

## **Innovative Sampling of Injured Microbial Cells in environmental microbiology: the “TAL” method**

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### Introduction

When microbial cells are treated with heat, cold, chemicals, dehydration, radiation or pressure, three populations of cells usually exist. One survives; another dies; a third survives but is injured. These injured cells can repair under favourable conditions, and later grow and metabolize as healthy cells. If we do not use the proper method to resuscitate injured cells, we may underestimate the existence of potentially pathogenic organisms in environmental samples. At KSU I led a group of researchers to work on a one-step Thin Agar Layers (TAL) method to recover injured cells.

### The “TAL” method

A layer of selective agar is first placed at the bottom of the Petri dish to solidify. Then, a layer of non-selective agar is poured over the selective agar. After solidification, the population of healthy and injured cells is spread above the thin agar layer. In 3 hours, the injured cells on top will repair while the inhibitory compounds from the selective agar at the bottom migrate upward. When the compounds reach the top of the non-selective agar, injured target cells are repaired and resist toxic effects of the selective agar.

### Test with “SAS Super 180” air sampler

Dr Crozier-Dodson modified the TAL method for use in the SAS Super 180 Microbial Air Sampler and collected data on the recovery of micro-organisms in the air. Typically, microbes in the air are injured due to lack of nutrients and dehydration. It is difficult to recover Gram-negative organisms directly from air due to the injury. Gram-positive organisms are more resistant to the air environment and can be recovered more easily. So, the recovery of airborne bacteria mostly consists of Gram-positive bacteria. In fact, many Gram-negative bacteria may be present and not recovered due to lack of a proper technique. Dr. Crozier-Dodson research found that the use of selective agar alone greatly underestimates the amount of Gram-negative organisms. The “TAL” method when used with the SAS Air Sampler, can improve the study of aeromicrobiology.

This “TAL” method has been tested on a variety of food pathogens such as *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Escherichia coli* 0157:H7, after these cells have been injured by heat, cold, organic acids, salt, radiation, and high pressure.