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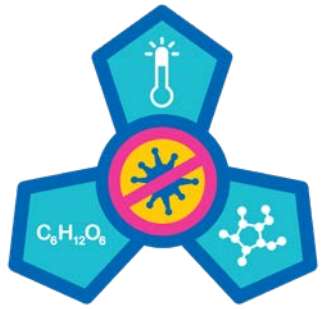
Pharma & Biopharma Raw Material Solutions

Application Note



High Temperature Short Time Pasteurization

Summary

We have expanded our Total Virus Safety portfolio to include HTST pasteurization at an industrial scale. Our Global sterile liquid capabilities, which produce liquid buffer and cell culture media, allow virally mitigated products to be shipped globally. HTST pasteurization can provide robust viral mitigation with an approximate 6 log reduction, for large volume high risk raw materials.

Introduction – From Pasteur to Perfusion

In 1864, French scientist Louis Pasteur discovered that subjecting beverages to heat for a specific period of time greatly reduced the rate at which spoilage occurred. This technique was further developed and has been widely adopted by the food and beverage industries. HTST has now come to the forefront of the biopharmaceutical industry as a viral risk mitigation strategy to prevent costly contamination events. This technology has been optimized to ensure that the potential for product impact is minimized by reducing the residence time within the high temperature phase of the process. This maintains product quality while providing high viral inactivation.

Monoclonal antibodies (mAbs) are typically produced using mammalian cell culture systems. These cell lines primarily utilize Chinese Hamster Ovary (CHO) cells, which are highly susceptible to viral contamination from viruses such as those carried by rodents.

Glucose and galactose are critical raw materials for mAb production, and given their sources (sugarcane or beet fields) coupled with in-process storage conditions, they are a high risk raw material for viral contamination as rodents are highly attracted to these materials.

Prevent with Pasteurization

Our HTST pasteurization technology allows customers to target specific pasteurization temperatures and residence times. We have generated optimal thermal processing set-points based on lab and pilot-scale trials.

Pasteurization of reactor feeds at the recommended set-points can provide inactivation of a worst-case virus of >6 logs (99.9999%). Pasteurization combined with post-HTST sterile filtration provides customers with ‘ready to go’ solutions.

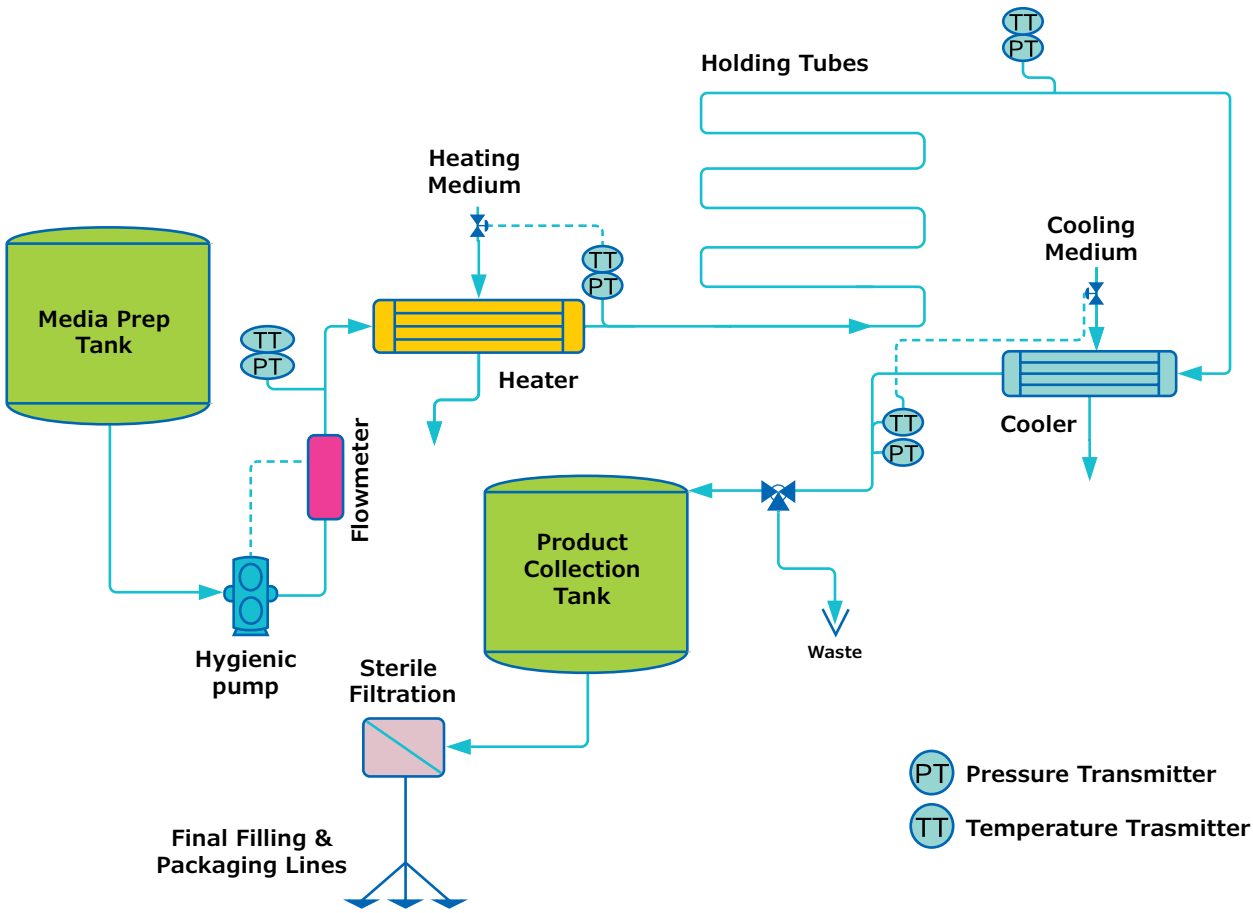
HTST Process Technology

The fully automated HTST process we have developed tracks critical process parameters and ensures that liquid products are subjected to predetermined pasteurization set-points. In-line instrumentation monitors process conditions and adjusts system operation to achieve reliable pasteurization performance.

We have researched the effects of HTST pasteurization in terms of both viral inactivation and cell culture performance post-pasteurization. This deterministic approach enables us to provide a viral safety strategy to each individual customer’s requirements, helping safeguard their bioreactors against the threat of infection from high risk raw materials.

We have developed a closely controlled process which continually assesses operating conditions (temperature and residence time) and automatically routes Out Of Specification (OOS) material to waste, ensuring that only material which has been subjected to the selected pasteurization set-points reach packaging lines and are released to customers.

Figure 1. HTST Simplified Process Flow Diagram



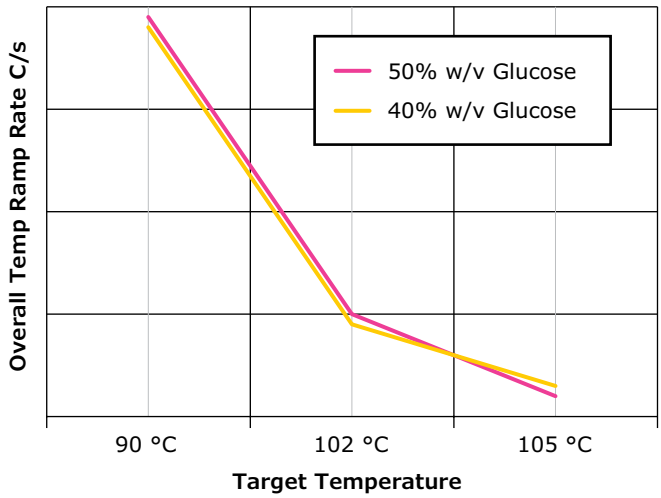
HTST Viral Inactivation

We have performed lab-scale virus inactivation trials which have been designed to be representative of the industrial scale pasteurization process. The analytical trials have subjected Minute Virus of Mice (MVM) to a series of HTST processing conditions to determine the viral Log Reduction Value (LRV) of different pasteurization temperatures and residence times.

As MVM is a highly physicochemical resistant parvovirus, and one of considerable risk to cell-based biopharmaceutical manufacturing, it is an ideal worst case virus to use in the viral inactivation study. The heat transfer rates of the pilot, mid-scale and large scale HTST processes were compared. The inactivation study was designed to subject virus to a comparable heat transfer rate across different residence times and pasteurization temperatures.

The heat transfer rates of different glucose solutions (40% w/v and 50% w/v) were analyzed. The analysis showed that there was a negligible difference in the rate at which the solutions increased in temperature during the lab-scale trials. This is shown in **Figure 2**.

Figure 2. Comparison of Temperature Heat Transfer Rate in 40% and 50% w/v Glucose Solutions



A series of water and oil baths were set at different operating conditions to mimic the industrial heating/cooling conditions used by the HTST process. The baths were temperature mapped so circulation could be optimized to ensure temperature homogeneity.

Stainless steel vials were used to contain the glucose/MVM test solutions. Glucose solutions were spiked with live MVM dilution and thoroughly mixed. Control vials were retained without pasteurization treatment as the hold samples for viral inactivation LRV determination.

The vials were mounted in a dip rack with surrogate vials to provide temperature data. The dip rack was moved through a series of baths and held at target pasteurization temperatures for specific residence times. After cooling, the contents of each vial were assayed for residual viral infectivity using the Tissue Culture Infectious Dose 50% ($TCID_{50}$) virus infectivity assay.

As the different glucose solutions heated up at a comparable rate, limited temperature runs (90 °C, 102 °C and 105 °C) on the 50% w/v solution were performed.

Experimental Results

Viral inactivation, expressed as LRV was determined by comparing the titer post-HTST treatment with the titer pre-HTST treatment. The results from the viral inactivation study on 40% glucose are shown in **Table 1** and the results from the study on 50% glucose are shown in **Table 2**.

Table 1. Viral Inactivation in 40% Glucose

Hold Temperature	Hold Time (s)	Mean Log Reduction Value
90 °C	10	3.5
	20	4.3
	30	4.2
	40	4.3
102 °C	10	6.5
	20	6.4
	30	6.4
	40	6.5
105 °C	10	6.5
	20	6.5
	30	6.6
	40	6.5
108 °C	10	6.2
	20	6.5
	30	≥6.5
	40	≥6.5
110 °C	10	≥6.5
	20	≥6.5
	30	≥6.5
	40	6.5

Table 2. Viral Inactivation in 50% Glucose

Hold Temperature	Hold Time (s)	Mean Log Reduction Value
90 °C	10	2.0
	40	3.2
102 °C	10	≥6.7
	40	≥6.7
105 °C	10	≥6.7
	40	≥6.7

Prevention with Performance

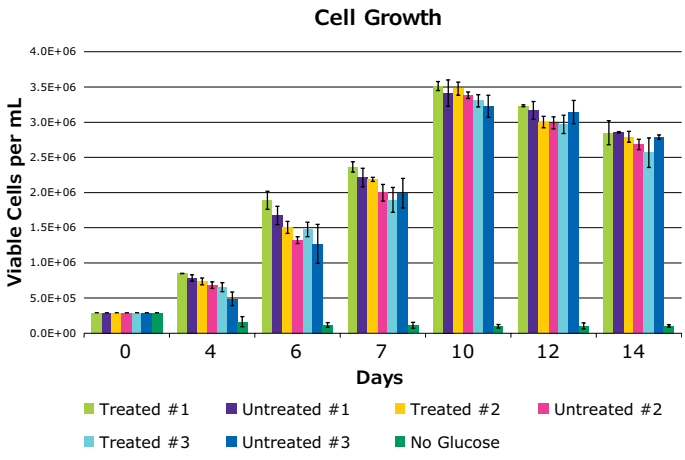
Post-treatment performance analysis has been performed to ensure that the safeguarding effect of pasteurization does not come at the cost of cell culture performance. **Figure 3** shows the results of cell growth and viability assessments.

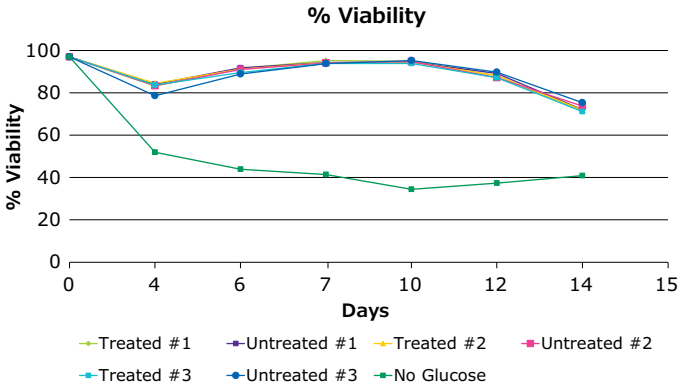
The impact of using a pasteurized 50% w/v glucose feed was assessed. Cell growth in systems fed with pasteurized and unpasteurized (referred to in **Figure 3** as Treated or Untreated) glucose solutions were compared all Treated runs were undertaken at 102.5oC at 10.5s with a tolerance of +/-0.5oC and 0.5s. Each glucose sample was contained in a 30 mL polypropylene tube and inoculated with CHOZN™ $GS^{-/-}$ ZFN-modified CHO cells at $3.0x10^5$ viable cells/mL. The tubes were held in a shaking Multitron incubator at 37 °C, 5% CO_2.

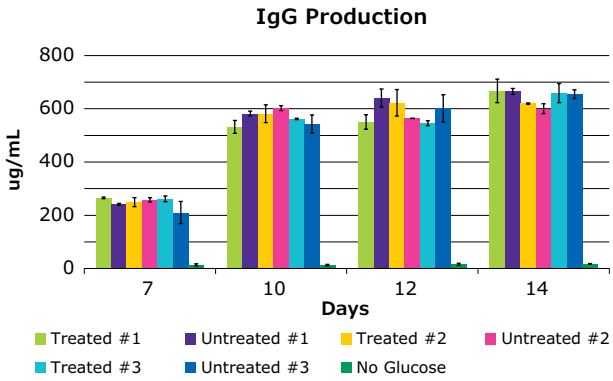
Cultures were counted daily using a Beckman Coulter Vi-CELL™ cell counter to determine growth and viability (trypan blue exclusion method). Spent media samples were collected on days 7, 10, 12 and 14 for IgG productivity analysis with a ForteBio Octet™ Interferometer system using Protein A biosensors.

Cell cultures fed with either HTST-treated or untreated glucose exhibited very similar growth characteristics and IgG production levels, demonstrating that HTST treatment did not significantly affect glucose in terms of cell growth, viability and productivity.

Figure 3. Growth, Viability and Productivity Results from 50% w/v Glucose Trials







Conclusion

Our HTST capability has been developed to achieve robust viral clearance performance on high risk raw materials. The technology does not impact feed viability or performance. Utilizing pasteurized reactor feed products can significantly reduce the probability of viral contamination events and contributes to a high level of virus safety assurance.

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