



Autoclave Validation

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Introduction

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
- Why we use autoclaves
- Identify the two main types used by us
- Detail how to validate an autoclave (including loading patterns)
- What to look for in an audit

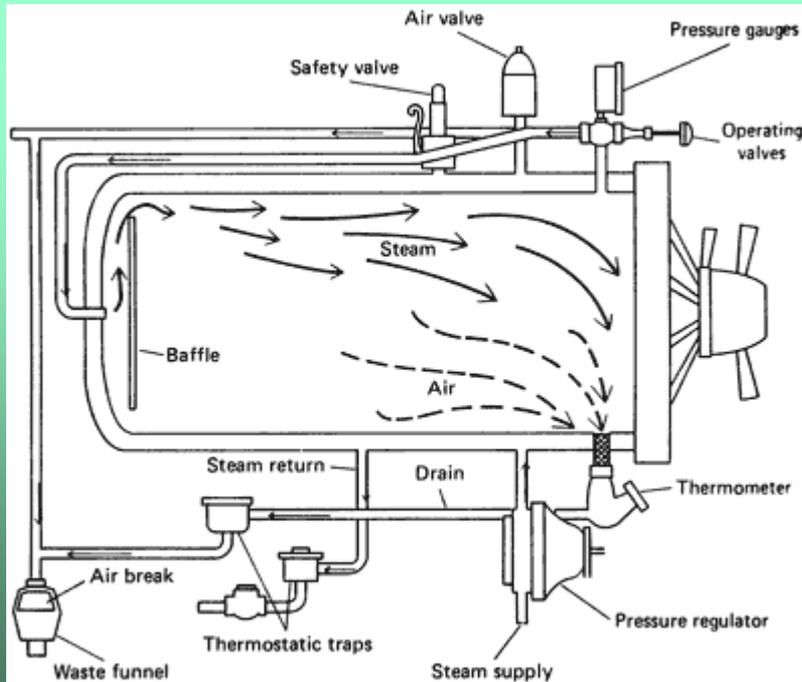


What is an Autoclave?

- How do they work
 - In general
 - In order to sterilize
- Types common to us
- Why use an autoclave
- What may affect sterilization
- Steam penetration

How 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 121°C (250°F). Overpressure protection is provided by a safety valve.



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



How Does An Autoclave Sterilize?

- Steam held at elevated temperature and pressure for 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.
- Heat acts to denature proteins, effectively killing all cells present.



Common Types

- Production autoclave.
 - Usually large
 - Loads one side, unloads the other
 - Used to sterilize production equipment
 - May be used to sterilize 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 - Sterilized items do not unload directly into production environment
 - Used to sterilize equipment as well as media. Also used to decontaminate materials before disposal



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 used to tell autoclaved and non autoclaved material apart – the temp/time/pressure trace is used to confirm sterilization occurred.



What may affect Sterilization

- Sterilization is dependant on:
 - initial bioload
 - microbe sensitivity to heat
 - time A/C held at sterilizing temperature
 - Ability of steam to penetrate items being sterilized

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?
 - 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



How To Validate

- The usual – URS. IQ, OQ, PQ.
 - This discussion will focus on PQ.
- IQ: does equipment meet the URS requirements? Is everything that was on the box, in the box? Is the unit installed properly. Are support programs in place for ongoing operation of A/C?
- OQ: does the A/C operate properly? Does the unit hold temp and pressure correctly?
- PQ: validation of autoclave cycles and loading patterns – need to show sterilization.



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 (WWQC 11.1.10).
- 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. As microbiologist, tend to use BI's (spore solution or impregnated strip).
- How does this all fit together?

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?
 - Sterilization 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?
 - Directive says annually 11.1.10 for terminal sterilisation.
 - 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

What to look for in an audit

- 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 sterilized 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 now know:
 - What is an autoclave and how it works
 - Why we use autoclaves
 - What the main autoclaves are used by microbiologists in the pharmaceutical industry
 - The basics behind autoclave validation
 - What to look for in an audit



Questions