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Pitcher's Name	Patricia Eats	**Purpose**	UQAPS Pitching Research Competition 2015 (A51)

(A) Working Title	De-mystifying the dark art of in vitro culture of bovine respiratory tissues
(B) Basic Research Question	Do complex, proprietary-formulated human respiratory tract tissue culture mediums promote superior bovine trachea explant viability in liquid-air interface culture systems?
(C) Key paper(s)	Reed, S.E. & Boyde, A., (1972) 'Organ Cultures of Respiratory Epithelium Infected with Rhinovirus or Parainfluenza Virus Studied in a Scanning Electron Microscope' Niesalla et al.(2009) 'Critical assessment of an in vitro bovine respiratory organ culture system: A model of bovine herpesvirus-1 infection'
(D) Motivation / Puzzle	Successfully developed in vitro models of living, explanted respiratory tract tissues of humans and other mammals have enabled research concerning cystic fibrosis, asthma and bacterial infection. Respiratory tract epithelium models using explant tissue provide the native structural characteristics and biological properties of in vivo tissue, so are the most accurate way to model respiratory diseases. Respiratory illnesses of intensively farmed cattle are significantly detrimental to industry, but published methods for culturing bovine respiratory tract are unreliable. Novel supplements used with success in formulated human respiratory tract culture mediums may improve viability in explanted bovine respiratory epithelium.
THREE	**Three** core aspects of any empirical research project i.e. the "IDioTs" guide
(E) Idea	Lonza laboratory supplies produce an upper and lower respiratory tract growth medium, specifically designed for human respiratory tissues in a liquid/air interface culture system that mimics conditions in the tract. Both growth mediums contain insulin and bovine pituitary extract, which are not present in other commercial mediums. No published research on the effect of inclusion of these novel ingredients is available, and their concentrations within the product are a proprietary secret. Explant culture of bovine respiratory tract may currently be unreliable due to lack of a key factor in the growth medium used. This inadequacy in conventionally used mediums may be the cause of failures of in vitro bovine respiratory tract. The hypothesis is that if Lonza supplemented growth mediums are used in the attempted culture of explant bovine respiratory epithelium, then epithelial ciliary beating will be maintained for longer and tissue necrosis will be delayed, enabling more reliable or longer term culture viability. More meaningful study of pathogenesis, pharmacology and virulence would be enabled. Observations will be made via visual appraisal of tissue under a light microscope. Key observation parameters will be the observation of coordinated ciliary beating motion, and evidence of necrosis. This study will follow successful models of respiratory tract explant to date, using micro-bead particles deposited on the explant surface within the liquid/air interface culture system. Viable cultures are able to fully disperse the beads via the healthy, coordinated beating motion of the cilia, whilst less viable cultures take longer or are unable to clear the micro-bead particles from their surface. The ability to clear micro-beads from the explant surface will be observed in the form of timed response to the stimulus at 12 hour intervals, for ten days following establishment of the explant

	culture. Clearance time is a continuous variable whilst presence of necrosis is a categorical variable. Observation of changed ciliary health is a continuous, qualitative observation parameter for which an appropriate classification scale will be developed. Five medium types will be used: Lonza Upper Respiratory Tract medium, Lonza Lower Respiratory Tract medium, Lonza base medium without bovine pituitary extract and insulin, Life Technologies Minimum Essential Medium with Earles salts and 5% and 10% Bovine Serum, all with added antibiotics and antimycotics (Niesalla et al. 2009). Tissue from all animals and tissue types will be grown in each culture medium.
(F) Data	Tissue explants will be from three respiratory tract sections, obtained within thirty minutes of death from abattoir slaughtered cattle. Tissue types will be trachea epithelium, bronchial bifurcation epithelium and lung lobe slice. Four to six explanted tissue piece replicates of one kind from one animal will be cultured in each culture dish with one medium type. E.g. Animal #001 will have five culture flasks of three tissue types, each with a different medium. All data will be observed and recorded by one technician in a University PC2 laboratory. Interpretation of observations will be standardized for future reference via employment of effective and specific protocols. No research assistance will be required, but funding will be sought for the purchase of air/liquid interface culture lab-ware. Scholarship and travel sponsorship has been sought for additional operator training, to promote a higher likelihood of success with the explant technique. The data collection period is concise, limiting risk of missed observation. Duplicated physical observation records will be stored in different locations and copies will also be entered into electronic spreadsheets, saved in an internal and an external hard-drive. Magnified images of cultures will also be collected during the study for comparative validation and illustration purposes. A good range of variance will be observed between treatment groups, ensuring experimental power adequacy. Lonza and other medium supply companies are multi-national, so no limitation of applicability of results is expected.
(G) Tools	Microscopy is required for the observation of explant tissue viability parameters. The QAAFI laboratories have suitable microscopes and imaging equipment. In the event that additional microscopy services are required, the University of Queensland Microscopy Service is convenient to the QAAFI laboratory, and may be accessed upon subscription. Standard ANOVA and further statistical analysis applications will be performed using existing subscriptions held by the research group.
TWO	**Two** key questions
(H) What's New?	Current emerging research initiatives inquire into the role of pathogen and cell-type specific host microRNA molecules, which are known to play significant roles in pathogenesis, virulence and other molecular cell biology processes. Cultured cell lines for study of disease are often highly reliable and commonly used models, but may produce inaccurate conclusions due to their immortalized

	form and non-native target cell type. Viable explant cultures would enable comparison with cultured cell research results, to define the limitations of bovine respiratory tract infection research undertaken to date.
(I) So What?	Reliable and replicable bovine respiratory tract epithelium models would open new research avenues and increase the accuracy of bovine respiratory infection research undertaken, whilst further reducing, replacing and refining the need for use of animals as models for disease and pharmacology research. It would also enable examination of microRNA factors of the bovine host that are specific to the respiratory tract, which are proposed to have huge influence in susceptibility to pathogens. This may inform about the potential to genetically select animals on the basis of resistance to infection and subsequently limit respiratory infection in cattle industry. Respiratory tract explant cultures could also be used in studies of pathogenesis factors and virulence, enabling improved knowledge of molecular biology factors of pathogens and thereby facilitate novel vaccine development.
ONE	**One** bottom line
(J) Contribution?	Knowledge of any beneficial effect of bovine pituitary extract and insulin in medium on the viability, reliability and replicability of bovine respiratory tract explant cultures.
(K) Other Considerations	Collaboration with the Queensland Brain Institute will enable access to microtome equipment which is capable of precision slicing of the lung tissue. This will confer improved replicability of lung lobe culture replication as a function of standardizing slice characteristics. Additional standardization and reliability of techniques used would be ensured by collaboration and the undertaking of a training course with the European Collection of Cultured Cells. Scholarships and travel grants have been sought for this purpose. Findings of this research will be submitted to the Journal of Virological Methods for publication. The low-level risks associated with the study include the risk that all explant cultures fail to be viable due to issues including contamination, potential differences associated with growing explant tissue in the presence of antibiotic/antimycotic, and a small risk that the individual animals sampled are not representative of the entire population and results are not repeatable on that basis. Animals from which samples of respiratory tract are obtained is assumed to provide a randomized sample of breed, genetic variation, gender. Animal ethics clearance is not required for the study, as tissue samples will be obtained as a by-product from animals slaughtered within a commercial abattoir facility.

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