ADDENDUM A

Procedures and possible projects emanating from the Psychrotrophic bacteria / Biofilm report of P J Jooste. (Read in conjunction with the Conclusions and Recommendations section)

- Possible follow-up studies or procedures to confirm the recommended bacterial count standards as stated in 2.3 of the report for the accelerated psychrotroph tests in milk as acceptable and attainable in collaboration with industry.This would entail inclusion of the accelerated psychrotroph tests (4.1.1 to 4.1.3 of the report) in the testing regime of DSA and larger processing plants.

- Outsourcing or creation of a facility for performing flow cytometry in conjunction with the fluorescence *in situ* hydridization technique for detecting or enumerating psychrotrophs in real time.

- Are procedures for detecting biofilms on the farm and in the processing plant in place? If so what are the procedures and who are they performed by?

- Are there companies that have installed *in situ* techniques, as referred to in the report, in the factory or on the farm for monitoring intact equipment for biofilms? Such methods are able to report on biofilm growth on-line, in real time and non-destructively.

- Facilities should be identified that can perform molecular (DNA based) techniques for the rapid identification of psychrotrophs and a project could be run to apply such techniques.

- A project (in my opinion) is sorely needed to evaluate tests for the proteolytical activity of bacterial proteases in milk and to propose acceptable and attainable standards in this regard. This should include a survey of the baseline proteolytic activity in milk after production and these samples should then be incubated at 7^{o}C until the samples flocculate with the alizarol test. The proteolytic activity should be measured at regular intervals and also when the milk flocculates. Accelerated proteolytic psychrotroph / *Pseudomonas* counts should be run concurrently to determine the statistical correlation between proteolytic activity, bacterial counts and time to flocculation.

- Although a procedure for determining the activity of indigenous plasmin activity has not been included in the report, such tests do exist and a study in this regard can be run simultaneously with the previous project on bacterial protease activity. It will be useful to determine the effect of indigenous plasmin activity on casein stability in milk in comparison with that of bacterial proteases.

- Projects are needed to prevent or combat biofilm contamination on the farm and the processing dairy.
    - A study on quorum sensor antagonists could be done at a Masters or PhD level.
    - A study should be done on the application and economic feasibility of applying enzymes to remove biofilms
    - An in depth study should be done to determine what sanitation reagents and sanitation procedures are applied in Souh African factories to eliminate biofilms on milk contact surfaces. Because of the economic implications of such procedures it should be a

collaborative study between a researcher(s), milk processors and reagent suppliers who are willing to collaborate and an economist to determine the costs involved.

-o0o-