Abstract: Quality Assurance in the PROSPECT Pilot

Citation: Lamarche D, Rossi L, Shah M, Zytaruk N, Clarke F, Hand L, Lee J, Lee Y, Smith O, Jakab M, Shah S, Breton SJ, Langlois H, Porteous R, Watpool I, Acres S, Foster F, Meade M, Marshall JC, Lauzier F, Mehta S, McIntyre LL, Pagliarello G, Henderson W, Johnstone J, Heyland D, Thebane L, Bowdish D, Cook DJ, Surette M for the PROSPECT Investigators and the Canadian Critical Care Trials Group. Quality Control In the Conduct of a Probiotic Randomized Trial. Can Crit Care Forum, Toronto, ON, October 28, 2014.

Rationale: Probiotics are defined as microorganisms, which when ingested, confer health benefits to the host. Probiotics are considered a natural health product in Canada. The imprecise definition of probiotics, and different standardization procedures for dietary supplements raise questions about dose consistency. Furthermore, once probiotics leave the manufacturer, typically no further analysis is performed to examine the integrity of the product. Randomized trials suggest that probiotics reduce the incidence of ventilator associated pneumonia and other acquired infections in the ICU, including *clostridium difficile*. To investigate these findings more rigorously, a randomized, blinded pilot trial is underway: PROSPECT (Probiotics to prevent Severe Pneumonia and Endotracheal Colonization Trial), which is evaluating enteral *Lactobacillus rhamnosus* GG versus placebo twice daily, to examine feasibility outcomes.

Objective: The objective is to evaluate the integrity of the probiotics administered in the PROSPECT Pilot Trial. We established a microbiological quality control procedure to evaluate whether the study product corresponds to 10^{10} Colony Forming Units (CFUs) of *Lactobacillus rhamnosus* GG per capsule.

Setting: 7 of 10 medical-surgical ICUs in Canada which participated in this quality control study.

Methods: From each site participating in the PROSPECT Pilot Trial, 1 of every 100 capsules scheduled for administration to patients are sent to the central Microbiome Laboratory of Dr. Surette at McMaster University, Hamilton, Ontario. Protocolized handling and shipping ensures the study product integrity during transportation. When the study product is received at the laboratory, we inoculate de Man, Rogosa and Sharpe (MRS) and Brain Heart Infusion (BHI) agar media with the appropriate dilution. MRS medium is selective for lactic acid bacteria such as *Lactobacillus,* while BHI media is a non-selective nutrient-rich medium. We determine the total CFU per capsule, and compare that to what is prescribed in the PROSPECT Pilot Trial, and to evaluate whether organisms other than *Lactobacillus* are recovered.

Results: To date, 18 probiotic capsules have been tested. Results show that every probiotic capsule contained at least 10^{10} CFU of *Lactobacillus rhamnosus* GG. No other microorganisms were recovered. We observed that the bacterial content of the capsules decreased over time but still remained above the established threshold for many months. Prior to the beginning of the trial in September 2013, 5 probiotic capsules were tested and showed a mean CFU count of 1.92×10^{14} CFU (standard deviation 1.76×10^{14} CFU). Then in June 2014, 5 more capsules were tested, and the counts were still elevated, surpassing the CFU threshold of the prescribe probiotic (10^{13} CFU per capsule). In September 2014, 10 more capsules were tested, yielding a mean count of 1.95×10^{10} CFU (standard deviation 3.76×10^{9}).

Conclusions: The importance of quality control is illustrated by this study, to ensure stable probiotic products over time. Viable organisms remained in amounts above the CFU threshold for over one year, such that dose attenuation was not observed. We identified no contamination. The PROSPECT Pilot Trial capsules tested during this quality control study reflect the doses prescribed by the protocol.

Funding: Hamilton Academic Health Science Organization, Physicians Services Incorporated of Ontario.