

Spore News™

Volume 8, No. 3

Caramelization or Darkening in the color of Autoclaved Liquid BI Ampoules

by Russ Nyberg

When autoclaving liquid agar or tryptic soy broth, many times the sterilized media takes on a darker than expected color due to the sterilization and resulting exposure to heat. This presents a concern when working with sealed glass ampoule type biological indicators (BI) that contain tryptic soy broth (TSB). With most ampoule type self-contained biological indicators, the bacterial spores are contained in the ampoule as a suspension of spores and TSB. Once the ampoule BI has been autoclaved in a typical cycle being monitored, the ampoule is then incubated to see if all the spores are dead or if some survived the cycle. This growth/no growth testing is evaluated by looking at the color and clarity of the TSB within the ampoule. The ampoules also contain a pH indicator that gives the TSB a purple color.



Figure 1: Ampoules with growth (right) and without.

Upon incubation, if the spores were not killed in the cycle they will germinate to vegetative cells, metabolize the TSB and begin to grow. As part of the metabolism of the sugars present in the TSB, acidic waste products are released and the color of the ampoule changes from purple towards a yellow color as a result of a lowering of pH. When one is using the self-contained ampoule the question arises as to whether the normal darkening of color that occurs when ampoules are exposed to extended or high temperature cycles will still promote growth and detect any badly damaged spores that may have survived the cycle. This is mainly being questioned since some individuals have stated that the darker color ampoules are *caramelized* and will no longer function due to an inability to promote growth.

Other than heat sensitive selective media, most other media, when sterilized according to manufacturer's directions, are to be sterilized at 121°C for 15 minutes. This time and temperature parameter for sterilization is made very clear in USP¹ to mean 'time at temperature of media'. Thus 15 minutes at 121°C means that the media is held at 121°C for a full 15 minutes. Considering that a come-up time could be 20 minutes for the media flask to reach temperature, the entire cycle would be set at 35 minutes: Twenty minutes for come-up and an additional 15 minutes at 121°C.

Longer or extended cycles along with cycles that are run at higher temperatures beyond the specified 15 minutes

at 121°C are likely to some degree or another cause a natural darkening or a brown color caramelization of the media. This is an occurrence simply due to the exposure of the media to extended or higher heat cycles other than specified by the manufacturer and by the physical presence of a purple colored pH indicator.

During caramelization the glucose and other sugars that may be present in the media slightly darken and the overall appearance of the media will look more of a dark honey or light brown color. Along with the media itself darkening, the sheer presence of the additional purple pH indicator used in these ampoules make the color look even darker than media would without the pH indicator.

Caramelization is a major concern with most users of self-contained sealed glass ampoule type biological indicators such as ProSpore Ampoule or MagnaAmp. Typically when these biological indicators are used in various situations where the ampoules are exposed to extended cycles or higher temperature cycles rather than just 15 minutes at 121°C, the color of the media tends to darken. Both of these types of BIs have bacterial spores suspended in an ampoule of TSB which contains sugar. The ampoules also contain a purple pH indicator for user ease in determining growth within the ampoule. If the BI was not killed in the sterilization cycle, upon incubation the surviving spores metabolize the sugars, release acid waste and when the pH goes down as a result, the pH indicator changes the ampoule color from purple to yellow.

If slight caramelization occurs while autoclaving these ampoules, due to the sugar presence along with the physical presence of a purple colored pH indicator, the ampoule will seem darker in color than would be expected and darker when compared to an unprocessed ampoule. This is why a negative control ampoule should be used for a visual processed ampoule color. The negative control ampoule is run along with the test ampoules and can be used as a future color reference of what a normal ‘processed’ ampoule color will be when it is removed from the autoclave.

Upon incubation, an ampoule that is demonstrating growth will change further in color to or towards a honey or yellow color and will exhibit turbidity. This additional color change can easily be noted when it is compared to the color of a processed control ampoule. A potential problem can occur when one person removes the ampoule from the autoclave after a cycle has been run and places the ampoule in an incubator without noting or taking notice that the ampoule color has already changed from its initial bright purple color. After 48 hours of incubation the ampoule is removed from the incubator by someone else who is unaware of the normal color change of the processed ampoule and thinks the color change of the ampoule being removed resulted from being incubated and records the color change as a positive test. This is a situation that can be avoided by keeping a processed ampoule as your color guide for a normal color change and as a color example for a negative growth ampoule test.

Will the caramelization compromise the media's ability to promote growth?

In an attempt to determine if caramelized TSB that is used in an ampoule BI will still promote growth, a study was done in 2009 by personnel at Mesa Labs' Omaha Manufacturing Facility². In this study, numerous test ampoules and control ampoules were autoclaved in an extended time and high temperature cycle. These ampoules were run in the autoclave for 2 hours at a temperature of 132°C. When removed, the ampoules were very dark in color. The growth promotion ability of the sterilized media was then tested by aseptically breaking the tops off the ampoules and inoculating each ampoule with *Geobacillus stearothermophilus* spores. This bacterial spore was chosen since it is the species initially present in the BI ampoules for testing. All ampoules were then incubated at 55 to 60°C for 48 hours. All twenty of the sterilized ampoules that were inoculated with viable spores showed signs of growth by turning the media color toward yellow within 48 hours of incubation. Thus the media's darkening or caramelization during exposure did not compromise the media's ability to promote growth and provide useful sterilization test

² Robert Bradley, ProSpore Media Integrity Study, Nov. 2, 2009, www.MesaLabs.com, Technical Papers.

data.

It should be noted that some caramelization with various media types may not promote the growth of other bacterial species if one is using bacterial species other than those used in ampoule BIs. Additional testing would need to be done with that media and/or those organisms. However, for the BI indicator organism *Geobacillus stearothermophilus* used in spore ampoules, the darkening and caramelization of the media will not affect the growth promotion ability of the media or the function of the BI.

Will the BI still function when the media is so dark? YES

How can I tell a positive ampoule when the media is so dark? Compare the exposed and incubated test ampoule to the exposed and incubated negative control ampoule for determination of color change.

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