use. If used as part of sterility testing…is used for EM sampling…is used for decon cycles…
- Loading patterns should be adhered to.
  - Worse case validated – can use less but not more equipment

Audit Considerations

- When auditing, look at:
  - Was validation conducted?
  - I would look mainly at PQ only if the unit is >15 years of age. If newer, the whole validation package. Also want to see a preventative maintenance program, SOPS, leak rate test data
  - Are all expiry dates current?
  - Cycle time – is it sufficient for tested D values?
  - Was validation equipment within calibration (pre and post use for thermocouples)
  - Temperature traces for validation and most recent cycle – Compare. Discrepancies?
  - Are vacuum cycles used appropriately?
  - Are the coolest and warmest positions clearly stated in the validation report?
  - Examine largest (bulkiest) loading pattern. Was the validation acceptable. Is anything thing not listed on the loading pattern present in the autoclave? Is there enough room for steam to circulate through the chamber?
  - Were there any deviations from the protocols. Are conclusions valid and justified?
  - *Can the site show that the product that has been terminally sterilised has retained efficacy?
  - *Could also check that the steam system is validated and that dye bathing (or some other integrity checking) occurs post sterilisation

*Not exactly on A/C audit scope.

Conclusion

- You will now know:
  - What is an autoclave and how it works
  - Why autoclaves are used in a manufacturing environment
  - What the main autoclaves are used by microbiologists in the pharmaceutical industry
  - The basics behind autoclave validation
  - What to look for in an audit

References

- In order of appearance
  - S.S. Block, Disinfection, Sterilization and Preservation, 2nd ed., Philadelphia, lea & Febiger, 1977)
  - https://www.ck12.org/physics/heat-temperature-and-thermal-energy-transfer/rwa/Boiling-Water/
  - PIC's PE009-14 Annex 1
  - https://www.sciencedirect.com/topics/immunology-and-microbiology/sterility-assurance-level