

In Focus: Specimen Integrity & Transport Issues

The 'Off' Lunch Box Sandwich

Harking back to your childhood days at primary school, most can recount the odd occasion when, for whatever reason, the sandwich your Mother had prepared so lovingly, was left in your lunch box or bottom of your school bag for a number of days. Upon opening the lunch box, a decomposed sandwich was revealed which normally carried with it a foul odour. The extent of decomposition was invariably related to the period of time the sandwich had been left in the lunch box or bottom of school bag. The particular season also had an effect, with warmer weather in the summer months leading to more gross effects! For many, this was most likely their first 'hands on' experimental experience in microbiology.

Decomposed Sandwich Likened To Specimen Integrity During Transport

This microbiology experiment, whilst comical for the most part, has a number of parallels with factors affecting integrity of stool specimens transported in nutrient broth vials for microbial identification using culturedependent techniques. Just like the school sandwich, fecal microbiotas are affected by oxygen concentration, available nutrients and temperature. It has long been recognized that a sample in transit can undergo significant changes in microbial balance¹.

The Old Way of Transport

In traditional stool culture analysis, it is imperative that organisms remain alive during transport to the laboratory where the sample is plated out for culture. This is achieved by transporting the stool specimen in specially developed nutrient broths. Such nutrient broths are designed to keep a wide a range of microorganisms alive as possible. However, the fecal flora is a dynamic ecological system with complex metabolic and environmental requirements. As such, it is not possible to have a nutrient broth that meets the nutritional requirements of all microorganisms. The majority of bacteria require anaerobic growth conditions and a specific pH environment. If these conditions are not present, alteration of the fecal flora because of starvation or bacterial overgrowth is inevitable¹.

The New Way

This is in contrast with the new methods of microbial detection based on DNA analysis. In molecular microbial analysis the stool specimen is placed in formalin or alcohol vials for transport to the laboratory. The formalin and alcohol immediately kill all organisms. When the specimens arrive at the laboratory for analysis, the balance present at the time of collection is unchanged, making for much more accurate and representative analysis of the stool specimen.

Metamatrix Specimen Transport Studies

Metamatrix recently undertook studies in their laboratory to demonstrate the effect that different transport mediums have on the balance of microorganisms in a stool specimen. One specimen was placed in two vials: one containing formalin and another a nutrient broth commonly used for transport. Both vials were incubated for three days at room temperature then DNA was extracted. The extract was incubated with three different restriction enzymes which cleave the DNA at specific base pair sequences.

Using PCR technology and special staining gels, it is possible to see a visual pattern of how the composition of each sample differed based on the banding patterns of each in the electrophoretic runs shown overleaf.

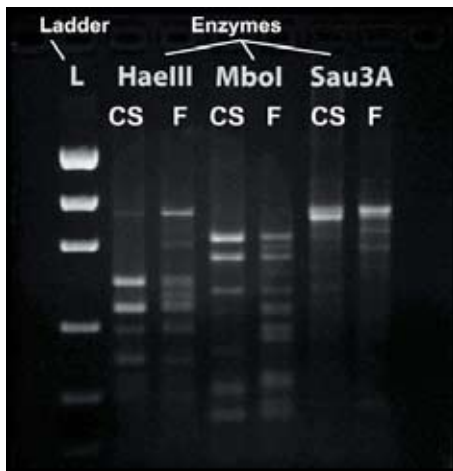


Figure 1. Haell, Mbol and Sau3A are the names of each restriction enzyme used to digest the same two samples. CS signifies the culture and sensitivity vial containing a nutrient broth, whereas F represents the formalin vial.

Two important features are seen in Figure 1 above. Firstly, the banding pattern for each restriction enzyme is different when comparing the culture and sensitivity vial with the formalin vial. This signifies that the microbial composition of each one is different. One must keep in mind that both specimens originated from the same individual. This illustrates the change in composition of the specimens maintained in culture and sensitivity vials over time.

The second important feature to note is the lower number of bands for each of the culture and sensitivity digestates. This loss of banding suggests that aerobic organisms have overgrown at the expense of the anaerobic populations leading to a loss of diversity. This data indicates that nutrient broth transport vials cannot provide the specimen integrity required for accurate measurement of gut microbes.

Effect of Transport in Culture and Sensitivity Vials on Multiple Microbes

Figures 2 through 4 illustrate the growth response of *Bifidobacteria*, *Candida* species and *Staphylococcus aureus* in one subject over three days. These results indicate that clinical laboratory data relying on transport of the specimen to the laboratory for culture are likely erroneous and can lead to inappropriate patient treatment. *Candida* species overgrowth is particularly notable. As these data indicate, *Candida* proliferates in nutrient broth transport media. This overgrowth in transport has likely fueled the common belief of many practitioners of the deleterious health effects of *Candida albicans* overgrowth in the gut. This may have resulted in inappropriate prescription of anti-fungal agents.

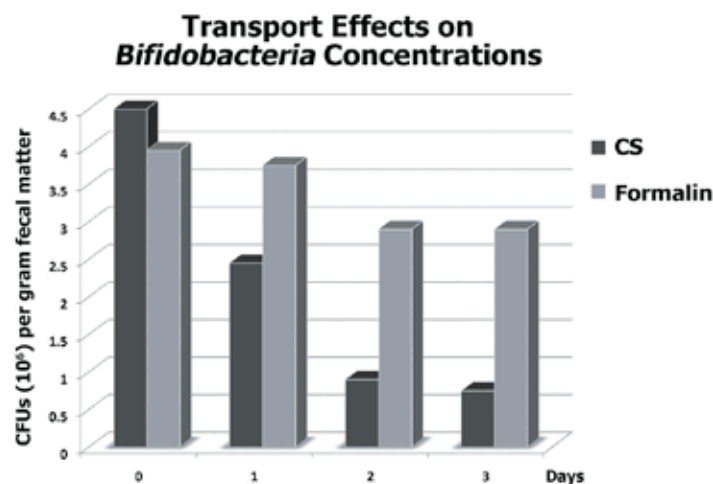


Figure 2. Significant decreases in Bifidobacteria occur over three days in standard nutrient broth vials (CS) used in traditional culture methodologies. Formalin-fixed samples show more consistent levels.

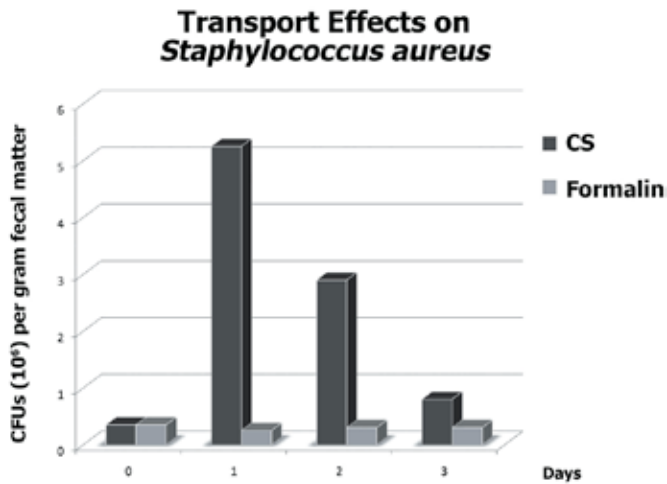


Figure 3. Potentially pathogenic, the opportunistic bacteria *Staphylococcus aureus* shows significant overgrowth during a three-day incubation in nutrient broth vial (CS). Formalin-fixed sample vial shows no growth. CFUs = colony forming units.

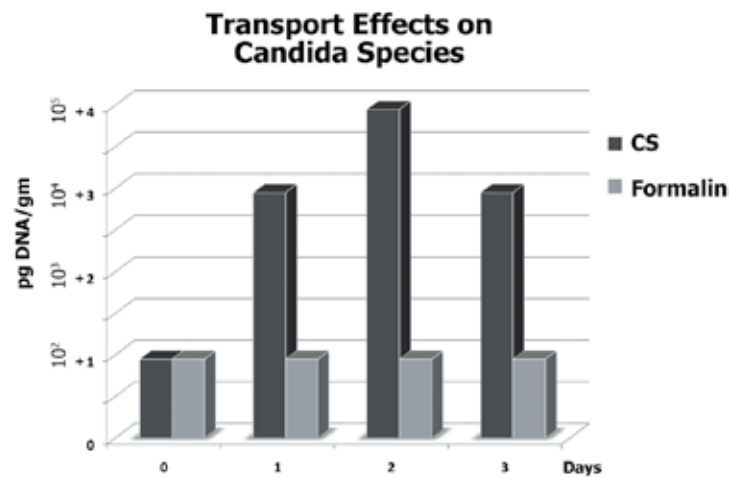


Figure 4. *Candida* species show significant overgrowth in nutrient broth vials (CS) over three days. Formalin-fixed sample vials do not exhibit growth and are at a level that would not be reported as abnormal overgrowth. The CS samples at 1, 2 and 3 days would be reported as abnormal overgrowth.

References

- Ott SJ, et al. In vitro alterations of intestinal bacterial microbiota in fecal samples during storage. *Diagnostic Microbiology and Infectious Diseases*. 2004;50(4):237-245.