Introduction to Vial Fogging and Mitigation Strategies
Kevin Bond, Research Associate II – Baxter International, Inc.
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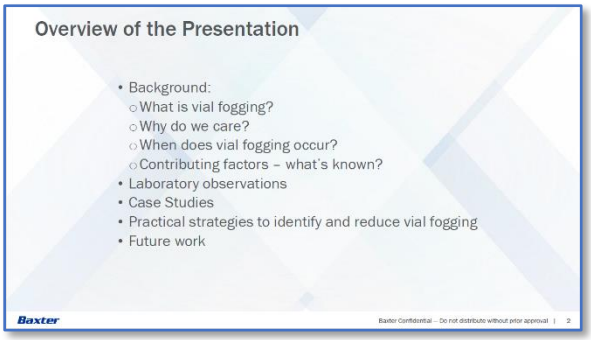
Before we get started, I wanted to take a minute to both introduce myself and introduce Baxter. My name is Kevin Bond. I'm a research associate at Baxter BioPharma Solutions. I have been with Baxter for a little over two and a half years now. Before Baxter, I received my PhD from Martin Gerald's group at Indiana University.

Our Baxter site is located in Bloomington, Indiana, and our site here in Bloomington is primarily dedicated to pharmaceutical contract manufacturing. Our R&D group here in Bloomington performs contract research and development to support incoming commercial projects to our manufacturing site. Our R&D group does a little bit of everything; supporting formulation, process, and analytical development activities for those projects.

Additionally, our group is also part of a lyophilization consortium called LyoHub, where members from academia and industry work together to advance freeze drying, as lyophilization is one of our group's specialties as well. Part of our research and development efforts in our group has been focused on vial fogging, which is the topic for my webinar today.

I also wanted to mention before we get started here that there should be plenty of time for questions at the end. I only expect this SlideDeck to take about 30 minutes or so. If anyone has questions, please feel free to drop them in the chat, and I will do my best to answer those at the end of our time. I know Brian will also be helping me keep an eye out for and keep track of questions. I believe those are all of our housekeeping items, so we can go ahead and get started.



Our time today will focus on providing a general overview and introduction to vial fogging for those who are not familiar with the phenomenon, as vial fogging is a concept that is unique to lyophilized drug products. We'll touch on what exactly vial fogging is, why we care about it, the timing of when vial fogging actually occurs, and some of what is known, or at least suspected, to influence the presence or severity of vial fogging in lyophilized products.

We'll also touch on some experimental observations from our lab, as well as two vial fogging case studies we have performed in our group. These laboratory observations and case studies provide us with some practical ways to limit the impact of vial fogging to development programs, and, ultimately, to your commercial drug products. We'll wrap up our discussion today reviewing key takeaways from our observations, and also by emphasizing the need for future research work to determine root causes and mechanisms of action for vial fogging.

With that, our most basic question is what exactly is vial fogging? Vial fogging presents as a haze of dried powder which is visible above the dried cake, where the dried cake meets the inner surface of that vial. Vial fogging is a phenomenon commonly observed in lyophilized drug products and has been widely reported in the pharmaceutical industry. The exact appearance of vial fogging varies from product to product, and can present as a dendritic or branching pattern, or as a uniform haze covering the interior of the vial. The photo on the left of our screen shows an example of vial fogging presenting as that dendritic branching pattern, while the photo on the right shows an example of vial fogging presenting as a more uniform haze.

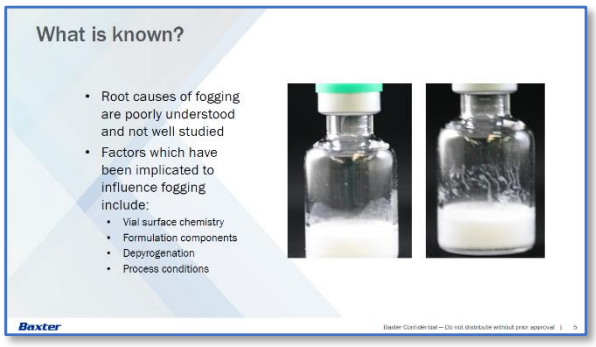
Our next question is, why do we care about vial fogging? The presence of vial fogging is often considered a cosmetic defect, but this can vary based on specific markets and client preferences. There is also the potential for product rejection during inspection if you're in a market which does not tolerate vial fogging, or if you are a client or have a client which does not consider vial fogging a defect.

Another reason that we care about vial fogging is if the dried product extends up above the shoulder and into the neck of the vial, like the example pictured here, this could raise concerns about container closure integrity, and is a risk to sterility failure. In those cases, typically, vials like that would be rejected during inspection just because of risk associated with potential sterility failure.

The above consequences of vial fogging can lead to rejection of batches of product or a large percentage of your product, which could be very costly to you due to the high cost of some biologic APIs and the cost of line time and just general costs associated with lyophilization. Therefore, the prevention and reduction of fogging becomes significant when considering the cost of lost product due to potential rejection.

We'll touch on a little bit of what is known about vial fogging and what is suspected to cause vial fogging. But first, we have to appreciate that the root causes of vial fogging are currently poorly understood. This is mainly because vial fogging is often considered a cosmetic defect, unless you have that product that gets up into the neck and shoulder of your vial. Generally, issues which are considered

cosmetic defects don't receive as much funding and are not as well studied as other issues in pharmaceuticals.



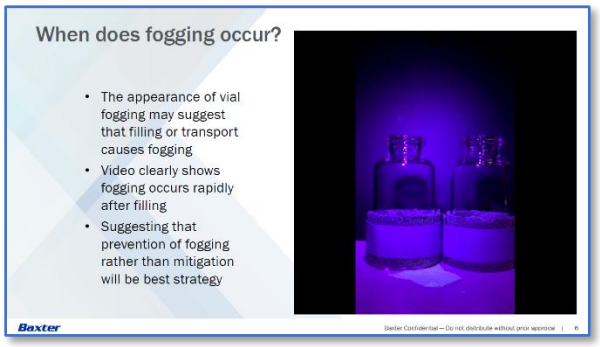
But even with this general lack of knowledge of the root causes surrounding vial fogging, there are a number of factors which have been reported in literature and which we have observed in our lab which will influence the severity and propensity of vial fogging. The first of those factors that I've included here is the vial's surface chemistry. Specifically, what I mean by that is the hydrophobicity of the inside of the vial will determine if fogging is present or not. For example, vial fogging is often not observed in vials which have a hydrophobic coating on the inside of the glass.

There are also certain formulation components which, when present in a drug product, increase the severity of vial fogging. Specifically, formulation components such as surfactants, like polysorbate 80, tend to make fogging much more severe when compared to those same formulations but without a surfactant. This is significant because, of course, many biologic formulations require a surfactant to reduce the propensity for aggregation during lyophilization.

Additionally, there have been studies which imply that depyrogenation increases the severity of vial fogging. But of course, not depyrogenating typical glass vials used in sterile injectable pharmaceuticals is not an acceptable practice, so that's one factor that we've really ruled out as a practical solution for reducing vial fogging.

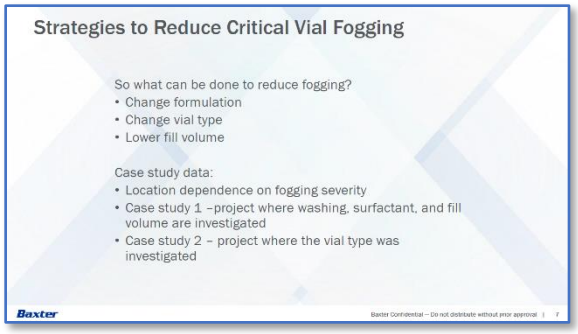
Finally, it has also been implied that processing conditions of your lyophilization cycle, (such as long pre-freeze hold steps at low temperatures or by using controlled nucleation in your cycle) could reduce the severity of vial fogging. However, in our experiences, we found that processing conditions of your cycle are at best unreproducible at reducing the presence or severity of vial fogging, and at worst have really shown no impact at all in reducing the presence or severity of vial fogging.

These are all areas which we continue to research and investigate. But I also want to touch on the last piece of our puzzle for the introduction of vial fogging. That's when exactly does vial fogging occur in the manufacturing process?



Some people say that vial fogging looks like it could've been caused by splashing of the drug product during filling, or by sloshing of the drug product in vials as they transverse filling lines or when they're being loaded onto the lyophilizer shelf. To touch on this and show you when this happens, and instead of just telling you about it, I think it's better to show you this in a video, so I'll go ahead and play that.

Here we have a sucrose mannitol solution which contains PS80 and a small amount of fluorescent dye that is back-lit by a black light. You can see that almost immediately after the solution leaves the pipette tip there is that drug product solution which creeps up the wall of the vial in those branching patterns. So this video pretty clearly demonstrates that fogging and solution creep occur very rapidly after solution is filled into a vial. Given that this creep of solution happens within seconds of the solution entering the vial, that would imply that strategies to prevent vial fogging in the first place rather than attempt to take steps to reduce it later, like processing conditions of your lyophilization cycle, would be more effective to eliminate vial fogging concerns.



What strategies can we employ to reduce vial fogging? Even though root causes are poorly understood, there are some strategies which we've used to help reduce the impact of vial fogging. If you're very early in development, you can experiment with formulation changes to see if you can reduce vial fogging while also keeping your drug substance stable, and not alternating any of your other critical quality attributes. However, most biologics will require the presence of a surfactant, so the formulation change options may be limited. Additionally, many vial fogging issues are discovered later in development programs after the formulation has been pretty well finalized, so that's not always an option.

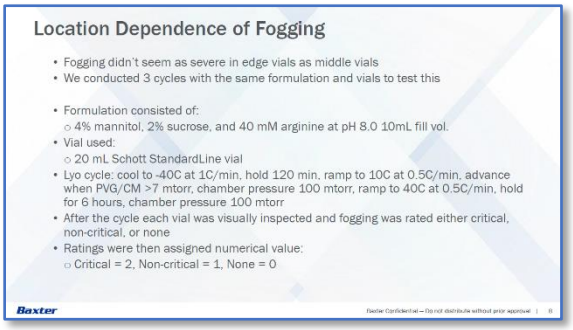
That leads us to our next, less intrusive change. If you already have a formulation selected or if your options to change formulation are limited, you can change your vial type for your drug product. If you're using a standard vial and you're able to change to a vial type that has some type of hydrophobic coating on the inside, then it may be worth your time to investigate a coated vial. There are also vials that have refinished glass inner surfaces, which also have been shown to reduce fogging. So, it would be worth your time to test whether either of those types of vials could help improve these fogging characteristics of your formulation.

Finally, if you're already locked into a specific vial or formulation, or your options to change those are limited, you can lower the fill volume of your drug product in your vial. When fogging does occur, that solution will have further to creep up the side of the vial before it reaches the shoulder and neck. This lower fill volume approach, again, accepts that fogging will happen, but that you would have less failures during inspection because that solution has further to travel up the vial. And again, as we previously said, when it reaches the shoulder and neck, then it's really a risk to container closure and sterility.

To illustrate some of these variables and strategies, I'll briefly touch on two case studies that we've conducted in our lab to highlight how vial fogging behavior changes with a variation of some of these factors we've discussed. First case study was a design of experiments where these factors were whether or not the vials were washed and depyrogenated, whether the formulation contained a surfactant or not, and finally that last input for the design of experiments was the fill volume. The second case study we'll touch on was investigating fogging in different vial types. So the formulation will stay the same in that, but the vial types will be changed.

But before we discuss these case studies, we need to touch on one additional observation that's come from our lab which we need to consider in order to execute these vial fogging experiments reproducibly.

Early on, when I started participating in these fogging experiments at Baxter, we began to notice that fogging didn't really seem to be uniform across the entire shelf. We noticed that fogging seemed to be worse in vials located in the center of the shelf compared to vials that were located along the edge.

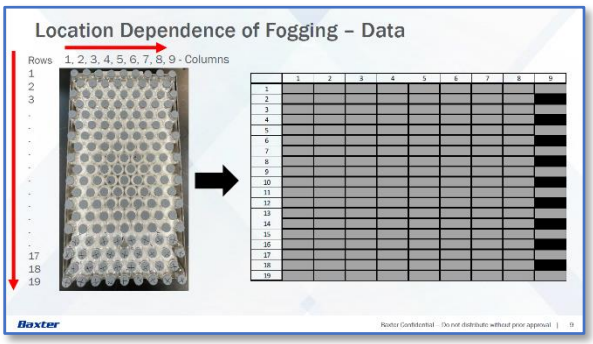


For a lot of our early experiments, we hadn't been tracking fogging data [inaudible 00:15:01] some of that fogging [inaudible 00:15:03] three cycles with identical formulations and processing parameters. The formulation was a 4% mannitol, 2% sucrose, and 40 millimolar arginine solution at pH 8. The fill volume was 10 milliliters in a 20-milliliter StandardLine Schott vial. The lyo conditions were as shown on the slide.

And then after completion of the cycles, the vials were removed from the lyo, visually inspected, and were classified as either having critical fogging, non-critical fogging, or no fogging. For this study, we defined no fogging as a vial which did not have any solution creep up the side of the vial above the height of the dried cake. We defined non-critical fogging as any vial which did show solution creep up the side of the vial above the dried cake, but that solution creep did not go above the shoulder of the vial. And then finally our last category is critical fogging, which we defined as vials which did have that solution creep up to the shoulder and above. We really care most about reducing the number of critical fogging vials, as again, those are the ones which would raise most concerns during inspection. We'll also use those same rating criteria for the other two case studies, the same critical, non-critical, and no fogging criteria.

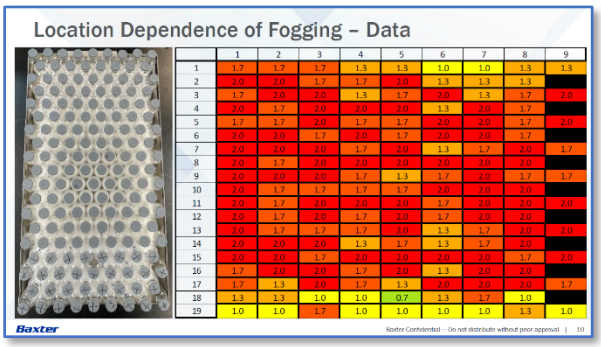
As we inspected and recorded the vial fogging for this study, we also kept track of where on the tray all the vials were located, so we had that positional data associated with fogging severity. And then to help easily just take averages for the fogging across the three cycles, we assigned the fogging ratings as either 2, 1, or 0, depending if it was critical, non-critical, or none.

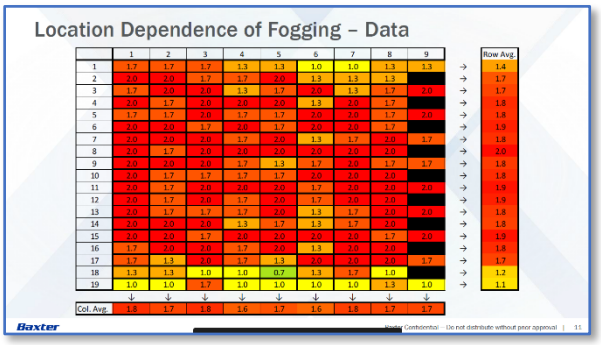
How we visualized this is we have a full tray of vials over here on the left, and there's a total of 19 rows, and then each row alternating between nine and eight vials, just due to the hexagonal packing of the tray. Again, we kept track of the fogging according to where they were located on the tray, so a row and column number. We did this in an Excel table which looked something like this, where each cell represents a vial over on the left.



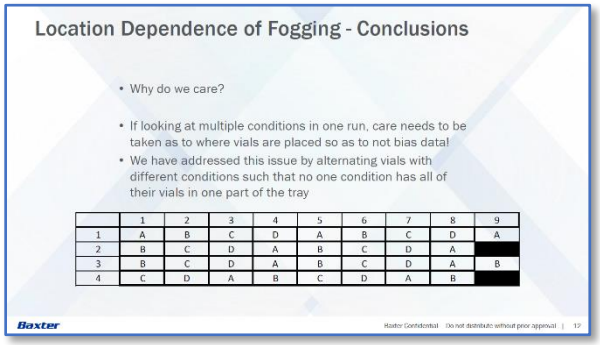
Now remembering that we scored these vials a 2, 1, or 0 depending on if it was critical, non-critical, or no fogging, we input all those values into Excel, and the results looked something like this. Remember, these are average fogging scores over three cycles for the entire shelf. Here we can see that the average vial fogging for vials in the front and back of the tray appear to generally be not as severe as vials in the center of the tray. This appears to be especially true for vials located in the front of the shelf.

Another helpful way to look at this data is to take the average fogging scores across the rows and columns of the tray. When we take the averages across the columns and rows, it looks like something like this, where we can see that the front two rows and the back row had lower fogging on average than vials located in the center of the tray. There, of course, were some vials located across the cycles in the center which didn't have as severe fogging, but again, just on average, the front two rows and the back row had lower fogging than vials which were located in any of the center rows.

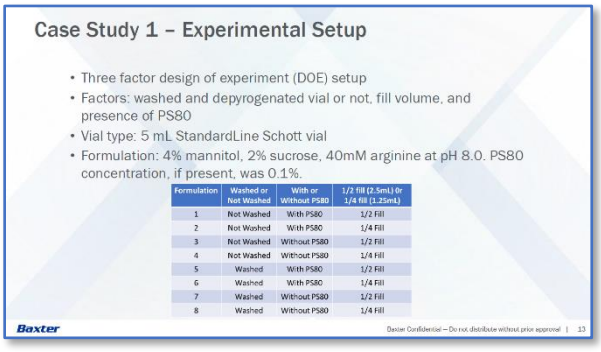




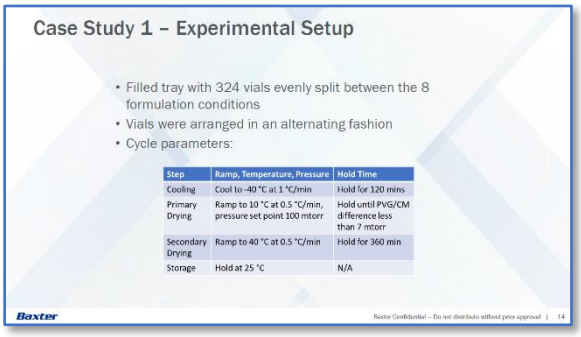
You might be asking, why do we really care about this? This apparent variant of fogging severity across the shelf becomes important when we begin to design experiments comparing multiple variables that are located on the same shelf at a given time. If you have multiple different vials all on one single lyo shelf, if you group all those vials for each condition together, whichever condition was in the front two rows of the tray might appear to have less severe fogging, when in reality they might not actually have less severe fogging, and that was just an artifact of where they were located on the shelf. So, we've taken to addressing this issue by alternating vials in the tray such that no single condition being investigated has all of their vials in a specific part of the tray. This table is an example of what the first four rows of a tray would look like if we were testing four different conditions arbitrarily named A, B, C, D. You can see that we alternate the vials such that they would be evenly distributed throughout the entire tray. This is a strategy that we'll use for the next two case studies, and is something that I treat as a best practice when performing fogging experiments looking at multiple variables on the same shelf.



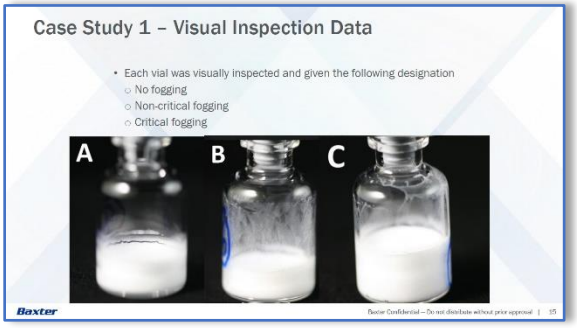
That brings us to our first case study, which consisted of a three-factor design of experiment setup. The factors were whether or not a vial was washed and depyrogenated, whether or not a formulation had PS80, and whether or not the vial was filled to a half or a quarter of its nominal capacity. The vial type was a 5 milliliter Schott StandardLine vial, and the formulation consisted of 4% mannitol, 2% sucrose, and the 40 millimolar



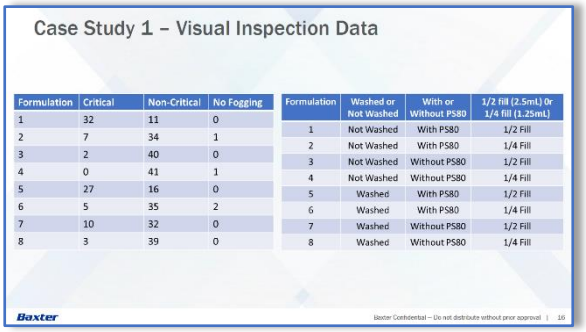
arginine at pH 8. If PS80 was present, it was present at 0.1%. The full factorial formulation combinations looked like this in the tray, or in the table located below, where you have eight distinct combinations for those three factors.



Using those eight formulation combinations from the table, a total of 324 vials were filled and evenly split among those eight formulations from the last slide. We also arranged them in an alternating fashion, so no one condition would be all located in the front, so we didn't introduce any bias based on the location of the vials. The vials were then lyophilized according to the cycle presented in the table, and then after the cycle was completed, each vial was visually inspected.



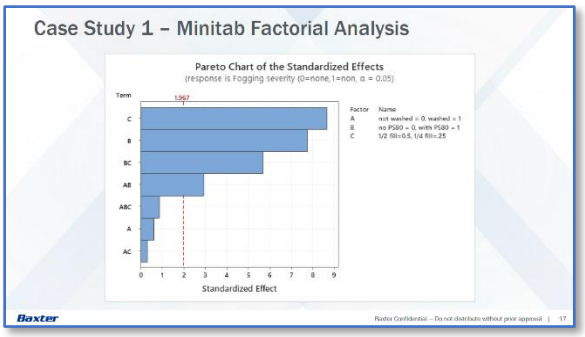
During inspection, the vials were again graded on the same rating that we had used before for the no fogging, non-critical, and critical fogging. We have some examples here of what those would look like. Here is an example of what no fogging would look like. Here is an example of non-critical fogging. Even though fogging is surrounding almost the entire vial and might look really bad, the fogging does not extend up above that bend, above the shoulder and up into that shoulder and neck area. That is, in our book and by our definition, still considered non-critical fogging. And then here we have a vial which we would consider that critical fogging, due to the product creep up above the shoulder and into the neck.

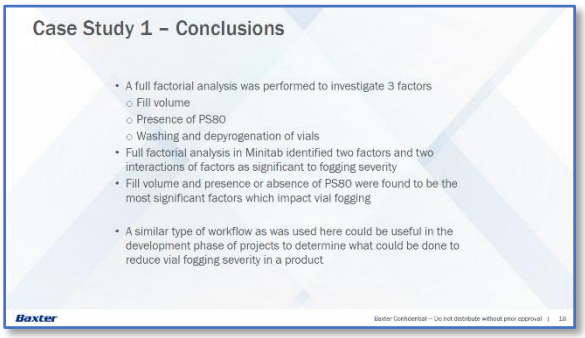


After inspection, we tabulated the results for each of the conditions, and the results are shown on the left. At a first glance, the data ... You can see that fogging was pretty prevalent across the board as there were only a total of four vials which did not exhibit any fogging at all. And then next, you might look and see that there may be two or three conditions that seem to have worse fogging than the others, those conditions being 1, 5, and 7. But it's hard to really tell what factor may or may not be significant just from that table. So the data from the left side table were put into Minitab in order to determine the significance of each of the factors we were testing.

Once that data were input and the DOE factorial analysis was performed, we were able to look at the Pareto chart for that data. This chart shows the main effects and those effects' interactions, and whether those effects and interactions were significant. All of the factors which are greater than that red dashed line present in the graph are considered significant.

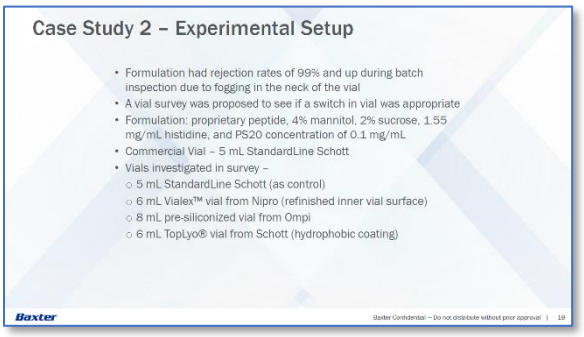
From this analysis, we've identified two factors and two interactions which significantly impacted the vial fogging present in the study. Those two individual factors were the fill volume and the presence or absence of polysorbate 80. Additionally, the interactions of the presence or absence of polysorbate 80 and fill volume and the interaction of the washing or not washing and depyrogenating and the presence of PS80 were also significant factors. So in this study, it didn't appear that depyrogenation alone was a significant factor based on this analysis.





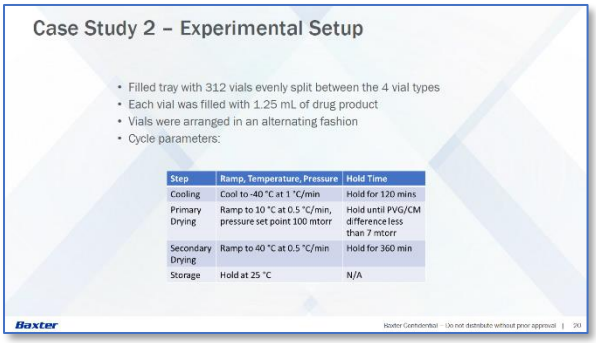
In our first case study, we examined three factors in a design of experiment setup which had eight unique combinations of those factors, and then based on the analysis of our data we determined that fill volume and the presence or absence of PS80 were two factors which individually were significant in the severity of vial fogging. Then we also see that interactions between different factors could be significant and shouldn't be ignored when evaluating vial fogging for other products and programs. We would reason that similar types of experimental designs could be useful during the formulation and primary container selection process of development programs just to help reduce the risk of vial fogging and later rejection due to vial fogging in commercial programs.

That brings us to our second case study. This case study was brought to our attention because there was a specific formulation which had experienced extremely high rejection rates during inspection of 99% and up for a number of batches. They were rejected because the fogging was observed above the shoulder and the neck. It was decided that we would do a survey of different vials to see if different vial types could help reduce the fogging in this drug product.

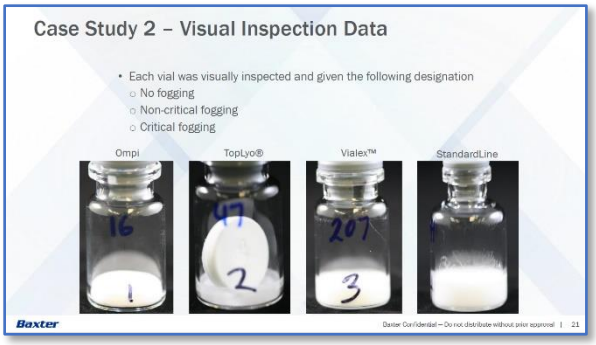


The formulation consisted of a proprietary peptide, a 4% mannitol, 2% sucrose, some histidine, and PS20 at a concentration of 0.1 mg/ml. In addition to the vial which was already in use, there were three new vials which were used as comparators. The vial which was already in use was a 5-milliliter StandardLine vial from Schott, and we used that as a control. The other three vials were a 6-milliliter vial Vialex vial from Nipro, which has a refinished inner glass surface, a 8-milliliter pre-siliconized vial from Ompi, and a 6-milliliter TopLyo vial from Schott, which has that hydrophobic coating that we've talked about before.

Using the formulation from the previous slide, a total of 312 vials were filled, evenly split between the vial types listed on the last page. As we've already discussed, we didn't want location to impact the fogging data, so the vials were alternated so they were evenly split across the shelf. The vials were then lyophilized according to the parameters presented in the table here. Then after the cycle was completed, each vial was visually inspected for fogging.

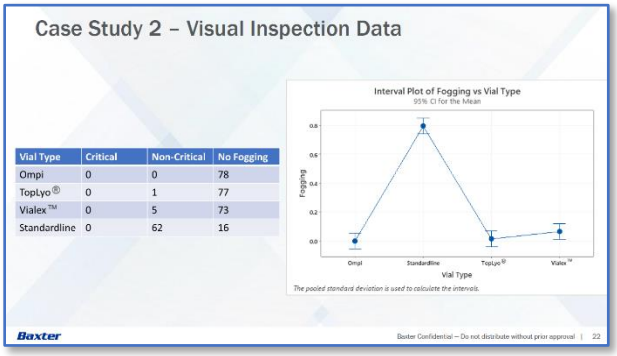


The same grading criteria that we've used for the previous studies was used here where no fogging, non-critical, and critical have the same definitions as before. We also have some representative photographs of the vials for the four vial types. From left to right, we have the Ompi vial, the TopLyo vial, the Vialex, and then the StandardLine vial. We can see that the first three vials show very clean inside walls, which would indicate that no fogging was present, while the fourth vial though, it is a little hard to see, there is vial fogging present in that StandardLine vial in the form of the dendritic pattern.



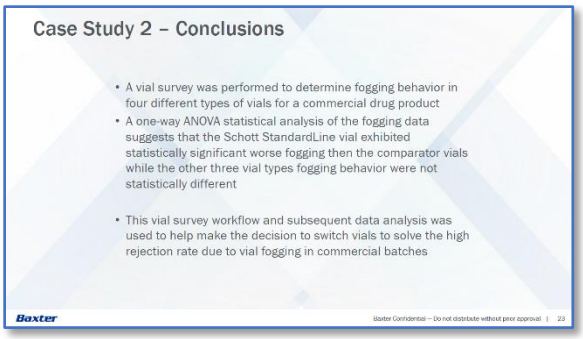
It's worth noting for this slide that the cake in the TopLyo vial was separated from the glass. The cake is still intact. We don't believe this to be anything more than, again, a cosmetic thing. And the cake separating from the vial does seem to be common, or at least not uncommon, for vials with a hydrophobic coating.

After our visual inspection and grading the vials, the results are shown here in this table. At a first glance, one can see that fogging was only prevalent in that StandardLine vial, and the other three vials were primarily fogging-free. To determine if the difference in the fogging between the StandardLine and the other three vials was significant, we performed a one-way ANOVA analysis, and the resulting interval plot looked something like this, where the analysis exhibited a significant difference between the StandardLine vial and the other vials, but no significant difference between the fogging behavior of the Ompi, TopLyo, and Vialex vials.

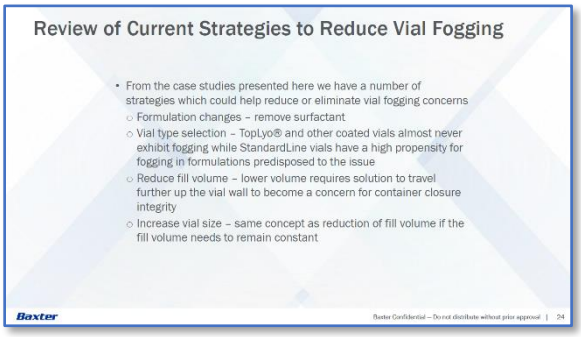


From our second case study, we were able to look at different vials and come to the conclusion that different vials definitely have an impact on the severity of vial fogging for a given formulation. Here, we performed a statistical analysis of that survey to measure those differences. That analysis suggests that the StandardLine vial had worse fogging behavior than the three comparators, which did not have

statistically significant fogging behaviors. And again, one could imagine that this type of experimental setup would be very useful in development programs when trying to select a vial for your drug product, especially if you know that your formulation has a risk for fogging, like if it's a biologic that contains a surfactant.



Here, we can touch on the current strategies that we use to reduce the severity and impact of vial fogging. The first one would be a formulation change, removing a surfactant. But those formulation changes can be difficult based on what your API is, and depending on how advanced your development program is. The next would be vial selection. We've seen from our case studies that TopLyo and other coated vials would almost never exhibit vial fogging, and some of the refinished glass surface vials also have a much better vial fogging performance than something like the StandardLine vials, which do have a high propensity for fogging in formulations which contain a surfactant or are predisposed to the fogging issue.



The other two strategies which you can practically implement would be to reduce the fill volume or increase the vial size, keeping the same fill volume. Both of those strategies effectively just increase the distance that your solution needs to travel up the side of the vial in order for the issue to go from cosmetic to quality-impacting.

With that, some of our key takeaways are that vial fogging's very common in lyophilized products. However, it's poorly understood. We will continue to keep investigating about fogging as a part of our research program, and we do have several experiments which are ongoing and planned for the near future, which we hope will provide even more detailed and specific information.

I also believe that vial fogging risk mitigation should be performed early to help ensure that you don't make it in a program to PPQ or commercial batches, and then figure out that you have severe vial fogging that leads to high rejection rates. Some studies that you might consider as a part of development program would be formulation studies, vial compatibility studies, and fill volume optimization, again, especially if you know that your formulation might have a risk for vial fogging.

And even though our case study didn't find depyrogenation to be a significant factor, I still think it's probably a best practice to depyrogenate any vials that you're going to use for a fogging study, just so that those vials are treated in as close of a manner as they would be in a GMP environment.

With that, I want to thank all of you for taking the time out of your day to be here with me today. And again, thank you to Millrock for hosting this webinar for us. In a second, I'll begin to start looking through the questions in the chat and see how many we can get to before our time's up. If I don't get to your question, or if something else comes to your mind after the webinar, I've also included my email address here on the slide. Please feel free to reach out to me if you would like to discuss vial fogging further. I'm always happy to discuss our observations and anything that we're working towards answering.