Enhanced Patient Serum Immunoreactivity to Recombinant Mycobacterium tuberculosis CFP32 Produced in the Yeast Pichia Pastoris Compared to *Escherichia coli* and Its Potential for Serodiagnosis of Tuberculosis

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CFP32 is a Mycobacterium tuberculosis complex-restricted secreted protein that was previously reported to be present in a majority of sputum samples from patients with active tuberculosis (TB) and to stimulate serum antibody production. CFP32 (annotated as Rv0577) was therefore considered a good candidate target antigen for the rapid serodiagnosis of TB. However, the maximal sensitivity of CFP32 serorecognition may have been limited in earlier studies because recombinant CFP32 (rCFP32) produced in *Escherichia coli* was used as the test antibody-capture antigen, a potential shortcoming stemming from differences in bacterial protein posttranslational modifications. To further investigate the serodiagnostic potential of rCFP32 synthesized in different heterologous hosts, we expressed rCFP32 in the yeast Pichia pastoris. Compared to E. coli rCFP32, yeast rCFP32 showed a higher capacity to capture polyclonal antisera in Western blot studies. Likewise, yeast rCFP32 was significantly better recognized by sera from TB patients, in enzyme-linked immunosorbent assay (ELISA), than *E. coli* rCFP32. In subsequent testing, the yeast rCFP32-based antibody-capture ELISA had a sensitivity of 85% and a specificity of 98% for the discrimination of active TB cases ($n = 40$) from BCG vaccinees ($n = 39$). The sensitivity was surprisingly high for a single-antigen TB serodiagnostic test compared to tests using *E. coli*-expressed antigens. Overall, the trans-production of rCFP32 in *P. pastoris* significantly improved the serologic detection of CFP32-specific antibodies in patient sera, thereby offering a new, possibly better, modality for producing antigens of diagnostic potential for use in the development of immunoassays for both TB and other infectious diseases (International patent request CA 2,551,537 of July 3rd, 2007).

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One year experience of APTIMA COMBO 2 Transcription Mediated Assay (TMA) for detection of Chlamydia trachomatis and *Neisseria* gonorrhoea in a large private pathology laboratory in Queensland

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Between June 2005 and July 2006, a total of 90898 samples including urine and non urine specimens were processed using the Aptima Combo 2 Assay (AC2) which has the ability to detect both Chlamydia trachomatis (CT) and Neisseria gonorrhoea (GC) in the same sample

tal specimens. CT was also detected in 69 tampon samples (12%), 34 Thin Prep samples (3.2%), 30 eye swabs (5.2%), 30 rectal swabs (8.7%), 14 throat swabs (1.4%) and 1 seminal fluid (1.1%).

Overall GC was detected in 0.7% of samples. GC was detected in 0.6% of urines specimens and 0.45% of genital swabs. GC was detected in 50 throat swabs (5.1%), 41 rectal swabs (12%), 11 tampons (1.9%) and 3 eye swabs (0.5%). Dual infections with CT and GC were detected in 0.7% of all samples. Overall prevalence of GC by culture was 0.3%. None had a positive GC culture and a negative AC2 assay result. 21% of specimens positive for GC by AC2 did not have a formal request for GC by AC2 (GCNR: GC Not Requested). 29% GCNR GC positive specimens by AC2 did not have a simultaneous culture request. 18% GCNR GC positive specimen had concurrent CT infection

Conclusions: CT and GC were detected by AC2 assay in a wide range of sample types. AC2 was more sensitive than culture for detection of GC. The ability of the assay to detect both CT and GC in one sample has emphasised the importance of testing for dual infections and enabled the un-requested GC to be detected. The data suggests that clinicians are opting for molecular- based testing for GC rather than culture- based methods.

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Application Molecular Methods on *Escherichia coli* and *Ornithobacterium rhinotracheale* Infectious in Commercial Flocks of Southern Khorasan Province in Iran

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Introduction: Ornithobacterium rhinotracheale (ORT) is a pleomorphic gram-negative Rod-shaped bacterium that has been isolated from chickens manifesting severe respiratory problems in many countries. ORT can be associated with high economic losses in poultry. Only 10 to 15% of *Escherichia coli* (*E. Coli*) intestinal coliforms are pathogenic (APEC). The aims of this study were the isolation of *Ornithobacterium rhinotracheale* and *Escherichia coli* from poultry and identification of the isolates by Polymerase chain reaction (PCR) for ORT and serology for *E. Coli*.

Materials: Samples. In this study Samples collected from lung and trachea from 13 commercially chicken flocks showing respiratory disease symptoms were pooled, homogenized and stored at -80 °C until required.

Bacteriology.

ORT: Samples were aseptically inoculated on blood agar supplemented with 7% sheep blood and 10 μg/ml gentamicin (to inhibit growth of other bacteria) to isolate and identify the causative bacteria from lung and trachea samples by routine both culture and PCR. The plates were incu-