Validation of a freeze dryer sterilisation cycle

Andrew Webb

AstraZeneca

Freeze dryer – steam sterilisation

- Basics of steam sterilisation
- Requirements for steam sterilisation for a freeze dryer
- Factory testing requirements
- Validation requirements
- A case study of the purchase and validation of a steam sterilisable freeze dryer

Basics of steam sterilisation

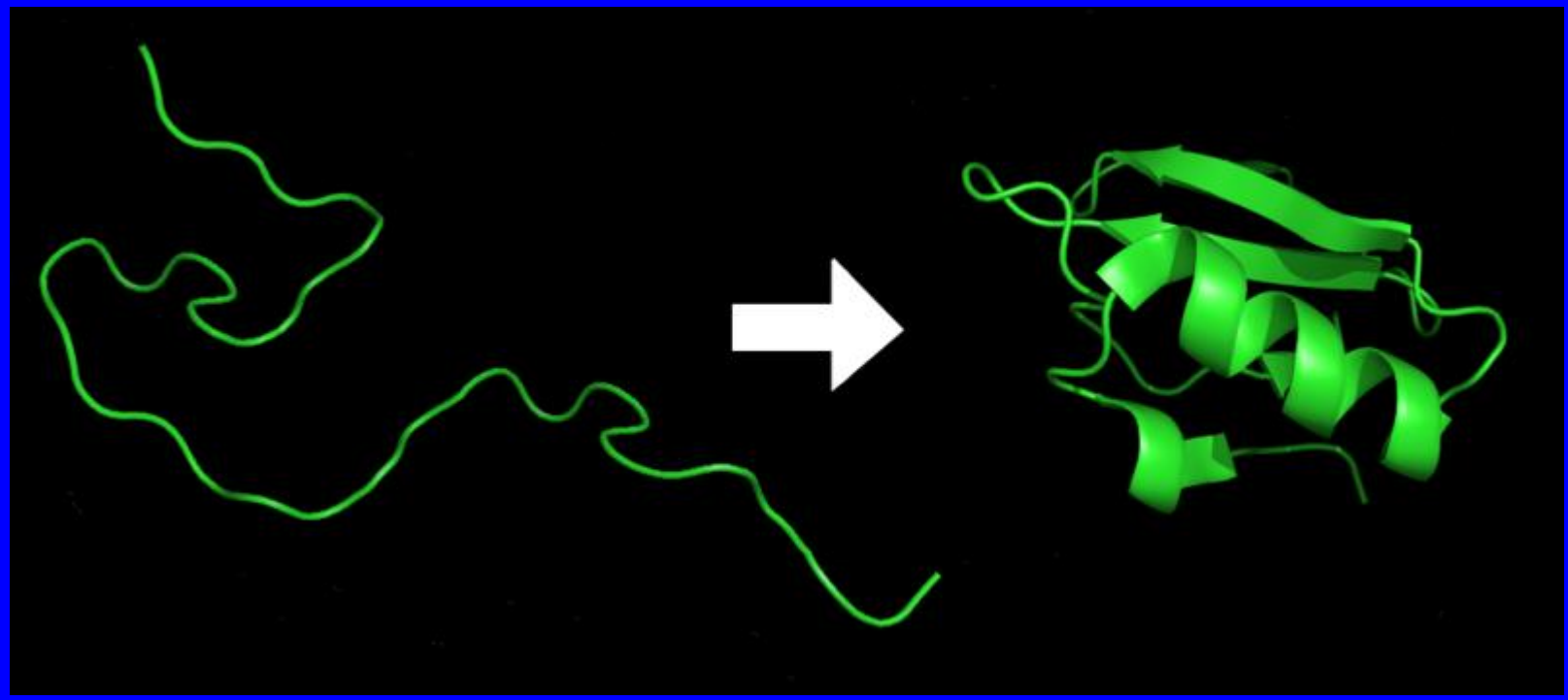
Sterilisation – typical general requirements

- **Steam sterilisation (SIP)** – minimum of 15 minutes at 121°C
- **Dry heat** – minimum of 30 minutes at 170°C
- Both use heat – but why is there a such a big difference in the times and temperatures required?

Why is wet heat more effective than dry heat?

- Proteins are relatively heat stable in the dry state, but break down easily in the wet state
- In the presence of water, proteins are easily broken down at fairly low temperatures ~ 60^{o}C
  - the protein structure (shape) changes
  - the proteins can no longer function
- Water **and** heat are necessary for steam sterilisation
- Dry heat sterilisation – oxidation of proteins/cellular components

Protein structure



Protein – non folded
(secondary structure)

Protein – tertiary
structure

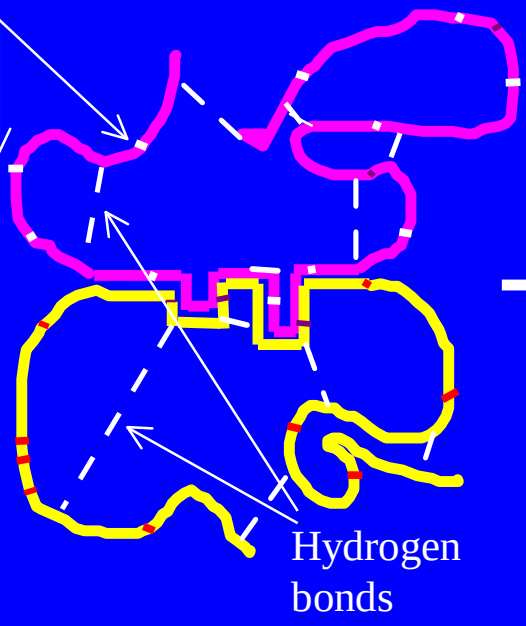
Effect of **heat** in the **presence** of **water** on the shape and functionality of proteins

FUNCTIONAL

i.e. can react

Protein A

Peptide bonds



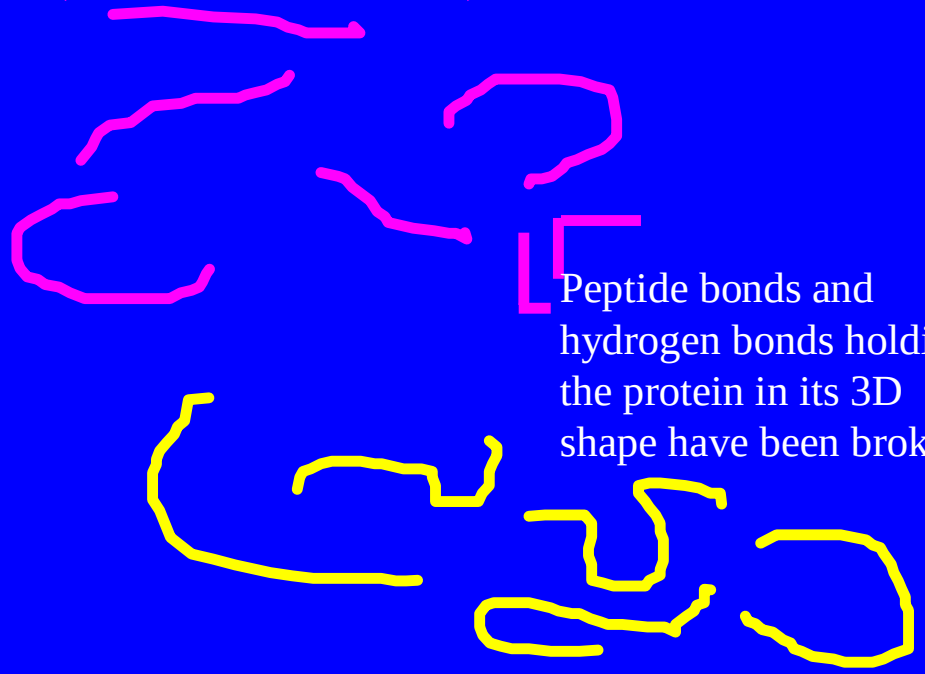
Protein B

Proteins A and B have a 3 dimensional shape - functional

Heat + water

NON - FUNCTIONAL

Fragments of protein A (non functional)



Peptide bonds and hydrogen bonds holding the protein in its 3D shape have been broken

Fragments of protein B (no 3 dimensional shape - non functional)

Coagulation of egg white (albumen - protein)

Water content (%)	Coagulation temperature (°C)
50	56
18	80 – 90
6	140 - 150
0	160 - 170

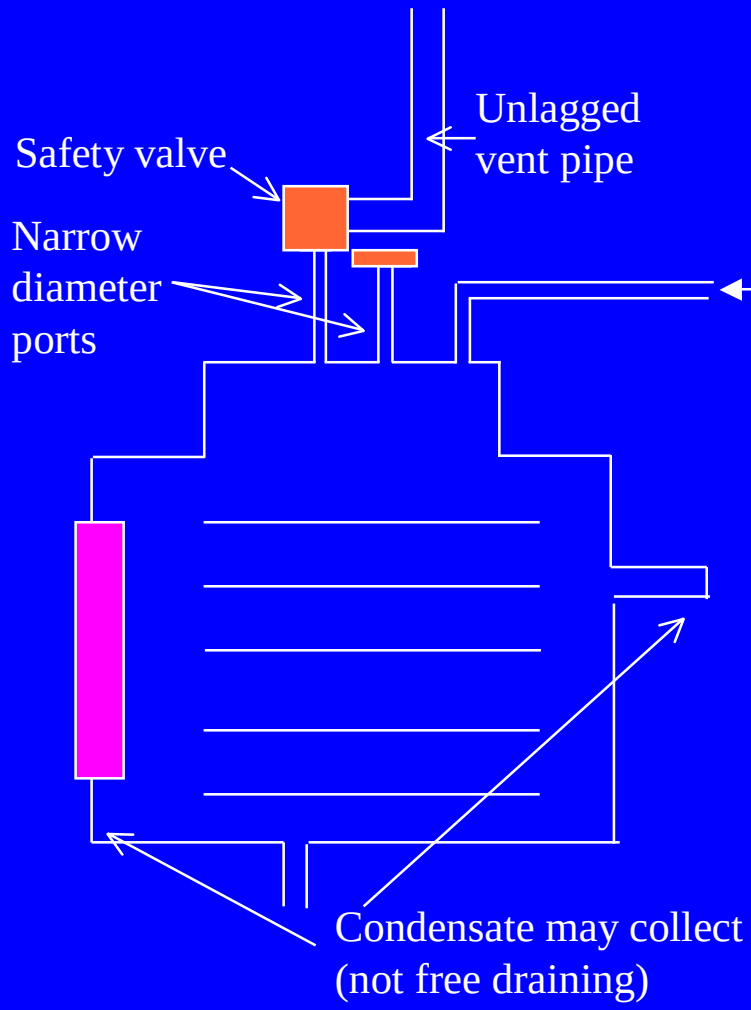
Proteins are **not** very **heat stable** in **solution**, *but* are relatively **heat stable** when **dry**.

Steam sterilisation – requirements for a freeze dryer

Steam sterilisation - requirements

- Need all the air to be removed from the chamber, condenser and filter
- The chamber must not have a significant leak (leak rate test required)
- The air is replaced with dry, saturated steam
- All surfaces must become hot AND wet
- A typical sterilisation cycle may be 20 mins at 121^{o}C (often higher temp for a longer time)
- The steam must not contain > 3.5 % non condensable gases (NCG's)
- The steam quality must be controlled

Problems seen in ports



Potential problems :-

(1) Narrow diameter pipe in ports makes it difficult to get steam in

(2) Heat sink effect of large mass pipe work & valves draws heat away from the ports

(3) Potential to trap air (or other NCG's) in the top of the port (ports act like an air detector)

(4) Condensate may collect in ports or on the base

Specification - URS

- The requirements for steam sterilisation are different to freeze drying requirements
- Need to involve users, Engineering, QA, validation personnel & sterilisation specialists
- Decide on the acceptance criteria required
- Ensure that the supplier fully understands the requirements

Understand the Functional Specification

- Will the machine deliver what is required (freeze drying & sterilisation)
- Does the supplier understand steam sterilisation?
- Do you understand how the control system works?
- Will the control system/chart recorder/data logging system deliver what is required?
- Has provision been made for thermocouple entry/pressure measurement?

Factory testing , on-site testing & validation

Factory testing - 1

- Must test at the factory
- Computer validation – involve sterilisation personnel
- Base the protocol for the factory testing of the steam sterilisation cycle on the production acceptance criteria/requirements
- Ensure that sufficient time is allowed for factory testing
- Whose equipment is to be used for the factory testing? Datalogger, thermocouples, pressure gauges etc.

Factory testing - 2

- Extensively temperature map the chamber, condenser and vent filter
- Be guided by the supplier BUT do challenge them
- Ensure that all locations are considered
- Actively look for difficult to sterilise locations – ports, places where condensate may collect etc
- Ensure thermocouples measure surface temperatures – not free space

Factory testing - 3

- Likely to have to perform more than one run
- Keep some T/C's in fixed positions for all cycles
- Where are the cold spots?
- Don't increase the temperature/extend the hold time to mask a problem
- Will the cold spot temperature be routinely monitored?
- Are the cycles reproducible?
- Is there a correlation between temperature and pressure (are the Steam Tables followed)

Factory testing - 4

- Where will the cycle be routinely controlled from? Is the cold spot in the drain?
- If the acceptance criteria are not met at the factory – will they be met on site?

On – site testing

- IQ
- Computer validation
- Validation of services
- Sterilisation cycle validation
- Freeze drying cycle validation

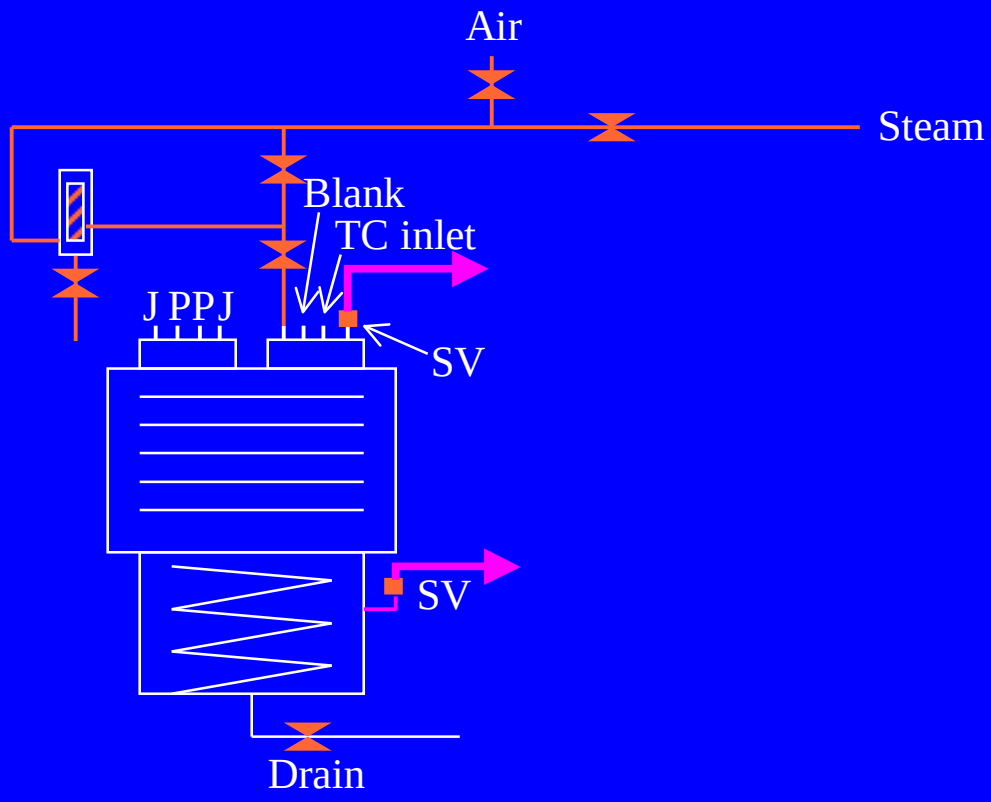
Validation of the sterilisation cycle

- Calibration of instruments and T/C's
- Enumeration of BI's
- Leak rate test (after T/C's fitted)
- Temperature mapping of chambers and vent filter + filter differential pressure (T/C's + BI's)
- Temperature reproducibility tests (T/C's + BI's)
- Formulation of acceptance criteria (routine cycles)
- Annual re-validation

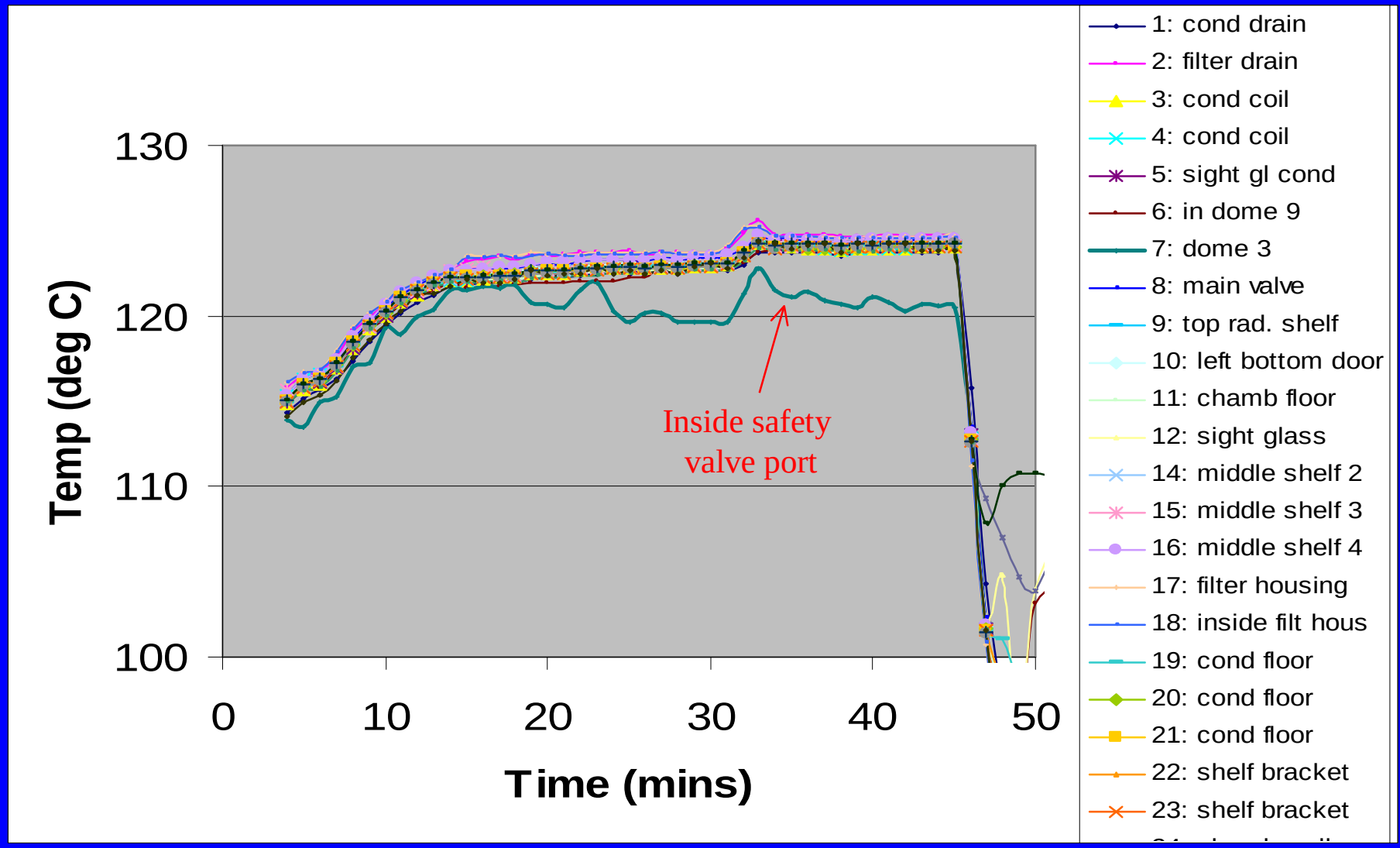
A case study

Validation of a freeze dryer sterilisation cycle and the modifications required to eliminate the cold spots

Machine - pipe work “as built”

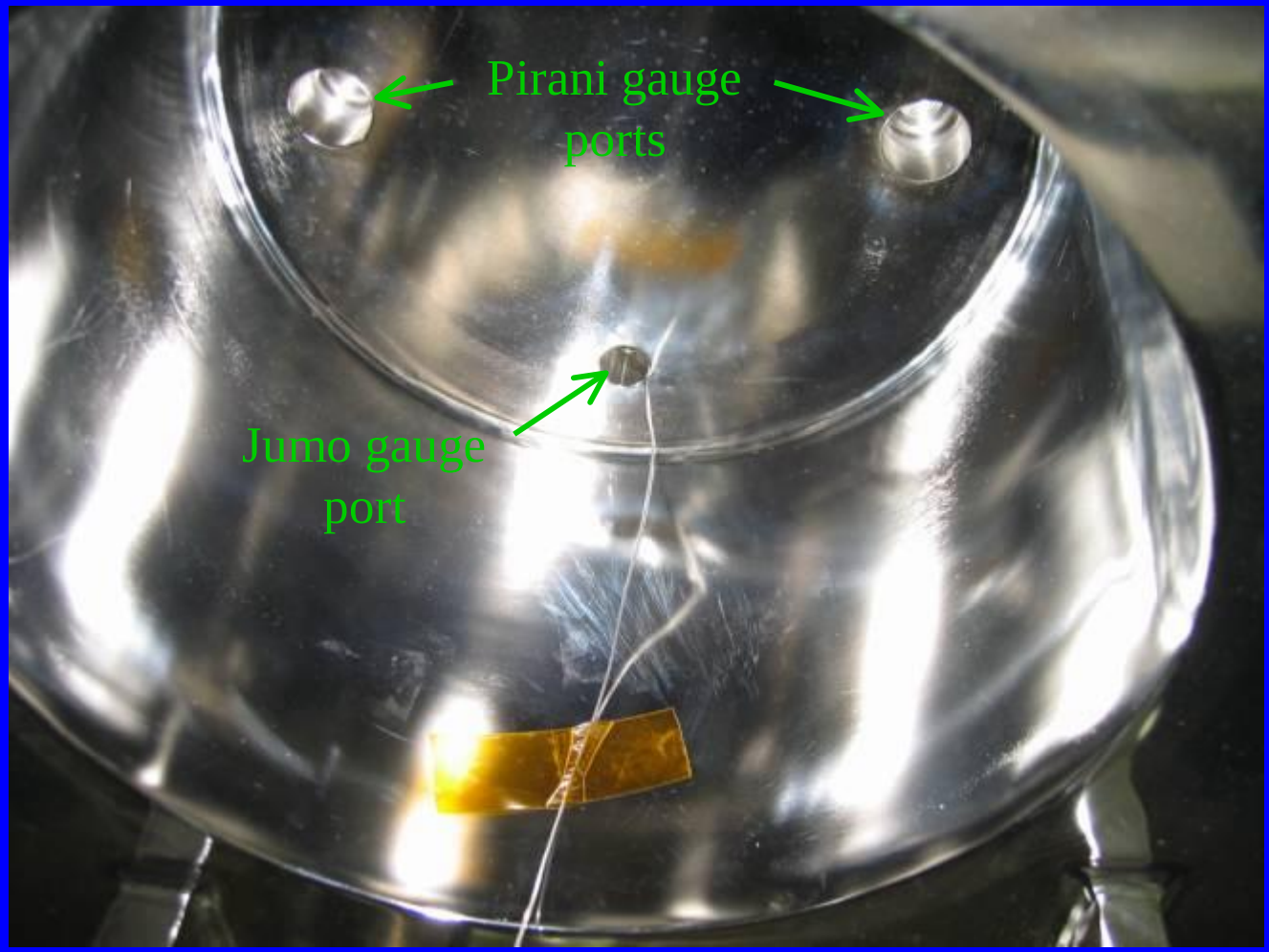


Factory testing – temperature mapping

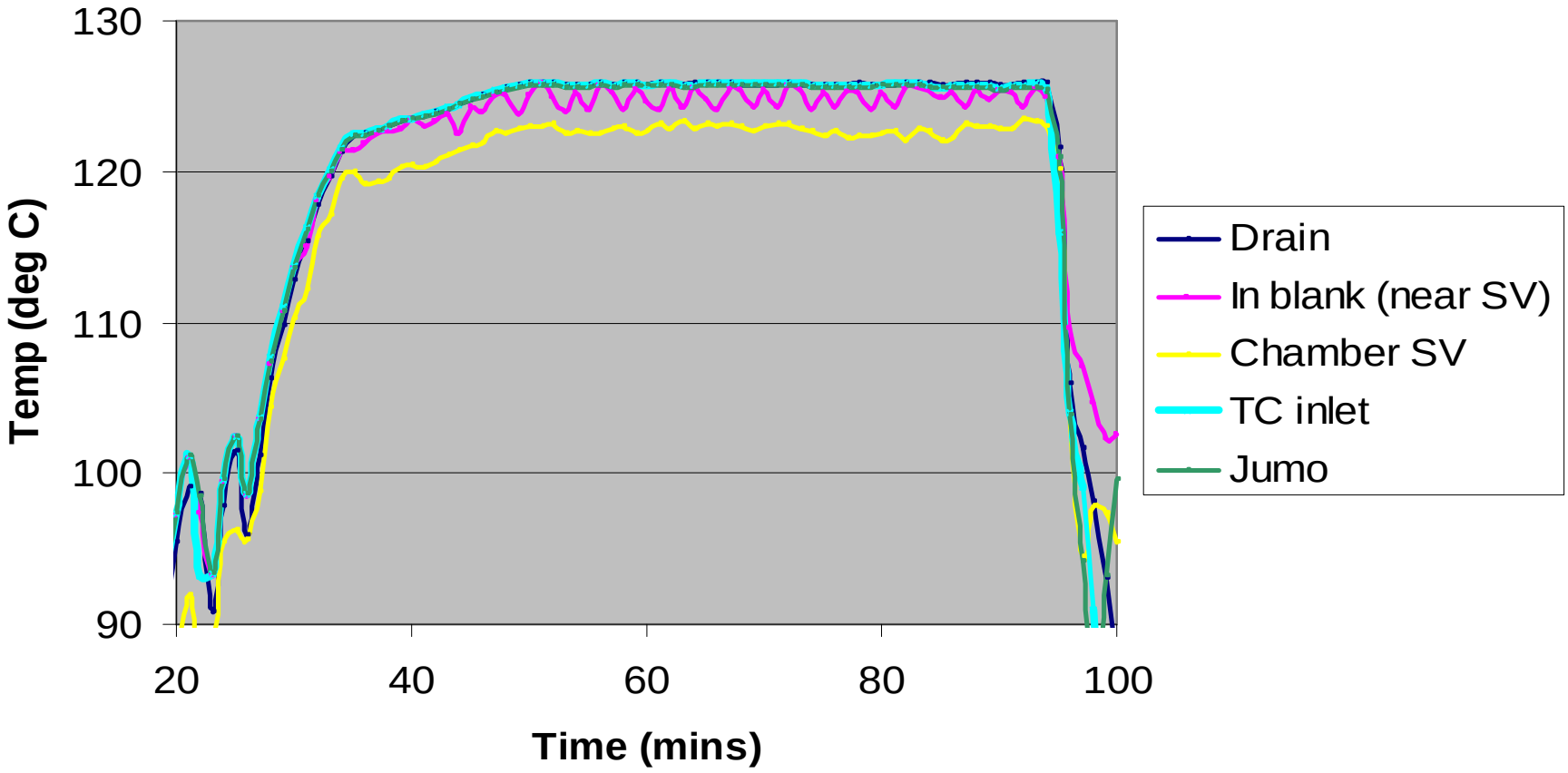


View inside dome A

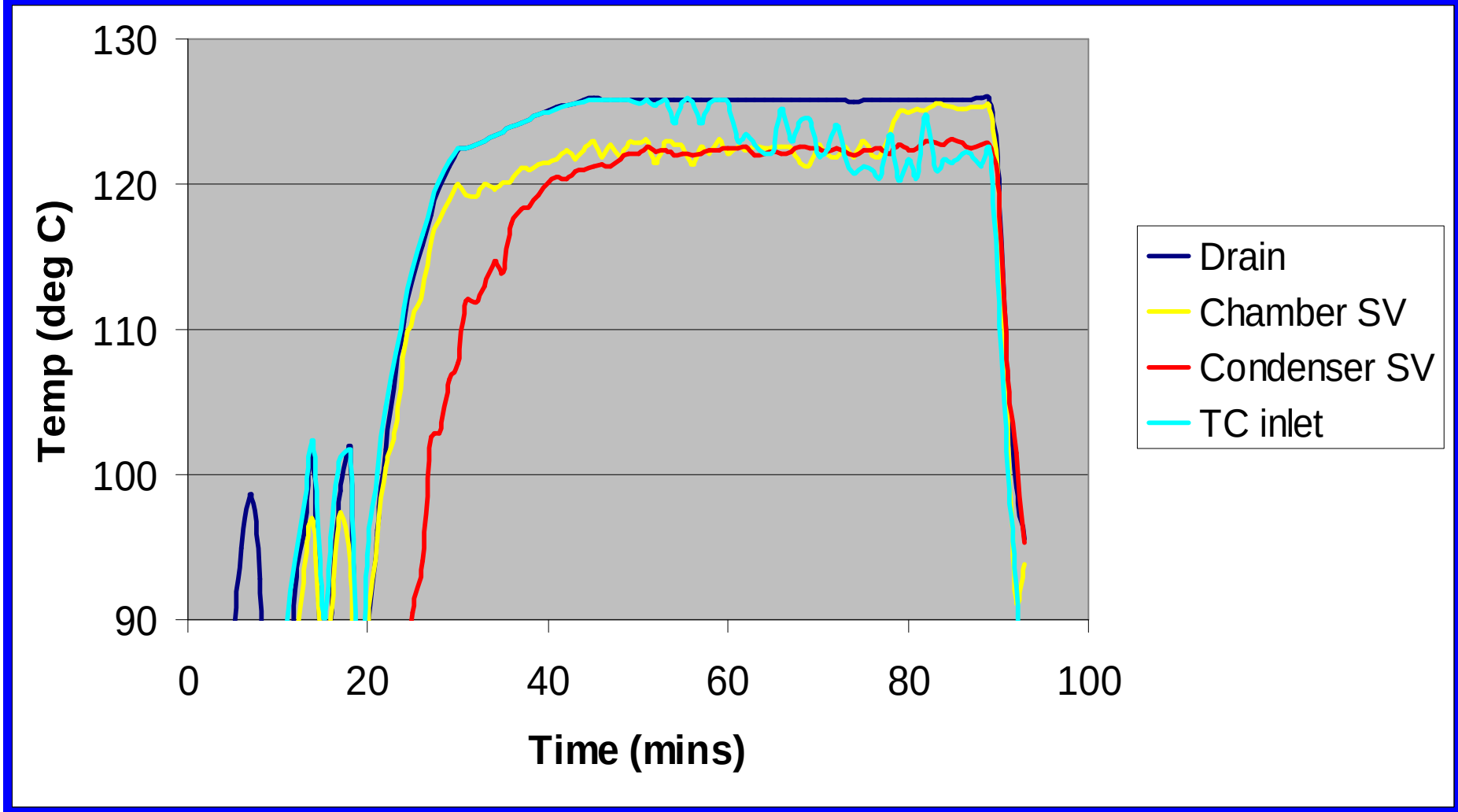
View inside dome B



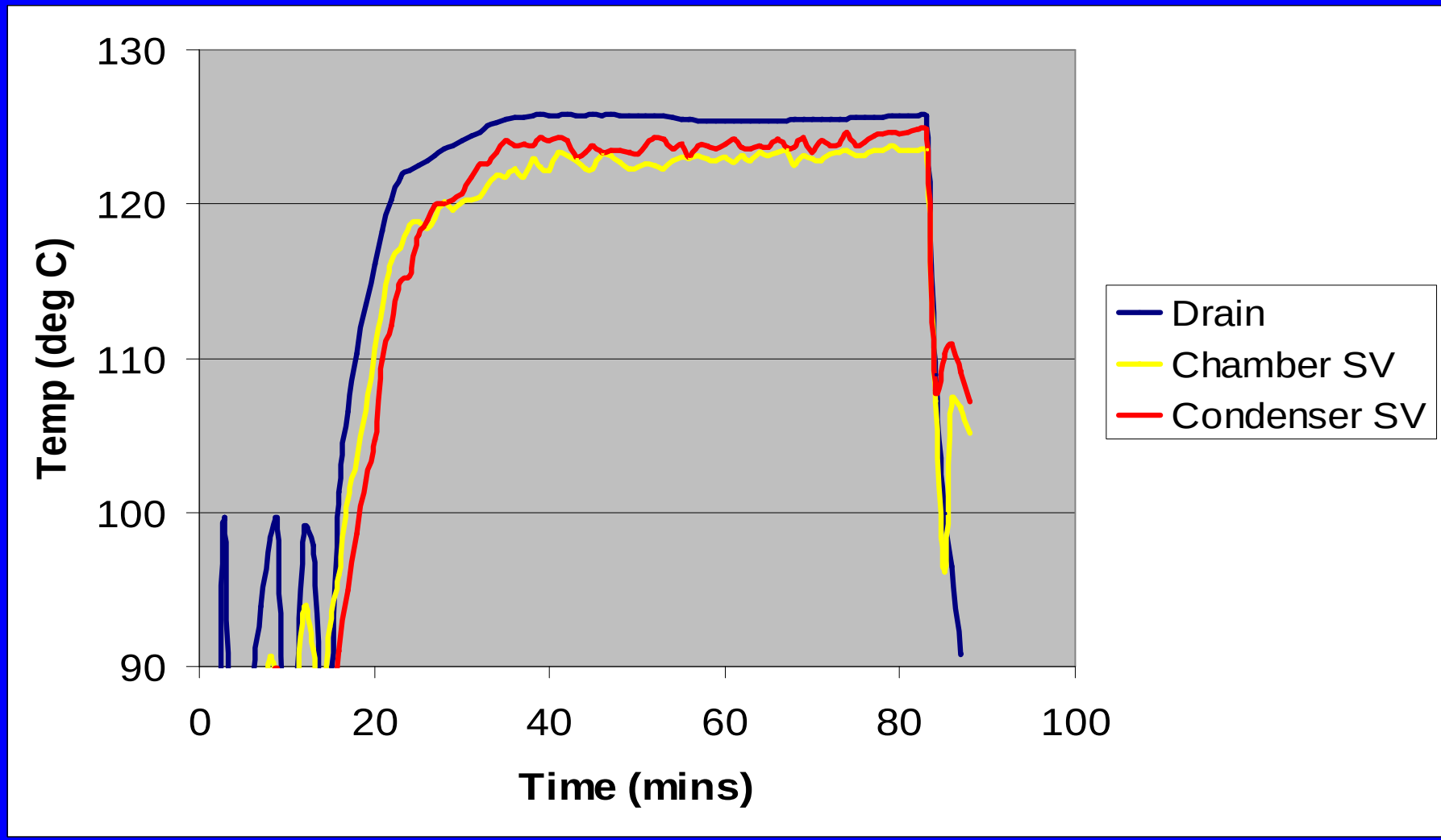
On site temperature mapping



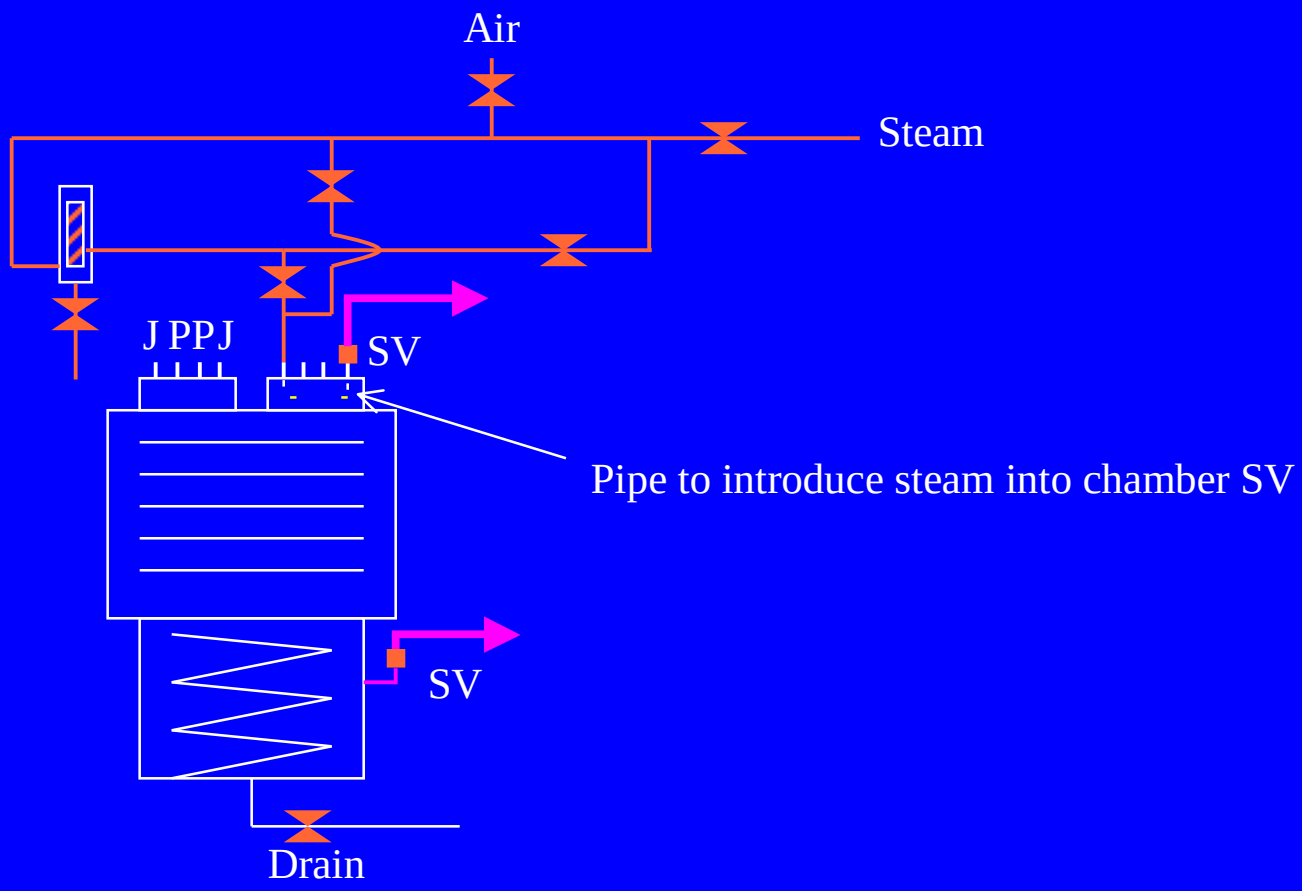
On site temperature mapping



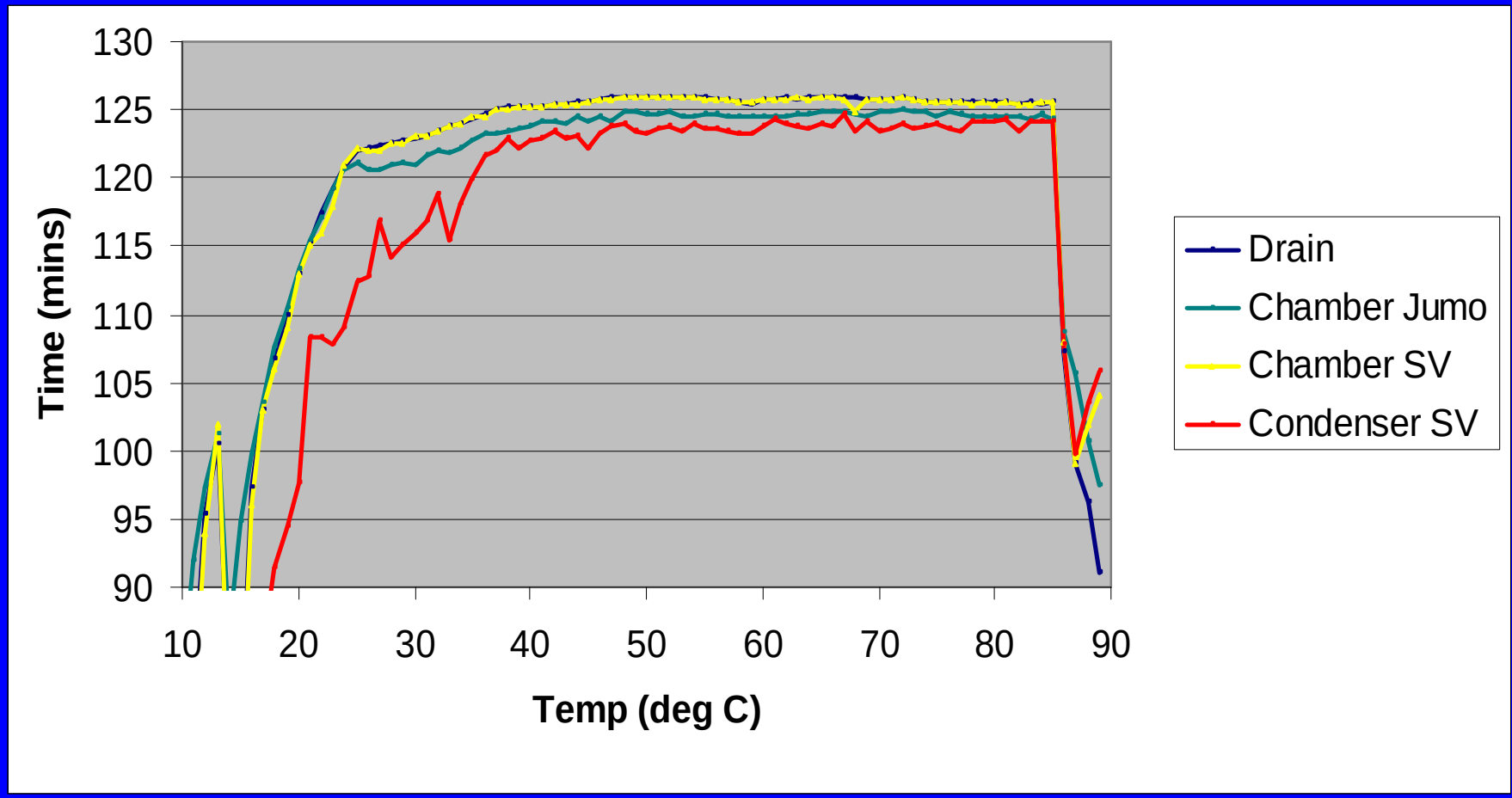
On site temperature mapping – test with safety valves lagged



Pipe work – initial modification to chamber safety valve



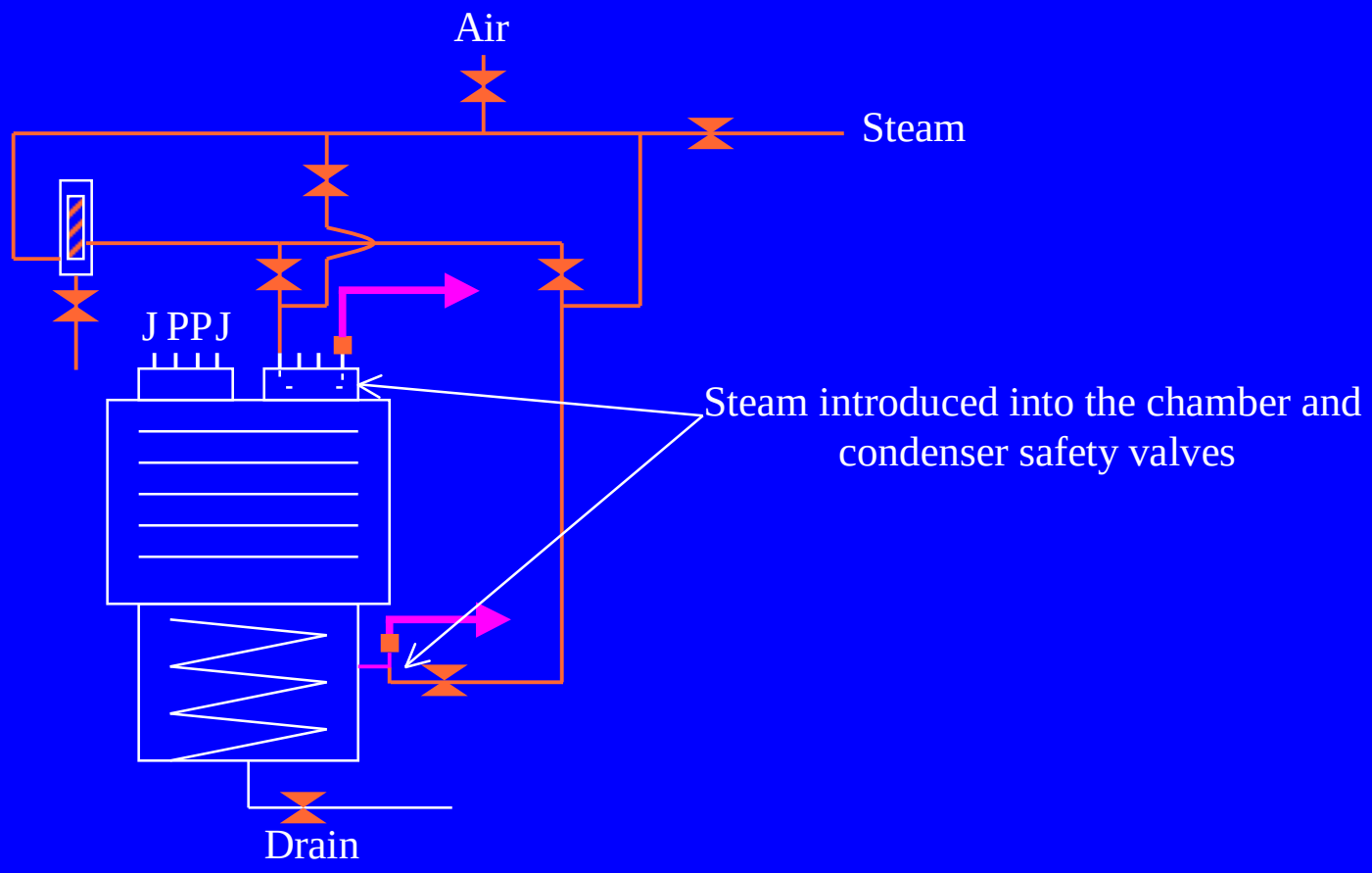
After steam introduction to the chamber safety valve



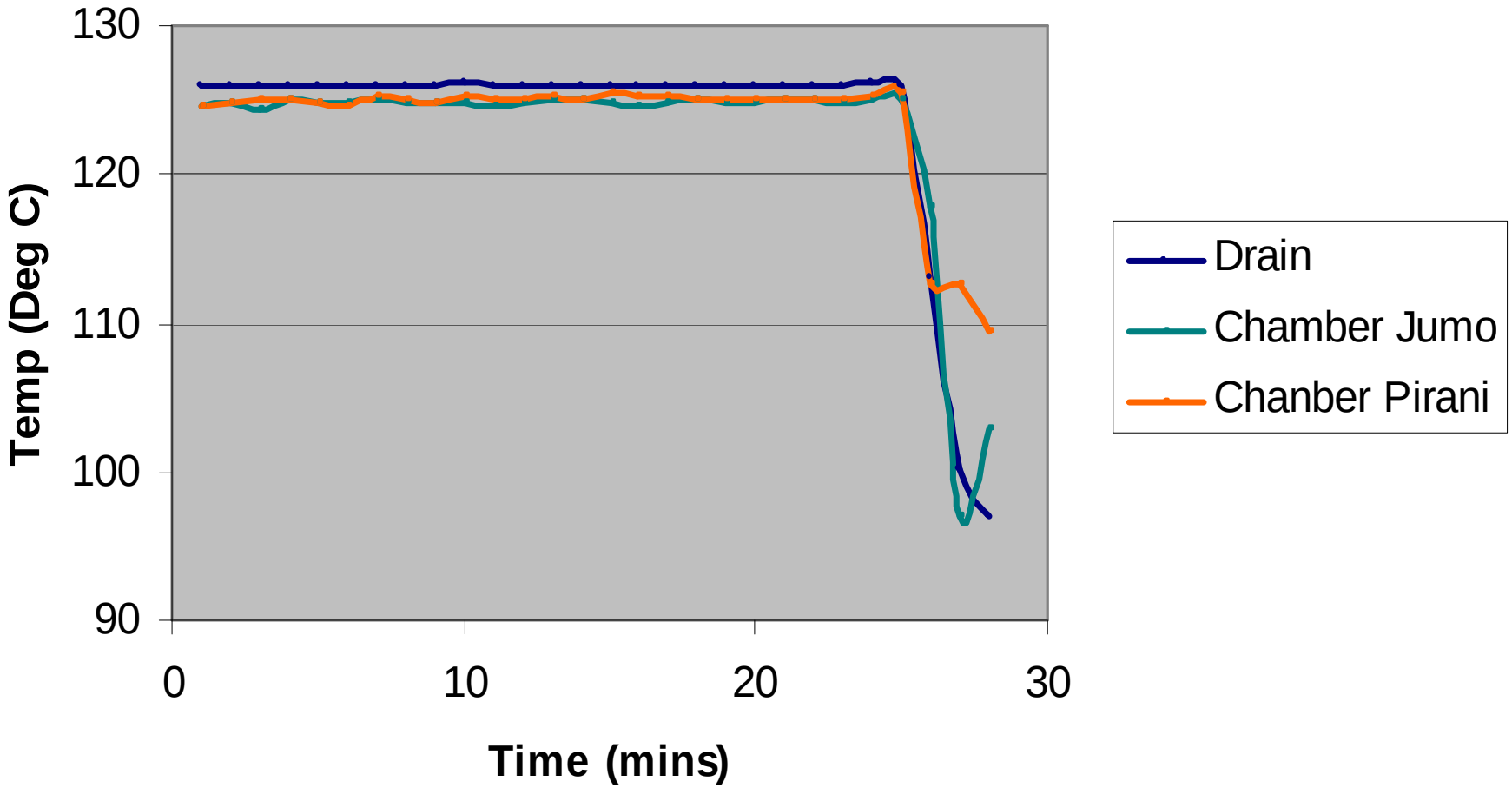
Summary of time at 122°C and Fo values (after mods to chamber SV)

Thermocouple location	Min time above 122°C (mins)	Min Fo during the sterilisation hold stage
Condenser drain	60	55
Jumo (chamber)	52	35
Pirani (chamber)	55	50
Shelf 3	60	53
Sight glass (chamber)	60	53
Shelf 2	60	54
Safety valve (chamber)	60	53
Shelf 1	61	54
Filter drain	61	56
Top shelf bracket (right)	60	53
Shelf 4	60	53
Door	60	51
Chamber floor	60	54
Bottom left shelf bracket	60	53
Condenser floor	61	55
Safety valve (condenser)	0	11

Pipe work – after modifications to chamber & condenser safety valves



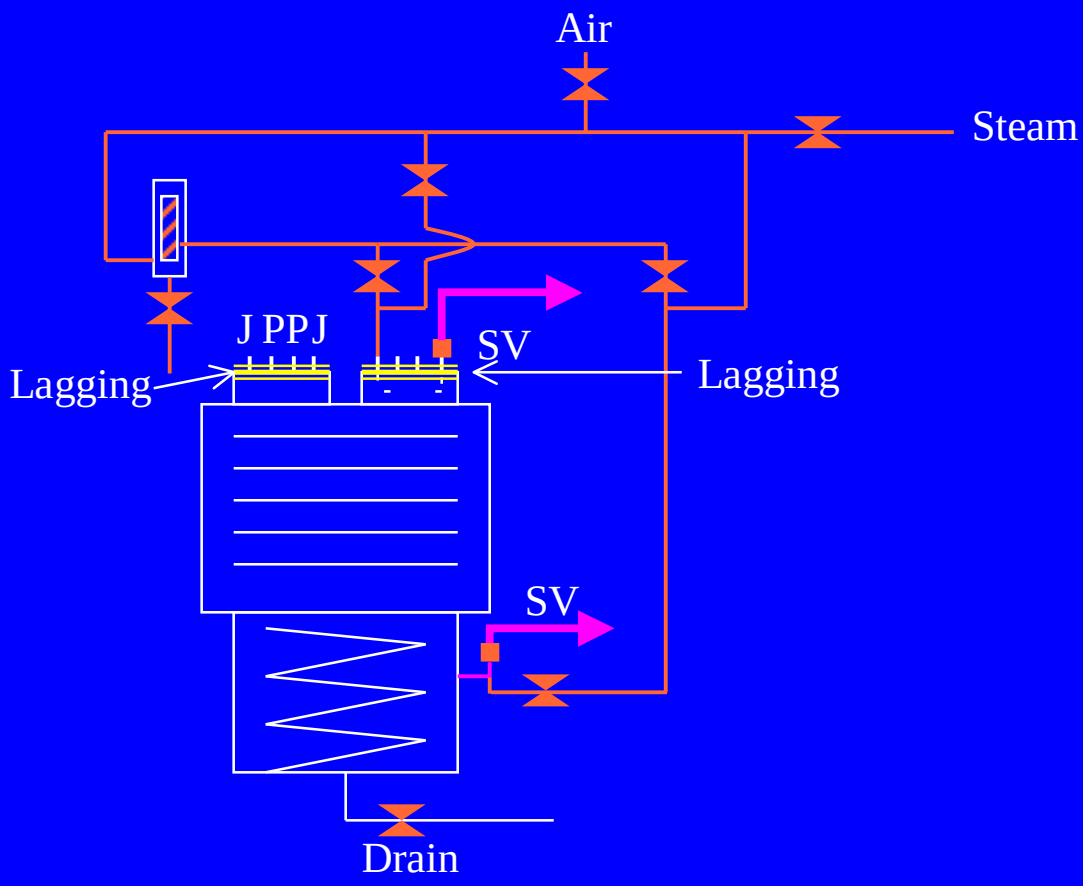
Temperature in Jumo and Pirani ports – after modifications to the safety valves



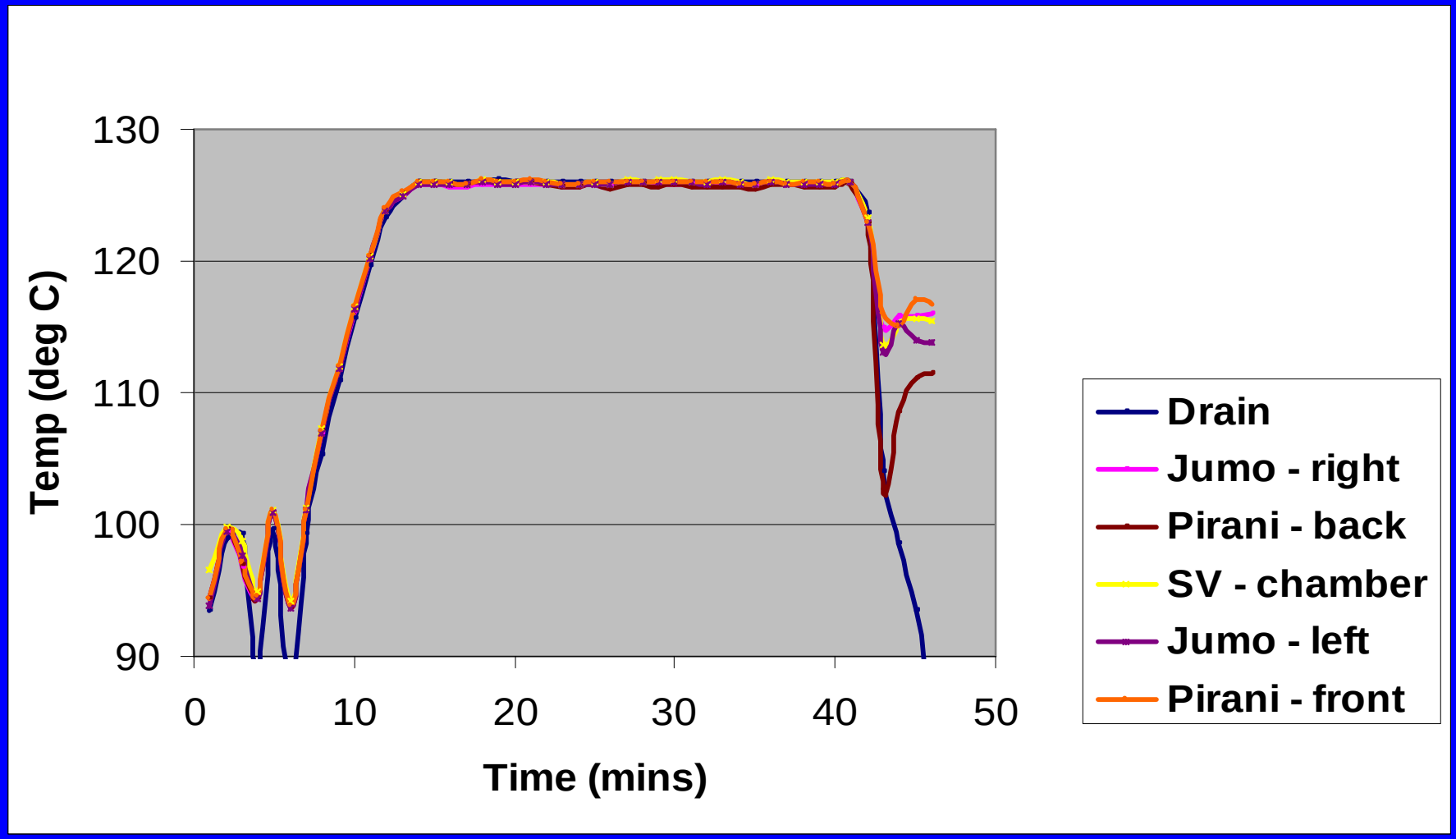
Summary of time at 122°C and Fo values (after mods to chamber and condenser SV)

Thermocouple location	Min time above 122°C (mins)	Min Fo during the sterilisation hold stage
Condenser drain	60	59
Jumo (chamber)	60	42
Pirani (chamber)	60	46
Safety valve (chamber)	60	59
Sight glass (chamber)	60	59
Shelf 2	60	59
Top shelf bracket (left)	59	60
Shelf 1	60	62
Filter drain	61	73
Top shelf bracket (right)	60	59
Shelf 4	60	59
Door	60	59
Chamber floor	60	60
Bottom left shelf bracket	60	59
Condenser floor	60	61
Safety valve (condenser)	60	62

After pipe work modifications to SV's and lagging to domes



After pipe work modifications to SV's and lagging to domes + reduction in length of sterilisation hold stage



Summary of time at 122°C and Fo values (after lagging & reduction to cycle time)

Thermocouple location	Min time above 122°C (mins)	Min Fo during the sterilisation hold stage
Condenser drain	30.3	61
Jumo (chamber)	30.5	59
Pirani (chamber)	30.5	54
Safety valve (chamber)	30.6	61
Sight glass (chamber)	30.5	60
Shelf 2	30.5	60
Top shelf bracket (left)	30.5	59
Shelf 1	30.5	60
Filter drain	30.5	84
Top shelf bracket (right)	30.6	60
Shelf 4	30.5	60
Door	30.5	59
Chamber floor	30.6	60
Bottom left shelf bracket	30.6	61
Condenser floor	30.4	57
Safety valve (condenser)	30.6	57

Conclusions

- Don’t assume that the freeze dryer manufacturer is expert in steam sterilisation
- ***You*** may have to present the validation data to the regulatory authorities
- Engineering solutions are better than ‘quick fixes’
- Be aware that any changes made may have a knock on effect
- Good validation takes time – but it’s worth it in the end!