5.1.2e

## Contents

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## 1. Executive Summary

This interim report documents the investigation relative to IDM # 154486 – Variable infectivity results obtained during RIVM testing of AZD1222, batch ABV3922.

On January 22, 2021, the National Institute for Public Health and the Environment (RIVM), The Netherlands, informed the AstraZeneca QPs of variable results obtained during government testing for AZD1222, batch ABV3922 manufactured at Catalent Anagni, Italy.

An in-depth investigation has taken place that consisted of :

- Extended additional testing of available samples from different sections of batch ABV3922.
- Extended additional absorbance testing of available samples from different sections of the batches manufactured after ABV3922.
- Detailed Root Cause Analysis evaluations performed by both Catalent Anagni and AstraZeneca using the Fishbone/Ishikawa and 5 Whys methodology.

As a result of the additional testing performed it can be concluded that :

- The results indicate an end of batch issue with ABV3922 that impacts Infectivity (ifu/ml), AEX (vp/ml), PS80 (%), and UV Absorbance.
- This issue is not observed on any of the vials tested coming from the beginning and middle of the batch up until approx. 85% of the batch.
- This issue has not been observed on any batches manufactured either before or after batch ABV3922 as confirmed by A260 Absorbance screening demonstrating batch homogeneity.

Based on the extensive root cause investigation, the most probable root cause has been identified as **"Machine – Chiller temperature probe failure"** during AZD1222 Drug Product manufacturing at Catalent Anagni. The chiller temperature probe was loosely connected resulting in an incorrect temperature reading. As a consequence, the chiller was autoregulating the cooling system based on a wrong temperature value. This failure was caused by a maintenance intervention that occurred in the shutdown after completion of PPQ and just prior to the manufacture of batch ABV3922.

During the manufacture of batch ABV3922 the maintenance technician observed frost on the tubing of the chiller. After the completion of the batch the technician together with the supervisor checked the chiller, observed the loose probe and corrected the situation before the start of the manufacture of the next batch.

The incorrect temperature regulation of the chiller of the jacketed tank may have led to supercooling of the refrigeration fluid and, therefore, of the tank wall. This may have resulted in local freezing of the final drug product bulk solution at the bag wall. During freezing solutes are expelled into the bulk so that the frozen material primarily consists of water. During filling when the liquid level in the bag goes down, the bag walls collapse from the side and fold on top of the liquid allowing the ice to melt causing a layer of water on top of the bulk solution that may have then resulted in the dilution of the product at the last part of the fill. Catalent Anagni already performed an experiment to mimic the

potential root cause and confirmed that freezing of the final bulk at the bag wall can occur when the tank wall is cooled to freezing temperatures.

Based on the most probable root cause identified, an impact analysis on batch ABV3922 is being performed to evaluate disposition.

It can also be concluded that PPQ batches manufactured before ABV3922 and AZD1222 batches manufactured after ABV3922 are not impacted by this event. This event is considered as an individual occurrence limited to AZD1222, batch ABV3922.

The list of CAPAs will be defined and communicated as part of the final investigation report.

## 2. Introduction

This interim report documents the investigation relative to IDM # 154486 – Variable infectivity results obtained during RIVM testing of AZD1222, batch ABV3922.

**The objective of this report is to document AstraZeneca's investigation of this event including** detailed root cause analysis (RCA), impact analysis and actions taken.

## 3. Description of the event

### 3.1. Initial Event Notification

On January 22, 2021, the National Institute for Public Health and the Environment (RIVM), The Netherlands, - Department for Biologicals, Screening & Innovation informed the AstraZeneca QPs of variable results obtained during government testing of batch ABV3922 manufactured at the Contract Manufacturing Organization (CMO) for AstraZeneca Catalent Anagni, Italy. Notification email text received is attached in 11.1.

In the initial test run (Run 1 in Table 1), RIVM tested two vials. The result was invalidated since the %RSD >30%. RIVM then retested these 2 first vials as well as 2 different new vials (Run 2 in Table 1).

The infectivity results reported by RIVM laboratory as part of this initial communication are the following :

*Table 1 : Initial Infectivity results from RIVM - January 22 2021*

| Number handwritten on the vial[1] | Result (ifu/mL) – Run 1 | Result (ifu/mL) – Run 2 |
|---|---|---|
| 658 | 5.06e8 | 4.11e8 (same vial - retest) |
| 470 | 10.9e8 | 10.8e8 (same vial - retest) |
| 658 (new vial) | - | 3.14e8 |
| 376 | - | 10.8e8 |

[1]The numbers reported by RIVM in Table 1 were written by hand on the vials. Details regarding Catalent Anagni's numbering system is described in section 4.2.2

The specification for Infectivity is **≥ 7.0 x $10^8$** ifu/ml. The results from both vials with the number 658 handwritten on the vials, including the retest result for the first vial were all out of specification. The other vials from ABV3922 were conform.

RIVM informed AstraZeneca that all other batches, results of approx. ten (10) batches tested up to that time, had potencies > 10 x $10^8$ ifu/ml.

Batch ABV3922 of AZD1222 was produced on the **"Biosuite 1" line at Catalent Anagni's manufacturing** site in Italy. The production of the batch under investigation has taken place from January 1, 2021 to January 3, 2021.

### 3.2. Immediate actions taken

- On January 22, 2021, Manufacturing Site Catalent Anagni has been notified and site investigation has been initiated. The manufacturing site deviation record TW #307662 to investigate the homogeneity issue (infectivity not conform) on batch ABV3922 – AZD1222 has been opened on January 29, 2021.
- On January 26, 2021, AstraZeneca TW IDM #154486 has been opened.
- Impacted Batch ABV3922 has been blocked from release pending further investigation.

### 3.3. Short term action taken

Enhanced Absorbance testing has been put in place in order to evaluate the potential impact on the other batches manufactured by Catalent :

- For all batches manufactured until February 3, 2021: retain B, M & E (Beginning, Middle and End) samples have been tested using A260 screening method. Results from this additional testing is presented in section 4.3.4.
- For all batches manufactured after February 3, 2021: additional enhanced screening has been implemented on vials from beginning, 25%, 50%, 75% and 100% of the filling process. This enhanced screening will be performed until closure of the investigation.

This enhanced testing is described in a memo sent by AstraZeneca to Catalent Anagni on February 3, 2021: Catalent_memo_Enhanced Sampling for AZD1222. Catalent Anagni initiated change control **TW#309573 "Additional Testing Request** - for Drug product AZD1222" to implement the UV relative homogeneity |A260-A320| as parameter to monitor the batch production.

# 4. Investigation

## 4.1. Problem statement

RIVM informed AstraZeneca on January 22, 2021, that the results taken from two different samples with the number 658 handwritten on the vials are non-conform for infectivity. The other vials tested from batch ABV3922 are conform. As the RSD percentage calculated was >30%, RIVM suggested a possible batch homogeneity issue.

## 4.2. Background Information

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#### 4.2.2. Catalent Anagni vials traceability

The handwritten numbers on the sample vials refer to Catalent vials traceability system that is used to ensure random sampling throughout the batch. The numbers refer to the box number that the vials are taken from after visual inspection. Box traceability is maintained throughout filling and visual inspection, so that vials from Box number 1 represent the very first vials filled and vials from the last box represent the very last vials filled. The total number of boxes per batch are determined by the total batch size (each box contains 220 vials).

For every test lab, the exact samples taken including the box number from which they were taken, are documented in the batch record.

Traceability related to the samples taken for batch ABV3922 is detailed for:

- Samples sent to RIVM in Appendix 11.2
- For retain samples kept at Catalent Anagni in Appendix 11.3.

### 4.3. Testing Results

#### 4.3.1. RIVM additional Test results

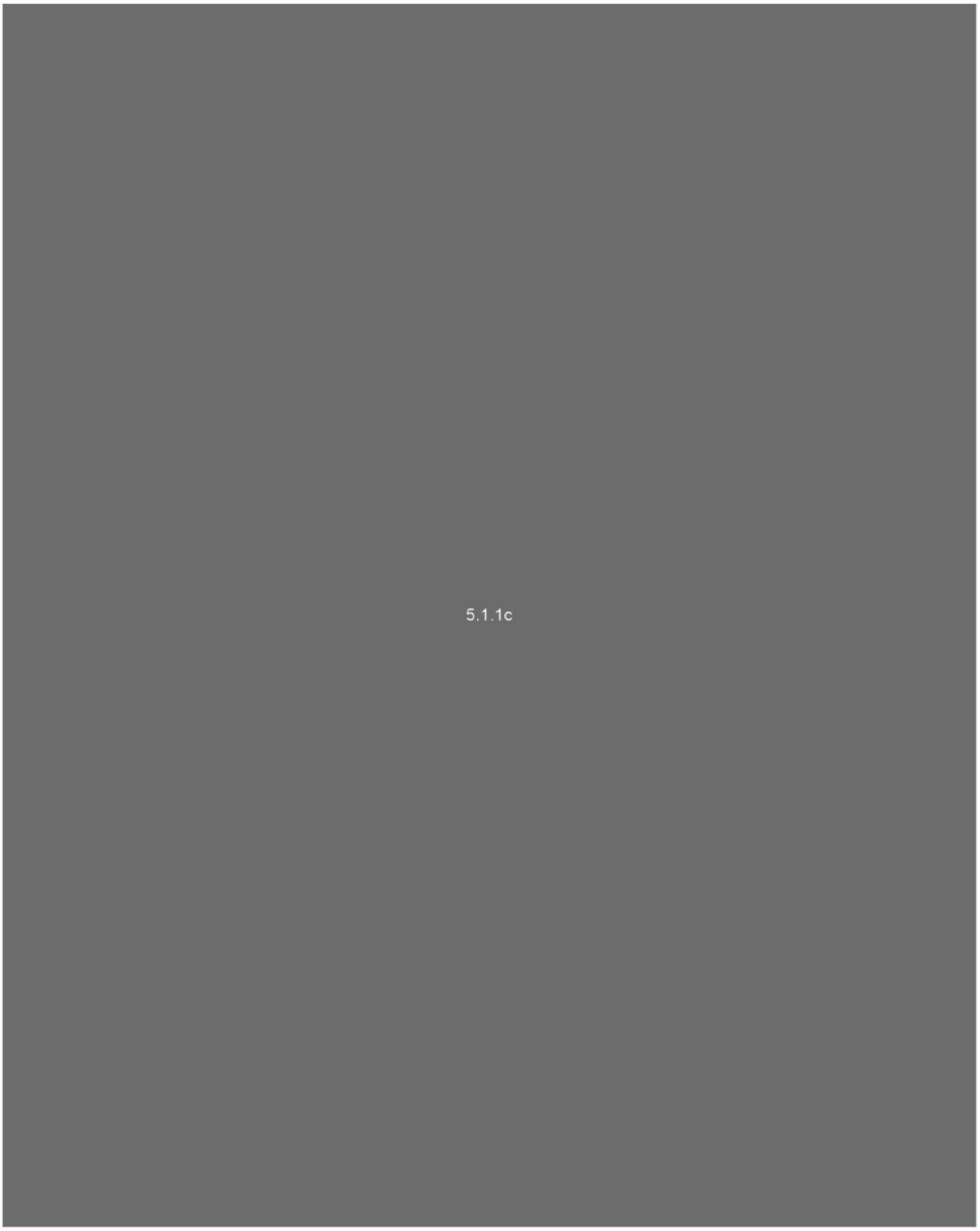
RIVM conducted additional Infectivity testing on the vials sent from Catalent Anagni for batch ABV3922 as well as on vials from other commercial batches.

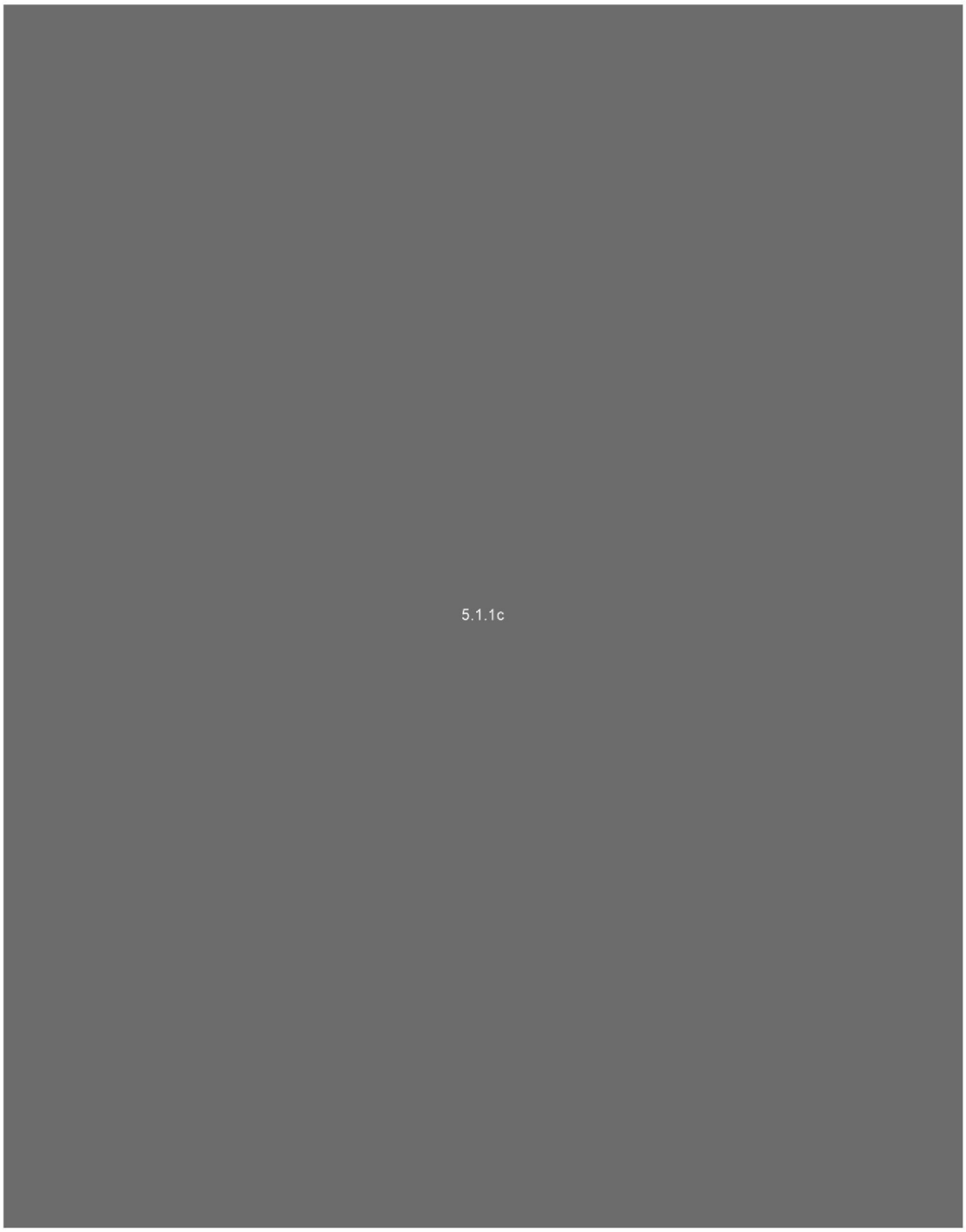
Results shared by RIVM are presented in Figure 2. The blue arrows point to the results for vials taken from box 658, near the end of batch ABV3922 (total number of boxes for ABV3922 is 663).


*Figure 2 : RIVM Infectivity and Absorbance (A280 and A260) Results shared*

From the RIVM results presented in Figure 2 the following observations can be made:

- At the time of signing this document, the only non-conforming Infectivity results communicated by RIVM are for the 2 vials of batch ABV3922 from box 658 (near the end of the Batch).
- Results from ABV3922 for vials from boxes 94 (two), 188, 282, 376, 470 and 564 (representing the first 85% of the batch) are conform to expected results;
- No other batches manufactured to date present the same pattern with a drop of infectivity at the end of the batch.





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#### 4.3.3. ABV3922 Release Results

ABV3922 QC release results are presented in Table 2 here-under. Release results are all conform to release specifications.

*Table 2 : ABV3922 Release Results.*

| Method | Results |
|---|---|
| Infectivity | 1.2e9 ifu/mL |
| Identity | Confirmed |
| Subvis | >/= 10 um is 253 particles/container; >/= 25 um is 0 particles/container |
| PS80 | 0.07% w/v |
| AEX | 9.7e10 vp/mL |
| P:I | 81 vp/ifu |
| Infectivity | 1.2e9 ifu/mL |
| Appearance | Conforms |
| pH | 6.5 |
| Osmo | 461 |
| EV | Conforms |
| Endo | <0.25 EU/mL |
| Sterility | No growth detected |

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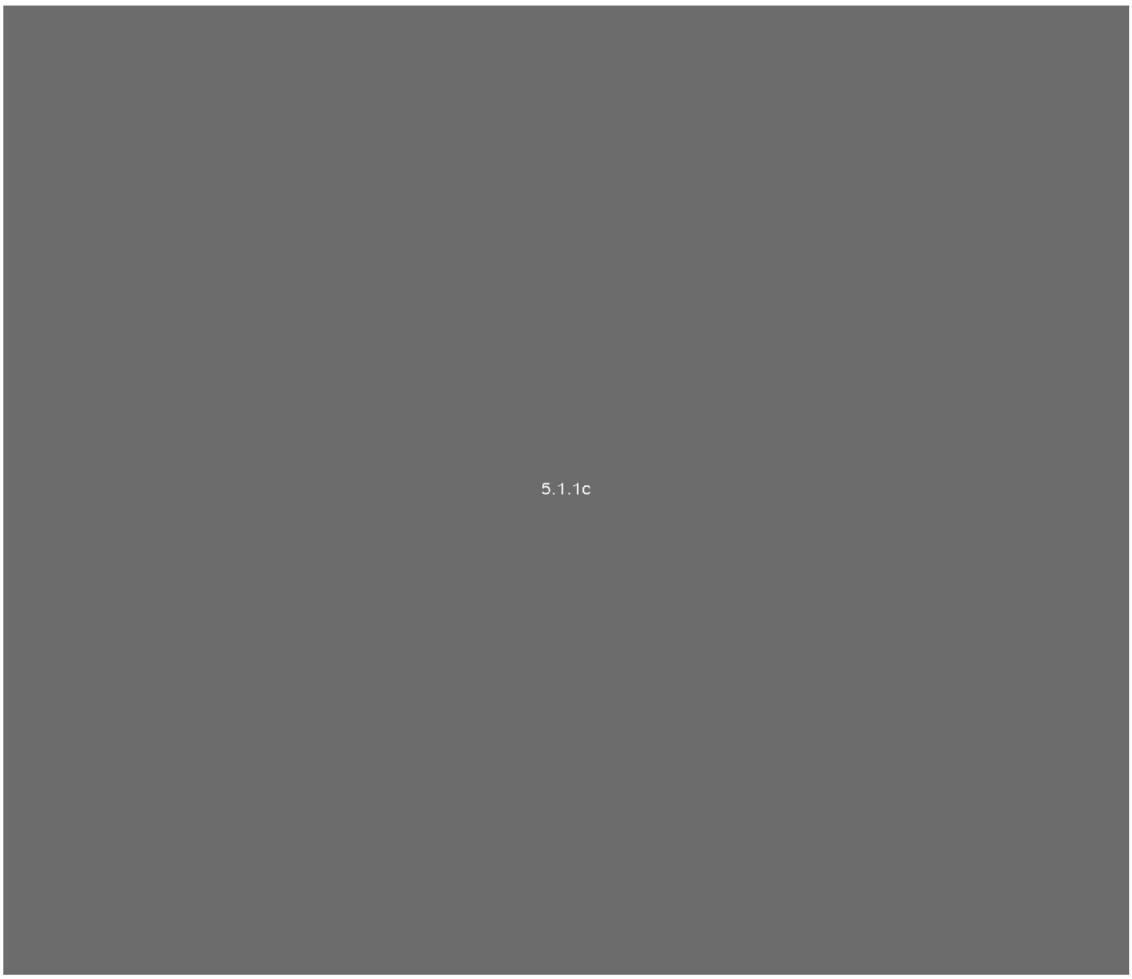



**4.3.5. Conclusion on overall test results**

From results presented we can conclude that :

- These results indicate an end of the batch issue with ABV3922.
- The testing of the end of batch vials of ABV3922 shows that there is an impact on :
  - Infectivity (ifu/ml)
  - AEX (vp/l)
  - PS80 (%)
  - Absorbance
- The effect of this issue is not observed on any of the tests done on the vials manufactured and filled up until 85% of the batch as the test results were conform to specifications.
- This issue has not been observed on any other batches than ABV3922.

### 4.4. AstraZeneca Investigation

*Investigation approach*

Upon notification of the issue by the RIVM, an investigation team was assembled consisting of subject matter experts within AstraZeneca. In the first meeting, the methodology of the Ishikawa (fishbone) diagram has been used to brainstorm potential causes for the observed low infectivity results at the end of bath ABV3922 and how these potential causes could contribute to the observed issue.

As a next step, the investigation team was broadened with members of Catalent Anagni to establish a joint Root Cause Investigation team and several sessions were held.

All potential causes mentioned in the Ishikawa diagram have been assessed. The Ishikawa (fishbone) diagram shared and discussed between the AstraZeneca and Catalent Anagni investigation teams is presented in Figure 8 and the final assessment of all possible root causes is detailed as part of Catalent Anagni's Investigation Report TW#307662.

### 4.5. Testing Site Investigation

As the non-conforming results have been confirmed from 2 different testing sites, RIVM the government testing site in the Netherlands and Catalent Anagni, Italy, a testing site error or sampling shipment issue are not considered probable root causes.

### 4.6. Manufacturing Site investigation

#### 4.6.1. Manufacturing Site Root Cause Analysis exercise

Catalent Anagni has performed a manufacturing site investigation tracked under *TW#307662 - Catalent Investigation Report & RCA*. This report is shared as a reference file to this document.

**Summary of the manufacturing site investigation:**

1. *Review of batch ABV3922 deviations:*
   - TW#296080 : Cryovault vent system and DS filling system different from conform work instruction.
   - TW#296553 : QC _Batch ABV3922_samples delivery

   ⇨ Due to the nature of the event, these deviations have been considered as not related.

2. *Laboratory investigation:*
   - Additional testing has been done to better understand the issue. Results are presented in section 4.3.

3. *Summary of production investigation:*

- **Methodology** : The Fishbone diagram methodology has been followed. Full detailed RCA including the evaluation of all possible root causes can be found in attached TW#307662 Catalent Anagni Manufacturing Site Investigation report.

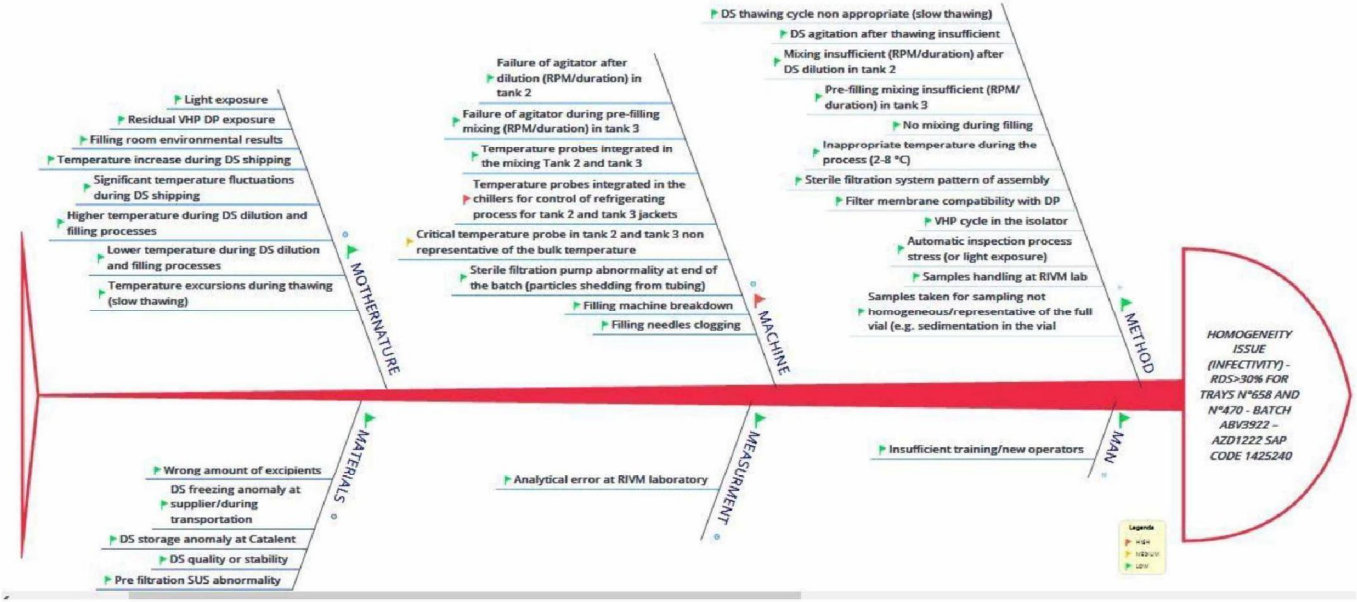
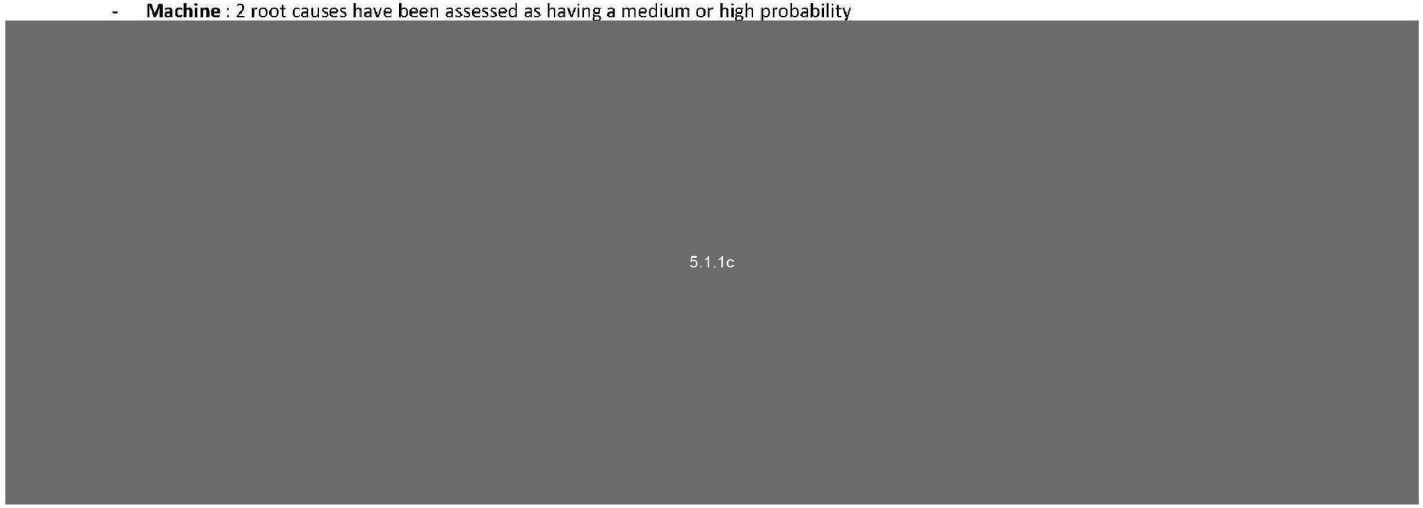



*Figure 8 : Fishbone Root Cause Analysis completed by Catalent Anagni - High Probability in red - Medium in yellow - Low in Green*

- **Results of the manufacturing site production root cause investigation :**

- **Environment (Mother nature):** All root causes evaluated have been assessed as low probability
- **Method :** All root causes evaluated have been assessed as low probability
- **Machine** : 2 root causes have been assessed as having a medium or high probability




- **Materials** : All root causes evaluated have been assessed as low probability
- **Measurement :** All root causes evaluated have been assessed as low probability

*4. Summary of maintenance investigation:*

On December 29, 2020, during the winter shutdown at Catalent Anagni, immediately after completion of the PPQ batches and before the production of the involved AZD1222 batch ABV3922 (which is the 1st commercial batch), maintenance was performed as per work order Maximo ref. 1010234878 to replace the protection cover of the cooling tubes connecting the Lauda chillers with the SU jacketed tanks.

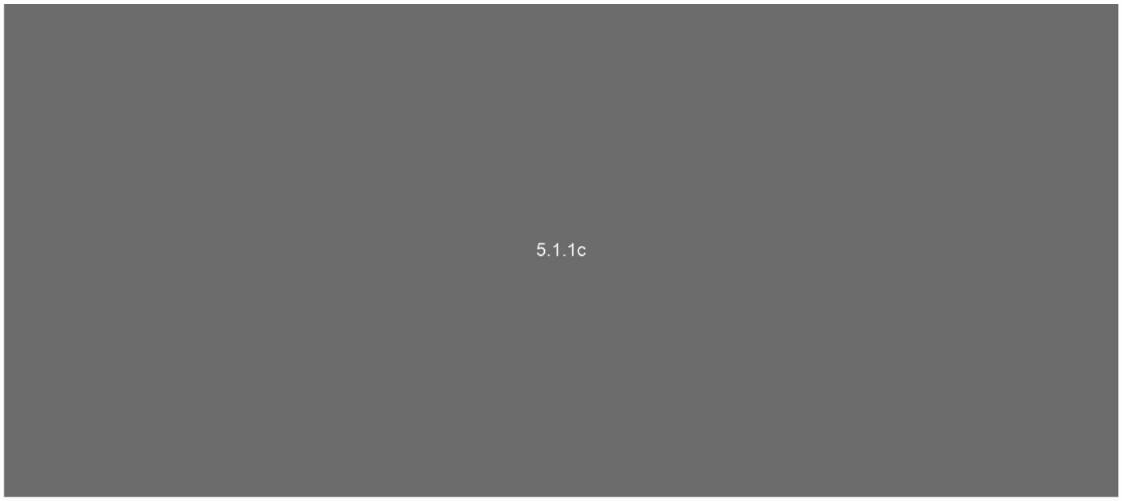
During the cover removal one tube tightener was slightly loosened generating a cooling liquid leak. The maintenance personnel refilled the liquid through the filling port on the top of the Lauda chiller. They filled more than necessary and there was a little spillage around the port. They assumed that the spillage might have also affected the internal part of the chiller because the port did not seem to guarantee the sealing.

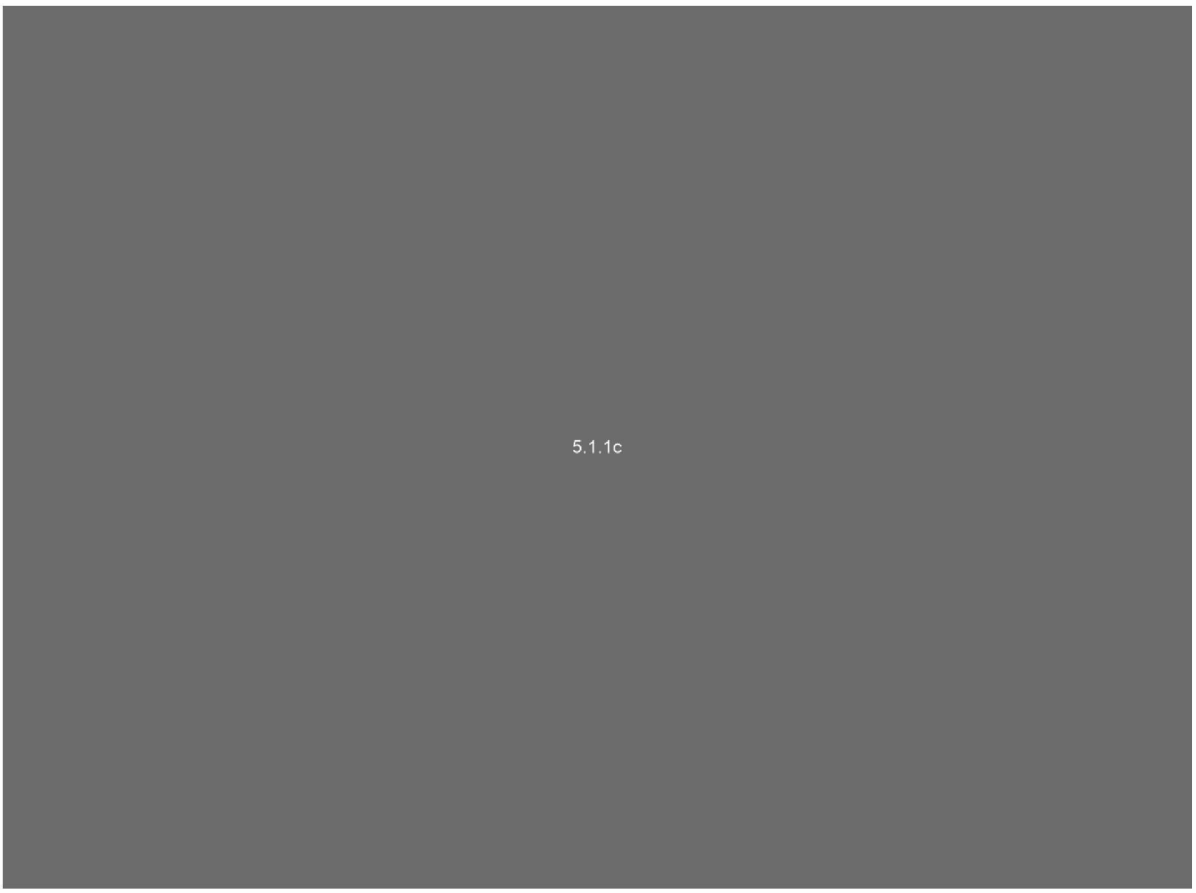
On December 30, 2020, a second maintenance intervention was executed (Maximo ref. 1010255664) to open the chiller covers and to verify the spillage effects on the internal components installed on top of the chiller cooling liquid tank, including the PT1000 probe.

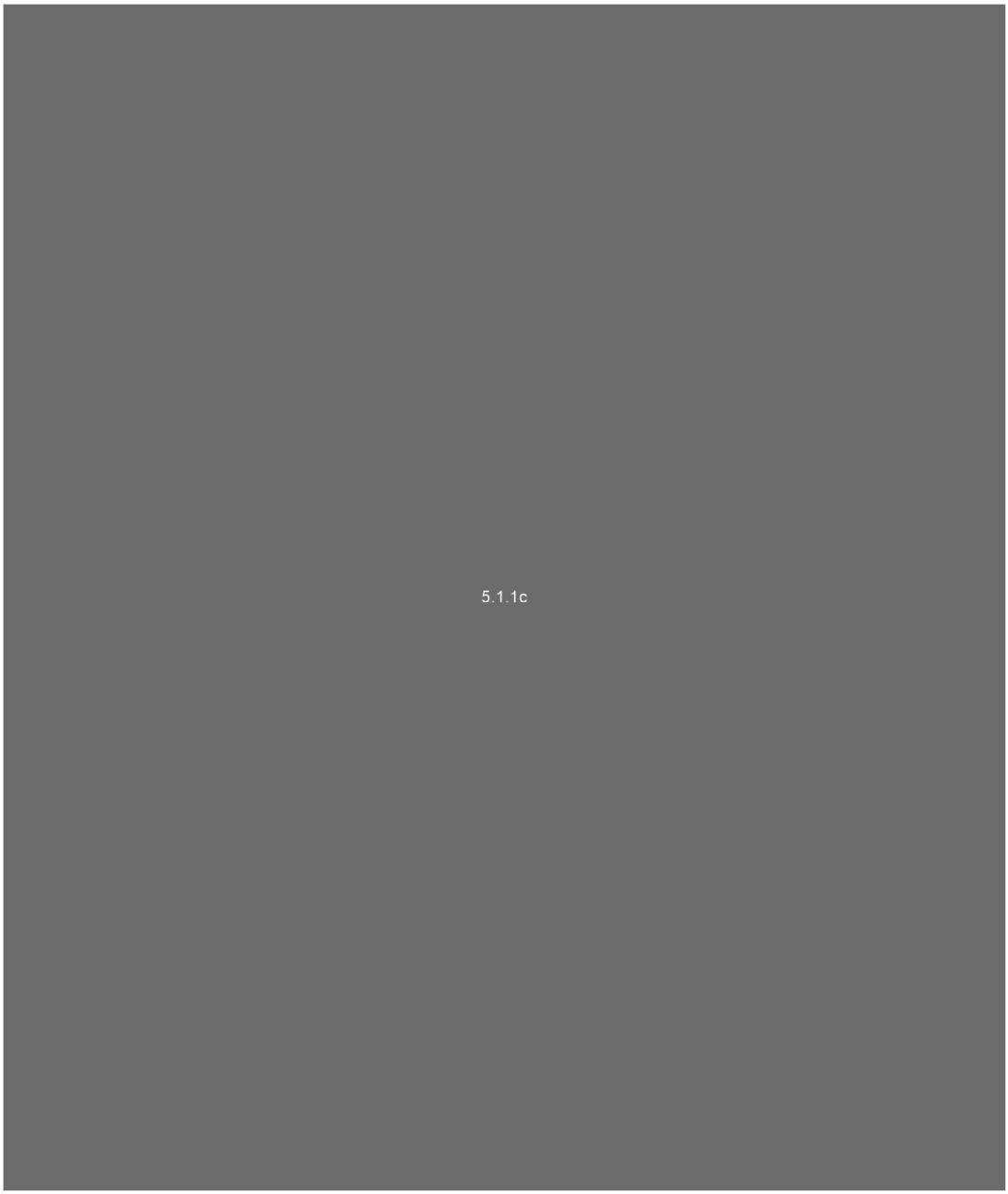
On January 2, 2021, during the manufacturing of batch ABV3922, the same maintenance technician was present to double check that the tube tighteners were fixed properly. He noticed that there was some frost on the cooling tubes. The tank 3 product temperature was displayed into the defined range (2 to 8°C), and considering that the production was on going and no additional evidence of anomalies were found, it was not possible to do further verifications on the chiller.

On January 3, 2021, after completion of the manufacture of batch ABV3922, the maintenance technician escalated the event to his supervisor to double check the situation at the end of the batch, before the next chiller utilization for the following batch. During this check a loose connection for the chiller PT1000 probe was detected. One clamp on the control panel card was in the wrong position (slightly open) as shown in Figure 9. Therefore, the maintenance technician correctly connected the chiller temperature probe.

The exact details on sequence of events as described above were obtained during an interview of the maintenance personnel on February 9, 2021. This interview is documented as part of the investigation at Catalent Anagni (***Intervista relativa a TW#307662 (AZD1222 "homogeneity issue").***





## 5. Impact Analysis

- Impact on ABV3922 :
  - AZD1222, batch ABV3922 is blocked – Based on the most probable root cause identified, an impact analysis on batch ABV3922 is being performed to evaluate disposition.

- Impact on Batches Manufactured before ABV3922 (PPQ Batches)
  - PPQ Batches have been fully tested for homogeneity and all results were conform.
  - The maintenance activities identified as the most probable root cause occurred after the manufacturing of the three PPQ batches.
  - Therefore, PPQ batches are not impacted by this event.

- Impact on Batches Manufactured after ABV3922 (Commercial Batches)
  - All Batches until closure of the investigation report are being screened using Absorbance method as agreed and discussed with EMA. All batches tested thus far using B, M and E sampling meet the agreed acceptance criteria demonstrating homogeneity.
  - On January 3, 2021, after the filling of commercial batch ABV3922 the temperature probe identified as being related to the root cause for this issue has been returned to the appropriate position by the maintenance operator and the maintenance supervisor isolating the event to only ABV3922.
  - Therefore, all batches manufactured after ABV3922 are not impacted by this event.

## 6. Actions

- **Immediate and Short term actions taken**

The immediate and short term actions taken are described in section 3.2 and 3.3.

- **Corrective Actions & Preventive Actions (CAPAs)**

Complete list of CAPAs to be defined by Catalent Anagni and AstraZeneca will be defined and listed as part of the final investigation report.

## 7. Conclusion

Following the notification of the issue on AZD1222, batch ABV3922 by RIVM to AstraZeneca on January 22, 2021, and the notification to the manufacturing site Catalent Anagni an in-depth investigation has taken place that consisted of:

- Extended additional testing of available samples from different sections of the batch ABV3922
- Extended additional absorbance testing of batches manufactured after ABV3922 using retain samples from beginning, middle and end of the batches
- Detailed Root Cause Analysis evaluations performed by both AstraZeneca and Catalent Anagni using the Fishbone/Ishikawa and 5 Whys methodology.

As a result the most probable root cause has been identified as **"Machine** – Chiller temperature probe **failure". The chiller temperature probe was** loosely connected and read an incorrect temperature. Therefore, the chiller was autoregulating the cooling system based on a wrong temperature value resulting in a supercooling of refrigeration fluid and as a result, of the tank wall. This may have resulted in local freezing of the final drug product bulk solution at the bag wall. During freezing solutes are expelled into the bulk so that the frozen material primarily consists of water. During filling when the liquid level in the bag goes down, the bag walls collapse from the side and fold on top of the liquid allowing the ice to melt causing a layer of water on top of the bulk solution that may have then resulted in the dilution of the product at the last part of the fill.

Based on the most probable root cause identified, an impact analysis on batch ABV3922 is being performed to evaluate disposition. The AZD1222 PPQ batches manufactured before batch ABV3922 as well as batches manufactured after batch ABV3922 are not impacted. This event is considered as an individual occurrence limited to AZD1222, batch ABV3922.

The list of CAPAs will be defined and communicated as part of the final investigation report.

## 8. Document change history

| Version | Description of Change | Reason for Change |
|---|---|---|
| 1.0 | New document | N/A |

## 9. Glossary & Definition of Terms

| Terms | Description |
|---|---|
| RIVM | National Institute for Public Health and the Environment, The Netherlands |
| CMO | Contract Manufacturing Organization |
| B, M & E | Beginning, Middle and End |
| AEX | Anion Exchange Chromatography |
| DS | Drug Substance |
| DP | Drug Product |
| RCA | Root Cause Analysis |
| Corrective Action/Preventive Action (CAPA) | Practice employed to address non-conformances or potential non-conformances within a process. A corrective action is a measure taken to address the underlying root cause of a discrepancy and prevent recurrence of a similar problem. A preventive action is a measure implemented to minimize or eliminate occurrence of a potential problem or nonconformance. |
| Deviation | Any departure from a GMP regulatory requirement or unplanned departures from a standard operating procedure, batch record, process standard, normal operating conditions, failure of a batch/equipment/system/result to meet its acceptance criteria or an environmental excursion, which could potentially impact product quality. |

| | |
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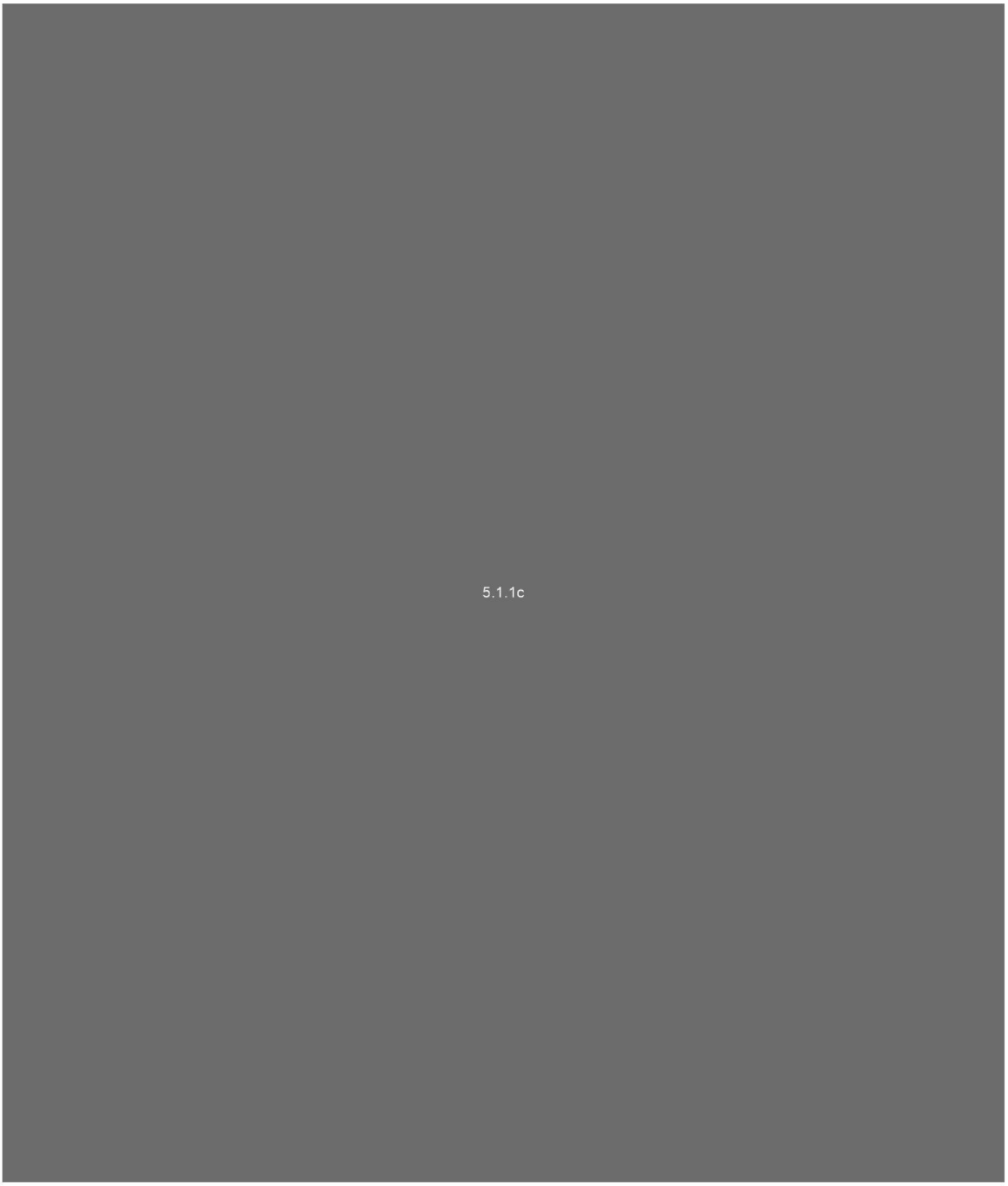
| Event | An unplanned departure from routine practices or processes that occurs during GMP activity but does not have any potential product impact, does not have an unexpected outcome, and is not a result of an unexpected breakdown of equipment or interruption to services. It is useful to record and trend an Event to monitor frequency and determine any potential reclassification to a deviation. |
| Good Documentation Practices (GDP) | Practices required in order to ensure proper documentation and compliance with CGMPs. |
| Current Good Manufacturing Practices (CGMP) | Current Good Manufacturing Practices followed by the pharmaceutical and biotech firms to ensure that the products produced meet specific requirements for identity, strength, quality, and purity. FDA regulates these industries to ensure CGMPs are being followed. |
| In Process Control (IPC) | A measured output that must be within a defined range or adjusted to a defined range to ensure product quality. |
| Master Batch Record (MBR) | Master document developed to ensure consistent manufacturing processes, result in batch to batch uniformity of intermediates and drug product |
| Validation | A method of establishing a high degree of assurance that a given operation will consistently and repeatedly produce a product to pre-determined specifications and quality attributes. |
| BPD | AstraZeneca BioPharmaceutical Development. The functional group responsible to develop biologic products. |
| CSD | Control Strategy Document. A document containing the necessary information for process validation including process parameter and output classification and proposed. |
| GTO | AstraZeneca Global Technical Operations. The functional group with technical responsibilities for Biologic products, analytical, drug substance, drug product, devices and packaging. |
| GTO-A | AstraZeneca Global Technical Operations Analytical Group |
| PV | Process Validation<br>A documented lifecycle program that provides a high degree of assurance (via design, data collection, evaluation, LCM, etc.) that a specific process consistently produces quality product meeting predetermined acceptance criteria. |

| | |
|---|---|
| PPQ | Process Performance Qualification<br>Documented actions proving that a process, operated within established parameters, performs effectively w/ reproducible results. Qualification is part of validation, but the individual qualification steps alone do not constitute process validation. |
| QA | Quality Assurance. |
| QC | Quality Control. |
| RU | Receiving Unit |
| TU | Transfer Unit (TU) in LDMS_001_00107146: 8-S27-CV-M: Standard for Technology Transfer |

## 10.References

- TW#307662 - Catalent Investigation Report & RCA
- MT-A/SP/SL/21/003 – Trial Buffer Temperature Excursion Simulation Study Report
- Catalent_memo_Enhanced Sampling for AZD1222



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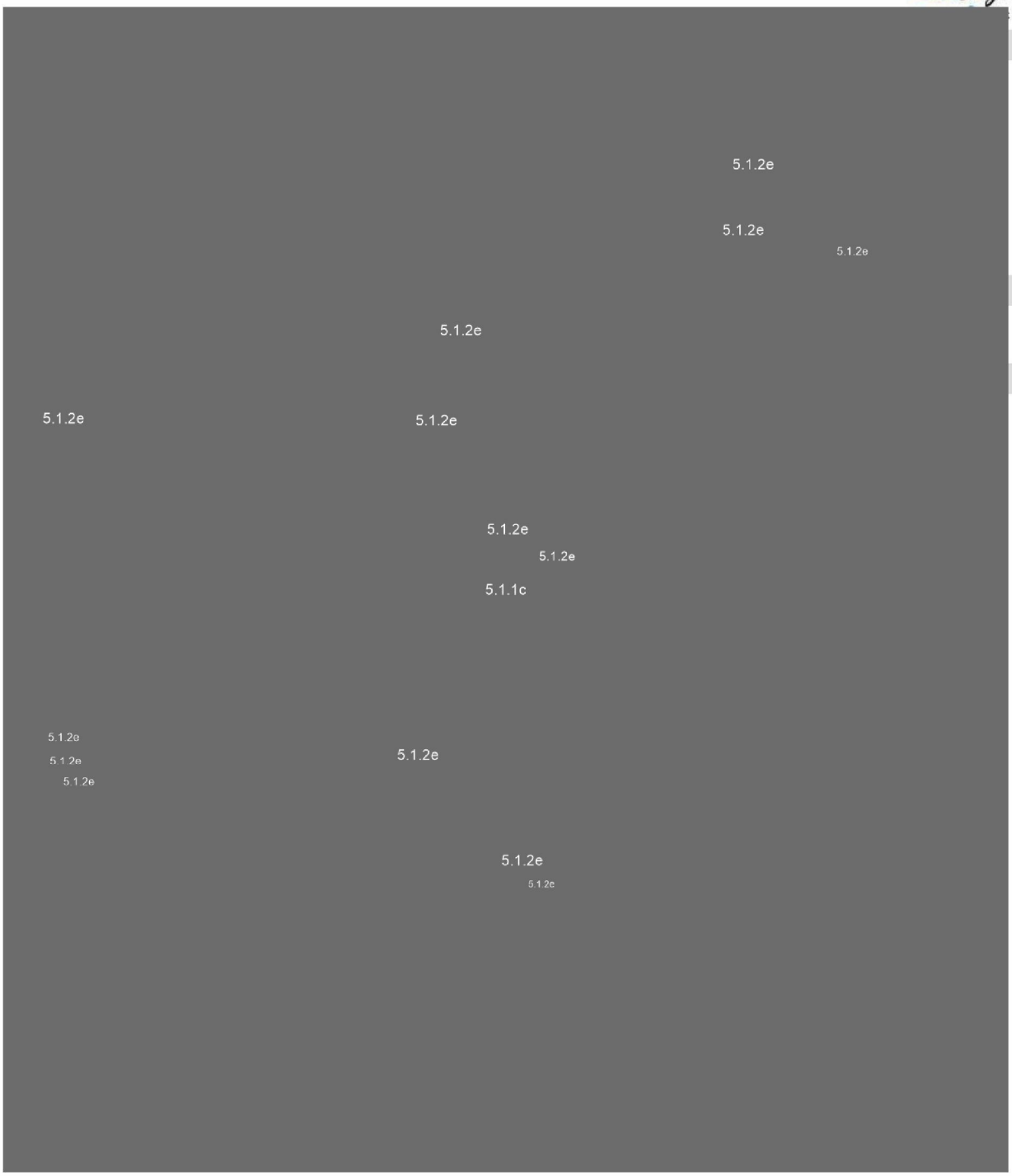
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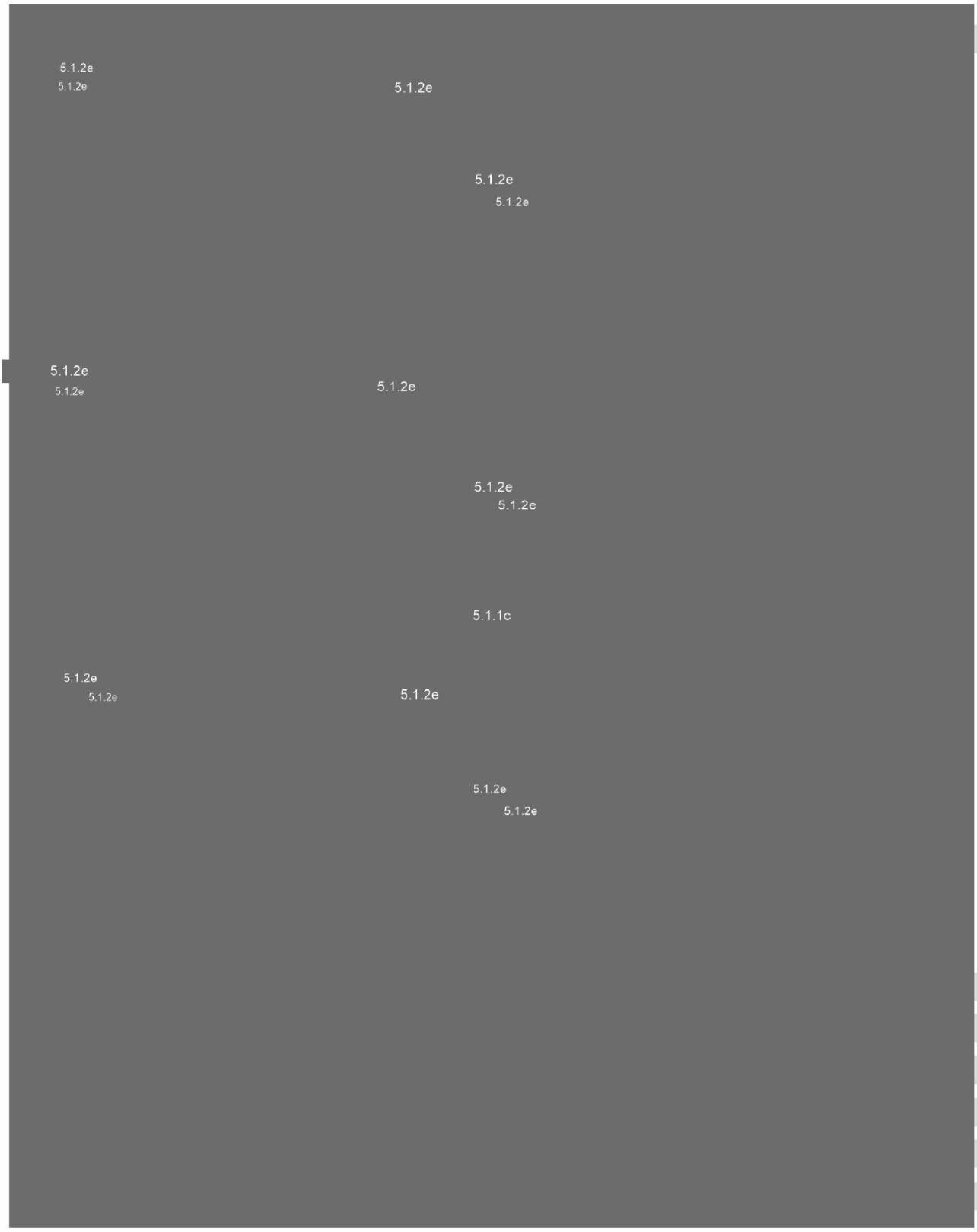
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**INVESTIGATION REPORT TW#307662**

Catalent

**ANAGNI**

| | |
|---|---|
| **Subject:** | ***TW#307662***<br>**Preliminary Investigation Report on *AZD1222 "homogeneity issue"***<br>**ABV3922 – *AZD1222 SAP code 1425240*** |

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# INVESTIGATION REPORT TW#307662

## I. Executive summary

On January 29th, 2021 the QA personnel opened the deviation TW#307662 to investigate on the homogeneity issue (infectivity not conform) occurred on the batch ABV3922 – AZD1222 SAP code 1425240.

The external laboratory, RIVM, performed the infectivity test on the impacted batch and communicated to Catalent Anagni on date January 22nd, 2021 that the result of vials relevant to the trays n°658 and n°470 was invalidated since the % RSD > 30% (VS RSD 1-15%)

On January 30th, **2021 the TW#307936 "Additional testing" was opened to allow the Anagni** QC laboratory to carry out the following tests on the final product as required by Astrazeneca:

- Determination of Viral Concentration (AEX) according to the method AZ Doc0172836
- Absorbance at 260 nm screening (memo AZ)
- Determination of PS80 Concentration with the method AZ DV-051226

The analyses performed by Anagni QC Laboratory confirmed the infectivity anomalous data of batch ABV3922 tray n°658. No anomalous results were highlighted for the other lots manufactured (evaluation performed from batch ABV0519 to ABV7764).

An in-depth investigation was performed using the Fishbone tool to evaluate any potential source. The Fishbone highlighted that most likely source is an undetected critical temperature drop induced the Final bulk (diluted drug substance) freezing on the tank 3 wall and, consequently, the dilution of the last part of the batch (cryoconcentration).

**The "5Why" tool helped the** probable **cause identification as "Machine - Chiller temperature probe failure".** The interviewed personnel reported that, during the pre-fill hold in Tank 3 and filling of the Drug Product relevant to the batch ABV3922, the chiller pipes were freezing. Considering that the production was on going and that the temperature check performed was compliant, it was not possible to do further verifications on the chiller. During the intervention, performed after the end of the ABV3922 filling activities, the maintainer found the chiller temperature probe loose connected contributing to a wrong temperature monitoring.

**Basing on the "5Why" tool, the causal factor of the event was "**Machine – **System not redundant". The** production personnel did not notice any issue since there is only one probe at the bottom of the tank in the center, as per Millipore design. Considering that the solution was not under mixing as required by process design, a temperature gradient was created: the product directly in contact with the probe had temperature within the limits.

As immediate action, on February 03trd, 2021 the ECC TW#309573 **"**Additional Testing Request - for Drug product AZD1222**" implemented the UV relative homogeneity** |A260-A320| as parameter to monitor the batch production.

As for the impact assessment performed (see par. VI - Impact/Risk assessment), the event impacted only the batch ABV3922.

The log sheet update will be performed to increase the process robustness regarding the tank and chiller temperature monitoring and the stirring management (see par. VIII – Proposed CAPAs).

# INVESTIGATION REPORT TW#307662

## II. Problem statement and description of the deviation

**Problem Statement**

***Defect***: Homogeneity issue (infectivity not conform)

***Object***: batch #ABV3922 – AZD1222 SAP code 1425240

**Description of the deviation**

**What:** the infectivity result of vials relevant to the trays n°658 and n°470 was invalidated since the % RSD > 30% (VS RSD 1-15%)

**Where:** batch #ABV3922 – AZD1222 SAP code 1425240 was produced on **"Biosuite 1"** line.

**When:** The production of the batch under investigation occurred from January 01st, 2021 to January 03trd, 2021

The deviation TW#307662 was opened in TW system on January 29th, 2021.

**Weight:** The RIVM laboratory (external laboratory) invalidated the infectivity result of vials labelled as 658 ($5.06 \times 10^8$ ifu/ml) and 470 ($10.9 \times 10^8$ ifu/ml) since RSD > 30% (VS RSD 1-15%). The RIVM laboratory repeated the infectivity test on two fresh samples (vial labelled as 658 (different vial) and vial labelled as 376) and including also the two old vials. The results for the fresh samples were $3.14 \times 10^8$ ifu/ml for vial labelled as 658 and $10.8 \times 10^8$ ifu/ml for vial labelled as 376. For the old vials reruns, the results obtained were $4.11 \times 10^8$ ifu/ml for vial labelled as 658 and $10.8 \times 10^8$ ifu/ml for vial labelled as 470 (RSD > 30%). Both test runs met all system suitability. Results are summarized in the table below:

| RIVM Test | Sample tray | Infectivity (ifu/ml) | Specification |
|---|---|---|---|
| #1 | 658 (first vial) | $5.06 \times 10^8$ | ≥ 7.0 x $10^8$ ifu/ml |
| | 470 (first vial) | $10.9 \times 10^8$ | |
| #2 | 658 (new vial) | $3.14 \times 10^8$ | |
| | 376 | $10.8 \times 10^8$ | |
| | 658 (first vial) | $4.11 \times 10^8$ | |
| | 470 (first vial) | $10.8 \times 10^8$ | |

**Table 1 Infectivity results of the involved vials.**

The infectivity results of the other tested vials for the same batch were within the expected range. The batch ABV3922 AZD1222 SAP code 1425240 is still under Catalent control.

All other lots tested from batch ABV0519 to ABV7764 had infectivity > $10 \times 10^8$ ifu/ml and RSD 1-15%.

***How big is the problem?*** Vials from tray 658 (out of 663) had infectivity results below the specification limits (≥$7.0 \times 10^8$ ifu/ml). The vials collected from the initial and the middle parts of the batch (trays n°94, 188, 282, 376, 470, 564) were within the specification limit.

***How often has it occurred?*** The trend was executed as required by SOPs MTMT006 and QOQO017 according to the following criteria:

- The event is related to a homogeneity issue
- The event occurred during the manufacturing of batch ABV3922
- The batch ABV3922 is the first batch manufactured after the three PPQ batches

No further event **related to "homogeneity issue (infectivity not conform)"** was noticed.

## III. Background

### 3.1 Process description

AZD1222 a recombinant ChAdOx1 adenoviral vector encoding the structural surface glycoprotein (Spike protein) antigen of the SARS-CoV-2. It is to be used as a prophylaxis against SARS CoV-2 infection (COVID-19 or Coronavirus Disease 2019).

The product is presented in A438 formulation buffer (10 mM Histidine/Histidine HCl, 7.5% (w/v) sucrose, 35mM NaCl, 1 mM MgCl2, 0.1% (w/v) PS80, 0.1 mM Edetate Disodium (EDTA), 0.5% (v/v) ethanol, pH 6.6 in Water for Injection) at a target concentration of $1.0 \times 10^{11}$ vp/mL.

AZD1222 filling process is designed to produce Drug Product (DP) at a target concentration of $1.0 \times 10^{11}$ vp/mL by dilution of the incoming Drug Substance (DS) using a Dilution Buffer. The process, outlined in Figure 1 consists of the following steps:

- Receipt of Drug Substance;
- Thawing;
- Pooling;
- Dilution of DS with the Dilution Buffer;
- Mixing to homogeneity to produce final bulk (i.e. diluted Drug Substance);
- Bioburden Reduction filtration (BBR) and hold of final bulk;
- Sterile filtration;
- Aseptic filling, stoppering and capping;
- 100% visual inspection of the Drug Product Vials.

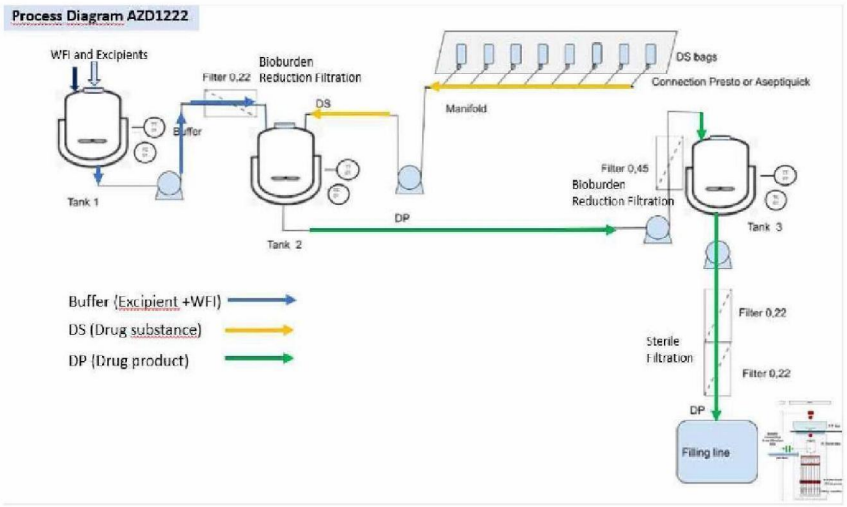


**Figure 1 AZD1222 Process diagram.**

The DS is shipped frozen from multiple DS manufacturing sites to the fill-**finish site and stored at** ≤-65°C.

The Dilution Buffer is prepared at the fill-finish site and equilibrated to 2-8 °C before transfer. The formulation operation starts by first transferring the DS to a mixing vessel followed by Dilution Buffer. The amount of dilution buffer is determined based on the amount and incoming virus particle concentration of the DS.

The dilution of DS is performed in two steps; in the first step approximately 50% of calculated dilution buffer is added. The DS and the Dilution Buffer are then mixed and an in-process sample is taken to determine virus particle concentration. The second dilution step utilizes virus particle concentration form in-process sample to make final dilution.

The intermediate product and the Dilution Buffer are then mixed to generate final bulk which is BBR filtered through a 0.45 µm filter into a holding bag.

The final bulk is semi continuously sterile filtered into a surge filling bag, keeping up with the filling line, through two 0.2 micron filters.

Figure of the tank 3, used for pre-fill hold and filling of the Drug Product, is shown below:

**Figure 2 On the left the tank bag during tank assembling, on the right tank temperature probe detail.**

Vials are aseptically filled, stoppered, and capped, and are held at 2-8°C in trays prior to 100% visual inspection. AZD1222 is a 6.5 mL nominal fill in a 10R glass vial. This allows ten 0.5 mL doses per vial with sufficient overfill.

The external laboratory, RIVM, analyzes product according to the specifications **AZDoc0172408 "AZD1222 Drug Product Master specification"**. To support the investigation, the report will focus on the following test:

- Infectivity: to monitor the virus activity;
- Virus particle concentration: performed by anion exchange chromatography (AEX) to monitor the virus amount;
- Polysorbate 80 (PS80) concentration

## IV. Investigation details

### 4.1 Batch record review

The manufacturing and filling activities of the batch under investigation were executed from January 01st, 2021 to January 03trd, 2021. The batch record review performed for the batch under investigation showed that it was produced according to all GMP and internal procedures.

The following deviations occurred during the production of batch ABV3922 – AZD1222 SAP code 1425240:

- TW#296080 Cryovault vent system and DS filling system different from conform work instruction. In particular, the vent and DS filling were on the same top side of the Cryovault and that had impact on the dilution step management of the DS.
- TW#296553 QC _Batch ABV3922_samples delivery. The production personnel delivered the samples collected for sterility test, endotoxins and CCIT in the sample receiving closet. These samples should be delivered in the fridge at 2-8 °C.

Considering the nature of the event under investigation, the present deviation is not linked to the ones listed above.

### 4.2 Laboratory investigation

On January 27th, 2021 Astrazeneca sent to Catalent Anagni a formal request to perform additional testing on drug product filled vials as part of an EMA commitment in order to evaluate batch homogeneity. Astrazeneca requested to Catalent Anagni to test all manufactured batches (from batch ABV0519 to batch ABV7764) for absorbance at 260 nm (A260) on two separate vials collected from beginning, middle and end of the filling. Astrazeneca authorized Catalent Anagni to use vials from chemical retains to perform the analyses. Moreover, Astrazeneca required for future manufactured batches additional sampling at 25% and 75% of the filling (sampling plan: at 0%, 25%, 50%, 75% and 100% of the filling) and the absorbance at 260 nm testing.

On January 30th, 2021 the **TW#307936 "Additional testing" was opened to allow the Anagni QC laboratory to** carry out the following tests on the final product as required by Astrazeneca:

- Determination of Viral Concentration (AEX) according to the method AZ Doc0172836
- Absorbance at 260 nm screening (memo AZ)
- Determination of PS80 Concentration with the method AZ DV-051226

On February 03trd, 2021 the ECC **TW#309573** "Additional Testing Request - for Drug product AZD1222" implemented the additional sampling (sampling plan: at 0%, 25%, 50%, 75% and 100% of the filling) and testing (absorbance A260) required by Astrazeneca. The ECC TW#309573 "Additional Testing Request - for Drug product AZD1222" **defined the** UV relative homogeneity |A260-A320| as parameter to monitor the batch production. The UV relative homogeneity |A260-A320| is defined as

$$\frac{MAX\,|A260-A320| - MIN\,|A260-A320|}{AVERAGE\,|A260-A320|}$$

and the following limits were set:

- **Action limit ≤ 0,40**
- **Alert limit ≤ 0,30**

The first batch involved by the ECC **TW#309573** "Additional Testing Request - for Drug product AZD1222" was ABV8139.

**Viral Concentration (AEX)** was determined for the PPQ batches (ABV0519, ABV1267 and ABV1953) and for the batch under investigation (ABV3922). For each batch 6 samples were tested (2 Beginning, 2 Middle and 2 End, each vial was injected twice), sampled from retain samples. Results are available in the attachment 1 ***"AEX results of the PPQ and of the involved batch"***. All the results obtained for the PPQ batches met the acceptance criteria. Moreover, no trend anomaly was observed. Only the vials related to the final part of the batch ABV3922 showed results not conform (AEX average: End 1 vial 0,31 $10^{11}$ Vp/mL End 2 vial 0,30 $10^{11}$ Vp/mL VS 0.7 – 1.3 $10^{11}$ vp/mL) confirming the anomalous infectivity data communicated by the external laboratory RIVM.

**UV Absorbance**

UV Absorbance screening at A260-A320 was reviewed for the batches ABV0519 to ABV7764 to monitor the protein content **(See attachment 2 "*UV absorbance screening at A260-A320 of the AZD1222 batches manufactured*"**. The UV test was executed on retain samples, 6 samples for each batch (2 Beginning, 2 Middle and 2 End). The results obtained are summarized in the chart below:

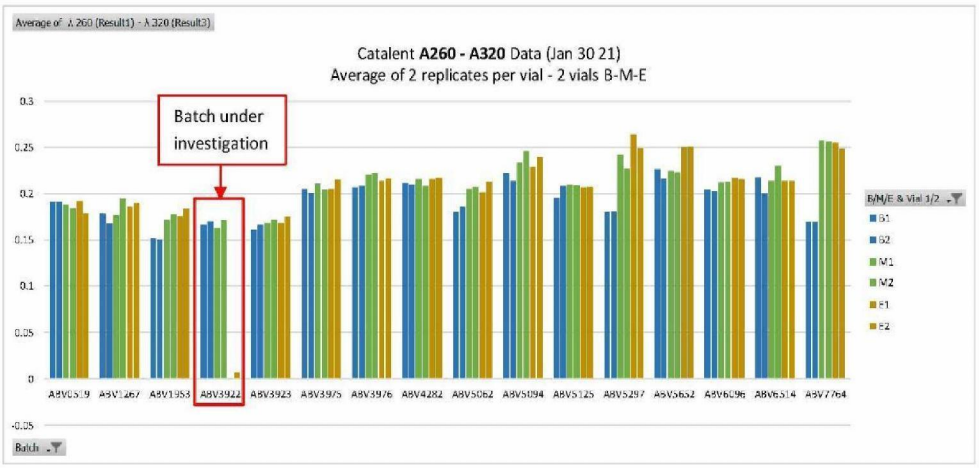


**Figure 3 UV relative homogeneity provided above each lot's UV absorbance results**

The results are reported as the average of values obtained at A260 corrected for the ones at A320 (Average |A260-A320|). The UV absorbance results confirmed the AEX and infectivity anomalous data for the batch under investigation. All the results obtained were within the action limit (≤ 0,40) except for the batch ABV3922, under present investigation. The results of batches ABV5297 and ABV7764 were above the alert limit (≤ 0,30). Therefore, the laboratory QC performed the AEX analysis on the same vials used for UV screening of the batches ABV5297 and ABV7764. The results obtained were within the specification limit (*See attachment 3 "AEX results of batches ABV5297 and ABV7764"*)

**PS80 Concentration**

The PS80 concentration was determined for the batch ABV3922 on the vials used for UV test (1 from beginning, 1 from the middle and 2 from the end). The result obtained (See attachment 4 ***"PS80 concentration result of batch ABV3922"***) are shown in the chart below:

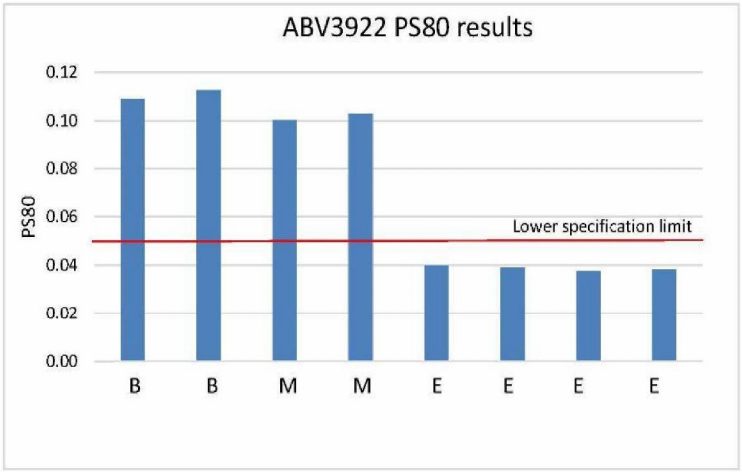


**Figure 4 ABV3922 PS80 results**

The results of the vials collected from the end part of the batch ABV3922 were below the lower specification limit (0,04 %w/v VS 0,05 %w/v).

### 4.3 Production investigation

The Fishbone diagram supported the homogeneity issue root cause analysis of batch ABV3922 – AZD1222 SAP code 1425240, as showed in the next figure

Tables 2 to 5 summarize all the investigation details, including rationale used to assess the probability of contributing to the deviation for each topic analyzed.

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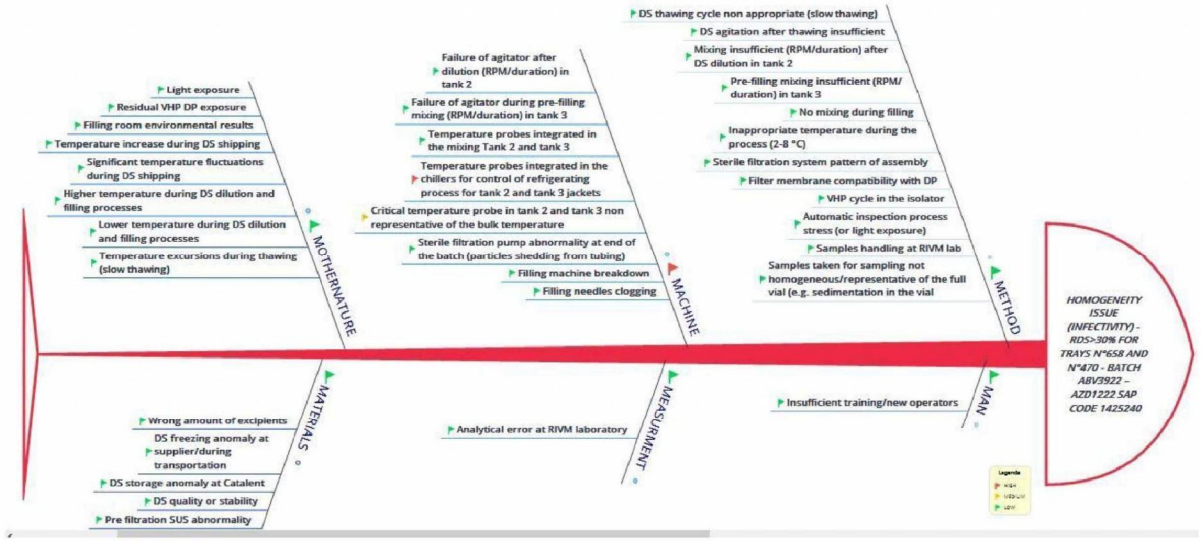



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**INVESTIGATION REPORT** TW#307662

| | Potential root causes | Direct effect | Final effect | PROBABILITY | WHY |
|---|---|---|---|---|---|
| MOTHER NATURE (environment) | *Light exposure* | Virus particles inactivation (decrease in infectivity) PS80 degradation | decrease in infectivity and in PS80 conc in the entire batch | LOW | - Only the end segment of the batch is impacted for vp/ml, PS80 and infectivity<br>- From beginning to at least 85% (based on sample from tray 564) of the batch the attributes are within specification. |
| | *Residual VHP DP exposure (VHP is used as decontaminant agent for isolator cleaning)* | Virus particles inactivation (Viricidal effect of VHP) | decrease in infectivity most likely at the beginning of the batch when potential residue is higher | LOW | - Only the end segment of the batch is impacted for vp/ml, PS80 and infectivity.<br>- From beginning to at least 85% (based on sample from tray 564) of the batch the attributes are within specification.<br>- After the VHP cycle, an aeration step is performed till the Draeger sensor detects VHP residue below 1 ppm<br>- The VHP cycle has been successfully qualified according to the protocols A-19-18-PQ-67 and A-20-18-PQ-20. |
| | *Temperature increase during DS shipping* | virus particles inactivation | decrease in infectivity in the entire batch | LOW | - Only the end segment of the batch is impacted for vp/ml, PS80 and infectivity.<br>- From beginning to at least 85% (based on sample from tray 564) of the batch the attributes are within specification.<br>- DS shipping documentation (including temperature monitoring data) reviewed at the time of DS delivery; no abnormalities observed (no deviations recorded for the DS batch 102152) |
| | *Significant temperature fluctuations during DS shipping* | virus particles agglomeration | Gradual decrease in vp/ml and PS80 conc because of agglomeration retention by BBR 0,45 filter and by 0,22 sterile filter | LOW | - DS shipping documentation (including temperature monitoring data) reviewed at the time of DS delivery; no abnormalities observed (no deviations recorded for the DS batch 102152)<br>- BBR 0,45 µ : comparing PPQ batch ABV1267 (batch size: theoretical vials 162751) and the impacted batch (batch size: theoretical vials 146751), for the step of continuous filtration between tank 2 and tank 3, no significant differences in time duration were observed (ABV1267: 02:18 -04:30 - 2 h 12 min ABV3922: 17:40 – 19:37 1h 57 min)<br>- Integrity test results (Bubble point) for the sterile filter for batch ABV3922 and ABV1267 (PPQ2 with similar batch size) were 2850 and 3250, showing an opposite behavior to the expected one in case of filter clogging. To be considered that only the IT of the main filter (closest to the filling) is requested by the MBR as post use IT.<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by a gradual clogging of the sterile filtration. |
| | *Higher temperature during DS dilution and filling processes* | virus particles inactivation potential for microbial growth | decrease in infectivity in the entire batch Potential for DP contamination in the entire batch | LOW | - Only the end segment of the batch is impacted for vp/ml, PS80 and infectivity<br>- From beginning to at least85% (based on sample from tray 564) of the batch the attributes are within specification.<br>- No temperature out of range was recorded in the Batch record |

Table 2 **Root cause analysis "MOTHER NATURE".**

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| | Potential root causes | Direct effect | Final effect | PROBABILITY | WHY |
|---|---|---|---|---|---|
| MOTHER NATURE (environment) | *Significant temperature drop during DS dilution and filling processes* | virus particles agglomeration filter clogging | Gradual decrease in vp/ml and PS80 conc because of agglomeration retention by BBR 0,45 filter and by 0,22 sterile filter | LOW | - No temperature out of range was recorded in the Batch record<br>- BBR 0,45 µ : comparing PPQ batch ABV1267 (batch size: theoretical vials 162751) and the impacted batch (batch size: theoretical vials 146751), for the step of continuous filtration between tank 2 and tank 3, no significant differences in time duration were observed (ABV1267: 02:18 -04:30 - 2 h 12 min ABV3922: 17:40 – 19:37 1h 57 min)<br>- Integrity test results (Bubble point) for the sterile filter for batch ABV3922 and ABV1267 (PPQ2 with similar batch size) were 2850 and 3250, showing an opposite behavior to the expected one in case of filter clogging. To be considered that only the IT of the main filter (closest to the filling) is requested by the MBR as post use IT.<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by a gradual clogging of the sterile filtration. |
| | *Temperature excursions during thawing (slow thawing)* | virus particles agglomeration | Gradual decrease in vp/ml and PS80 conc because of agglomeration retention by BBR 0,45 filter and by 0,22 sterile filter | LOW | - No temperature out of range was recorded in the Batch record<br>- BBR 0,45 µ : comparing PPQ batch ABV1267 (batch size: theoretical vials 162751) and the impacted batch (batch size: theoretical vials 146751), for the step of continuous filtration between tank 2 and tank 3, no significant differences in time duration were observed (ABV1267: 02:18 -04:30 - 2 h 12 min ABV3922: 17:40 – 19:37 1h 57 min)<br>- Integrity test results (Bubble point) for the sterile filter for batch ABV3922 and ABV1267 (PPQ2 with similar batch size) were 2850 and 3250, showing an opposite behavior to the expected one in case of filter clogging. To be considered that only the IT of the main filter (closest to the filling) is requested by the MBR as post use IT.<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by a gradual clogging of the sterile filtration. |

Table 3 **Root cause analysis "MOTHER NATURE".**

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**INVESTIGATION REPORT TW#307662**

| | Potential root causes | Direct effect | Final effect | PROBABILITY | WHY | WHY |
|---|---|---|---|---|---|---|
| METHOD | *DS thawing cycle non appropriate (slow thawing)* | virus particles agglomeration | Gradual decrease in vp/ml and PS80 conc due to agglomeration retention by 0,22 sterile filter | LOW | - Thawing cycle successfully validated in the PPQ batches (see report A-20-18-P-04 ver.00) and conform data for subsequent commercial batches)<br>- No abnormalities were observed during thawing of batch ABV3922 | |
| | *DS agitation after thawing insufficient* | Nonhomogeneous DS suspension | Potential for highly concentrated hold up volume in the Cryovault | LOW | - Agitation process successfully validated in the PPQ batches (see report A-20-18-P-04 ver.00 and conform data for subsequent commercial batches)<br>- IPC for titer (vp/ml) by AEX method after first dilution was aligned with the theoretical data (actual: 1,9E11 vp/ml; theoretical: 1,86 E+11 vp/ml). The process was conformed at least till the first dilution | |
| | *Mixing insufficient (RPM/duration) after DS dilution in tank 2* | Nonhomogeneous DP suspension<br>Nonhomogeneous Temperature in the bulk<br>Virus inactivation in higher temperature areas | Significant variability in vp/ml and in infectivity over the batch | LOW | - Mixing step successfully validated in the PPQ batches (see report A-20-18-P-04 ver.00 and conform data for subsequent commercial batches)<br>- IPC for titer (vp/ml) by AEX method after first dilution was aligned with the theoretical data (actual: 1,9E11 vp/ml; theoretical: 1,86 E+11 vp/ml). The process was conformed at least till the first dilution | |
| | *Pre-filling mixing insufficient (RPM/duration) in tank 3* | Nonhomogeneous DP suspension<br>Nonhomogeneous Temperature in the bulk<br>Virus inactivation in higher temperature areas | Significant variability in vp/ml and in infectivity over the batch | LOW | - Mixing step successfully validated in the PPQ batches (see report A-20-18-P-04 ver.00 and conform data for subsequent commercial batches)<br>- From beginning to at least 85% (based on sample from tray 564) of the batch the attributes were within specification. | |
| | *No mixing during filling* | Virus particles sedimentation.<br>Temperature stratification in the bulk<br>Virus inactivation in higher temperature areas | Significant variability in vp/ml and in infectivity over the batch | LOW | - Mixing step successfully validated in the PPQ batches (see report A-20-18-P-04 ver.00 and conform data for subsequent commercial batches)<br>- 72 h DP Hold Time in tank 3 challenged during PPQ exercise with successful results. No inhomogeneity was observed (see report A-20-18-P-04 ver.00) | |
| | *Inappropriate temperature during the process (2-8 °C)* | virus particles agglomeration | Gradual decrease in vp/ml and PS80 conc because of agglomeration retention by 0,22 sterile filter | LOW | - Temperature setting successfully validated in the PPQ batches (see report A-20-18-P-04 ver.00 and conform data for subsequent commercial batches)<br>- No temperature anomaly was reported in the Batch record | |

Table 4 **Root cause analysis "METHOD".**

**INVESTIGATION REPORT TW#307662**

| Potential root causes | Direct effect | Final effect | PROBABILITY | - WHY |
|---|---|---|---|---|
| *Sterile filtration system pattern of assembly* | Back flow from flushing bag into the filling line at the end of filling – Study available for VP/PS80 adsorption at the beginning of the filtration when membrane is not properly wetted – DP in the flush bag might be diluted | Lower vp/ml and PS80 conc in the vials filled with the flushing volume | LOW | - All SUS equipment assembly and relevant procedures successfully validated. Back flow cannot occur. The flushing bags are hung below each filter either for 0,45 µ and for 0,22 µ filters. The vent bags of the filter capsules are secured by a valve.<br>- Characteristics of DP product from the flush bag have been investigated by AZ and this root cause can be ruled out according to the study data (see ref ML03135-09) |
| *Filter membrane compatibility with DP* | Vp-PS80 (PS80 bound to the vp) retention by the membrane | Gradual decrease in vp/ml and PS80 conc because of retention by 0,22 sterile filter | LOW | - Filter membrane successfully validated in the PPQ batches (see report A-20-18-P-04 ver.00and conform data for subsequent commercial batches)<br>- Sterile filtration validation executed for a DP batch size 1100 lt<br>- Astazeneca is currently working on a filter blockage/ clogging test study |
| *VHP cycle in the isolator* | Virus particles inactivation (Viricidal effect of VHP) | decrease in infectivity most likely at the beginning of the batch when potential residue is higher | LOW | - The effect of the VHP residue in the isolator evaluated successfully during PPQ (see report A-20-18-P-04 ver.00 and conform data for subsequent commercial batches)<br>- From beginning to at least 85% (based on sample from tray 564) of the batch the attributes were within specification. |
| *Automatic inspection process stress (or light exposure)* | virus particles inactivation | decrease in infectivity in the entire batch | LOW | - The effect of the Automatic inspection evaluated successfully during the PPQ (see report A-20-18-P-04 ver.00 and data for subsequent commercial batches)<br>- Samples taken from the newly implemented two steps inspection are conform for the subsequent commercial batches<br>- The deviated batch was run in a single step inspection |
| *Samples handling at RIVM lab* | virus particles inactivation | decrease in infectivity in the vial | LOW | - All samples tested by RIVM compliant for the PPQ batches;<br>- Samples results by RIVM confirmed by Catalent Anagni lab (AEX and PS80 method) |
| *Samples taken for sampling not homogeneous/representative of the full vial (e.g. sedimentation in the vial)* | Nonhomogeneous DP suspension | Low vp/ml in the sample aliquot analyzed (decrease in infectivity observed) | LOW | - All samples tested by RIVM compliant for the PPQ batches<br>- Samples results by RIVM confirmed by Catalent Anagni lab (AEX and PS80 method) |

**Table 5 Root cause analysis "METHOD".**

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**INVESTIGATION REPORT** TW#307662

| | Potential root causes | Direct effect | Final effect | PROBABILITY | WHY |
|---|---|---|---|---|---|
| MACHINE | *Failure of agitator after dilution (RPM/duration) in tank 2* | Nonhomogeneous DP suspension<br>Nonhomogeneous Temperature in the bulk<br>Virus inactivation in higher temperature areas | Significant variability in vp/ml and in infectivity over the batch | LOW | - No anomaly was reported in the Batch record<br>- No specific interventions were reported in the Batch record<br>- Maintenance work orders log were reviewed and no intervention linked to the agitator was found.<br>- Only the end segment of the batch is impacted for vp/ml, PS80 and infectivity<br>- IPC for titer (vp/ml) by AEX method after first dilution was aligned with the theoretical data (actual: 1,9E+11 vp/ml; theoretical: 1,86 E+11 vp/ml). The process was conform at least till the first dilution |
| | *Failure of agitator during pre-filling mixing (RPM/duration) in tank 3* | Nonhomogeneous DP suspension<br>Nonhomogeneous Temperature in the bulk<br>Virus inactivation in higher temperature areas | Significant variability in vp/ml and in infectivity over the batch | LOW | - No anomaly was reported in the Batch record<br>- No specific interventions were reported in the Batch record<br>- Maintenance work orders log were reviewed and no intervention linked to the agitator was found<br>- From beginning to at least 85% (based on sample from tray 564) of the batch the attributes were within specification. |
| | *Failure of Temperature probes integrated in the mixing Tank 2 and tank 3* | DP Temperature reading not accurate: a drop in bulk DP temperature not detected<br>virus particles agglomeration | Gradual decrease in vp/ml and PS80 conc because of agglomeration retention by BBR 0,45 filter and by 0,22 sterile filter | LOW | - No anomaly was reported in the Batch record<br>- No specific interventions were reported in the Batch record<br>- Maintenance work orders log were reviewed and no intervention linked to the temperature probes was found<br>- Last calibration of the probe compliant<br>- BBR 0,45 µ: comparing PPQ batch ABV1267 (batch size: theoretical vials 162751) and the impacted batch (batch size: theoretical vials 146751), for the step of continuous filtration between tank 2 and tank 3, no significant differences in time duration were observed (ABV1267: 02:18 -04:30 - 2 h 12 min ABV3922: 17:40 – 19:37 1h 57 min)<br>- Integrity test results (Bubble point) for the sterile filter for batch ABV3922 and ABV1267 (PPQ2 with similar batch size) were 2850 and 3250, showing an opposite behavior to the expected one in case of filter clogging. To be considered that only the IT of the main filter (closest to the filling) is requested by the MBR as post use IT.<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by a gradual clogging of the sterile filtration. |
| | *Failure of Temperature probes integrated in the mixing Tank 2 and tank 3* | DP Temperature reading not accurate: a peak in bulk DP temperature not detected<br>Virus inactivation in higher temperature areas | decrease in infectivity in the entire batch | LOW | -<br>- No specific interventions were reported in the Batch record<br>- Maintenance work orders log were reviewed and no intervention linked to the temperature probes was found<br>- Last calibration of the probe compliant<br>- From beginning to at least 85% (based on sample from tray 564) of the batch the attributes were within specification. |

Table 6 **Root cause analysis "MACHINE".**

**INVESTIGATION REPORT TW#307662**

| | Potential root causes | Direct effect | Final effect | PROBABILITY | WHY |
|---|---|---|---|---|---|
| MACHINE | *Failure of Temperature probes integrated in the chillers for control of refrigerating process for tank 2 and tank 3 jackets* | Drop in temperature<br>Bulk DP cooling/freezing – cryoconcentration-<br>virus particles agglomeration | Gradual decrease in vp/ml and PS80 conc because of agglomeration retention by BBR 0,45 filter and by 0,22 sterile filter | LOW | - Maintenance work orders (WO. 1010234878 and 1010255664) were performed before the production of the involved batch<br>- The probe was reading an incorrect temperature due to lose connection<br>- No anomaly was reported in the Batch record<br>- No specific interventions were reported in the Batch record<br>- Integrity test results (Bubble point) to be collected<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by a gradual clogging of the sterile filtration.<br>- Freezing/thawing studies on DS showed cryo-concentration, hence an agitation step was added after thawing to homogenize the DS solution bags to be pooled. |
| | *Failure of Temperature probes integrated in the chillers for control of refrigerating process for tank 2 and tank 3 jackets* | Drop in temperature<br>Bulk DP freezing on the tank 3 walls — dilution of the last part of the batch due to melting of frozen material potentially lower in viral particles and other excipients | Sudden decrease in vp/ml and PS80 conc | HIGH | -<br>- Maintenance work orders (WO. 1010234878 and 1010255664) were performed before the production of the involved batch<br>- The chiller probe was reading an incorrect temperature due to lose connection<br>- No anomaly was reported in the Batch record<br>- No specific interventions were reported in the Batch record<br>- The log sheet S373 requires to check the chiller temperature tank 3 only twice: before and after the DP BBR filtration from tank 2 to tank 3<br>- No check of the tank 3 chiller temperature during filling is performed<br>- The DP temperature is checked during filling as conform/Not conform, no actual value is reported. Also the DP Temperature value of 5°C in S373 was taken just after transfer to Tank 3 and could have changed with time (almost a day) |
| | *Failure of Temperature probes integrated in the chillers for control of refrigerating process for tank 2 and tank 3 jackets* | Increase in temperature<br>Virus particle inactivation | decrease in infectivity in the entire batch | LOW | - Maintenance work orders (WO. 1010234878 and 1010255664) were performed before the production of the involved batch<br>- The probe was reading an incorrect (higher) temperature due to lose connection<br>- No anomaly was reported in the Batch record<br>- No specific interventions were reported in the Batch record<br>- Only the end segment of the batch is impacted for vp/ml, PS80 and infectivity.<br>- From beginning to 71% of the batch the attributes are within specification. |
| | *Critical temperature probe in tank 2 and tank 3 non representative of the bulk temperature* | Temperature reading not representative: a drop in bulk DP temperature not detected (thermal gradient)<br>Virus particles agglomeration | Gradual decrease in vp/ml and PS80 conc because of agglomeration retention by BBR 0,45 filter and by 0,22 sterile filter | LOW | - Only one probe at the bottom of the tank in the center, as per Millipore design<br>- Maintenance work orders log were reviewed and no intervention linked to the temperature probes was found<br>- No anomaly was reported in the Batch record<br>- No specific interventions were reported in the Batch record<br>- All temperature data in the BR were compliant<br>- Integrity test results (Bubble point) for the sterile filter for batch ABV3922 and ABV1267 (PPQ2 with similar batch size) were 2850 and 3250, showing an opposite behavior to the expected one in case of filter clogging. To be considered that only the IT of the main filter (closest to the filling) is requested by the MBR as post use IT.<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by a gradual clogging of the sterile filtration.<br>- Freezing/thawing studies on DS showed cryo-concentration, hence an agitation step was added after thawing to homogenize the DS solution bags to be pooled. |

Revisione n. : 0 Stato: Effective 2021-02-18T14:45:45 Data Stampa: 2021-02-18T14:48:10



| | | | | | |
|---|---|---|---|---|---|
| | *Critical temperature probe in tank 2 and tank 3 non representative of the bulk temperature* | Temperature reading not representative: a drop in bulk DP temperature not detected Bulk DP freezing on the tank 3 walls – cryoconcentration – dilution of the last part of the batch | Sudden decrease in vp/ml and PS80 conc | MEDIUM | - Only one probe at the bottom of the tank in the center, as per Millipore design<br>- Maintenance work orders log were reviewed and no intervention linked to the temperature probes was found<br>- No anomaly was reported in the Batch record (temperature was monitored each hour as required by log sheet S374)<br>- No specific interventions were reported in the Batch record<br>- All temperature data in the BR were compliant |
| | *Sterile filtration pump abnormality at end of the batch (particles shedding from tubing)* | Filter clogging | Gradual decrease in vp/ml and PS80 conc because of retention by 0,22 sterile filter | LOW | - No anomalous events reported<br>- Integrity test results (Bubble point) for the sterile filter for batch ABV3922 and ABV1267 (PPQ2 with similar batch size) were 2850 and 3250, showing an opposite behavior to the expected one in case of filter clogging. To be considered that only the IT of the main filter (closest to the filling) is requested by the MBR as post use IT.<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by a gradual clogging of the sterile filtration |
| | *Filling machine breakdown* | Filling process duration too long, longer DP storage in the tank – sedimentation longer DP-filter membrane interactions – filter clogging | Gradual decrease in vp/ml and PS80 conc because of retention by 0,22 sterile filter | LOW | - Batch record review for filling stops: no significant stops observed<br>- Maintenance work orders log were reviewed and no intervention linked to the filling machine breakdown was found<br>- Integrity test results (Bubble point) for the sterile filter for batch ABV3922 and ABV1267 (PPQ2 with similar batch size) were 2850 and 3250, showing an opposite behavior to the expected one in case of filter clogging. To be considered that only the IT of the main filter (closest to the filling) is requested by the MBR as post use IT.<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by a gradual clogging of the sterile filtration |
| | *Filling needles clogging* | Virus particles kept by the clogging Partially filled vials Drift in fill weights | Gradual decrease in vp/ml and PS80 conc | LOW | - 100% weight check on the filling machine<br>- Filling weights report reviewed: no drift observed |

Table 7 **Root cause analysis "MACHINE".**

**INVESTIGATION REPORT** TW#307662

| | Potential root causes | Direct effect | Final effect | PROBABILITY | - WHY |
|---|---|---|---|---|---|
| MATERIALS | *Wrong amount of excipients* | Low PS80 conc<br>Viral particle aggregation | Gradual decrease in vp/ml and PS80 conc because of retention by 0,22 sterile filter | LOW | - No anomaly was reported in the Batch record<br>- BBR 0,45 µ : comparing PPQ batch ABV1267 (batch size: theoretical vials 162751) and the impacted batch (batch size: theoretical vials 146751), for the step of continuous filtration between tank 2 and tank 3, no significant differences in time duration were observed (ABV1267: 02:18 -04:30 - 2 h 12 min ABV3922: 17:40 – 19:37 1h 57 min)<br>- Integrity test results (Bubble point) for the sterile filter for batch ABV3922 and ABV1267 (PPQ2 with similar batch size) were 2850 and 3250, showing an opposite behavior to the expected one in case of filter clogging. To be considered that only the IT of the main filter (closest to the filling) is requested by the MBR as post use IT.<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by gradual clogging of the sterile filtration. |
| | *DS freezing anomaly at supplier/during transportation* | virus particles agglomeration<br>filter clogging | Gradual decrease in vp/ml and PS80 conc because of agglomeration retention by 0,22 sterile filter | LOW | - DS documentation by CAT BWI was reviewed and no anomaly was found<br>- DS shipping documentation (including temperature monitoring data) reviewed at the time of DS delivery; no abnormalities observed (no deviations recorded for the DS batch 102152)<br>- BBR 0,45 µ : comparing PPQ batch ABV1267 (batch size: theoretical vials 162751) and the impacted batch (batch size: theoretical vials 146751), for the step of continuous filtration between tank 2 and tank 3, no significant differences in time duration were observed (ABV1267: 02:18 -04:30 - 2 h 12 min ABV3922: 17:40 – 19:37 1h 57 min)<br>- Integrity test results (Bubble point) for the sterile filter for batch ABV3922 and ABV1267 (PPQ2 with similar batch size) were 2850 and 3250, showing an opposite behavior to the expected one in case of filter clogging. To be considered that only the IT of the main filter (closest to the filling) is requested by the MBR as post use IT.<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by a gradual clogging of the sterile filtration. |
| | *DS storage anomaly at Catalent* | virus particles agglomeration<br>filter clogging | Gradual decrease in vp/ml and PS80 conc because of agglomeration retention by 0,22 sterile filter | LOW | - Delta V system Emerson data were reviewed for the storage freezer EACNG028 and no excursions were found<br>- BBR 0,45 µ : comparing PPQ batch ABV1267 (batch size: theoretical vials 162751) and the impacted batch (batch size: theoretical vials 146751), for the step of continuous filtration between tank 2 and tank 3, no significant differences in time duration were observed (ABV1267: 02:18 -04:30 - 2 h 12 min ABV3922: 17:40 – 19:37 1h 57 min)<br>- Integrity test results (Bubble point) for the sterile filter for batch ABV3922 and ABV1267 (PPQ2 with similar batch size) were 2850 |

**INVESTIGATION REPORT TW#307662**

| | | | | | |
|---|---|---|---|---|---|
| | | | | | and 3250, showing an opposite behavior to the expected one in case of filter clogging. To be considered that only the IT of the main filter (closest to the filling) is requested by the MBR as post use IT.<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by gradual clogging of the sterile filtration. |
| | *DS quality or stability* | virus particles agglomeration filter clogging | Gradual decrease in vp/ml and PS80 conc because of agglomeration retention by 0,22 sterile filter | LOW | - Review of DS documentation by CAT BWI on going<br>- Comparing PPQ batches and the impacted batch, for the step of continuous filtration (BBR 0,45 µ) time duration between tank 2 and tank 3 is no longer than for the PPQ batches<br>- Integrity test results (Bubble point) for the sterile filter for batch ABV3922 and ABV1267 (PPQ2 with similar batch size) were 2850 and 3250, showing an opposite behavior to the expected one in case of filter clogging. To be considered that only the IT of the main filter (closest to the filling) is requested by the MBR as post use IT.<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by gradual clogging of the sterile filtration. |
| | *Pre filtration SUS abnormality* | Adsorption/particles shedding | Filter clogging | LOW | - Review of CoA of SUS compliant<br>- Integrity test results (Bubble point) for the sterile filter for batch ABV3922 and ABV1267 (PPQ2 with similar batch size) were 2850 and 3250, showing an opposite behavior to the expected one in case of filter clogging. To be considered that only the IT of the main filter (closest to the filling) is requested by the MBR as post use IT.<br>- Surge bag re-filling duration times do not show a decrease in flowrate that could be explained by gradual clogging of the sterile filtration. |

Table 8 **Root cause analysis "MATERIALS".**

| | | | | | |
|---|---|---|---|---|---|
| MAN | *Insufficient training/new operators* | Operations not aligned with Sop; MBR and working instruction | Incorrect operations could affect batch quality | LOW | - Production personnel was trained to operate on "BIOSUITE 1" line |
| MEASUREM | *Analytical error at RIVM laboratory* | Inaccurate infectivity data | OOS | LOW | Analytical data by RIVM were confirmed on the vial from the same tray by QC lab CAT |

Table 9 **Root cause analysis "MAN" and "MEASUREMENT".**

### 4.4 Maintenance investigation

On December 29th, 2020, during the winter shut down (PPQ batches production completed), maintenance personnel made an intervention (Maximo ref. 1010234878) to replace the protection cover of the cooling tubes connecting the Lauda chillers with the SU jacketed tanks


**Figure 5 Chiller cooling tubes cover**

On February 09th, 2021 the maintenance personnel were interviewed to detail the activities. During the cover removal one tube tightener was slightly loosed generating a cooling liquid leak.

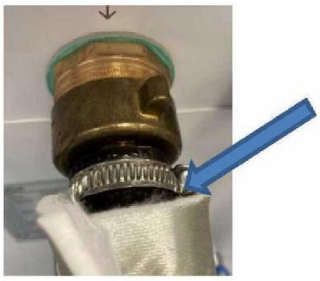


**Figure 6 Chiller cooling tubes detail**

The maintenance personnel refilled the liquid through the filling port on the top of the Lauda chiller. They filled more than necessary and there was a little spillage around the port. They assumed that the spillage might have also affected the internal part of the chiller because the port seems not guarantee the sealing


**Figure 7 Chiller cooling liquid port**

As result of the cooling liquid spillage, on December 30th, 2020 maintenance intervention (Maximo ref. 1010255664) was executed to open the chiller covers and to verify the spillage effects on the internal components installed on top of the chiller cooling liquid tank, including the PT1000 probe.

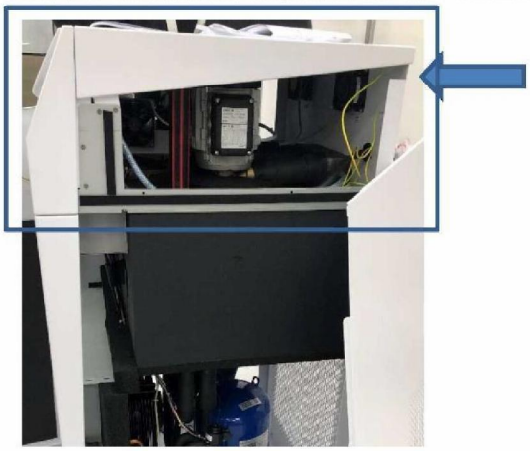


**Figure 8 Involved chiller section detail**

The interviewed maintenance technician confirmed to have removed all the carters on top of the chiller, where internal components are installed, including the electrical parts and the control panel electronic card (see ***"Intervista relativa a TW#307662 (AZD1222 "homogeneity issue")" – "TW#307662 (AZD1222 "homogeneity issue") Interview***). Everything was checked and dried (a small infiltration was found). The chiller covers were reassembled. This intervention was done during the line shut down. Neither the chiller nor the connected tank was in operation.

The same maintenance technician was present during the following production run, batch ABV3922, (in the night between January 2nd and 3rd) to double check that the tube tighteners were fixed properly. He noticed that there was some frost on them. The tank 3 product temperature was displayed into the defined range (2-8°C), considering that the production was on going and no additional evidence of anomalies were found it was not possible to do further verifications on the chiller.

Figure 9 Cooling pipe connection to tank 3

The maintenance technician escalated the event to his supervisor to double check the situation at the end of the batch, before the next chiller utilization for the following batch. During this check, performed before the production of batch ABV3923, it was detected a loose connection for the chiller PT1000 probe. One clamp on the control panel card was in the wrong position (slightly open) as shown below. Therefore, the maintainer correctly connected the chiller temperature probe.

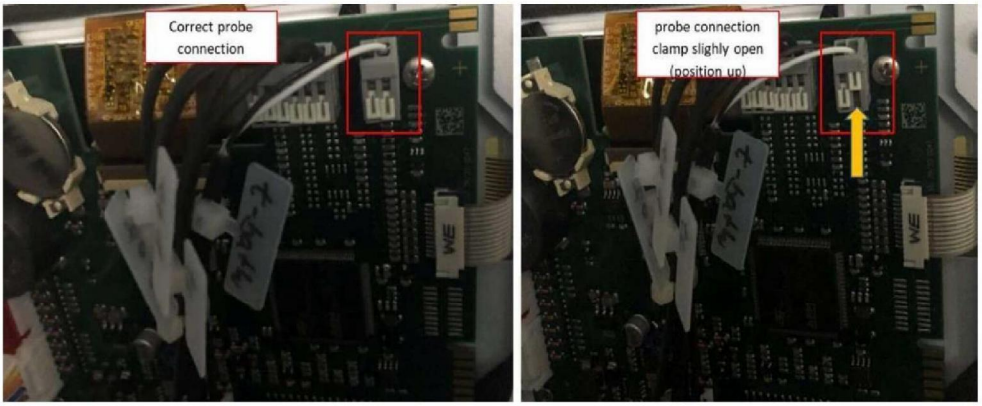


Figure 10 On the left, example of correct probe connection and on the right example of loose probe connection

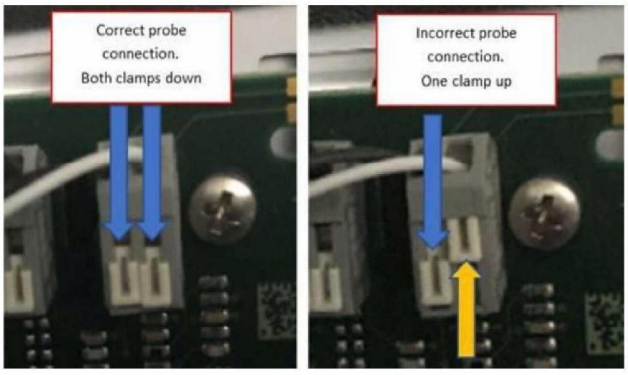


Figure 11 Correct probe connection details (zoom of Figure 9).

Even if the probe wire connection was loose, the wire remained inserted into the connector still in contact with the inner metal plate (if no contact at all, an alarm is generated). The loose contact generated a higher resistance (Ohm) detection which was translated in a higher temperature reading.

### V. Root cause Analysis

The investigation **on the event "homogeneity issue (infectivity not conform)" of the batch** ABV3922 – AZD1222 SAP code 1425240 was performed using the Fishbone diagram to evaluate any potential source.

**The "5Why" tool helped the** analysis of the most probable causes, highlighted by the Fishbone diagram, to determine the root cause of the event:

| ***Defect*** | **Homogeneity issue** (infectivity not conform) | | |
|---|---|---|---|
| *Why?* | Vials from tray 658 (out of 663) had infectivity results below the specification limits (7.0 x $10^8$ ifu/ml). The vials collected from the initial and the middle parts of the batch were within the specification limit | | |
| *Why?* | A drop of assay vp/ml and PS80 concentration was observed at the end of the batch. This can explain the decrease of infectivity. | | |
| *Why?* | The assay vp/ml decrease was most likely due to dilution of the last part of the batch | | |
| *Why?* | The dilution of the last part of the batch was most likely due to Bulk DP freezing on the tank 3 walls | | |
| *Why?* | Bulk DP freezing on the tank 3 walls occurred due to an undetected critical temperature drop | | |
| *Why was the issue generated?* | Interview to personnel highlighted that the chiller inlet and outlet pipes were freezing supporting the hypothesis that the chiller temperature control system was incorrectly regulating the parameter | *Why was the issue not observed?* | Even if the operator checked the Tank 3 temperature each hour as required by log sheet S374, the Tank 3 temperature was not representative of the whole batch volume (temperature of the product close to the surface of the walls was lower) |
| *Why was the issue generated?* | The chiller was regulating the temperature basing on an incorrect probe reading | *Why was the issue not observed?* | The probe position does not facilitate the temperature reading |
| *Why was the issue generated?* | The probe was reading an incorrect temperature due to loose connection | **CAUSAL FACTOR** | **MACHINE – System not redundant** |
| **PROBABLE CAUSE** | **MACHINE – Chiller temperature probe failure** | | |

**Table 10 5 Whys tool applied to the present event**

### VI. Impact/risk assessment

Considering that:

- Vials from tray 658 (out of 663) of the batch ABV3922 had infectivity results below the specification limits ($7.0 \times 10^8$ ifu/ml)
- The vials collected from the initial and the middle parts of the batch ABV3922 (trays n°94, 188, 282, 376, 470, 564) were within the specification limit
- No anomalous results were highlighted for the other lots manufactured for infectivity (evaluation performed from batch ABV0519 to ABV7764)
- The event was most likely generated due to the chiller temperature probe failure
- The chiller temperature probe was fixed after the batch ABV3922 production

It is possible to assess that the event impacted only to the batch ABV3922 – AZD1222 SAP code 142524. Final batch ABV3922 disposition is under Astrazeneca responsibility. No risk was highlighted for the quality of the other batches manufactured.

### VII. Conclusion

On January 29th, 2021 the QA personnel opened the deviation TW#307662 to investigate on the homogeneity issue (infectivity not conform) occurred on the batch ABV3922 – AZD1222 SAP code 1425240.

An in-depth investigation was performed using the Fishbone tool to evaluate any potential source. The Fishbone highlighted that most likely an undetected critical temperature drop induced the Bulk DP freezing on the tank 3 wall and, consequently, the cryoconcentration of the batch.

**Basing on the "5Why" tool, the** probable cause of the event was **"Machine** – Chiller temperature probe **failure"**. The chiller temperature probe was loose connected and read an incorrect temperature. Therefore, the chiller was autoregulating the cooling system basing on a wrong temperature value.

**The "5why" tool contributed to identify the causal factor of the event as "Machine – System not redundant".** The production personnel did not notice any issue (temperature was monitored each hour as required by log sheet S374) since there is only one probe at the bottom of the tank in the center, as per Millipore design. Considering that the solution was not under mixing as required by process design, a temperature gradient was created: the product directly in contact with the probe had temperature within the limits.

### VIII. Proposed CAPAs

The log sheet update will be performed to increase the process robustness regarding the tank and chiller temperature monitoring and the stirring management. The update will focus on the following subjects:

**Implementation of new controls:**

- The temperature check frequency during the hold time in tank 3 before the filling activities will be established
- The log sheet will require to report the chiller set point when currently a range is allowed
- The log sheet will require the visual check of the stirrer functioning after the switch on
- A second operator will verify the stirrer switch off

- Feasibility evaluation of the use of additional external temperature probe to check the Tank (containing Buffer or DP) temperature

**Improvement of the existing controls:**

- The log sheet will require to report the temperature of the Tank (containing Buffer or DP) every time that the chiller temperature is checked
- The log sheet will require to report the temperature value each time that the check of temperature is required
- The log sheet will require to report the Tank 3 and chiller temperature values checked each hour during the filling activities (currently the log sheet requires only to flag if Tank 3 temperature is conform or not).

**Immediate action**

- On January 30th, **2021 the TW#307936 "Additional testing" was opened to allow the Anagni QC** laboratory to carry out the following tests on the final product as required by Astrazeneca:
  - Determination of Viral Concentration (AEX) according to the method AZ Doc0172836
  - Absorbance at 260 nm screening (memo AZ)
  - Determination of PS80 Concentration with the method AZ DV-051226
- On February 03trd, 2021 the ECC **TW#309573** "Additional Testing Request - for Drug product AZD1222" **implemented the UV relative homogeneity** |A260-A320| as parameter to monitor the batch production. The ECC TW#309573 planned also the additional sampling of the batch (at the beginning, at 25%, 50%, 75% and 100% of the filling process)

## IX. Attachments & References

Attachment 1 - *AEX results of the PPQ and of the involved batches*

Attachment 2 - *UV absorbance screening at A260-A320 of the AZD1222 batch manufactured*

Attachment 3 – *AEX results of batches ABV5297 and ABV7764*

Attachment 4 - *PS80 concentration result of batch ABV3922*

Attachment 5 – *AZD1222 commercial batches data*

Attachment 6 - *Surge bag replenishment duration ABV3922 VS ABV1267*

Attachment 7 - *Filling weight data for ABV3922 and ABV1267*

Attachment 8 – *Temperature data of batch ABV3922 VS PPQ batches*

Attachment 9 - *TRIAL BUFFER TEMPERATURE EXCURSION SIMULATION STUDY REPORT MT-A/SP/SL/21/003*

## X. List of abbreviations

- AEX = Anion Exchange Chromatography
- BBR = BioBurden Reduction
- DP = Drug Product
- DS = Drug Substance
- ECC = Electronic change control
- PPQ = Process Performance Qualification
- PS80 = Polysorbate 80
- RSD = Relative Standard Deviation
- VHP = Vaporized Hydrogen Peroxide

ATTACHMENT TO INVESTIGATION REPORT TW#307662

1. *AEX results of the PPQ and of the involved batch*

| | STEP | Single Inj (Vp/mL $10^{11}$) | Average (Vp/mL $10^{11}$) | Specification limit (Vp/mL $10^{11}$) |
|---|---|---|---|---|
| ABV0519 | Begin 1 | 0,99 | 0,98 | 0,7 – 1,3 |
| | Begin 1 | 0,98 | | |
| | Begin 2 | 0,95 | 0,95 | |
| | Begin 2 | 0,95 | | |
| | Middle1 | 0,97 | 0,96 | |
| | Middle1 | 0,96 | | |
| | Middle2 | 1,03 | 1,03 | |
| | Middle2 | 1,03 | | |
| | End1 | 1,03 | 1,03 | |
| | End1 | 1,03 | | |
| | End2 | 1,03 | 1,03 | |
| | End2 | 1,03 | | |
| ABV1267 | Begin 1 | 1,00 | 1,00 | |
| | Begin 1 | 1,01 | | |
| | Begin 2 | 1,03 | 1,03 | |
| | Begin 2 | 1,03 | | |
| | Middle1 | 1,04 | 1,04 | |
| | Middle1 | 1,04 | | |
| | Middle2 | 1,04 | 1,05 | |
| | Middle2 | 1,05 | | |
| | End1 | 1,05 | 1,05 | |
| | End1 | 1,06 | | |
| | End2 | 1,06 | 1,06 | |
| | End2 | 1,05 | | |
| ABV1953 | Begin 1 | 0,96 | 0,95 | |
| | Begin 1 | 0,95 | | |
| | Begin 2 | 0,87 | 0,87 | |
| | Begin 2 | 0,87 | | |
| | Middle1 | 0,98 | 0,97 | |
| | Middle1 | 0,97 | | |
| | Middle2 | 0,94 | 0,94 | |
| | Middle2 | 0,95 | | |
| | End1 | 1,11 | 1,11 | |
| | End1 | 1,11 | | |
| | End2 | 0,97 | 0,97 | |
| | End2 | 0,97 | | |
| ABV3922 | Begin 1 | 0,85 | 0,85 | |
| | Begin 1 | 0,85 | | |
| | Begin 2 | 0,97 | 0,96 | |
| | Begin2 | 0,96 | | |
| | Middle1 | 0,92 | 0,92 | |
| | Middle1 | 0,91 | | |
| | Middle2 | 0,87 | 0,86 | |
| | Middle2 | 0,86 | | |
| | End1 | 0,31 | 0,31 | |
| | End1 | 0,31 | | |
| | End2 | 0,30 | 0,30 | |
| | End2 | 0,30 | | |

ATTACHMENT TO INVESTIGATION REPORT TW#307662

*2. UV absorbance screening at A260-A320 of the AZD1222 batches manufactured*

| Lot | Average \|A260-A320\| | Max \|A260-A320\| | Min \|A260-A320\| | UV relative homogeneity \|A260-A320\| | Alert limit on UV relative homogeneity \|A260-A320\| | Action limit on UV relative homogeneity \|A260-A320\| |
|---|---|---|---|---|---|---|
| ABV0519 | 0,187115 | 0,191525 | 0,178075 | 0,071881 | ≤ 0,3 | ≤ 0,4 |
| ABV1267 | 0,182080 | 0,194260 | 0,167905 | 0,144744 | | |
| ABV1953 | 0,168456 | 0,183195 | 0,150460 | 0,194324 | | |
| ABV3922 | 0,112687 | 0,17118 | 0,000128 | 1,517939 | | |
| ABV3923 | 0,168328 | 0,174205 | 0,161090 | 0,077914 | | |
| ABV3975 | 0,207526 | 0,214790 | 0,200795 | 0,067437 | | |
| ABV3976 | 0,214320 | 0,221935 | 0,206235 | 0,073255 | | |
| ABV4282 | 0,212946 | 0,210975 | 0,208420 | 0,040175 | | |
| ABV5062 | 0,198477 | 0,212340 | 0,180175 | 0,162059 | | |
| ABV5094 | 0,230513 | 0,245445 | 0,213935 | 0,136695 | | |
| ABV5125 | 0,205808 | 0,209540 | 0,194930 | 0,070988 | | |
| ABV5297 | 0,223536 | 0,263930 | 0,179770 | 0,376494 | | |
| ABV5652 | 0,231619 | 0,250455 | 0,216095 | 0,148476 | | |
| ABV6096 | 0,210303 | 0,216510 | 0,202265 | 0,067736 | | |
| ABV6514 | 0,215012 | 0,230030 | 0,200055 | 0,139411 | | |
| ABV6522 | 0,255138 | 0,263810 | 0,243955 | 0,077821 | | |
| ABV7279 | 0,226957 | 0,238570 | 0,218675 | 0,087660 | | |
| ABV7764 | 0,225556 | 0,256800 | 0,168710 | 0,390546 | | |

*3. AEX results of batches ABV5297 and ABV7764*

| | STEP | Single Inj (Vp/mL $10^{11}$) | Average (Vp/mL $10^{11}$) | Specification limit (Vp/mL $10^{11}$) |
|---|---|---|---|---|
| ABV5297 | Begin 1 | 0,72 | 0,71 | 0,7 – 1,3 |
| | Begin 1 | 0,70 | | |
| | Begin 2 | 0,69 | 0,69 | |
| | Begin2 | 0,69 | | |
| | Middle1 | 0,91 | 0,89 | |
| | Middle1 | 0,88 | | |
| | Middle2 | 0,82 | 0,82 | |
| | Middle2 | 0,82 | | |
| | End1 | 0,97 | 0,97 | |
| | End1 | 0,96 | | |
| | End2 | 0,89 | 0,89 | |
| | End2 | 0,89 | | |
| ABV7764 | Begin 1 | 0,70 | 0,70 | |
| | Begin 1 | 0,70 | | |
| | Begin 2 | 0,71 | 0,70 | |
| | Begin2 | 0,70 | | |
| | Middle1 | 0,96 | 0,96 | |
| | Middle1 | 0,96 | | |
| | Middle2 | 0,96 | 0,96 | |
| | Middle2 | 0,96 | | |
| | End1 | 0,93 | 0,93 | |
| | End1 | 0,93 | | |
| | End2 | 0,93 | 0,93 | |
| | End2 | 0,93 | | |

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4. *PS80 concentration result of batch ABV3922*

| Lot | Batch portion | PS80 (%w/v) | | Average per vial (%w/v) | Specification limit (%w/v) |
|---|---|---|---|---|---|
| ABV3922 | Begin | PREP 1 | 0,11 | 0,11 | 0,05 – 0,15 |
| | | PREP 2 | 0,11 | | |
| | Middle | PREP 1 | 0,10 | 0,10 | |
| | | PREP 2 | 0,10 | | |
| | End | PREP 1 | 0,04 | 0,04 | |
| | | PREP 2 | 0,04 | | |
| | End | PREP 1 | 0,04 | 0,04 | |
| | | PREP 2 | 0,04 | | |

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5. *AZD1222 commercial batches data*

| SUPPLIER BATCH | MAN/FILL BATCHES | KG DS 1 | Assay 1 | KG DS 2 | Assay 2 | THEORICAL QUANTITY | FLUSH bag KG MBR S373 point 60 S373 | FLUSH bag KG MBR S373 point 97 S373 | FLUSH bag KG MBR S373 point 113 S373 | FLUSH bag KG MBR S373 point 117 S373 | Initial rejected vials for set up (line flushing and load cells calibration) | THEORICAL QUANTITY (TANK 2) | QUANTITY (TANK 3) | YIELD MANUFATURING | THEORICAL VIALS FILLING | FILLED VIALS | Rejected vs TANK3 | YELD FILLING |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 102152 | ABV3922 | | | 48,9 | 20,0 | 146363 | 1,3 | 1,3 | 1,3 | 1,4 | 192 | 145735 | 145196 | 99,6% | 145196 | 143264 | 1932 | 98,7% |
| 102160 | ABV3923 | | | 47,8 | 15,0 | 107303 | 1,3 | 1,3 | 1,3 | 1,3 | 192 | 114427 | 113798 | 99,5% | 113798 | 111852 | 1946 | 98,3% |
| 102326 | ABV3975 | | | 48,8 | 15,0 | 109548 | 1,3 | 1,3 | 1,3 | 1,3 | 115 | 110237 | 109697 | 99,5% | 109697 | 107643 | 2054 | 98,1% |
| 0142000111 | ABV3976 | | | 61,8 | 10,5 | 97112 | 1,3 | 1,3 | 1,4 | 1,5 | 156 | 111733 | 111014 | 99,4% | 111014 | 108705 | 2309 | 97,9% |
| 102158 | ABV4282 | | | 49,1 | 14,0 | 102873 | 1,3 | 1,2 | 1,3 | 1,3 | 665 | 93027 | 92158 | 99,1% | 92158 | 90274 | 1884 | 98,0% |
| 102333 | ABV5062 | | | 51,2 | 18,0 | 137923 | 1,4 | 1,3 | 1,4 | 1,6 | 155 | 124513 | 123914 | 99,5% | 123914 | 120552 | 3362 | 97,3% |
| 0142000112 | ABV5094 | | | 63,6 | 12,0 | 114217 | 1,3 | 1,3 | 1,3 | 1,3 | 155 | 123257 | 122837 | 99,7% | 122837 | 120792 | 2045 | 98,3% |
| 102338 | ABV5125 | | | 52,9 | 12,0 | 95001 | 1,3 | 1,4 | 1,3 | 1,3 | 115 | 91829 | 91559 | 99,7% | 91559 | 89208 | 2351 | 97,4% |
| 102343 | ABV5297 | | | 51,0 | 13,0 | 99222 | 1,3 | 1,3 | 1,3 | 1,3 | 196 | 101137 | 100748 | 99,6% | 100748 | 98637 | 2111 | 97,9% |
| 142000114 | ABV5652 | | | 62,0 | 13,2 | 122478 | 1,4 | 1,3 | 1,3 | 1,3 | 765 | 98713 | 98174 | 99,5% | 98174 | 92789 | 5385 | 94,5% |
| 102329 | ABV6096 | | | 51,5 | 15,0 | 115609 | 1,5 | 1,3 | 1,3 | 1,2 | 201 | 104579 | 103861 | 99,3% | 103861 | 102148 | 1713 | 98,4% |
| CTMA0184 | ABV6522 | | | 65,4 | 13,0 | 127237 | 1,9 | 1,5 | 1,3 | 1,5 | 116 | 136067 | 135168 | 99,3% | 135168 | 134058 | 1110 | 99,2% |

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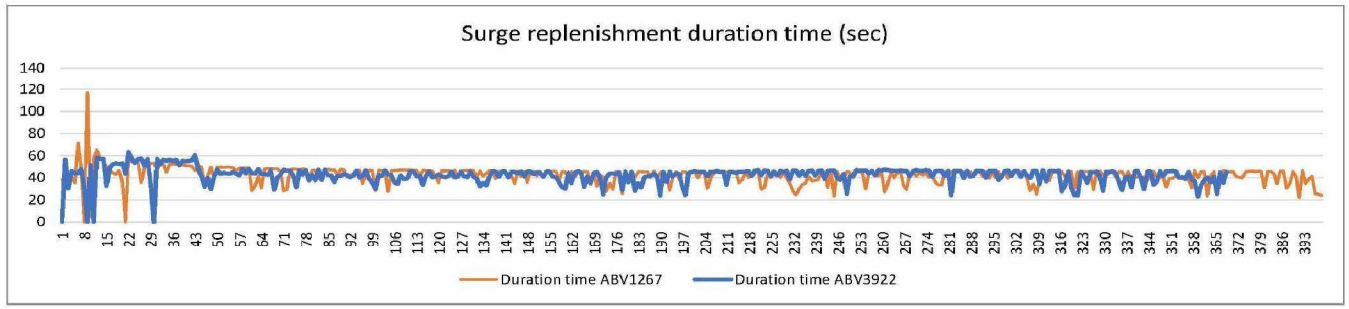
***6. Surge bag replenishment duration ABV3922 VS ABV1267***



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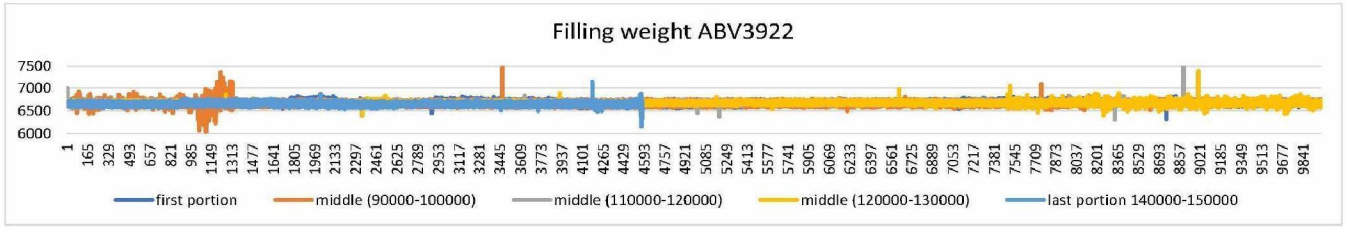
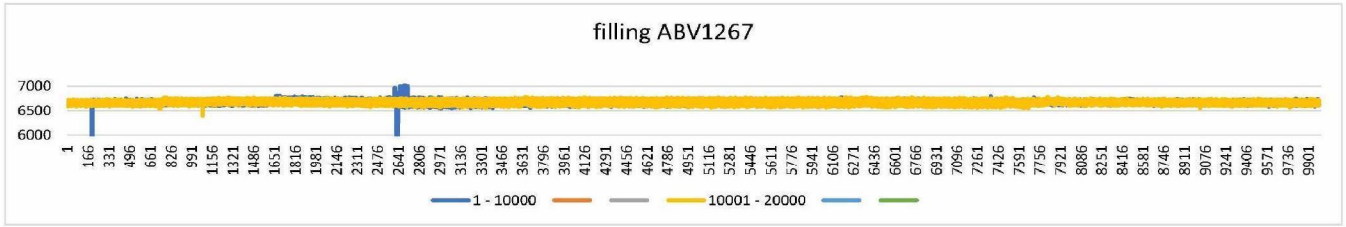
7. ***Filling weight data for ABV3922 and ABV1267***



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8. ***Temperature data of batch ABV3922 VS PPQ batches***

| | | Temperature (°C) | | | |
|---|---|---|---|---|---|
| **Step** | **Activity** | ABV3922 | ABV0519 | ABV1267 | ABV1953 |
| Tank 2 assembly and 0,45 BRR filtration | Temperature verification chiller tank 2 | 4 | 0 | 0 | 4 |
| Temperature buffer verification before transfer | Temperature buffer verification before transfer | 5 | 2 | 2 | 3 |
| DP temperature verification on tank 2 | DP temperature verification on tank 2 | 2 | 3 | 4 | 4 |
| Chiller temperature tank 3 verification | chiller temperature tank 3 | 5 | 0 | 0 | 0 |
| Chiller temperature tank 2 verification | chiller temperature tank 2 | 3 | 4 | 4 | 2 |
| Chiller temperature tank 3 verification | chiller temperature tank 3 | 5 | 5 | 5 | 4 |

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| | | |
|---|---|---|
| CONFIDENTIAL | **DATE ISSUED: 15/02/2021** | |
| DEPARTMENT:<br>Manufacturing Technology Anagni –Italy | PRODUCT<br>AZD1222 | REPORT NUMBER:<br>MT-A/SP/SL/21/003 |
| **TITLE:** | **BUFFER TEMPERATURE EXCURSION SIMULATION STUDY REPORT** | |
| AUTHOR(S): | 5.1.2e | |

SUMMARY:

The objective of this document is to describe the simulation performed to observe how a significative temperature variation of the chiller system could have effects on the physical properties of the AZD1222 buffer solution held in a holding tank.

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**FOR INTERNAL APPROVAL**

5.1.2e

5.1.2e

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## 1. PURPOSE

The purpose of this document is to provide a description of the executed simulation study on the AZD1222 buffer solution to observe how a significative temperature excursion of the chiller system could determine a change in the buffer properties.

Ref to deviation TW# 307662

**Homogeneity issue (infectivity not conform) lot: ABV3922 product AZD1222 SAP code 1425240**

The trial was carried out on a residual amount of buffer solution from a commercial batch, buffer that had to be discarded at the end of the activity. The study involved just a visual inspection of the buffer at different times with the chiller set at T=-6°C. An additional PH reading was performed with the Mettler Toledo PH meter in the production dept, was performed on the freezed portion of the buffer that resulted from the trial.

## 2. STUDY DESCRIPTION AND SAMPLING PLAN

The study was focused on the chiller and tank temperatures to simulate extreme conditions that might have an impact on the DP physical properties.

The work instructions provided a list of all the activities that had to be performed by production operators, together with the rationale for the selection of the test conditions.

Below the results of the activities executed and related pictures.

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### 3. ACTIVITIES DESCRIPTION AND OUTPUT

| STEP | EXECTUED ACTIVITIES | OUTPUT DATA/VISUAL INSPECTION RESULTS | IMAGE | |
|---|---|---|---|---|
| 1. | Record buffer residual volume from previous commercial batch | 547 kg | | Assuming the lot is good till 564 tray (220 vials per tray, approximately 807 L), the problem occurred between 944 L and 807 L. Almost 140 L impacted. |
| 2. | Set the chiller at T set | At 06:30<br>The Chiller was set at T set = - 6 at. | | |
| 3. | Record the tank temperature at the bottom probe | At 06:30 the tank bottom temperature<br>T tank=4,2°C | | |
| 4. | DO NOT OPEN THE TANK VESSEL DOORS DURING THE TRIAL | | | Tank 3 bag is directly filled and the doors are never opened during production once DP is inside |
| 5. | DO NOT MIX THE SOLUTION IN THE TANK DURING THE TRIAL | | | Tank 3 was kept on hold for 7 hours and 33 minutes without agitation |
| 6. | Record the time when T int of the chiller reaches -6°C | T int = -6<br>time: 9:00 potency 100% | | |
| 7. | Record the tank temperature at the bottom probe | At 09:00 the tank bottom temperature<br>T tank=2,3°C | | Tank 3 remained at 2-8°C at the end of DP transfer and for all filling duration |



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| STEP | EXECTUED ACTIVITIES | OUTPUT DATA/VISUAL INSPECTION RESULTS | IMAGE | RATIONALE FOR THE TEST CONDITIONS (AS PER TW#307662) |
|---|---|---|---|---|
| 8. | Inspect chiller outlet and inlet tubing for frost or ice or condensation presence at Tint -6°C | The chiller tubing had few ice drops at the chiller inlet. |  Ref# picture 1 | Frost was observed at some point on chiller inlet and outlet tubings |
| 9. | Observe through the tank window if there is ice on the tank walls or floating on the buffer | No ice was observed on the buffer surface, inspection was performed from tank window and from tank gasket. |  Ref# picture 2 | |

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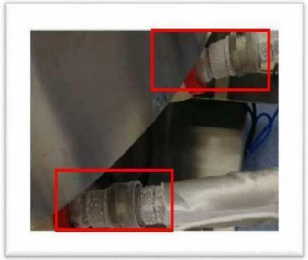
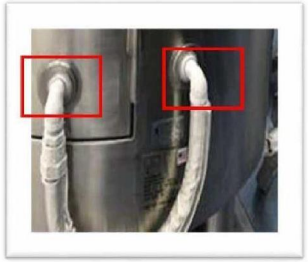
| **APPROXIMATELY AFTER 4 HOURS, AT 12:40, A VISUAL INSPECTION WAS PERFORMED** | | | | |
|---|---|---|---|---|
| **STEP** | **EXECTUED ACTIVITIES** | **OUTPUT DATA/VISUAL INSPECTION RESULTS** | **IMAGE** | **RATIONALE FOR THE TEST CONDITIONS (AS PER TW#307662)** |
| 10. | Inspect chiller outlet and inlet tubing for frost or ice or condensation presence at Tint -6°C | The chiller outlet and inlet were covered with ice frost |   Ref# picture 3-4 | Frost was observed at some point on chiller inlet and outlet tubings |

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| APPROXIMATELY AFTER 4 HOURS, AT 12:40, A VISUAL INSPECTION WAS PERFORMED | | | | |
|---|---|---|---|---|
| **STEP** | **EXECTUED ACTIVITIES** | **OUTPUT DATA/VISUAL INSPECTION RESULTS** | **IMAGE** | **RATIONALE FOR THE TEST CONDITIONS (AS PER TW#307662)** |
| 11. | Observe through the tank window if there is ice on the tank walls or floating on the buffer, do not open the tank doors during the trial | Some water -ice slurry was observed on the buffer surface, inspection was performed from tank window and from tank gasket. |  Ref# picture 5 | |

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| APPROXIMATELY AFTER 4 HOURS, AT 14:35, A VISUAL INSPECTION WAS PERFORMED | | | | |
|---|---|---|---|---|
| **STEP** | **EXECTUED ACTIVITIES** | **OUTPUT DATA/VISUAL INSPECTION RESULTS** | **IMAGE** | **RATIONALE FOR THE TEST CONDITIONS (AS PER TW#307662)** |
| 12. | Observe through the tank window if there is ice on the tank walls or floating on the buffer, do not open the tank doors during the trial | Ice stratified on the bag walls. The ice walls probably were already formed but they were not easily visible until they reached 1 cmm thickness |   Ref# picture 6 | |
| 13. | Record the tank temperature at the bottom probe | The tank temperature did not go under 2°C. The T tank was between 2.1°C and 2.3°C for all trial duration. |   Ref# picture 7 | The temperature at bottom probe was between 2-8°C at the end of DP transfer and for all filling duration. |

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| **APPROXIMATELY AFTER 6 HOURS, AT 14:35, A VISUAL INSPECTION WAS PERFORMED** | | | | |
|---|---|---|---|---|
| **STEP** | **EXECTUED ACTIVITIES** | **OUTPUT DATA/VISUAL INSPECTION RESULTS** | **IMAGE** | **RATIONALE FOR THE TEST CONDITIONS (AS PER TW#307662)** |
| 14. | Disconnect the bag from the tank hooks | The bag was disconnected, and the detachment of ice was observed from the bag walls. The ice block floated on the top of the solution. The height of the block was around 1- 2 cm |  Ref# picture 8 | Tank 3 was filled directly with fluid so emptying is guided by bag folding on itself. |
| 15. | In order to have a further information of what was the composition of the ice block formed, a block of ice was taken and let thaw in a plastic beaker. When the solution thawed, only for informative purposes the PH meter in production, Mettler Toledo Seven pH mV meter, was used to read the value. **The pH was 6,9.** Buffer solution has a target pH 6.6. The buffer higher limit is 6,8. | | | |

Note 1: Executed test sheet will be attached to the current document

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## 4. TECHNICAL CONSIDERATION

The mobius tank full volume capacity is approximately 1100 liters.

A product DP volume of 944 liters would cover approximately 0.95m height in the tank.

Tab.1: Ice volume consideration

| Mobius Bag base diameter<br>Ref ( drawing PLPMIX1000L1121) | Bag lateral surface area in contact with DP<br>Considering 1mt height | ice volume thickness 20 mm<br>water volume | indicative volume of DP frozen if the ice thickness was around 20 mm thick |
|---|---|---|---|
| 1104,9 mm | 3579876 sq mm | 65,72 L | **60-70 L** |

Given an ice block of 0,02 m, this could create at maximum a total mass of 60-70 liters of solution that could detach from the wall at the end of filling and be transferred to the vials. The bag unfolding during filling could help ice detachment from wall and thawing from ice to liquid.

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Diagram 1: Tank overview side, top and bottom view

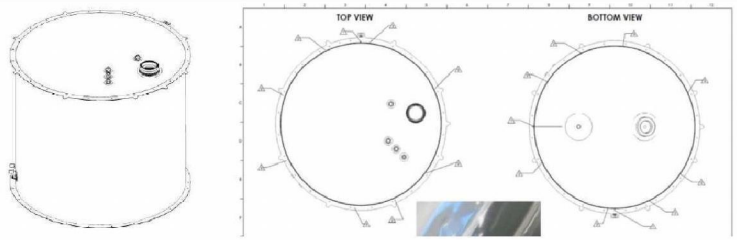
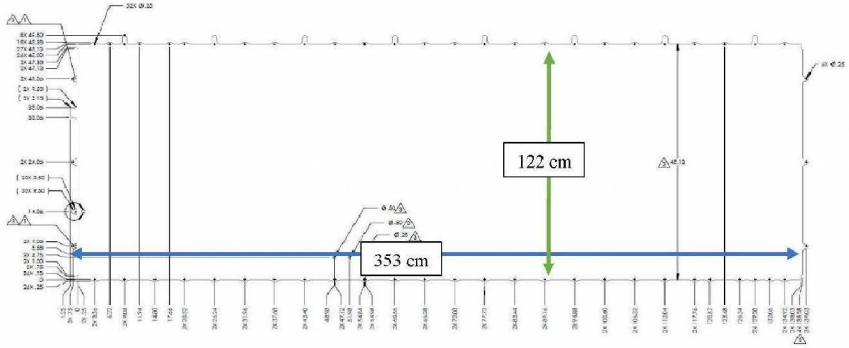



Diagram 2: Tank side panel



## 5. CONCLUSIONS

Based on the trial performed it is possible to conclude that:

- Chiller temperature deviation from the 0°C set point could create DP ice formation on the tank bag walls
- Even if there is ice formation on the tank bag walls the bottom probe of the tank could not be affected by the local DP temperature decrease and freezing at the tank wall, the solution at the center could still be between 2-8°C
- The ice walls could detach from the bag when this last collapse, possibly during the transfer. The ice block remains on the surface of the liquid.

Given all this consideration, strongly supported by the trial performed, it is possible that something similar occurred during the lot ABV3922. The change of physical state from liquid to ice could have impacted the physical properties of the solution, confirmed also by the pH higher value observed (pH 6,9 vs a buffer higher limit 6,8).

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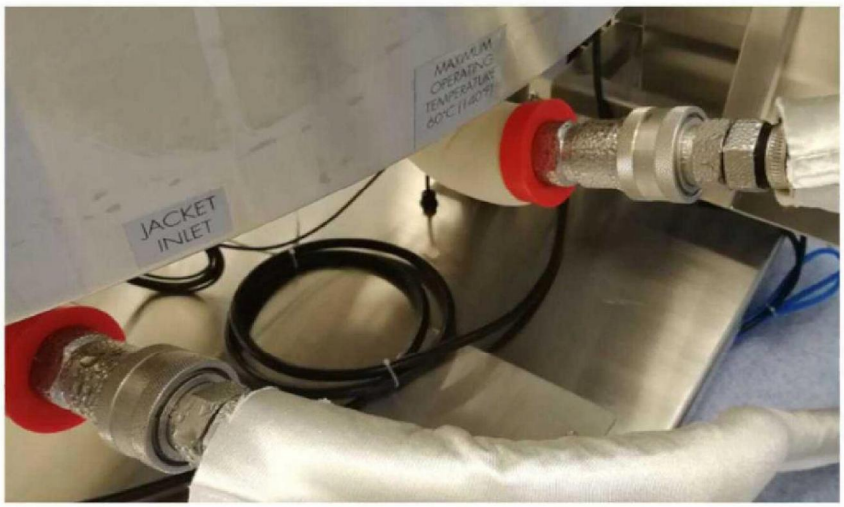
Attachment 1: trial pictures.




Picture 1

Picture 2



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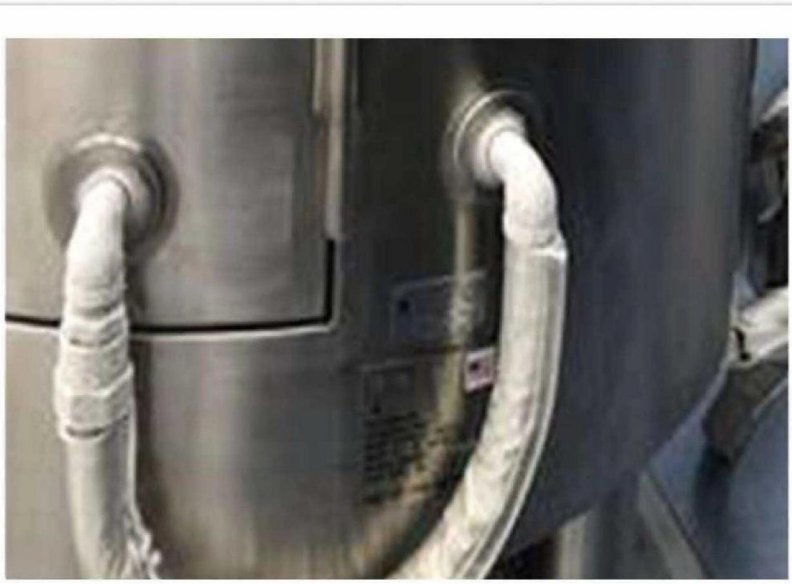
Picture 3



Picture 4

Picture 5


Picture 6


Picture 7

Picture 8