Vaccine Carrier Water-Pack Freezing

Results from studies on visual inspection methods to assess freeze status and on water-pack temperature and expansion during freezing

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Contents

Executive summary

This report summarizes experiments to develop a visual inspection method for determining whether water-packs are frozen, partially frozen, or unfrozen when removed from a freezer for use in vaccine carriers. The evaluation involved training water-pack evaluators in visual inspection techniques using water-packs of known temperature history and uses a checklist of tests that water-packs must pass to be classified as frozen. The resulting visual inspection test was shown to accurately classify water-packs into one of the three categories when used by novice water-pack evaluators.

The report also examines water-pack temperature and expansion during freezing. Temperatures recorded by thermocouples placed inside the water-packs are shown to be an unreliable measure of when a water-pack is completely frozen. Cessation of water-pack expansion is a better marker of the completion of the water-pack freezing process.

A significant number of AOV International water-packs developed cracks during the visual inspection and expansion tests, whereas water-packs from other manufacturers did not develop cracks. The cracking is likely the result of the geometry of the upper indentation on the AOV water-pack. A redesign of the AOV water-pack's upper indentation geometry could solve the cracking problem.

1. Introduction

This report presents the results from experiments to develop a visual inspection method for evaluating the degree of freezing of water-packs used in vaccine carriers. We also examine the expansion of water-packs during freezing as a potential indicator of degree of water-pack freezing and the failure of one brand of water-pack over repeated freeze/thaw cycles. We divide the report into three main areas:

- **Section 2** examines the materials and equipment.
- **Section 3** gives our results and discusses interpretations and recommendations.
- **Section 4** concludes the report.

2. Materials and methods

We used the following equipment and supplies from the PATH product development shop during our evaluation:

- Nilkamal Plastics BCVC-46 2.86 L passive vaccine carrier.
- Nilkamal 0.6 L water-packs, compliant with PQS/E005/IP01.
- AOV International 0.6 L water-packs, compliant with PQS/E005/IP01.
- Blowkings 0.6 L water-packs, compliant with PQS/E005/IP01.
- Omega type-T thermocouples, 5SRTC-TT-T-30-72.
- Thermo Scientific Revco-CXF 120V chest freezer.
- Press plates.
- National Instrument Lab View SignalExpress data logging software.

All water-packs were filled with tap water for the experiments. To measure the temperature of the water in the water-packs, type-T thermocouples were installed so that the tip of the thermocouple was located approximately halfway into the water-pack and midway between the indents and the side of the water-pack, as shown in Figure 1. To expose water-packs to similar conditions while freezing, water-packs were arranged as shown in Figure 2.

Figure 1. Approximate location of thermocouple tip in water-pack.

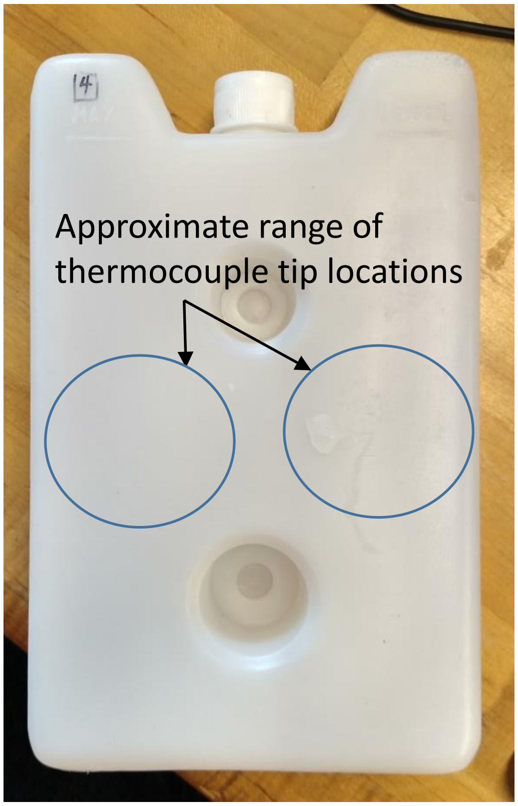
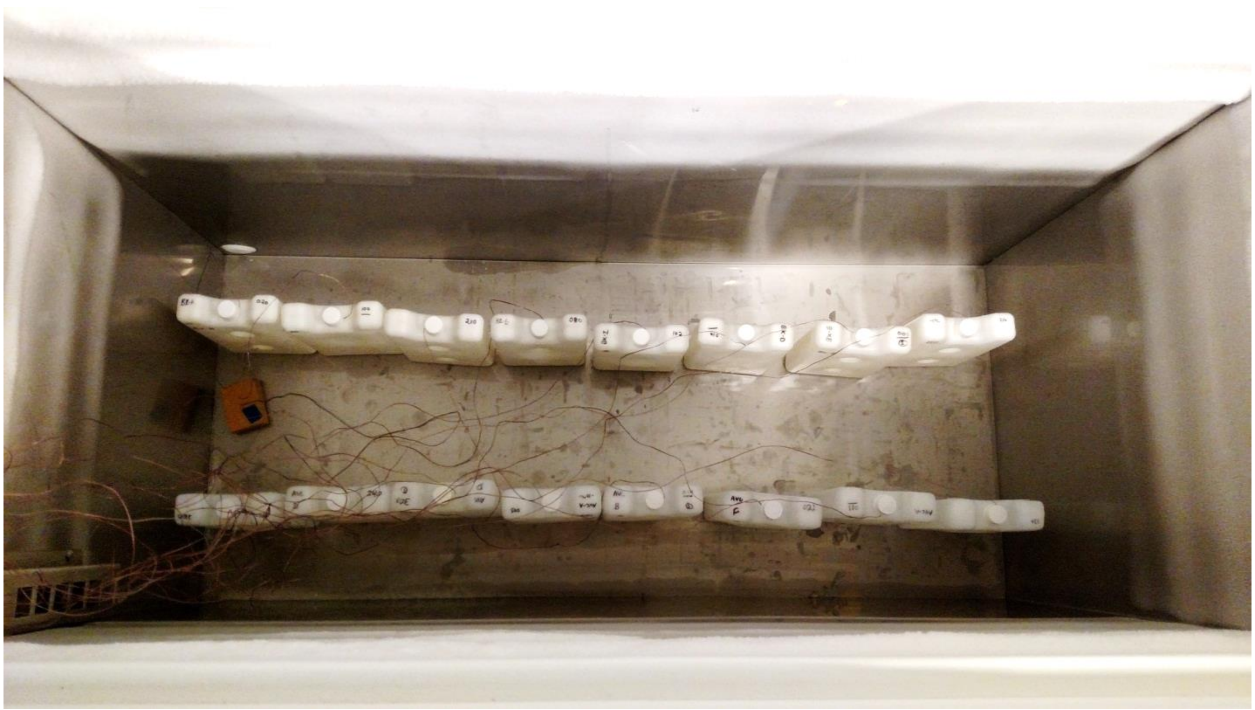


Figure 2. Arrangement of water-packs in freezer during experiments.



To measure expansion of a water-pack during freezing, the water-pack was placed between press plates and the press plates were closed until the plates made contact with the water-packs. The separation of the press plates was measured using digital calipers, as shown in Figure 3.

Figure 3. Measuring water-pack expansion with press plates and digital calipers.

3. Results and discussion

Visual inspection method

Prior studies in PATH's product development shop had evaluators conduct a visual inspection method and attempt to classify the degree of freezing of a water-pack in 10% or 25% increments. The results showed that evaluators could not accurately classify partially frozen water-packs. Furthermore, holdover testing of water-packs conditioned to -25°C and -10°C showed that water-packs must be completely frozen to achieve World Health Organization performance, quality, and safety–specified holdover times in long-range vaccine carriers. To address the requirement that water-packs be fully frozen when used in vaccine carriers and the fact that evaluators could not accurately classify water-packs using specific percent frozen values, we investigated a visual inspection test using simplified categories. The categories were:

1. **Frozen:** Water-pack was 100% ice and 0% liquid water by volume.
2. **Unfrozen:** Water-pack was 0% ice and 100% liquid water by volume.
3. **Partially frozen:** Water-pack contained both ice and liquid water in any ratio.

Evaluators were also trained prior to classifying water-packs by giving them water-packs frozen for 2, 6, or 8 hours at -30°C or conditioned to -25°C, -10°C, or -1°C. Evaluators were informed of the freezing history of each water-pack. We asked the evaluators to examine the practice water-packs and suggested the following tests to help them classify the water-packs:

1. Shake a water-pack and listen for the sound of water sloshing in the water-pack.
2. Tilt the water-pack and look for water (liquid) or bubbles moving in the water-pack. Hold the water-pack up to the light when tilting to help observe water or air movement.
3. Squeeze the water-pack near the edges. A water-pack should flex if only slightly frozen. Completely frozen water-packs will not flex near the edges. The middle of the water-pack will not compress regardless of how frozen the pack is due to the reinforcement of the water-pack.
4. Ice expands when it freezes. Completely frozen water-packs will bulge significantly between the areas of reinforcement and the sides of the water-pack.

The water-packs used to test the visual inspection method were conditioned from an unfrozen state to -25°C, -10°C, -1°C or were frozen at -30°C for 2, 6, or 8 hours. Zero-hour, unfrozen water-packs were cooled to 5°C. Three water-packs at each condition were prepared. The water-packs used were AOV (transparent) and Blowkings (opaque) 0.6 L water-packs. Four volunteer evaluators without prior experience in classifying water-packs classified each water-pack without knowledge of its condition or freezing history. The four evaluators classifying three water-packs at each temperature condition yielded 12 classifications per condition per water-pack type (transparent or opaque). Tables 1 and 2 below show the results of the classification with each percentage of responses being out of 12 classifications. The percent frozen measurements are from results of a different experiment where the pour test[1] was conducted on water-packs with the same temperature histories.

Table 1. Evaluator classification of transparent water-packs by freeze time or stabilization temperature.

Freeze time or stabilization temperature	**Percent frozen**	**Percentage of responses**		
		Unfrozen	**Partially frozen**	**Frozen**
0 hour	0	100	0	0
2 hour	28	0	100	0
6 hour	71	0	50	50
8 hour	91	0	0	100
-1°C	100	0	42	58
-10°C	100	0	0	100
-25°C	100	0	0	100

[1] To measure the percent frozen using the pour test, the mass of the empty water-pack and the mass of the water-pack when filled with water were measured before the start of conditioning. After conditioning the water-pack in the freezer, the liquid water was poured out and the mass of the water-pack with the remaining ice was measured. When the liquid water in the water-pack was surrounded by ice, a drill was used to create holes to the water pocket so that the liquid water could be emptied from the water-pack. The empty water-pack mass was subtracted from the preconditioning filled water-pack mass and the water-pack mass after pouring out the liquid water to obtain the initial mass of water and the final mass of ice respectively. The percent frozen was calculated by dividing the final mass of ice by the initial mass of water.

Table 2. Evaluator classification of opaque water-packs by freeze time or stabilization temperature.

Freeze time or stabilization temperature	**Percent frozen**	**Percentage of responses**		
		Unfrozen	**Partially frozen**	**Frozen**
0 hour	0	100	0	0
2 hour	28	0	100	0
6 hour	71	0	50	50
8 hour	91	0	25	75
-1°C	100	0	33	67
-10°C	100	0	0	100
-25°C	100	0	8	92

The results showed that evaluators could accurately classify 0-hour, 2-hour, -10°C, and -25°C water-packs. Evaluators had significant trouble classifying water-packs that had been frozen for 6 hours or conditioned to -1°C.

The visual inspection method was refined by making the set of suggested tests a required checklist; to classify a water-pack as frozen, the water-pack would have to pass each of the tests suggested above. If a water-pack failed any of the tests, it could not be classified as frozen. A water-pack failing one or more of the tests would be classified as unfrozen or partially frozen based on the presence or absence of ice. The checklist of tests was written as follows:

1. Shake the water-pack. If you hear the sound of water sloshing in the water-pack, the water-pack is not frozen and fails this test.
2. Tilt the water-pack and look for liquid water or bubbles moving in the water-pack. If you can see bubbles moving in the water-pack, the water-pack is not frozen and fails this test.
3. Squeeze the water-pack between the edge and the indentations in the middle of the water-pack. If the water-pack flexes, the water-pack is not frozen and fails this test.
4. Water expands when it freezes. Look for significant localized bulging near the centerline of the water-pack when viewing the water-pack from the side. If no bulging is present, the water-pack is not frozen and fails this test.
5. If an water-pack fails one of the above tests, classify it as partially frozen or unfrozen based on whether ice is present (partially frozen) or not (unfrozen). If a water-pack passes all of the above tests, classify it as frozen.

Repeating the experiment with seven volunteer evaluators using the rigorous checklist method in addition to training yielded the results shown in Tables 3 and 4 below.

Table 3. Evaluator classification of transparent water-packs by freeze time or stabilization temperature using a checklist.

Freeze time or stabilization temperature	Percent frozen	Percentage of responses		
		Unfrozen	Partially frozen	Frozen
0 hour	0	86	14	0
2 hour	28	38	62	0
6 hour	71	0	95	5
8 hour	91	0	19	81
-1°C	100	0	86	14
-10°C	100	0	10	90
-25°C	100	0	14	86

Table 4. Evaluator classification of opaque water-packs by freeze time or stabilization temperature using a checklist.

Freeze time or stabilization temperature	Percent frozen	Percentage of responses		
		Unfrozen	Partially frozen	Frozen
0 hour	0	95	5	0
2 hour	28	10	90	0
6 hour	71	0	100	0
8 hour	91	0	19	81
-1°C	100	0	71	29
-10°C	100	0	19	81
-25°C	100	0	19	81

Having the evaluators use the test checklist and training them significantly improved evaluator classifications at the 6-hour level when compared to just training them. When just training the evaluators, 50% of respondents classified 6-hour water-packs as fully frozen. When using the checklist method, 95% of 6-hour, transparent and 100% of 6-hour, opaque water-packs were accurately classified as partially frozen.

A significant percentage of responses classified water-packs conditioned to -1°C as partially frozen even though the -1°C water-packs were completely frozen when removed from the freezer. We hypothesized that melting of the -1°C water-packs was occurring as the water-packs sat in the insulated carriers waiting to be classified. We repeated the classification test with the modification that the water-packs were removed from the freezers immediately before classification instead of sitting in carriers until they were ready to be used. In this experiment, we used Nilkamal transparent water-packs since the AOV water-packs developed leaks over repeated freeze-thaw cycles. Tables 5 and 6 below give the responses of seven new evaluators.

Table 5. Evaluator classification of transparent water-packs by freeze time or stabilization temperature when removed immediately from freezer.

Freeze time or stabilization temperature	Percent frozen	Percentage of responses		
		Unfrozen	Partially frozen	Frozen
0 hour	0	100	0	0
2 hour	28	48	52	0
6 hour	71	5	95	0
8 hour	91	5	90	5
-1°C	100	0	90	10
-10°C	100	0	29	71
-25°C	100	0	10	90

Table 6. Evaluator classification of opaque water-packs by freeze time or stabilization temperature when removed immediately from freezer.

Freeze time or stabilization temperature	Percent frozen	Percentage of responses		
		Unfrozen	Partially frozen	Frozen
0 hour	0	86	14	0
2 hour	28	33	67	0
6 hour	71	5	95	0
8 hour	91	0	81	19
-1°C	100	0	67	33
-10°C	100	0	10	90
-25°C	100	0	5	95

The evaluators were able to accurately classify water-packs during this round of tests. The 8-hour water-packs in this test, unlike the previous tests, were not completely frozen by the time the test began, so the high percentage of partially frozen classifications was accurate for this round of testing. The reason for the 8-hour water-packs' failure to freeze completely is unclear, as the freezing was carried out in the same manner as for previous tests. Despite modifying the experiment so that the water-packs were removed from the freezer immediately before being given to the evaluators for classification, significant melting of the -1°C water-packs occurred while the water-packs were handled by the evaluators. The melting resulted in a large percentage of the -1°C water-packs being classified as partially frozen even though they were completely frozen when removed from the freezer. Lastly, an analysis of the evaluator classifications showed that the squeeze test was not yielding accurate water-pack classifications; thus the squeeze test was dropped from the test checklist. The visual inspection test was documented in the draft protocol *Visual Inspection Procedure for Classification of Icepacks for Vaccine Carriers* submitted to the World Health Organization in September 2015.

Water-pack expansion

During the freezing tests, we noted that water-packs were expanding significantly. The monitoring of water-pack temperatures with thermocouples during freezing also indicated that the time at which a water-pack's temperature dropped below 0°C varied widely from water-pack to water-pack when freezing multiple water-packs at the same time, suggesting that monitoring the temperature of a water-pack might not be an accurate way to determine when a water-pack is completely frozen. To compare water-pack expansion monitoring versus internal temperature monitoring to determine the completion of water-pack freezing, we froze 16 water-packs simultaneously at -30°C. AOV water-packs were numbered 1 to 8, and Blowkings water-packs were numbered 11 to 18. All water-packs had type-T thermocouples installed. During freezing, we removed the water-packs from the freezer every hour to measure their thickness.

The time at which water-pack temperatures dropped below 0°C varied considerably as shown in Figure 4. The earliest water-pack started to drop below 0°C at approximately 4 hours into the test. The latest water-pack to begin dropping below 0°C did so approximately 9 hours into the test. Previous experience with measuring the mass percent frozen of a water-pack using the pour test over time showed that water-packs took approximately 8 hours to freeze at -30°C. This suggested that water-packs whose temperature dropped below 0°C only 4 to 6 hours into freezing were unlikely to be fully frozen.

Figure 4. Water-pack and freezer temperature versus time during expansion experiment.

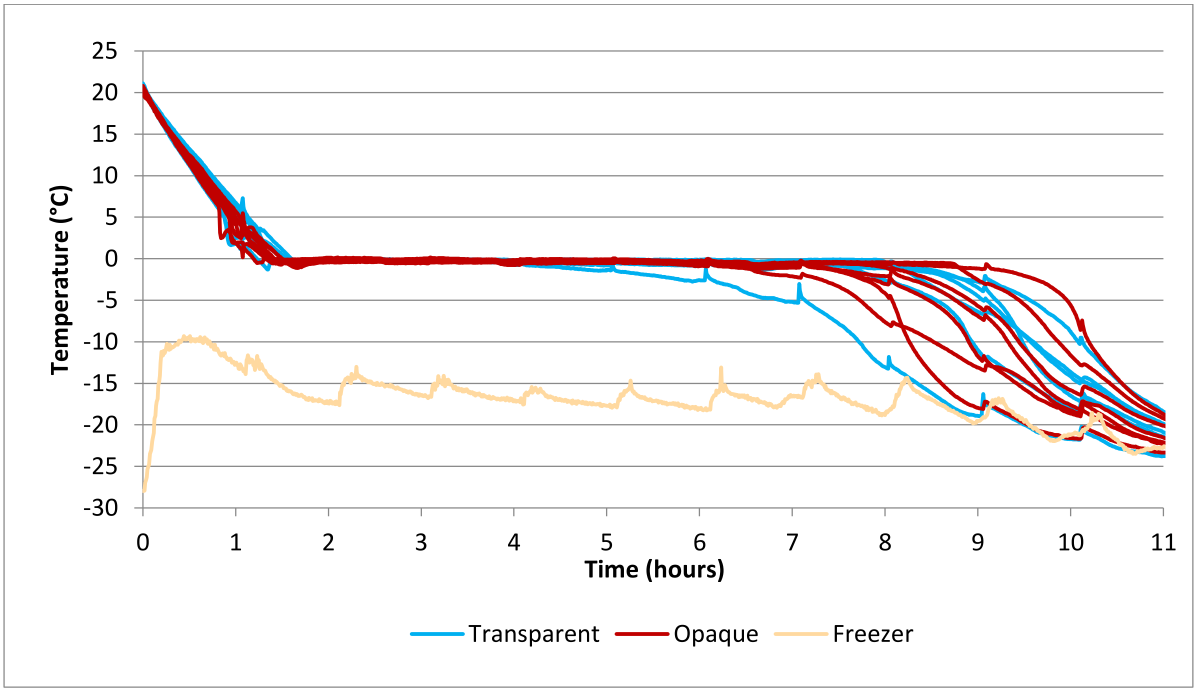
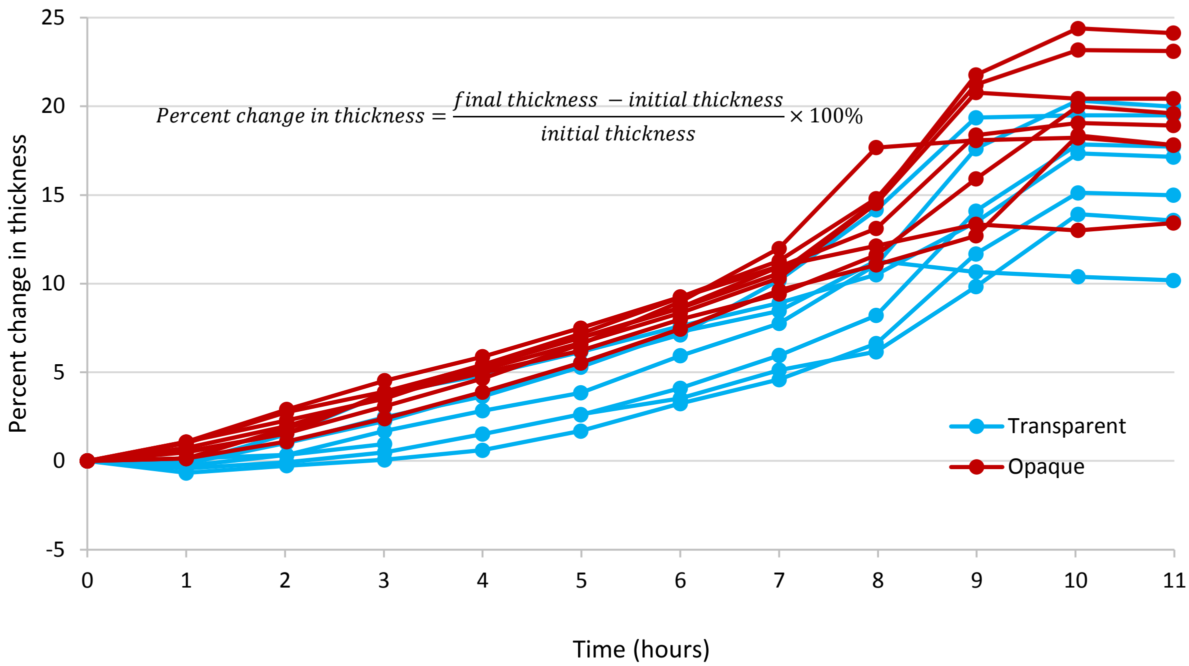


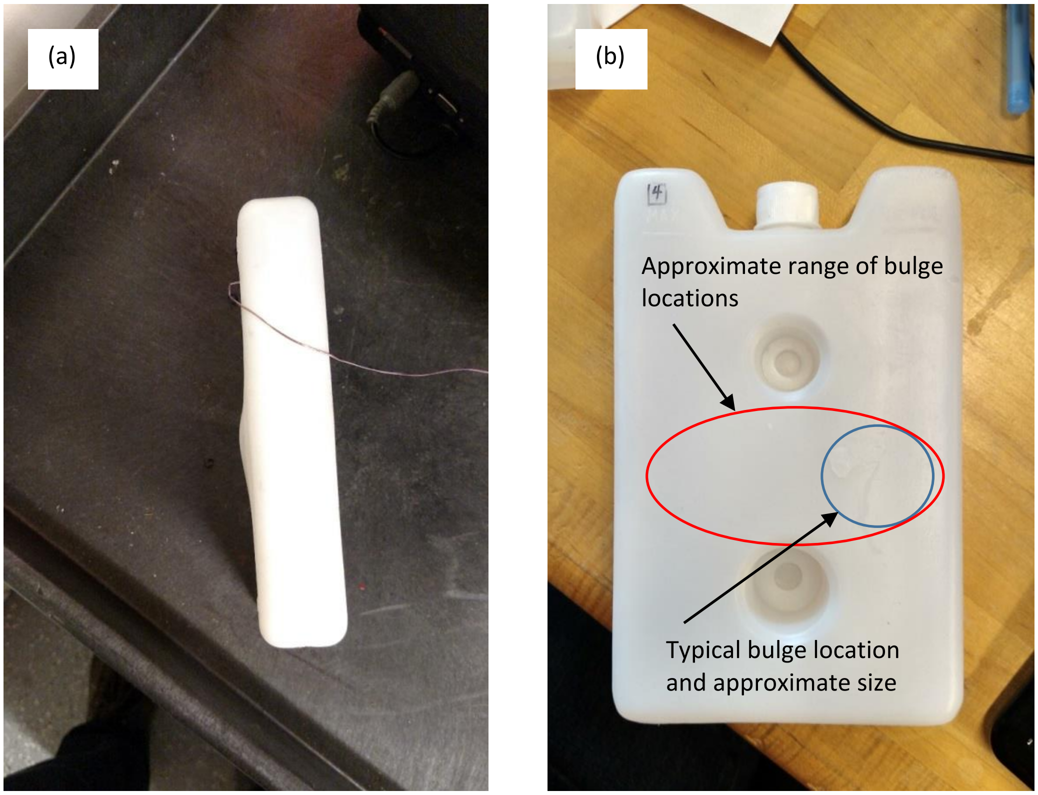
Figure 5 shows the measurement of the expansion of the water-packs. Water-packs would begin to expand within 2 hours of being placed in the freezer. The rate of expansion would slowly increase with continued freezing and then accelerate rapidly as the freezing time approached 8 hours. Then water-pack thickness would abruptly stop increasing with time. Water-pack thickness would remain constant or decrease slightly for the remainder of the experiment.

Figure 5. Percent change in water-pack thickness versus time.



Our observations of water-packs after freezing showed the consistent presence of a localized bulge in the water-pack. The exact location of the bulge varied from water-pack to water-pack, but it was usually located midway between the sidewall of the water-pack and indentations and between the indentations. An end on view of a water-pack exhibiting the bulge is shown in Figure 6. We hypothesize that the bulge forms in the location where the last liquid water in the water-pack freezes. This last pocket is surrounded by thick sections of ice. The ice is forced to expand outward, toward the front and back of the water-pack where the ice is at its thinnest.

Figure 6. (a) View of water-pack lying on its side with opening to the top of the picture showing bulge. (b) Schematic drawings of typical bulge locations and approximate size.

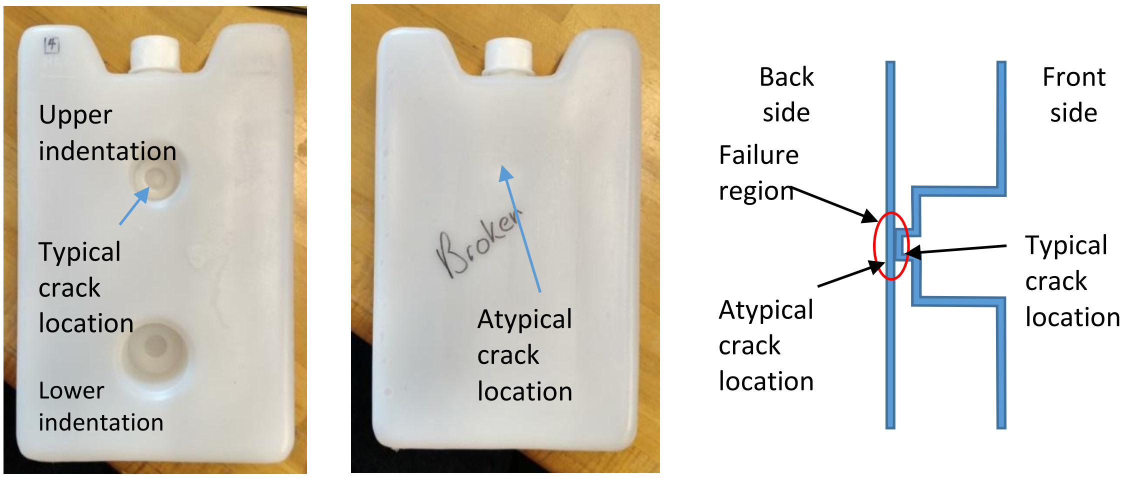


If the hypothesis is correct, the bulge formation would explain both the variation in the time at which water-pack temperature drops below 0°C and the acceleration of water-pack expansion nearing the completion of freezing. If the last liquid water to freeze in the water-pack is located in a discrete pocket, then a thermocouple would need to be immersed in that pocket in order to remain at 0°C throughout the freezing process. A thermocouple that becomes embedded in ice earlier in the freezing process would no longer be in thermal equilibrium with the remaining liquid water and would start to read a temperature below 0°C. Temperature measurements of the inside of a water-pack are therefore an inconsistent indication of the completion of freezing. Conversely, the acceleration of expansion of the water-pack due to the bulge followed by a cessation of expansion is a reliable indicator of the completion of freezing.

Water-pack failures

In the course of performing holdover testing and developing the visual inspection test, a significant number of the 0.6 L AOV water-packs failed during freezing. The water-packs failed by breaking where the indent near the opening of the water-pack connects with the backside of the water-pack. Figure 7 shows photos of frontside and backside failures as well as a schematic cross section with the locations of the failures. The typical failure mode is for the front side of the indent to crack. The atypical failure mode is for a crack to form in the backside of the water-pack. Either failure mode results in water leaking out of the water-pack. The failure likely results from stresses in the plastic created by the expansion of water when it freezes.

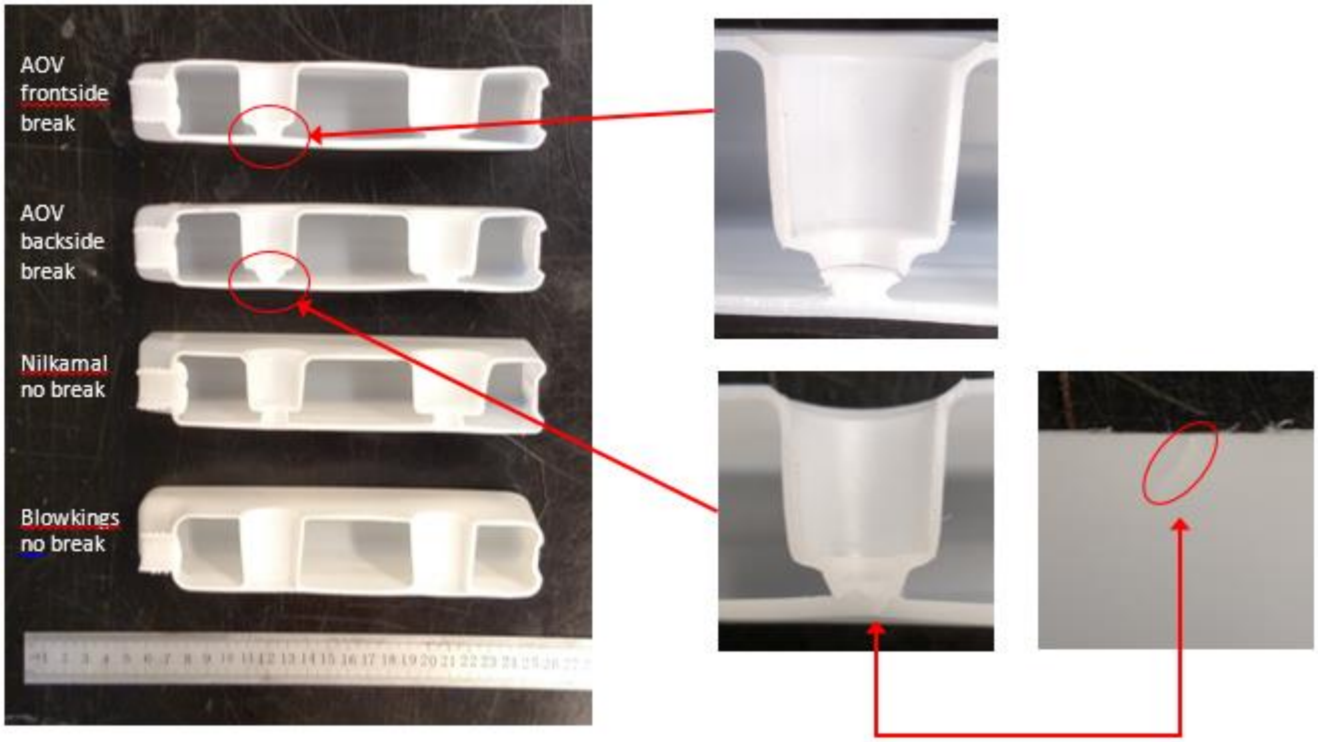
Figure 7. Photos of broken AOV water-packs showing typical and atypical locations of cracks with a schematic cross section through the location of a crack.



During holdover testing, none of the 0.6 L Blowkings water-packs failed. While the Nilkamal 0.6 L water-packs were not used in the holdover testing or most of the visual inspection testing, the Nilkamal water-packs were run through ten freeze-thaw cycles with freezing at -30°C and thawing at room temperature. None of the Nilkamal water-packs failed during this cycling experiment, whereas some of the AOV water-packs failed after one or two cycles. These results suggested that something specific to the AOV water-packs was causing the failures.

To investigate the failures further, samples of Blowkings water-packs, Nilkamal water-packs, and failed AOV water-packs were sectioned using a band saw to compare the construction of the water-packs. Figure 8 shows the cross sections of the water-packs and AOV water-pack failures. A comparison of the diameter of the upper indentation at the location where it comes into contact with the backside of the different water-packs shows that the diameter of the upper indentation for the AOV water-packs is smaller than for the Nilkamal and Blowkings water-packs. The smaller diameter means that the area of the indentation over which the forces from ice expansion are spread is smaller for the upper indentation of the AOV water-packs than for the indentations on the Nilkamal or Blowkings water-packs, or even the lower indentation on the AOV water-packs. The smaller area leads to higher stresses in the indentation at the joint, which causes the failure. Only failures of the upper indentation on the AOV water-packs have been observed; the lower indentations have not been observed to fail. The absence of lower indentation failures suggests that the failure is a result of the geometry of the joint between the front and backsides of the water-pack in the upper indentation and not a result of material flaws. Redesigning the indentation to increase the diameter of the indentation where the frontsides and backsides join could reduce water-pack failures in this location.

Figure 8. Cross sections of three manufacturer water-packs including detailed AOV water-pack failures.



4. Conclusion

We developed a visual inspection test for evaluating water-packs to determine whether they are frozen, partially frozen, or unfrozen when removed from a freezer for use in vaccine carriers. The method developed involved training water-pack evaluators in visual inspection techniques using water-packs of known temperature history and using a checklist of tests that water-packs must pass to be classified as frozen. The resulting visual inspection test was shown to accurately classify water-packs into one of the three categories when used by novice water-pack evaluators. The visual inspection test was used to evaluate water-packs that contained completely liquid water when freezing had begun.

The report also examined water-pack temperature and expansion during freezing. Temperatures recorded by thermocouples placed inside the water-packs were shown to be an unreliable measure of when a water-pack was completely frozen. Cessation of water-pack expansion more closely coincided with the completion of water-pack freezing. When starting with completely thawed water-packs, the last remaining pocket of liquid water in the water-pack expanded as it froze, forming a distinct, localized bulge in the water-pack. If the thermocouple was not located in the last remaining water pocket, then the temperature measured by the thermocouple dropped below 0°C before the water-pack completely froze. A bulge formation caused an acceleration of water-pack expansion followed by an abrupt cessation in expansion with the completion of freezing, both of which were easily measured.

The experiments done on the visual inspection test and the water-pack expansion were for water-packs that were completely thawed at the beginning of freezing. Neither the inspection test nor the water-pack expansion experiments looked at water-packs that were partially frozen when the water-packs were placed in the freezer. While we would generally expect the visual inspection test to work well with these water-packs, it is unclear whether the water-pack bulge would form in the same way as with water-packs that start the freezing process in a partially frozen state. Expansion of the water-pack should still occur, though the acceleration of expansion near the end of freezing may not occur if the bulge does not form. Further work could involve evaluating the accuracy of the visual inspection test when used with water-packs that are partially frozen when freezing commences and looking at water-pack expansion and bulge formation with these water-packs.

A significant number of AOV water-packs developed cracks during the visual inspection and expansion tests, whereas water-packs from other manufacturers did not. Comparisons of the construction of the AOV water-packs with water-packs from Blowkings and Nilkamal suggested that the small area of contact where the front and back walls of the AOV water-packs were joined in the upper indentation resulted in high stresses during freezing expansion, which led to the crack formation. A redesign of the AOV water-pack upper indentation geometry could solve the cracking problem.