MesaLabs

Browning Of Media From Prolonged Thermal Insult

White Paper

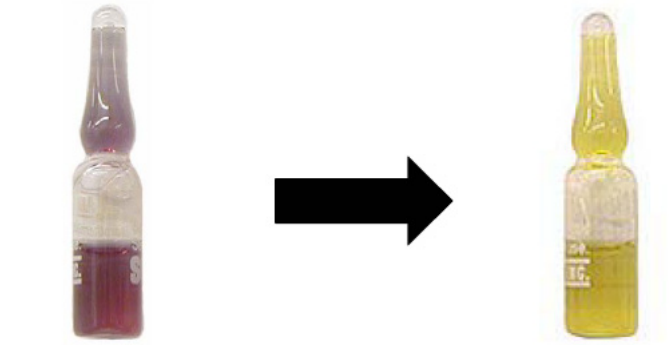
Questions are often asked regarding the color of media contained inside Mesa's SterilAmp and MagnaAmp biological indicators after prolonged sterilization cycles. Studies were performed to answer questions and address the concerns customers had in observing changes in color while using these products. Media contained inside SterilAmp and MagnaAmp is identical except for volume used.

Why is my ampoule brown (or commonly referred to as "bourbon")? The "bourbon" color seen is a result of thermal degradation of the media, which results in a lowering of media pH due to prolonged thermal exposure. The time needed to kill the spore challenge is significantly less than the thermal insult required to change the ampoule color to "bourbon". The media retains its purple color at the exposure time necessary to kill the spore challenge. Displayed below in Figure 1 is a color comparison of an unexposed ampoule, an ampoule exposed at F_o= 65, and a positive control after incubation.

Figure 1

Will the thermally degraded media still support growth of low numbers of spores? Yes! All biological indicators are systems. Spores cannot be separated from the carrier or container. However, Mesa's performed growth promotion testing in the spirit of USP by inoculating 10-100 spores into media that had been thermally stressed to levels needed to achieve complete spore lethality. Tests were positive with all samples showing turbidity and a bright yellow color. Displayed below in Figure 2 is an example of an ampoule exposed to a F_o = 30 which is beyond the thermal insult required to kill all spores present. The color change to yellow appeared after inoculation with 10-100 spores and incubation.

Figure 2



Inoculated with 10-100 spores

Inoculated with 10-100 spores

The degraded media will support growth of low numbers of uninjured spores but will it support growth of low numbers of spores injured in the thermal process? Mesa exposed a set of negative control ampoules to sufficient heat to achieve the "bourbon" color. Two different lots of biological indicators were exposed to the longest time still allowing total survivors, that point at which all subsequent exposures resulted in fraction negative or complete kill, to produce heat-injured spores. The low levels of viable spores at this treatment level were ascertained by plating on tryptic soy agar. The "bourbon" media from each lot was inoculated in triplicate with the injured spores and incubated. All replicates for both lots turned positive for growth (turbidity and pH indicator color change to yellow) within 48 hours. See figure 3. Therefore, "bourbon" media will support the growth of injured spores as well.

Is the functionality of the pH indicator compromised by the heating process? No! Media was exposed to F_0's of 30 and 50. At each condition, including unexposed media, a pH measurement was taken (unexposed @ 6.19, 30 minutes @ 5.89 and 50 minutes @ 5.78). The media was titrated with 1N HCL and a bright yellow color was achieved in all samples. Approximately the same volume of acid was necessary to titrate all the different exposures regardless of starting shade of purple or "bourbon". pH measurements were also taken after titration. These pH's ranged from unexposed @ 4.43, 30 minutes @ 4.27 and 50 minutes @ 4.10. See figure 3 for an overview of color appearances as testing was being performed.

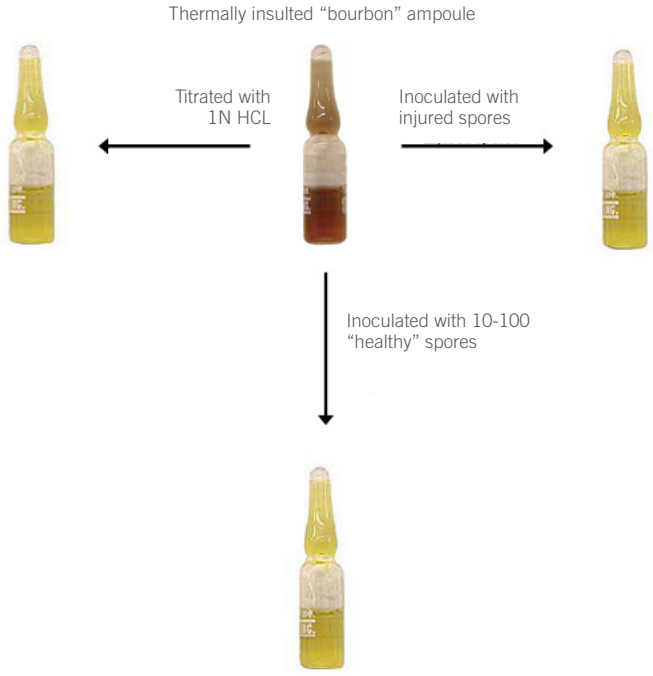


Figure 3 displays examples of ampoule color appearances seen while performing the testing to answers for the above questions.

My ampoule appears to have a yellow hue when I remove it from the incubator. Is this a positive sample that is just starting to turn yellow? Probably not. It is always good practice to incubate a negative control (an ampoule without spores) and a positive control (an ampoule containing spores) with an exposed ampoule for color comparison. The negative control ampoule needs to be subjected to the same thermal insult as the testing ampoule for correct comparison. Sometimes the "bourbon" ampoule will appear to have a yellow hue, but when compared side by side to the positive control ampoule that has turned positive, the color difference is very obvious. Any ampoule that has turned positive from spores surviving the sterilization cycle will still show a vibrant yellow color as that displayed in the positive control. Actual positive ampoules make the color change from purple to yellow in about 2 to 3 hours. Over this time period, the yellow continues to gain intensity. If in doubt, continue incubation for another 2 or 3 hours. Figure 4 displays a side-by-side comparison of how a thermally insulted ampoule may appear when removed from the incubator and the color of a positive control after incubation.

Figure 4

Thermally insulted "bourbon" ampoule

Positive control

Summary

Testing performed demonstrates that SterilAmp and MagnaAmp units exposed to conditions resulting in the loss of any shade of color purple: 1) are still able to promote the growth of low levels of injured indicator organisms, 2) still have a functioning color indicator allowing for 48 hour readout time, and 3) do not have any surviving spores. Therefore, it can be concluded that both MagnaAmp and SterilAmp are fully functional as biological indicators when exposed to any conditions described. The use of negative controls (supplied with every lot) is encouraged when a sterilization cycle is of sufficient length to discolor media. The negative control is only useful when it is exposed to the same conditions as the ampoules containing the spores. A direct comparison between the ampoules can then be made to determine if a color change is the result of media degradation or biological activity.

Learn more at biologicalindicators.mesalabs.com

bi-support@mesalabs.com | +1.406.585.9535 ext. 10042