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## Freezing Rate Controlled Validation

### Introduction:

It is well understood that freeze/thaw processes affect the product quality of biopharmaceuticals.<sup>1-3</sup> It was reported that there is no consistent published literature of controlled freezing and thawing rates for biological formulations.<sup>4</sup> Traditionally, freezing storage chambers were used to provide an energy state for the environment surrounding the product with very little excess capacity to change the state of the product.

This study shows how controlled rate freezing can be achieved. It also includes the effect of load and container's position on freeze rates. The freeze/thaw controlled rate chamber used in this study was a Model 4002 manufactured by Farrar Scientific. It permits rapid, uniform bulk freezing and/or thawing of products. It is also equipped with programmable profiles, which allow flexibility to meet unique product conditioning.

### Experimental Procedure:

In this study, the biological formulation was sensitive to freezing rate and rate of cooling below the 1<sup>st</sup> phase transition to -35°C. The client needed to reduce the product temperatures from a range of 5°C - 25°C to -35°C between 11 hours (660 minutes) and 24 hours (1440 minutes), plus the cooling rate below the 1<sup>st</sup> phase transition (0°C) to -35°C needed to be ≤ 0.9°C/min, with 0.3°C/min to 0.9°C/min more desirable.

Tap water was used to simulate the water-based biological formulation. Bottles (10 l capacity) were used in this study. The standard 4 bottle arrangement is shown in Figure 1. Bottle positions with respect to number labels, are shown in Figure 2. Each bottle contained 7.7 l of water. Thermocouples were placed at the geometric center of the bottle. They were attached to a metal rod that was routed through the center of the closure.

In order to meet the freezing requirements, the controlled rate chamber was set up with the following program.

- Step 1. Timed Event to -20°C in 10 seconds
- Step 2. Wait for process
- Step 3. Soak for 9 hours
- Step 4. Timed Event to -60°C in 10 seconds
- Step 5. Wait for process
- Step 6. Soak for 11 hours (2<sup>nd</sup> Plateau Temp.)
- Step 7. Timed Event to -75°C in 10 seconds
- Step 8. Wait for process
- Step 9. Soak for 4 hours
- Step 10. Wait for event

Figure 1. Standard Four Bottle Positions in Chamber.

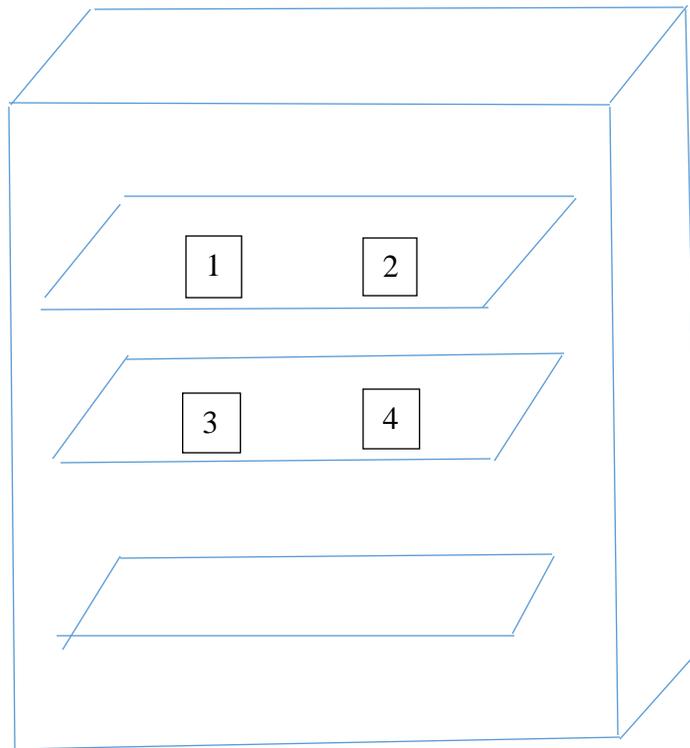


### Results and Discussion:

Controlled Freeze Test 1 results are shown in Figure 3. Notice that bottles 3 and 1 break from the phase change temperature ( $0^{\circ}\text{C}$ ) before bottles 2 and 4. Air flow in the chamber enters from the left air duct and passes out through the right air duct. Air flow in contact with bottles 2 and 4 is partially shielded. Table 1 shows the cooling rate at different temperature ranges. All four bottles had cooling rates that were over the allowable limit from  $-5^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$ . Therefore, Test 2 was conducted where the 2<sup>nd</sup> plateau temperature was changed from  $-60^{\circ}\text{C}$  to  $-45^{\circ}\text{C}$ .

Figure 4 shows the Test 2 freeze rates. Compared to Test 1, Test 2 results show increased time for all bottles to break from the phase change temperature. In addition, Test 2 has a greater range for all bottles to break from the phase change. Table 2 displays the cooling rate at different temperature ranges.

**Figure 2. Numbering of 4 Bottle Controlled Freeze Tests.**



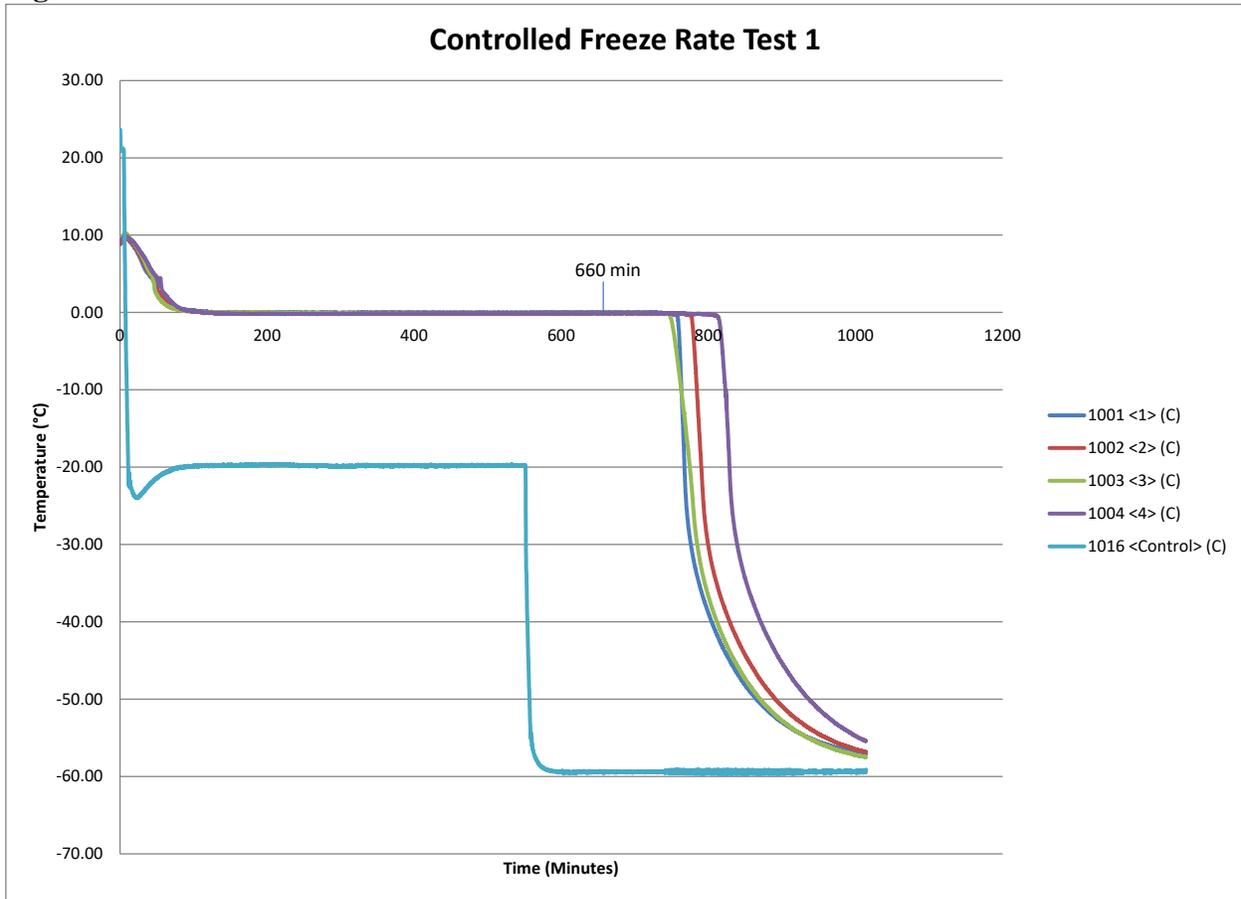
All bottles have cooling rates within the allowable cooling rate ( $0-0.9^{\circ}\text{C}/\text{min}$ ) except bottle 4 in the temperature range of  $-10^{\circ}\text{C}$  to  $-20^{\circ}\text{C}$ . Thus, Test 3 was conducted where the 2<sup>nd</sup> plateau temperature was changed from  $-45^{\circ}\text{C}$  to  $-40^{\circ}\text{C}$ .

The results from Test 3 are shown in Figure 5. In this case, Test 3 results showed that the break from phase change time of bottle 1 did not change in Test 3 compared to Test 2. However, all other bottles in Test 3 had had increased times where the phase change started to break. Additionally, the Test 3 has a greater range for all bottles to break from the phase change. Table 3 displays the cooling rate at different temperature ranges. The cooling rate of all bottles fall within the allowable limit throughout the entire range from  $-5^{\circ}\text{C}/\text{min}$  to  $-35^{\circ}\text{C}/\text{min}$ . However, the cooling rate was undesirably slow from  $-20^{\circ}\text{C}$  to  $-35^{\circ}\text{C}$ .

In order to determine the effect of the minimum product load on cooling rate, Test 4 with one bottle, was conducted. The bottle was positioned in the chamber as shown in Figure 6. The 2<sup>nd</sup> plateau temperature was set to  $-45^{\circ}\text{C}$ . Figure 7 and Table 4 shows the Test 4 freeze rates. The cooling rate was in the allowable range throughout the entire temperature range.

The single bottle had a freezing profile very similar to bottle 1 from the 4-bottle freeze study. This shows that the freeze chamber has excess energy capacity to provide similar freeze profiles regardless of the load size. Test 5 was conducted to further test the effect of load on product freezing rates. The 2<sup>nd</sup> plateau temperature was set to  $-45^{\circ}\text{C}$ . Arrangement and numbering of the 12 bottles for Tests 5 and 6 is shown in Figure 8. Figure 9 and Tables 5 and 6 display the Test 5 freeze rates. The average cooling rate from  $-5^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  is within the allowable range for all

**Figure 3.**



**Table 1. Test 1 Cooling Rates.**

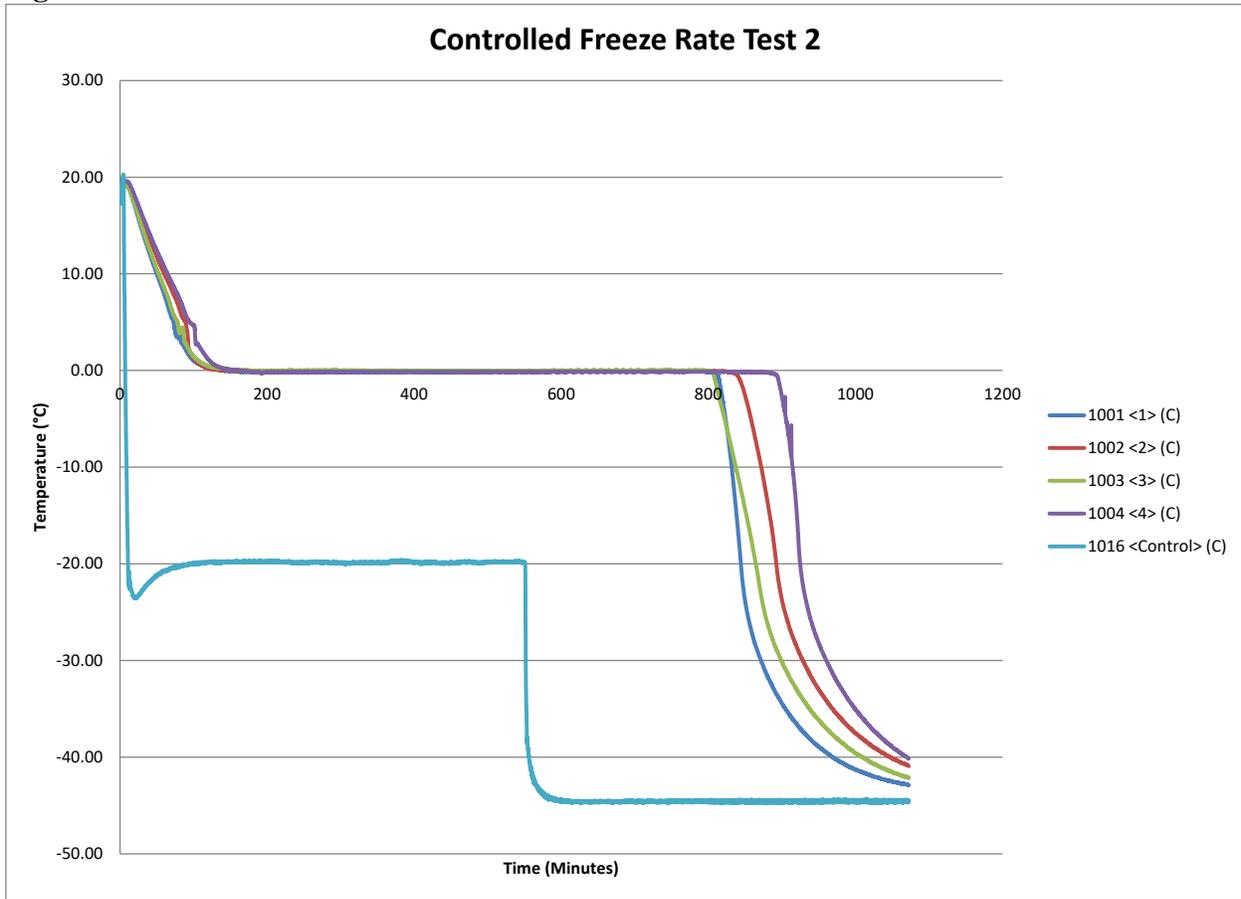
Temp. Range (°C)	Cooling Rate (°C/min)			
	Bottle #			
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
-5 to -10	2.50	1.25	0.71	1.25
-10 to -20	2.50	2.00	0.83	1.67
-20 to -30	1.11	1.00	1.00	1.00
-30 to -35	0.45	0.45	0.50	0.38

bottles. However, bottles 5, 8, and 12 had cooling rates faster than 0.9°C at some point in the temperature range of -5°C to -30°C.

The unusually slow cooling rate for bottle 11 is unexpected. It appears that the thermocouple shifted during freezing, resulting in a premature end to the phase change. In fact, after the bottles were thawed the thermocouple was located in between the bottle wall and the geometric center.

Since the cooling rates for bottles 5, 8, and 12 were higher than the maximum allowed, Test 6 was conducted with a slightly higher 2<sup>nd</sup> plateau temperature (-42.5°C). Figure 10 and Tables 7

**Figure 4.**

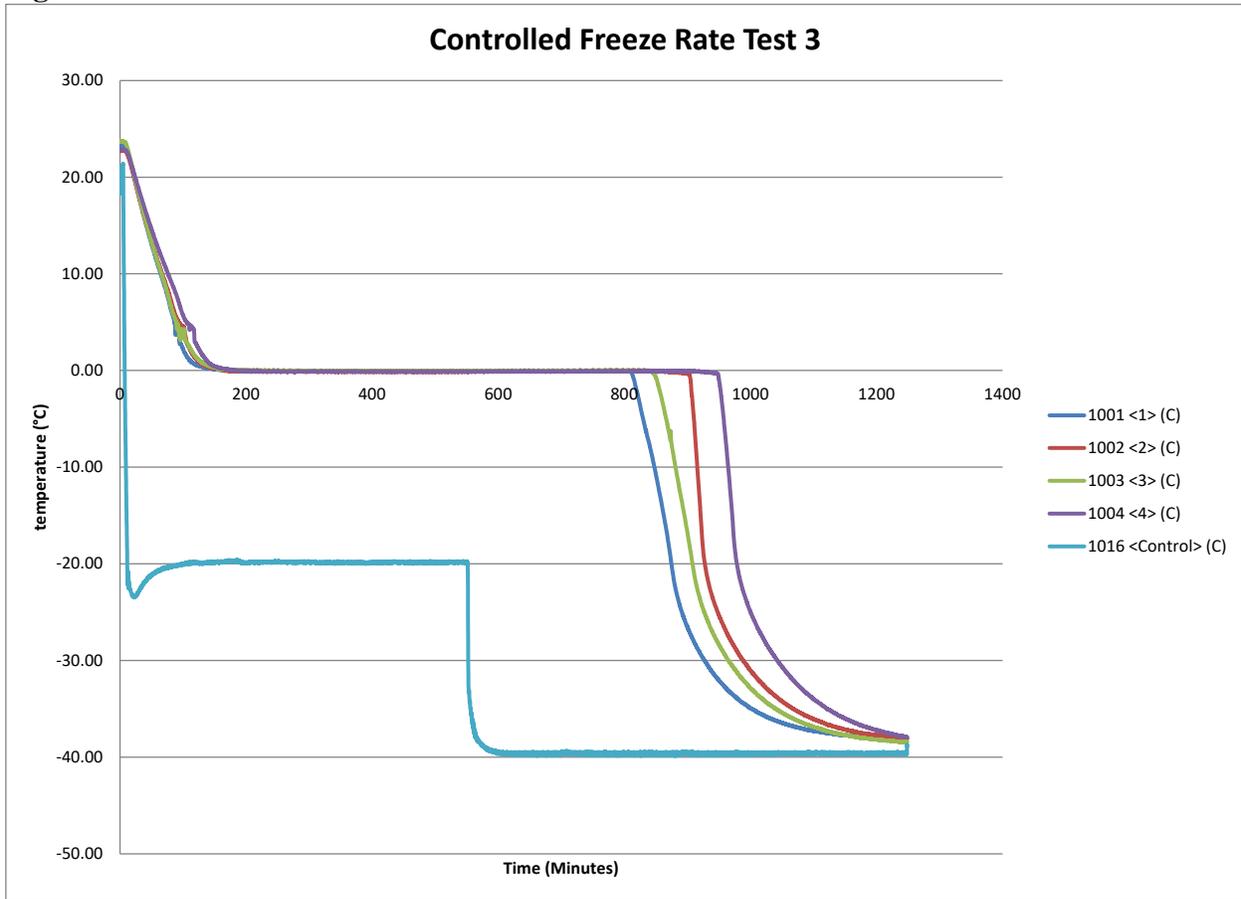


**Table 2. Test 2 Product Cooling Rates.**

Temp. Range (°C)	Cooling Rate (°C/min)			
	Bottle #			
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
-5 to -10	0.56	0.36	0.33	0.50
-10 to -20	0.80	0.50	0.37	0.97
-20 to -30	0.36	0.28	0.29	0.29
-30 to -35	0.15	0.12	0.13	0.13

and 8 display the Test 6 freeze rates. The range of time needed for all bottles to break from the 1<sup>st</sup> phase change was 756 minutes to 979 minutes. The range of time needed for all bottles to reach -35°C was 875 minutes to 1,132 minutes. The one requirement of all bottles reaching -35°C in the range of 660-1,440 minutes was met. Regarding the second requirement of all bottles having a cooling rate below the 1<sup>st</sup> phase transition of  $\leq 0.9^\circ\text{C}/\text{min}$ , only one cooling rate was barely above the  $0.9^\circ\text{C}/\text{min}$  limit (Bottle 3 from -10°C to -20°C) throughout the entire temperature range (-5°C to -35°C). However, the heat capacity of the actual biological to be

**Figure 5.**



**Table 3. Test 3 Product Cooling Rates.**

Temp. Range (°C)	Cooling Rate (°C/min)			
	Bottle #			
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
-5 to -10	0.28	0.91	0.33	0.71
-10 to -20	0.80	0.85	0.37	0.73
-20 to -30	0.19	0.17	0.18	0.15
-30 to -35	0.07	0.06	0.07	0.06

formulation was expected to be higher than ice. Therefore, the biological formulation cooling rate is expected to be  $\leq 0.9^{\circ}\text{C}/\text{min}$  for all bottles from  $-5^{\circ}\text{C}$  to  $-35^{\circ}\text{C}$ .

A small amount of buffer solution, which had similar thermal properties to the biological formulation, was acquired from the client. The buffer crystalline melting point ( $-3.2^{\circ}\text{C}$ ), heat of fusion ( $261 \text{ J/g}$ ), and glass transition temperature ( $-37.2^{\circ}\text{C}$ ) were determined by differential scanning calorimetry. Water has a heat of fusion of  $334 \text{ J/g}$ . The buffer heat of fusion is 78.2% that of water. The lower buffer heat of fusion indicates that the time to get through the phase change is less. However, the lower buffer melt temperature and  $-37.2^{\circ}\text{C}$  glass

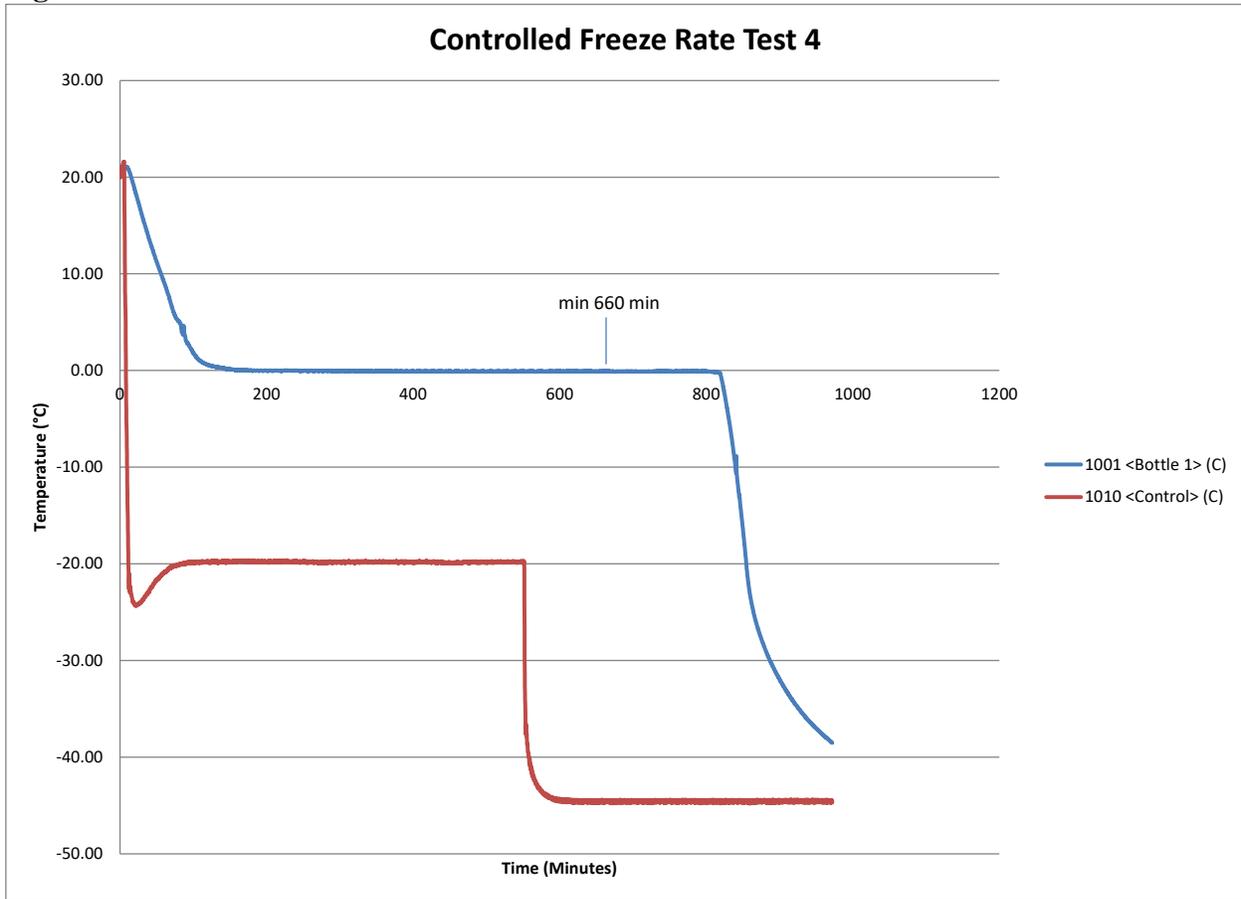
Figure 6. Standard Single Bottle Position in Chamber.



transition temperature should give a slower cooling rate at temperatures less than the melt point. Test 7 was conducted to prove out these conclusions. The 2<sup>nd</sup> plateau temperature was the same as Test 6 (-42.5°C). In addition, the minimum amount of buffer (5 kg) was placed in a single bottle to simulate the smallest load in the freeze chamber. Also to simulate a minimum freeze time, the buffer was cooled to between 5°C to 10°C prior to starting the test.

The cooling rate results from Test 7 are shown in Figure 11 and Table 9. The time needed for the buffer to break from the 1<sup>st</sup> phase change and reach -35°C was 747 minutes and 860 minutes, respectively. The one requirement of product reaching -35°C in the range of 660-1,440 minutes was met. In addition, the other product cooling rate requirement of  $\leq 0.9^{\circ}\text{C}/\text{min}$  below the 1<sup>st</sup> phase transition to -35°C was met as well.

**Figure 7.**

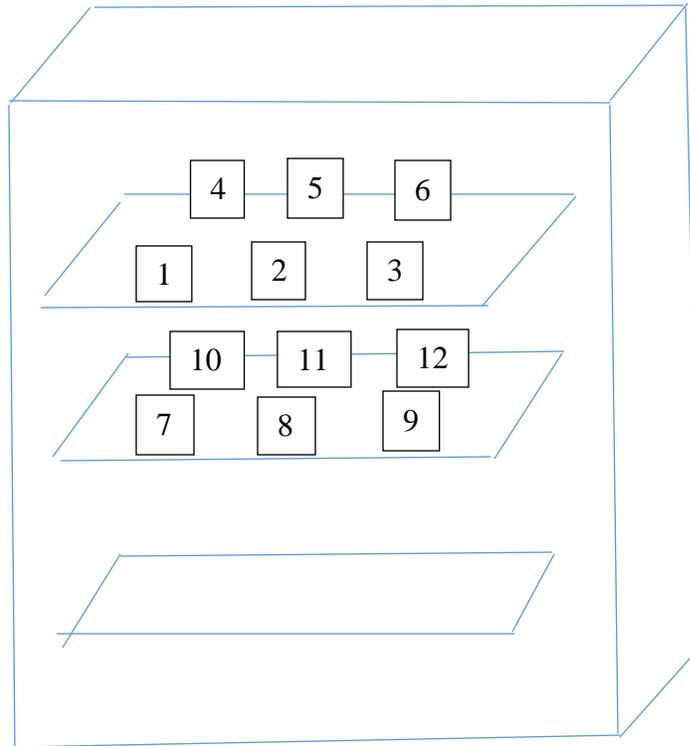


**Table 4. Test 4 Product Cooling Rates.**

	Cooling Rate (°C/min)	
	Bottle #	
Temp. Range (°C)	<u>1</u>	
-5 to -10	0.56	
-10 to -20	0.67	
-20 to -30	0.31	
-30 to -35	0.13	

Results of Test 7 (single bottle of buffer) were compared to Test 4 (single bottle of water) even though the 2<sup>nd</sup> plateau temperature for Test 4 was slightly lower -45°C compared to -42.5°C. As predicted, the time needed for the buffer solution to break from the 1<sup>st</sup> phase change was less (747 minutes) compared 819 minutes for water. Also as expected, the buffer cooling rate was slower throughout the temperature range of -5°C to -35°C even though the buffer load was 35% less.

**Figure 8. Numbering for 12 Bottle Controlled Freeze Tests 5 and 6.**



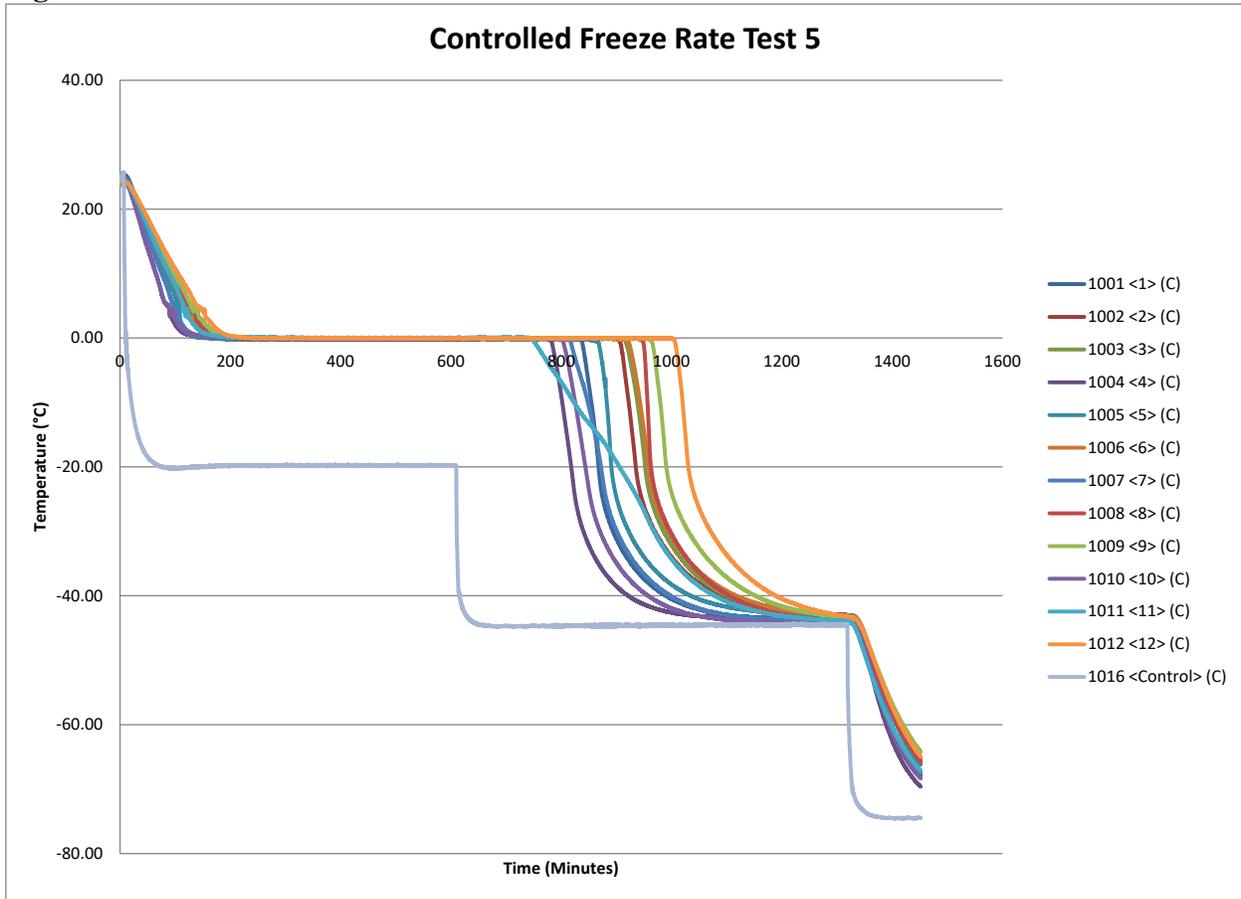
### **Conclusions and Recommendations:**

A method was successfully developed to control the desired freezing rate using a Farrar Scientific Model 4002 Controlled Rate Freeze/Thaw Chamber. It was validated with different loads (1, 4, and 12 bottles) and product positions in the chamber. A program was developed that the client can use to reliably and uniformly bulk freeze their biological formulation.

### **References:**

1. Cao E, et al. Effect of Freezing and Thawing Rates on Denaturation of Proteins in Aqueous Solutions. *Biotechnol. Bioeng.* 82(6) 2003: 684-690.
2. Radmanovic N, et al. Understanding the Freezing of Biopharmaceuticals: First-Principle Modeling of the Process and Evaluation of Its Effect on Product Quality. *Journal of Pharma. Sci.* 102(8) 2013: 2495-2507.
3. Reinsch H, et al. Examining the Freezing Process of an Intermediate Bulk Containing an Industrially Relevant Protein. *Enzyme and Microbial Tech.* 71 (2015): 13-19.
4. Puri M, et al. Evaluating Freeze-Thaw Processes in Biopharmaceutical Development – Small-Scale Study Designs. *BioProcess Int.* 2015: 1-16.

**Figure 9.**



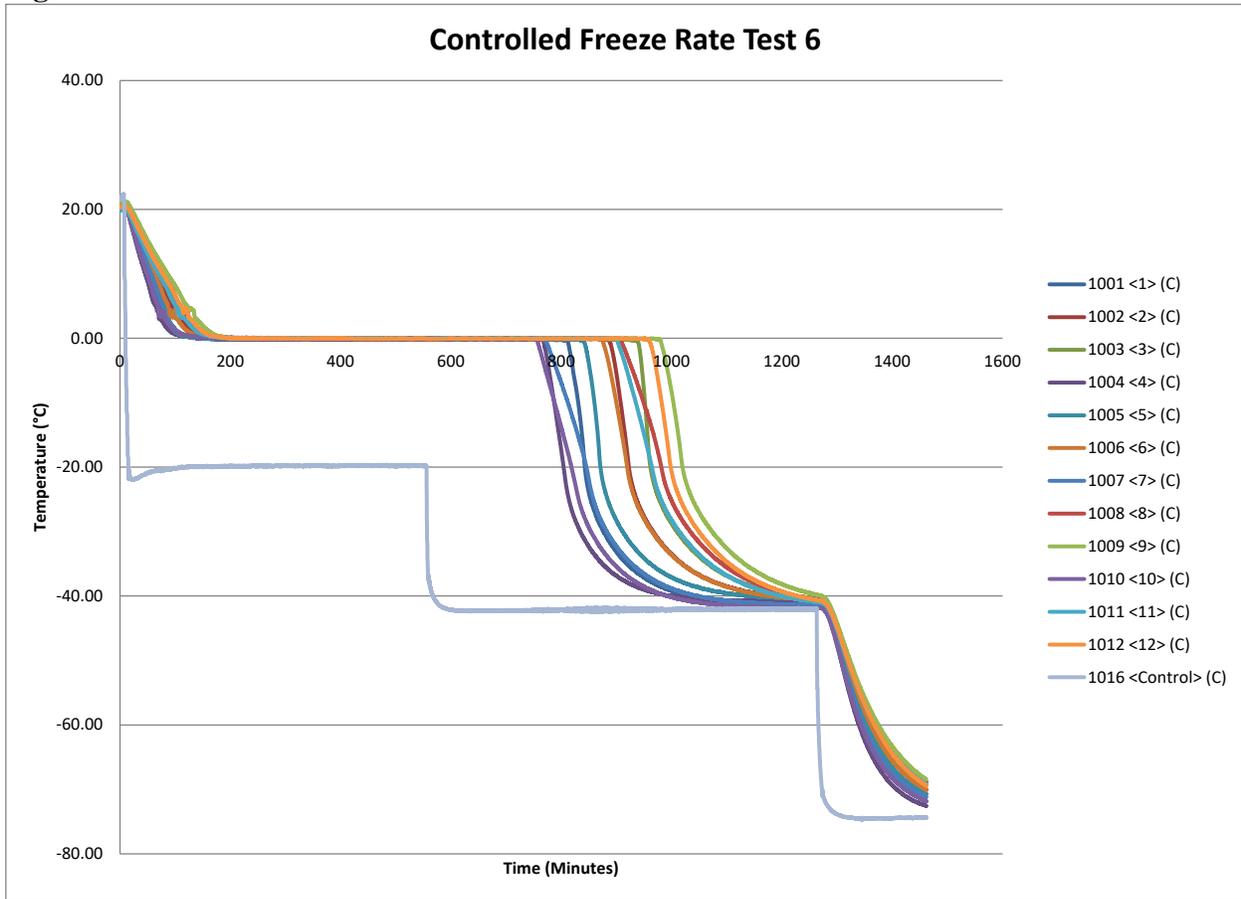
**Table 5. Test 5 Product Cooling Rates for Bottles 1-6.**

Temp. Range (°C)	Cooling Rate (°C/min)					
	Bottle #					
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
-5 to -10	0.56	0.71	0.50	0.56	0.71	0.56
-10 to -20	0.77	0.77	0.71	0.63	1.22	0.67
-20 to -30	0.38	0.29	0.29	0.45	0.32	0.28
-30 to -35	0.15	0.13	0.13	0.19	0.14	0.12

**Table 6. Test 5 Product Cooling Rates for Bottles 7-12.**

Temp. Range (°C)	Cooling Rate (°C/min)					
	Bottle #					
	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>
-5 to -10	0.31	1.58	0.71	0.50	0.13	0.81
-10 to -20	0.42	1.58	0.92	0.53	0.12	0.97
-20 to -30	0.34	0.29	0.25	0.38	0.16	0.24
-30 to -35	0.15	0.13	0.12	0.16	0.13	0.12

**Figure 10.**



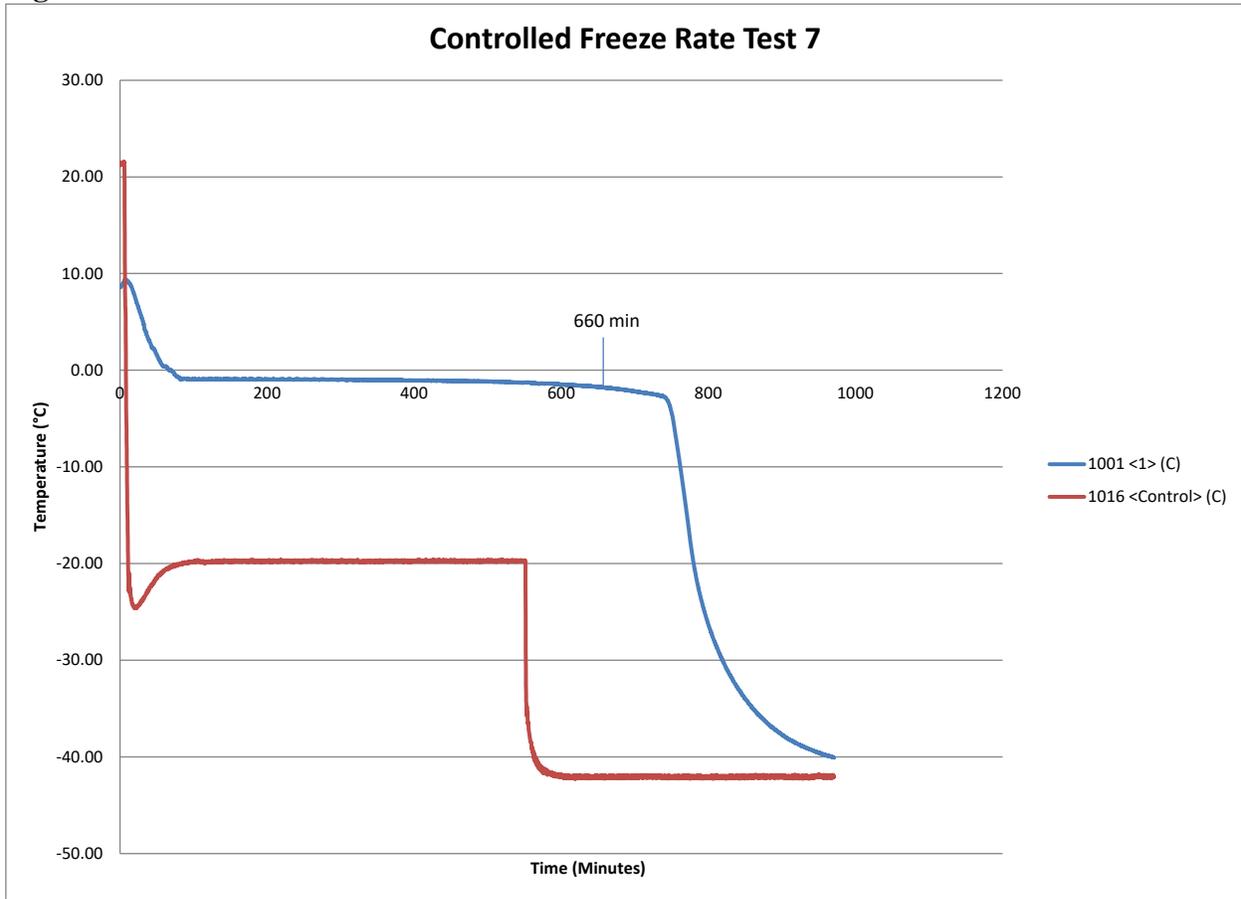
**Table 7. Test 6 Product Cooling Rates for Bottles 1-6.**

Temp. Range (°C)	Cooling Rate (°C/min)					
	Bottle #					
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
-5 to -10	0.50	0.56	0.94	0.56	0.63	0.45
-10 to -20	0.85	0.67	1.00	0.59	0.92	0.53
-20 to -30	0.29	0.23	0.21	0.32	0.25	0.22
-30 to -35	0.12	0.09	0.09	0.13	0.10	0.09

**Table 8. Test 6 Product Cooling Rates for Bottles 7-12.**

Temp. Range (°C)	Cooling Rate (°C/min)					
	Bottle #					
	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>
-5 to -10	0.25	0.25	0.50	0.31	0.31	0.56
-10 to -20	0.29	0.31	0.59	0.33	0.34	0.63
-20 to -30	0.27	0.19	0.19	0.28	0.20	0.20
-30 to -35	0.11	0.09	0.08	0.12	0.09	0.09

**Figure 11.**



**Table 9. Test 7 Product Cooling Rate.**

Temp. Range (°C)	Cooling Rate (°C/min)	
	Bottle #	
	<u>1</u>	
-5 to -10	0.50	
-10 to -20	0.55	
-20 to -30	0.25	
-30 to -35	0.12	

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