

## Upcoming event

### Virology SIG Meeting

Date: Monday 15<sup>th</sup>  
September 2008

Time: 6.30pm Light dinner;  
7.00pm Talks

Venue: Edmund Blackett  
Building Function Room,  
Prince of Wales Hospital

Contact: Gillian Scott (02)  
9382 9096 or email  
[Gillian.Scott@sesiahs.health.nsw.gov.au](mailto:Gillian.Scott@sesiahs.health.nsw.gov.au)

Please note the following  
deadline for submissions to  
Syntrophy Volume 9:8:2008  
**closes 18<sup>th</sup> September  
2008**. Email all contributions,  
as well as any suggestions or  
comments, to the  
Administrative Officer, Natasha  
Pavic, at  
[natashapavic@hotmail.com](mailto:natashapavic@hotmail.com).

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articles.

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## From the Editor

Welcome to our new 'financial year'! The AGM is over and we have lost two and gained one new committee member for the coming year. Thank you again to Sacha and Shona who have been great and welcome William Crozier who has returned to the Committee. Previously on the Committee in the 90's...not so long ago really (my opinion!).

We can now announce a dual award of the James Vincent Scholarship for 2008 to Nathan Saul and to Nicholas Scott, both from The University of Sydney. Well done to you both.

Our focus article this week is by BD award winner Anna Lau covering a new platform for molecular detection of fungal pathogens by a new multiplex PCR format. Of note also is Anna's other interests and a strong family connection!

Reports from recent events include Christmas in July (the 'real one' isn't that far away either) with the ACT's own TGA man Dr Gary Grohmann heading north (report by Sacha), a Clinical SIG meeting at Royal North Shore (good science and interesting presentations.. and food and wine) (report by Tom), more Virology on CMV by Prof Chou (report by Jenna), our own AGM with Dr Whitchurch (ex-Victorian) presenting a great talk on Biofilms (report by Peter). Thanks for the reports and photos and keep up the excellent work all you SIGs.

Don't forget the Sydney University talks on a Thursday afternoon (Dr Tim Newsome) and September and October has plenty more talks and topics and functions for all to keep that calendar jam-packed

and full of interesting items.

Our generous sponsors again have some new products and one has changed names to Pharmaceutical & Medical Professionals.... nothing stands still for long in Microbiology!

Microbes in the News... how many can we dredge up... unbelievable diversity and interest. I hope something grabs someone's attention... hey when is grant time? I've got a few ideas!

Cheers, Ian Carter

## Focus *MT-PCR: New Diagnostic Platforms for Invasive Fungal Infections* **By Anna Lau**

The frequency of invasive fungal infections (IFIs) has increased in recent years as a result of medical advances and the rising number of immunocompromised patients. Disease-associated morbidity and mortality remains high despite developments in antifungal therapy. Rapid and accurate identification is important for patient management, given the increasing incidence of antifungal-resistance in yeasts and moulds, including *Candida glabrata*, non-*fumigatus*

*Aspergillus* spp. and the Zygomycetes. Diagnosis, however, is often delayed because the 'gold standard' practices of histopathology and culture are insensitive, non-specific and slow.

To overcome these limitations, numerous molecular assays have been developed to improve the speed and accuracy of fungal identification. However, routine implementation of these tests into diagnostic microbiology laboratories has been poor due to

difficulties in performing the assay or the need for sophisticated, expensive equipment (as in the case of microarray platforms). In this study, we aim to develop new diagnostic platforms using multiplex tandem PCR (MT-PCR) technology (2) to provide simple, rapid, real-time identification of numerous pathogens (up to 72 targets) directly from clinical specimens. MT-PCR utilises a short-cycle, multiplex amplification followed by multiple,

*Continued on page 7*

## Focus continued

simultaneous single-plex PCR reactions. Results are available within 2 h and the process can be automated.

To date, four MT-PCR assays targeting 16 yeasts and 13 moulds (including eight Zygomycetes) have been developed to identify pathogens directly from blood culture, EDTA whole blood, tissue and fluid specimens, and culture plates. Assay sensitivity was 10 cfu/ml blood and specificity was maintained against >300 negative control samples, including DNA from 49 clinically relevant microorganisms.

Seventy-seven blood culture specimens from 49 patients that flagged positive and were found to contain yeast by Gram stain were tested. MT-PCR results were concordant with culture for 47 (96%) patients (1). For the remaining two patients, no MT-PCR result was achieved due to the absence of detection targets for *Candida lambica*, *Candida nivariensis* and *Kodamaea ohmeri*. The latter two species were initially misidentified biochemically as *Candida glabrata* and *Candida guilliermondii*, respectively.

Blood cultures require  $\geq 72$  h for fungal detection and identification and are negative in up to 60% of cases. To determine whether the time to diagnosis could be expedited, we applied MT-PCR directly to EDTA blood from patients with proven candidemia. A total of 123 EDTA specimens were drawn before blood culture positivity from 42 patients. Of these, MT-PCR was positive for 32 (76%) patients. Unexplained discrepancies in species identification occurred in 3 (7%) patients. MT-PCR demonstrated its potential as a screening tool for high risk patients, detecting fungaemia up to four days before positive blood culture. The assay is currently being assessed in a prospective study comprising critically ill and haematology patients.

MT-PCR also proved useful for the rapid identification of fungi directly from culture. Correct identification was made for all 183 cultures studied without the need for DNA extraction (submitted). Colony MT-PCR reduced time to identification to <2h, significantly faster than biochemical identification, which requires a pure culture and an additional 4-48 h to reach biochemical endpoints.

With the growing number of organisms being 'genetically' reclassified and the limitations of traditional phenotypic tests, fungal identification using molecular methods has become increasingly important. Implementation of MT-PCR into routine diagnostic laboratories has the advantage of accurate and simultaneous identification of microbial infections, leading to prompt initiation of targeted therapy and better clinical outcomes. Cost per assay is ~\$18 plus 30 minutes labour. Work on including targets for antifungal resistance detection is underway.

**Anna would like to thank Prof. Tania Sorrell, Dr Catriona Halliday, Dr Sharon Chen and Dr Jon Iredell, all from Westmead Hospital and Prof. Keith Stanley from AustDiagnosics for their valuable contribution to this study.**

### References

1. Lau, A., T. C. Sorrell, S. Chen, K. Stanley, J. Iredell, and C. Halliday. 2008. Multiplex-Tandem PCR: A Novel Platform for the Rapid Detection and Identification of Fungal Pathogens from Blood Culture Specimens. *J. Clin. Microbiol.* (in press)
2. Stanley, K. K., and E. Szewczuk. 2005. Multiplexed tandem PCR: gene profiling from small amounts of RNA using SYBR Green detection. *Nucleic. Acids. Res.* **33**:e180.

### About the author

Anna is a 3<sup>rd</sup> year PhD student at the Centre for Infectious Diseases and Microbiology, University of Sydney. Her research is supervised by Prof. Tania Sorrell and Dr Catriona Halliday. In her spare time, she performs gigs with the 4 Seasons Quartet and Balmain Sinfonia and loves to karaoke, dance and play ping pong.

## \*\*\*BRANCH AWARD WINNERS ANNOUNCEMENT

**James Vincent Scholarship 2008:  
Nicholas Scott and Nathan Saul,  
The University of Sydney**

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