143 Volatile organic compound analysis in the early detection of *Pseudomonas aeruginosa* in cystic fibrosis

S. Smith[1], C.S. Probert[2], M. Williams[3], D.G. Downey[3]. [1]*University of West of England, Bristol, United Kingdom;* [2]*University of Bristol, Bristol, United Kingdom;* [3]*Adult CF Centre, Bristol Royal Infirmary, Bristol, United Kingdom*

Background: Infection with *Pseudomonas aeruginosa* (PA) is a major cause of morbidity and mortality in patients with cystic fibrosis (CF). We have investigated emission of volatile organic compounds (VOCs) from sputum, saliva and bacterial cultures in order to identify VOCs associated with PA.
Methods: Fifteen samples of sputum and saliva from stable patients with CF were studied; 11 had PA. Sputum and saliva samples, 0.2–1 ml, were decanted in to septum topped headspace vials and analysed on the day of collection. VOC content was measured using solid phase micro-extraction fibres (SPME) and gas chromatography mass spectrometry (GCMS), following incubation for 30 mins at 60ºC and SPME headspace extraction for 20 mins. Sputum samples were also cultured on blood agar for 3 days. Following incubation, samples were allowed to equilibrate at 22ºC for 1 hr prior to headspace SPME extraction for 1 hr. VOCs were assigned an identification using Perkin Elmer Turbomass proprietary software and NIST 2005 mass spectral database.
Results: VOCs from sputum of patients with PA were similar to those of patients without, except for 2-Butanone which was present in 9/11 PA patients and 1/4 patients without PA. VOCs emitted from culture and saliva samples were similar, irrespective of the presence of PA.
Discussion: The point of care recognition of PA in the sputum of patients with CF has the potential to influence clinical care. Our initial results suggest that 2-butanone would yield a sensitivity of 82% and a specificity of 75% for PA. This marker may be identifiable from fresh samples using a sensor device. This small study needs further work.
Supported by: A grant from the David Telling Charitable Trust.

144 Comparison of the sensitivity of six PCR formats for the detection of *P. aeruginosa*

P. Deschaght[1], T. De Baere[1], L. Van Simaey[1], S. Roels[1], P. Schelstraete[2], S. Van Daele[2], F. De Baets[2], M. Vaneechoutte[1]. [1]*Laboratory Bacteriology Research (LBR), University Ghent, Ghent, Belgium;* [2]*Cystic Fibrosis Center, Ghent University Hospital, Ghent, Belgium*

Introduction: Colonization of the airways of CF patients with *Pseudomonas aeruginosa* results in faster decline of the lung function and decreased life expectancy. Detection of infection with this pathogen as early as possible enables to start the eradication treatment as soon as possible.
Aim: To compare the sensitivity of six PCR formats for a dilution series of *P. aeruginosa* in sputum.
Materials and Methods: Sputa from CF patients positive for *P. aeruginosa* were liquefied by adding v/v Sputasol and were 1/5 serially diluted in a pool of liquefied sputa from CF patients negative for *P. aeruginosa*.
DNA was extracted with the bioMérieux easyMAG Nuclisens extractor. Conventional PCR was performed using the Veriti 96-Well Thermal Cycler, with *oprL* as the target, followed by capillary resp. agarose gel electrophoresis. Different real-time PCR formats on the LightCycler 1.5, i.e. SYBR Green I, HybProbes and Taqman probe, were compared with the TaqMan® *P. aeruginosa* detection kit on the ABI7000.
Results: For conventional PCR, *P. aeruginosa* could be detected up to dilution 6 using agarose gel electrophoresis and up to dilution 7 with capillary electrophoresis. For real-time PCR, *P. aeruginosa* could be detected with SYBR Green I up to dilution 7 and up to dilution 8, i.e. up to 20–40 cfu/ml, with the HybProbes, TaqMan probe and the TaqMan® *P. aeruginosa* detection kit.
Conclusion: Real-time PCR, using the HybProbes, the TaqMan probe or the TaqMan® *P. aeruginosa* detection kit is 5–25 fold more sensitive for the detection of *P. aeruginosa* than conventional PCR and real-time PCR using SYBR Green I.

145 Early detection of *Pseudomonas aeruginosa* (PA) in patients with cystic fibrosis (CF) by real-time PCR (RT-PCR): original method developed for a preliminary study

G. Héry-Arnaud[1,2], R. Le Berre[2], M.L. Abalain[1,2], J. Hardy[2], S. Gouriou[2], C. Payan[1,2]. [1]*Département de Microbiologie, CHU, Brest, France;* [2]*LUBEM, Faculté de Médecine, Brest, France*

Background: Early detection of PA, the most threatening pathogen in CF patients, is crucial. To date, there is no agreement about the usefulness of PCR in routine probably due to the lack of cohort studies.
Objective: To establish an optimised protocol for the accurate molecular detection of PA directly from CF sputum.
Methods: The reliability of four widely used DNA targets (*opr*L, *opr*I, *ecf* X and *rrl*) was evaluated: 1) On a set of 30 PA isolates, and on 14 different *Pseudomonaceae strains* (except PA) commonly found in CF patients 2) On 154 sputum samples from 95 CF patients.
Results: The *opr*L PCR showed the best specificity (100%) and sensitivity (100%). RT-PCR produced a linear quantitative detection range of 8 logs (r = 0.99857), with a lower detection limit of 10 colony-forming units (CFU)/mL (Ct=38.6±1.33). The standardized Ct and fluorescence of the DICO Extra r-gene detected inhibitors for 18.5% of clinical samples.
Discussion: It is the first time that DICO Extra r-gene has been used as an internal control for the PA molecular detection in CF sputum. This universal control prevents defaults in extraction process and provides accurate detection of inhibitors. With a lower detection limit of 10 CFU/mL as opposed to 100 CFU/mL which is usually found with culture, this study encourages the use of molecular screening for PA in CF patients.
Prospects: The development of this original protocol was the prerequisite of a multicenter prospective study, which began in September 2008 and is running for 3 years in 3 centres (CRCM Roscoff, CRCM Nantes, and CHU Brest) specialized in the management of CF patients (n = 310).
Supported by: Vaincre la Mucoviscidose, DIRC Grand Ouest.

146* Paranasal sinuses are a focus for *P. aeruginosa* (PA) lung infection in CF

H.K. Johansen[1], K. Aanaes[2], T. Pressler[3], S.K. Hansen[4], M. Skov[3], K.G. Nielsen[3], S. Molin[4], N. Høiby[1], C. Buchwald[2]. [1]*Dept. Clin. Microbiol., Rigshospitalet, Copenhagen, Denmark;* [2]*Dept. Otolaryngol., Rigshospitalet, Copenhagen, Denmark;* [3]*Cph. CF Centre, Rigshospitalet, Copenhagen, Denmark;* [4]*Biomed. Microbiol., DTU, Lyngby, Denmark*

Chronic sinusitis is a common complication in CF patients. It has been shown that patients who have had a lung transplant are recolonised in their lung grafts with the same clones as those that were cultured from their old lungs. We therefore hypothesised that the sinuses may serve as a place for bacterial adaptation and focus for lung infections.
We treated 47 CF patients (median age 16 years, range 6–50) for chronic sinusitis with Functional Endoscopic Sinus Surgery (FESS). We evaluated whether their sinuses serve as bacterial reservoir. Nineteen patients were chronically infected (14 with PA, 2 with Burkholderia, 2 with Achromobacter, 1 with Moraxella) and 28 were intermittently colonised (17 with PA and 11 with other CF pathogens) in their lungs.
In chronically infected patients there was complete agreement between lung and sinus bacteriology and genotypes. In children intermittently colonised with PA there was agreement in 15/17 (88%) cases. After FESS 5/17 (30%) of these children are still free from PA in their lungs (median 219 days, range 42–431). PA were organised in biofilm structures similar to those seen in sputum. In contrast to biofilms from the lungs, we found that biofilms from the sinuses were only surrounded by few inflammatory cells. The sinus colonisation therefore seems immunologically silent. In intermittently colonised patients PA are most often cultured from throat flora and not from lower respiratory secretions and anti-PA antibodies are not increased. That is why we think that the paranasal sinuses are the focus for PA infection and that the lungs are infected by aspiration.