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REVIEW

BIOTECHNOLOGY PROGRESS

Review: High temperature short time treatment of cell culture media and feed solutions to mitigate adventitious viral contamination in the biopharmaceutical industry

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Abstract

Events of viral contaminations occurring during the production of biopharmaceuticals have been publicly reported by the biopharmaceutical industry. Upstream raw materials were often identified as the potential source of contamination. Viral contamination risk can be mitigated by inactivating or eliminating potential viruses of cell culture media and feed solutions. Different methods can be used alone or in combination on raw materials, cell culture media, or feed solutions such as viral inactivation technologies consisting mainly of high temperature short time, ultraviolet irradiation, and gamma radiation technologies or such as viral removal technology for instance nanofiltration. The aim of this review is to present the principle, the advantages, and the challenges of high temperature short time (HTST) technology. Here, we reviewed effectiveness of HTST treatment and its impact on media (filterability of media, degradation of components), on process performance (cell growth, cell metabolism, productivity), and product quality based on knowledge shared in the literature.

KEYWORDS
contamination, flash pasteurization, high temperature short time, precipitation, virus

1 | INTRODUCTION

Viral contaminations are a severe issue in the frame of the production of biopharmaceuticals by mammalian cells. The production of therapeutic proteins from living organisms, such as mammalian cells, requires a set of components essential for cell survival and cell growth. Those components are provided by cell culture media. Formerly, bovine serum was widely used in cell culture media as a source of amino acids, proteins, vitamins, carbohydrates, lipids, hormones, growth factors, inorganic salts, trace elements, and other compounds. The use of serum or in general of any animal or animal derived raw materials is associated with a higher risk of viral contaminations. For safety and economic reasons, serum-free media, sometimes supplemented with protein hydrolysates, have been developed to replace the use of serum.[1] However, animal component free raw materials also carry a viral contamination risk if they were in contact with animal or animal-derived material during cultivation, manufacturing process, storage, or shipment. This risk of viral contamination by cell culture media raw materials was pointed out early in 1977 by Nuttall and al.[2] They reported the high risk of occurrence of bovine viral diarrhea virus (BVDV) contamination in untreated fetal bovine serum. Some examples of viral contamination in the pharmaceutical industry were publicly reported and are presented in Table 1. Those virus contamination events become rare as only 26 virus contaminations were reported over the past 36 years.[3] Raw materials were the likely source of contamination in most cases. In 11 of the 12 reported

TABLE 1 Examples of viral contamination events in the pharmaceutical industry

Year	Viruses	Cell	Identified or most likely cause	Reference
1988	EHDV[a]	CHO[b]	Animal derived raw material (Fetal Bovine Serum)	38, 39
1993; 1994	MMV[c]	CHO[b]	Raw material (Unknown)	40, 41
1999	Reovirus	Newborn kidney	Unknown	42, 43
Not disclosed	Reovirus	CHO[b]	Fetal bovine serum	44
2000	CVV[d]	CHO[b]	Animal derived raw material (Fetal Bovine Serum)	45
2002	Human Adenovirus	HEK 293[e]	Non animal derived raw material (Unknown)	46
2003	Vesivirus 2117	CHO[b]	Not disclosed	47
2003; 2004	CVV[d]	CHO[b]	Animal derived raw material (Fetal Bovine Serum)	45
2008; 2009	Vesivirus 2117	CHO[b]	Raw material (Not disclosed)	48, 49
2009	MMV[c]	CHO[b]	Non animal derived raw material (recombinant media additive—contamination outside of the facility)	50
2010	PCV-1[f]	Vero	Animal derived raw material (Trypsin)	51
2010	PCV-1; PCV-2[f]	Vero	Animal derived raw material (Trypsin)	52

[a]Epizootic hemorrhagic disease virus.
[b]Chinese Hamster Ovary.
[c]Mouse Minute Virus.
[d]Cache Valley Virus.
[e]Human Embryonic Kidney.
[f]Porcine Circovirus.

contaminations in CHO cell culture, a raw material or medium component was identified or suspected to be the source.[3] Such an event often leads to serious economic and safety consequences.[4] Costs associated with a viral contamination event can range from one to hundreds of millions of dollars depending on its severity.[3] For example, the costs associated with a vesivirus 2117 contamination were higher than $200 million including a fine of $175 million from the U.S. Food and Drug Administration.[4] To reduce the risk of introducing viruses during production, preventive steps are carried out such as detection tests on cell banks and on raw materials of animal origin. Detection of potential viral contaminations is also performed on the unprocessed bulk. Downstream viral clearance steps allow one to inactivate or remove most of viruses during the purification of the therapeutic proteins depending on the type and the final amount of viruses present in the unprocessed bulk.[5] Given the inhomogeneity of distribution of potential adventitious agents, the sensitivity and specificity limits of detection of viral testing, it remains difficult to identify a viral contamination in a raw material or in the cell culture media before the cell culture step.[6] It was also reported that using cytopathic assays is not 100% effective in assuring that the cell culture is free of viruses.[6,7] Therefore, it is necessary to better mitigate this risk by implementing a virus preventive treatment of the cell culture media and feed solutions. Several techniques can be considered such as high temperature short time treatment, nanofiltration, gamma, or ultraviolet-C (UV-C) irradiation. Briefly, UV-C irradiation has the advantage of being compatible with serum-based media. However, this technology is associated with tremendous challenges regarding exposure uniformity, and maintenance of the equipment.[8-10] Alterations of vitamins, carboxylic acids, and proteins were also often reported.[8-11] Gamma irradiation is widely used for viral inactivation of serum, but it is not practically a point of use technology and it requires application by the supplier. Its efficacy is virus-dependent, and the technology is also very challenging to be implemented for large cell culture media volumes.[12,13] Nanofiltration of media is considered effective for virus removal, but costs can be significant and media composition can sometimes lead to low flow rate and filter clogging.[14,15] Pasteurization is historically performed in food industry on milk, juice, and some meat products to inactivate potential pathogens and extend shelf life. Pasteurization of plasma products for the pharmaceutical industry was first performed in the 50s of the last century after cases of hepatitis reported in the US Armed Forces troops were linked to a vaccine manufactured with human serum.[16] In 1948, a study, published by Gellis et al., showed the effectiveness of pasteurization as a viral inactivation technique on human serum albumin with hepatitis B virus.[17] In this review, the high temperature short time (HTST) technology will be discussed as a mitigation step for cell culture media and feed solutions against viral contamination in the biopharmaceutical industry.

2 | HIGH TEMPERATURE SHORT TIME TREATMENT

HTST treatment is also well known as flash pasteurization. In the biopharmaceutical industry, it is important to highlight the difference between heat treatment of culture media and heat treatment of viruses for vaccine applications. The first one only lasts a few seconds to preserve media integrity and is a preventive treatment. The second one can last longer as it is crucial for pathogenicity suppression.[18,19] Flash pasteurization was often the chosen technology put in place by biopharmaceutical industries that have experienced one or more viral

contamination events in the 90s. In general, flash pasteurization of cell culture media is performed with continuous systems composed of two heat exchangers separated by an insulated retention tube. The medium is heated to the targeted temperature in the first heat exchanger and then held at this temperature for a short period of time in an insulated retention tube (e.g., 100°C for 5 s). The heating section is mainly divided in two parts: pre-heating and heating. The medium is then cooled through the second heat exchanger and sterilized by a standard microfiltration step. Inactivation temperature and holding time are the most important controlled parameters to reduce impacts on cell culture media while ensuring an effective viral inactivation.

Two types of heat exchangers are used in the industry. One of them is the shell and tube heat exchanger, which typically works by countercurrent circulation of fluids in the tubes and the shell. Shell and tube heat exchangers are the most common type used in the pharmaceutical industry. Thanks to their resistance to high pressure, they are ideal candidates for high viscosity media. Generally, shell and tube heat exchangers have welded tube sheets that prevent the removal of the tube bundled for inspection and cleaning, although there are many designs and some modern hygienic exchangers will allow the removal of the tube sheet. Borescope inspections are commonly used as well as standard pressure testing for inspection and maintenance purposes. The second type, plate and frame heat exchangers, is smaller, cheaper, and more efficient in terms of heat transfer. Plate and frame exchangers can have additional plates installed easily to increase the flow path (or removed to decrease the flow path). However, they are more prone to pressure drop issues due to plate fouling as the flow path is narrow. Maintenance is typically faster as the plate packs can easily be removed for cleaning or replacement. Selection of plate and frame heat exchanger is, therefore, fluid dependent.[20]

3 | VIRAL INACTIVATION EFFECTIVENESS

High temperature short time treatment is known to be able to inactivate effectively a broad range of viruses. Boschetti et al. reported the principle of virus inactivation by heat, by measuring endonuclease activity after different heat treatments. A high level of endonuclease activity measured by polymerase chain reaction (PCR) after heat treatment at 90°C for 10 min illustrated accessibility of viral DNA for endonucleases, and therefore, the disintegration of the viral capsid. Heat inactivation of viruses prevents the interaction of viral capsid proteins with cell-surface receptors that facilitate their entry into the cells.[21]

Heat sensitivity of viruses after short time exposure at high temperature (>90°C) was reported in scientific articles (Table 2). Larkin et al. highlighted that due to their chemical composition, enveloped viruses are more sensitive to heat, while non-enveloped parvoviruses and picornaviruses show the highest resistance to heat treatment.[22] Mouse Minute Virus (MMV), a non-enveloped parvovirus, was often used as a worst case viral model given its high resistance to heat treatment, its small size (18–24 nm), and its demonstrated capability to contaminate recombinant protein cell culture operations.[3,5,23] Media spiked with viruses were put into small containers and exposed to a specific temperature and time of exposure. This method is commonly applied for viral inactivation studies, although alternative technologies can be used, like the microflow system experimented by Murphy et al.[24] The comparison of data from literature allows to highlight some convergences and divergences related to heat sensitivity of viruses. A robust inactivation of viruses (≥4 LRV) was not achieved by only treating media for a few seconds below 90°C for MMV based on references presented in Table 2. At 90°C, at least 10 min of heat treatment was required to reach an effective MMV inactivation (≥4 LRV).[21] Heating to 97°C and immediate cooling was efficient to provide complete inactivation (0.7 log10 TCID50 limit of detection) for MMV.[25] A similar finding was made by Schleh et al. who highlighted that at a temperature above 95°C, culture media spiked with MMV and exposed for a minimum of 2 s to heat treatment could be effectively cleared of viruses (≥4.56 LRV).[26] Therefore, if MMV is inactivated under certain conditions, it is indeed very likely for other viruses under the same conditions to be effectively inactivated as well (≥4 LRV). For example, above 95°C and with a holding time as short as 5 s, an effective viral clearance (≥5.6 LRV) was demonstrated for Porcine Circovirus 2 (PCV2).[27] This leads to high degree of virus safety regarding known and emerging human pathogens.

It is important to note that experimental conditions (device uses, composition of media, virus load) may have a significant impact on the assessment of viral clearance. For example, different heat transfer materials were used (e.g. water, steam, sand, oil). Depending on the material used, the time required to heat media to the targeted temperature might vary. Some materials are associated to high heat conductivity while others can cause media to heat more slowly. The media composition also has an impact on the viral inactivation.[26] Media properties (e.g., high media viscosity) may have protective effects by impacting heat transfer and the microenvironment of viruses. Viruses suspended in plasma showed a higher temperature resistance than viruses suspended in distilled water.[28] The effectiveness of HTST treatment on cell culture media may also depend on the number of viral contaminants as Murphy et al. reported that the probability of achieving complete inactivation (100%) decreases as MMV contaminants increase.[24]

4 | IMPACT ON CULTURE MEDIA

Culture media are partially composed of heat sensitive components, which can be damaged by HTST treatment (Table 3). Therefore, every medium treated with this technology should be analyzed to ensure that media can still feed the cells with all necessary nutrients.

The major issue encountered in the literature with HTST treated media is component precipitation. While Schleh et al. and Kiss indicated that heat treatment did not cause any precipitation, such events have been highlighted by Cao et al, Pohlscheidt et al., and Shiratori et al.[25,26,29-32] When precipitation occurred, the precipitates were

TABLE 2 Assessment of viral inactivation by heat treatment based on data available in the literature

Virus	Media	Technology	Conditions tested	Conclusions taken from respective publications	Reference
BPV[a]	Distilled water or human plasma	Fusion-sealed ampoules of 0.1 ml fully immersed in agitated water bath	Media were incubated for durations between 0 and 48 h at 60°C	Viruses suspended in plasma show a higher temperature resistance than viruses suspended in distilled water. Inactivation of 7 log was obtained after 28 h in water and 48 h in plasma.	28
MMV[b], BEV[c], SINV[d], PRV[e], HIV-1[f]	Solution of 2.5% apolipoprotein A-I, 3 M guanidine hydrochloride, and 0.75 mM EDTA	Not given (probably a standard incubator)	Media were treated for 0, 60, 120, 180, 240, 300, 360 min at 60°C and immediately diluted fivefold in ice cold cell culture medium.	Robust inactivation ≥4 log was obtained for BEV[c], PRV[e],and HIV-1[f] after 3 h, inactivation of ≥8 log was obtained for Sin after 3 h, and inactivation of ≥6 log was obtained for MMV[b] after 10 h.	23
MMV[b]	Water	Homemade device, wet-heat treatment, and ice bath.	Water were incubated for 0, 1, 2, 5, 10, 20, and 60 min at 70, 80, 90°C. media were immediately cooled on ice until titration.	Robust inactivation (≥4 log) was only achieved at 90°C for at least 10 min.	21
PCV2[g] PPV[h]	Eagle's MEM with non-essential amino acids containing antibiotics and 10% of fetal calf serum	Media in glass containers immersed in a heated shaking water bath.	Media were incubated for 5 s, 5 min, and 15 min at 80°C, 90°C, and 95°C, then placed after treatment on an ice bath.	The highest reduction of infectivity, ≥6.0 and ≥5.6 log10, respectively, could be seen after 5 s at 95°C for PCV2[g]. In this study PPV[h] was also studied. The results indicated that PCV2[g] is even more heat resistant than PPV[h].	27
MMV[b]	Two cell culture media with slightly different compositions	Apparatus designed and constructed in house	Media were incubated for 2, 5, 10, 15, 30 s at 95°C, 105°C, 115°C. Media were then cooled to ~17° before cooling.	Logarithm virus inactivation in these conditions was ≥3.24 by heating media 1 at least 5 s and ≥4.56 logs for media 2 under all conditions tested.	26
MMV[b]	Tests performed with a serum containing (10%, 2% or serum free media) cell culture media sample	Heating tube within a hot oil bath, held at that temperature by flowing through an insulated retention coil, rapidly cooled by a refrigerated water bath	Media were incubated for 0, 2, 6, 8, 10 s at 87°C, 92°C, 97°C.	Act of heating to 97°C and immediately cooling was enough to provide complete inactivation for media containing 10% serum. Similar inactivation results were obtained with 2% serum containing medium and serum free medium at 102°C over the range of hold times. Based on a modeling work, a design target of 102°C and 10 s exposure was selected for evaluation of media compatibility studies.	25
MMV[b]	Eli Lilly's proprietary medium LM7105	AFRICA microflow system (syrris Ltd)	Media were treated for 10, 20, 30, 40, 50, 60 s at 80, 90, and 100°C.	To receive ≥3 log10 of reduction, MMV[b] spiked medium had to be treated at 100°C for 60 s.	24

[a]Bovine papillomavirus.
[b]Mouse minute virus.
[c]Bovine enterovirus.
[d]Sindbis virus.
[e]Pseudorabies virus.
[f]Human immunodeficiency virus.
[g]Porcine circovirus.
[h]Porcine parvovirus.

mainly calcium phosphates. This has been chemically explained by Cao et al.[29] The solubility of calcium complexes in water, which is already low at 25°C (K_{sp} = 10^{-33}), decreases when temperature increases. These precipitations can lead to serious consequences such as clogging of filters or loss of trace elements, which can further alter product integrity (e.g. incomplete glycosylation) and production process yields.[31] In addition, precipitates may disrupt temperature control by fouling the heat exchanger surfaces. Key factors associated with

TABLE 3 Assessment of media impacts by heat treatment based on data available in the literature

Media	Technology	Conditions tested	Conclusions taken from respective publications	Reference
Media Similar to the common DMEM/Hams F12 medium formulations supplemented with protein hydrolysates Glucose solution Trace elements solution	Heat sterilization for media and glucose solution. Autoclave sterilization for trace elements solution.	102°C for 10 s for media and glucose solution.	10%–20% loss of insulin No significant loss of amino acids, vitamins, methotrexate. No significant impact on glucose concentration. Trace elements solutions treated by autoclave sterilization did not significantly affect the metal concentration. No significant impact on media or feed filtration performance.	25
Medium containing a growth factor, a complex plant hydrolysate, vitamins, amino acids, methotrexate, and other nutrients	Screening of media with a bench scale using a fluidized sand bath. Series of heat exchangers.	102 ± 2°C for approximately 10 s and then cooled to 37°C	At elevated temperature, calcium and inorganic phosphates in the media form insoluble particles, such as hydroxyapatites. Significant back pressure due to filter clogging Significant precipitation and residue on filter and production equipment Calcium and inorganic phosphate salts were removed from the basal media and added after the HTST treatment. Media prepared with all components except calcium salts showed negligible precipitation when heated to 102°C.	30
Three different media in term of composition	Custom-fabricated module. The heater is a steam heated, shell-and-tube heat exchanger.	Media heated at 102°C for 10 s and then cooled to 25°C	Precipitation of components during the heat treatment was mainly metal phosphates (tricalcium phosphate, trimagnesium phosphate, and iron [III] phosphate). Need to reduce or remove some or all heat labile components Prevent precipitations by lowering the pH before treatment and by adding NaOH solution right after the HTST treatment.	29
Four cell culture media, including one containing fetal bovine serum (FBS)	Bench-scale processing system. The unit is a tubular heat exchanger with sections configured for preheating, heating, and cooling of the solution to be treated.	102°C for 10 s and 115°C for 30 s	FBS containing media was not compatible with HTST treatment	34
Seven CHO media formulations including three chemically defined media and four supplemented with soy hydrolysates	Small scale HTST system developed in-house for continuous-flow media processing	100°C for 10 s and then cooled to 20°C	No visible alteration by heat treatment Very modest shifts in pH values associated to the partial release of CO_2 during heat-treatment Stable back pressure Increased presence of Maillard products in HTST-treated formulations, particularly for hydrolysate-supplemented formulations, which introduce more carbohydrates and amino-acids	33
Roche/Genentech proprietary cell culture media	Study 1. HTST units designed for bioprocessing applications with shell and tube heat exchangers Study 2. Fluidized Sand-bath model system (extreme conditions)	102 ± 2°C for 10 ± 2 s	Study 1. no significant effect on typical cell culture media amino acids, most of the vitamins, selection agents, some growth factors, glucose, and salts, among others. ∼10%–20% loss of recombinant human insulin and ∼10%–20% loss of highly heat-labile vitamins.	31

(Continues)

TABLE 3 (Continued)

Media	Technology	Conditions tested	Conclusions taken from respective publications	Reference
			Formation of a precipitate highly enriched with phosphate and calcium ($Ca_5(PO_4)_3OH$(s) 2. Up to 60% loss of some heat-labile vitamins (e.g., vitamin B12). In both study, trace elements losses were observed for some condition (specifically iron). Precipitation mitigation by lowering the phosphate concentration, the media pH, or both the pH and the phosphate concentration before HTST treatment	
Undefined medium formulations supplemented with soy hydrolysates	Bench-top HTST device	100°C, 150°C, and 200°C for 10.8 s, 110°C for 21.6 and 43.2 s, and then cooled to 20°C	Prolonged holding times of 43 s at temperatures of 110°C did not adversely impact medium quality while significant degradation was observed upon treatment at elevated temperatures (200°C) for shorter time periods (11 s). At 150°C, 10.8 s, precipitates, considerable browning, pH shifts were observed	36
Basal production media and feed medium with varying pH Levels	Fluidized sand bath	102°C for 10 s pH from 5.9 to 7.5 Calcium from 0 to 3.5 mM Phosphate from 0 to 6.5 mM Samples cooled in a water bath	In several cases, lowering pH without lowering calcium and phosphate concentrations led to lower turbidity after heat treatment Formulations without calcium or with low calcium concentrations neither showed precipitation events nor had high turbidity even at neutral pH. Importance of the ratio between phosphate and calcium concentrations	32

the risk of precipitation of calcium phosphates with HTST treatment are temperature, pH, and concentrations of calcium and phosphate.[31] Reducing the temperature may not be the best choice to prevent precipitations, as the viral inactivation may then not be effective (≤4 LRV). However, lowering the pH was shown to be a promising strategy that resulted in reduced turbidity of the media.[32] Another option to mitigate precipitation events is to supply the medium with calcium and phosphate only after the HTST treatment step (these additions would nevertheless need to undergo another type of viral inactivation) depending on the overall viral risk of the sources of $CaPO_4$. Methods such as fluidized sand bath or oil bath can be used to predict if precipitation is going to occur with a specific media during HTST treatment.[30,31] Shiratori et al. used a design of experiments to assess when precipitation events occurred depending on the nature of the solution treated, pH, and phosphate and calcium concentrations.[31,32]

Apart from precipitation, HTST treatment might have other impacts on media, especially on heat labile components. For instance, a loss by 10%–20% of insulin and highly heat labile vitamins was reported by Kiss et al., while no significant losses of amino acids, methotrexate, glucose concentration were detected.[25,31,32] Floris et al. reported that heat treatment of CHO media formulations was associated with increased presence of Maillard products, particularly for hydrolysate-supplemented formulations, which introduce more carbohydrates and amino-acids.[33] Furthermore, Weaver and Rosenthal observed that a medium containing fetal bovine serum was not compatible with heat treatment.[34] However, as HTST was not performed on the same medium without serum, it cannot be assessed whether this incompatibility came from the serum itself or another component of the basal medium. Kiss reported no impact on a medium containing serum.[25] In conclusion, tests must be performed for each medium composition to elucidate possible incompatibilities with HTST, which would eventually require alternative viral clearance methods to be considered.

5 | IMPACTS ON PROCESS PERFORMANCE AND PRODUCT QUALITY

In addition to the evaluation of the impact of heat treatment on media component degradation, and due to the complex nature of cell culture media and feed solutions, functional tests are also required to assess the impact on cell growth, productivity, and product quality. Kiss et al., reported no impact on specific growth rate of a Chinese hamster ovary (CHO) cell line with treated culture growth medium over 4 days containing 2% serum and treated production culture growth for 10 s at 97, 102, 107, and 115°C in a 2 L model system.[25] Same conclusions were made for media treated at 102°C for 60 s. No significant impacts were observed on titer or on the product quality attributes. In the same way,

Pohlscheidt et al., reported at small and large-scale comparable cell growth, cell metabolism, product yield, and product quality (e.g., charge and size variants attributes) for a CHO cell line after heating media containing a growth factor and complex plant hydrolysates in addition to other nutrients at 102°C for 10 s.[30] Weaver and Rosenthal, only identified a minor impact on cell growth under extreme treatment conditions (115°C for 30 s), but no impact was observed under standard conditions of exposure at 102°C for 10 s.[34] In addition, they demonstrated the absence of impact on measured protein quality attributes following media heat treatment by analyzing sialylated glycan profile, Lys-C peptide map, and size-exclusion high-performance liquid chromatography (SE-HPLC) profile. Shiratori and Kiss reported no negative effects on the CHO cell culture performance or on measured product quality attributes even though a loss of 10%–20% of recombinant insulin and 10%–20% of highly heat labile vitamins was assessed in heat-treated media.[31]

6 | HIGH TEMPERATURE SHORT TIME TREATMENT IN THE BIOPHARMACEUTICAL INDUSTRY

Viral inactivation of cell culture media and feed solutions in a biomanufacturing process is a preventive treatment that is not viewed as mandatory by regulatory agencies. HTST is a technique, which can be quite easily implemented at a large-scale without being excessively costly. Genentech was the first company to have implemented HTST treatment to mitigate potential virus contamination events after they experienced a contamination by MMV in 1993 and 1994.[25,30] Since then, more commercial processes have been adapted to include this preventive treatment. In 2011, Genentech/Roche had established 16 HTST processes (six commercial processes, including a 16,500 L scale- and 10 clinical processes) at nine different sites, including those of contract manufacturing organisations or partners.[25,30] In 2013, 22 HTST operating units were reported in the pharmaceutical industry.[35] Among them, some are known to be owned by Amgen, and Biomarin.[26,29,33,34,36] Eli Lilly also showed interest in this technology with a study at small-scale.[24] The Life Science business of Merck KGaA offers the opportunity for outsourcing HTST treatment of high-risk raw materials such as glucose at their two redundant large-scale production sites. Outsourcing HTST treatment can save high upfront capital investments, mitigates production risks, and saves operational footprint.

7 | CONCLUSION

The HTST treatment, as discussed in this review, acts as an effective preventive step regarding the risk of a viral contamination. The growing number of studies on this subject have brought to light a recurring problem regarding the impacts that HTST treatment can have on culture media. Media composition generally include calcium and phosphate components, which precipitate above a certain concentration due to a decreasing solubility with temperature. In addition of creating mechanical problems with the heat exchanger or with downstream filtration of media, this could induce a coprecipitation of other metal salts containing for instance iron, magnesium, manganese, or copper. As a result, the major impact on the process is a loss of trace elements, originally present in the media formulation that could eventually alter critical quality attributes of the product (e.g. glycosylation, charge variants, drug substance color) and thereby its efficacy.[37] Considering potential impacts on the media, temperature can be lowered to a minimum of 95°C, but given that the efficiency of the heat treatment highly depends on the temperature, it is not the best strategy to explore. Precipitations can be overcome by temporarily lowering the pH of culture media and by reducing (ideally to zero) the phosphate or calcium concentration prior to HTST treatment. Compounds with phosphate and/or calcium can, for example, be added to the culture after heat treatment. Likewise, it might be also advisable to only add trace elements after the treatment to ensure that they do not coprecipitate by using an alternative type of viral barrier such as nanofiltration. For efficient pre-screening, easily available techniques for HTST treatment such as oil bath or fluidized sand bath allow the rapid screening of numerous media compositions at small scale, in order to determine the best composition for preventing precipitation during HTST treatment.[30-32] In times where new viral threats arise, the implementation of additional viral clearance barriers for cell culture media become more important than ever, especially for cell and gene therapies. They require special focus for upstream viral safety since downstream viral filtration is not possible.[3]

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AUTHOR CONTRIBUTIONS

Veronique Deparis: Writing-review and editing. **Leïla DJEMAL:** Writing-original draft; writing-review and editing. **Joerg von Hagen:** Writing-review and editing. **Harald Kolmar:** Writing-review and editing. **Clemence Fournier:** Writing-original draft.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

PEER REVIEW

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All data generated or analysed during this study are included in this published article.

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