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Verification of the BioFire FilmArray Pneumonia Plus Panel for pathogen screening of respiratory specimens

Sir,

Pneumonia-like illnesses cause significant morbidity and mortality in the hospital and community setting.¹ The most common aetiological agents, bacteria (intracellular or extracellular) and viruses, often exhibit similar clinical presentations. Empiric broad spectrum antimicrobial therapy is recommended for suspected bacterial infections; however, it is unnecessary in many cases where the aetiological agent is

proven to be viral, contributing to its overuse. Rapid laboratory diagnostic testing, as such, forms a critical component of the clinical response to lower respiratory tract infections (LRTIs), as well as an effective antimicrobial stewardship strategy. Pathogen identification by culture-based methods and antimicrobial susceptibility testing can take 48–72 hours.² Ancillary specific rapid diagnostic methods have been developed for some of the most common aetiological agents of LRTIs; however, these may be ineffective in diagnosing infections with a complex aetiology.

Rapid multiplex polymerase chain reaction (PCR) panels that detect a range of aetiological agents and the most common antimicrobial resistance targets have emerged to meet this clinical gap.³ One system that has recently been approved is the BioFire FilmArray Pneumonia Plus (PNplus) Panel (bioMérieux, France). This assay incorporates automated testing for 18 bacteria (11 Gram-negative, 4 Gram-positive, 3 atypical) and nine viruses that cause LRTIs as well as seven common antibiotic resistance markers.⁴ Validation studies in various settings are emerging, revealing high sensitivity (91–98%) and specificity (76–87%) for targets available for testing.^{5,6}

This study aimed to assess the accuracy of the PNplus as part of a verification process at the Nepean Microbiology Laboratory, Penrith, Australia, which provides microbiology diagnostic services to the Nepean Blue Mountains Local Health District. Sputum and bronchoalveolar (BAL) samples were selected from inpatients who had organisms or antimicrobial resistance genes of interest identified by National Associating of Testing Authorities Australia (NATA) approved conventional techniques. In the case of rarely encountered viral and atypical targets, previously stored sputum and BAL frozen specimens in normal saline were used. For rarely encountered bacterial and antimicrobial resistance targets, a single colony of interest was inoculated into an inpatient specimen which had been processed via conventional methods and had no pathogens isolated.

Sputum and BAL samples from hospital inpatients were processed ideally within 2 hours of collection. Samples were inoculated onto 5% horse blood agar, chocolate agar, Brilliance UTI Clarity agar (ThermoScientific, Australia) and incubated at 37°C on 5% CO₂. Plates were examined at 24 and 48 hours for bacterial growth. Predominant pathogens were searched for and identified using matrix assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Germany). Results were considered negative if there was no significant growth or a non-pathogenic normal respiratory flora was isolated. Susceptibility testing was performed by VITEK 2 (bioMérieux, France) and/or disk diffusion methods as dictated by the Clinical and Laboratory Standards Institute (CLSI) guidelines. Etest (bioMérieux, France) was used as a substitute for broth microdilution and CLSI guidelines were used to interpret results. Phenotypic susceptibility patterns were reviewed, and if consistent with multi-drug resistance, cultured isolates underwent additional testing using in-house PCRs performed on the Rotor-Gene 3000 or Rotor-Gene Q (Qiagen, Germany). All *Staphylococcus* were PCR tested for the presence of *mec* and *nuc* genes using primers and probes adapted from previous reports.^{7,8} CTX-M and IMP were detected in Enterobacteriaceae using an assay adapted from previous reports.^{9,10} The GeneXpert Carba-R (Cepheid, USA) was used for detection of NDM.

For viral pathogens and atypical bacterial, AusDiagnostics Respiratory Pathogens B (16 well) and AusDiagnostics Atypical Pneumonia (8 well) (AusDiagnostics, Australia) were used, respectively. Positive or negative results were recorded, and Ct values for samples where a target was detected were detailed and compared.

Prepared PNplus pouches were loaded onto the BioFire FilmArray 2.0 or BioFire FilmArray Torch for nucleic acid extraction, PCR amplification and analysis. Organism identification was recorded for conventional methods and PNplus. Discordant results were investigated by repeating conventional microbiology methods and both results were documented. In cases where bacterial organisms included on the panel were identified via conventional methods and not on the PNplus, isolates were referred to a reference laboratory for 16S rRNA identification.

Thirty-nine specimens were included in this evaluation, with 27 sputum samples and 12 BAL samples assessed. Of these, four (2 sputum and 2 BAL samples; 10.2%) had no organisms detected on the PNplus. Single pathogen types were detected in 26/39 (66.7%) specimens, divided between bacterial (17/39; 43.6%), viral (6/39; 15.4%), and atypical bacterial (3/39; 7.7%) agents. Infections with >1 organism were also detected including either bacteria and viruses (7/39; 17.9%) or atypical bacteria and bacteria (1/39; 2.6%). PNplus revealed that BAL samples were more likely to be mono-microbial, while sputum samples were more likely poly-microbial (Fig. 1).

PNplus detection of microorganisms (bacteria and viruses) in BAL and sputum samples are presented in Table 1. The PNplus detected 83 pathogens in 35/39 samples (89.8%), including bacteria (53/83; 63.8%), viruses (24/83; 28.9%) and atypical bacteria (6/83; 7.2%). The most common pathogens detected, included *Staphylococcus aureus* (11), *Haemophilus influenzae* (7), *Pseudomonas aeruginosa* (5), *Streptococcus pneumoniae* (6), parainfluenza (5) and rhinovirus/enterovirus (7). Even numbers of atypical bacteria were detected (2) including *Chlamydia pneumoniae*, *Legionella pneumophila* and *Mycoplasma pneumoniae*. Five resistance genes were detected across seven samples, including NDM (1), CTX-M (2), IMP (2), *mecA/C* and MREJ (2).

Culturing detected bacterial pathogens in 17 of 22 samples (77.3%) where bacteria were detected. Comparative screening for viral pathogens and atypical bacteria with the AusDiagnostics PCRs detected 10/13 (76.9%) and 6/6

(100%) positive samples, respectively. The most frequent bacteria detected by culture were again *S. aureus* (7) and *P. aeruginosa* (5). Similar to the PNplus results, rhinovirus/enterovirus (7) and parainfluenza (5) were the most common, with several cases of adenovirus (4) also noted. Identical types of atypical bacteria and antibiotic resistance markers were detected by the in-house comparison PCRs to the PNplus results.

Organisms with higher copies per mL as determined by PNplus ($>10^7$ or 10^6) had a higher association with growth and identification using conventional methods, than those with lower copies per mL (10^5 and 10^4). Sputum samples with $>10^7$ or 10^6 copies per mL were concordant with conventional methods in 21/28 cases (75.0%), while sputum samples with 10^5 and 10^4 were concordant in only 1/9 cases (11.1%). All discrepant cases had more than one organism identified on the PNplus. For the BAL samples, 4/7 (57.1%) with $>10^7$ or 10^6 copies per mL calculated on the PNplus were concordant with conventional methods, whilst 1/8 (12.5%) were concordant with 10^5 and 10^4 copies per mL. Only one sample not identified via conventional methods in the high colony count BAL group was determined to be monomicrobial on the PNplus (data not shown).

Comparing the PNplus to conventional methods, one or more bacteria were detected by PNplus in 81.8% of specimens (18/22), with only 41.2% (7/17) concordance observed using conventional methods in sputum sample analysis, and 20.0% (1/5) complete concordance in BAL samples for the identification of all bacteria detected on the PNplus. One hundred percent agreement was observed for specimens containing *P. aeruginosa* (5/5), *Serratia marcescens* (3/3), and *Acinetobacter baumannii* (1/1). The most discordant results were observed for observed for *H. influenzae* (7:1 PNplus:conventional), *S. pneumoniae* (6:2), *S. aureus* (11:7) and *Enterobacter cloacae* complex (3:1). Two samples where an organism was available on the PNplus were detected by conventional methods, but not by the former method. The first was a sputum sample where *P. aeruginosa* and *E. cloacae* complex were detected on the PNplus; however, *P. aeruginosa* and *Klebsiella aerogenes* were identified using conventional methods, and the latter was confirmed using 16S rRNA sequencing. A BAL sample also initially demonstrated discrepant results, whereby *H. influenzae* was identified on the PNplus; however, *S. pneumoniae* was identified using conventional phenotypic methods. This

Fig. 1 A comparison of the number of agents detected in sputum and BAL specimens via the FilmArray Pneumonia Plus Panel or conventional methods.

Table 1 Comparison of the results of BioFire Pneumonia Plus panel screening of respiratory samples (n=39) versus results derived from conventional microbiology and in-house PCR screening

Target	Species	BioFire PN Plus, n	Conventional microbiology, n	In-house PCRs, n
Bacteria	<i>Acinetobacter calcoaceticus</i> — <i>baumanii</i> complex	1	1	—
	<i>Enterobacter cloacae</i> complex	3	1	—
	<i>Escherichia coli</i>	4	2	—
	<i>Haemophilus influenzae</i>	7	1	—
	<i>Klebsiella aerogenes</i>	1	2	—
	<i>Klebsiella oxytoca</i>	2	1	—
	<i>Klebsiella pneumoniae</i> group	2	1	—
	<i>Moraxella catarrhalis</i>	2	1	—
	<i>Proteus</i> spp.	2	1	—
	<i>Pseudomonas aeruginosa</i>	5	5	—
	<i>Serratia marcescens</i>	3	3	—
	<i>Staphylococcus aureus</i>	11	7	—
	<i>Streptococcus agalactiae</i>	3	2	—
	<i>Streptococcus pneumoniae</i>	6	2	—
	<i>Streptococcus pyogenes</i>	1	1	—
	<i>Streptococcus mitis/oralis</i> ^a	0	1	—
	<i>Streptococcus pseudopneumoniae</i> ^a	0	1	—
	<i>Stenotrophomonas maltophilia</i> ^a	0	1	—
	<i>Citrobacter koseri</i> ^a	0	1	—
	<i>Candida albicans</i> ^a	0	2	—
	<i>Candida dublenesis</i> ^a	0	1	—
Virus	Adenovirus	2	—	4
	Coronavirus	1	—	0
	Human metapneumovirus	2	—	2
	Influenza A	3	—	3
	Influenza B	2	—	2
	Parainfluenza	5	—	5
	Rhinovirus/enterovirus	7	—	7
	Respiratory syncytial virus	2	—	2
Atypical bacteria	<i>Legionella pneumoniae</i>	2	—	2
	<i>Chlamydia pneumoniae</i>	2	—	2
	<i>Mycoplasma pneumoniae</i>	2	—	2
Resistance genes	NDM	1	—	1
	CTX-M	2	—	2
	IMP	2	—	1
	<i>mecA/C</i> and MREJ	2	—	0

^a Organism not on PN Plus Panel.

organism was subsequently confirmed to be *Streptococcus pseudopneumoniae* using 16S rRNA sequencing.

Concordance between the PNplus and the comparison antibiotic resistance gene PCR assays was 57.1%. Discrepant results included: (i) one sample where IMP was detected by PNplus, however, no organism was identified via conventional methods; and (ii) two samples where *S. aureus mecA/C* and MREJ were identified by PNplus but were missed by the in-house PCR of *S. aureus* cultures isolated. For virus detection, there was a high concordance (88.6%) between PNplus and other PCR panels with the following exceptions: (i) a coronavirus (synthetic control sample) identified on the PNplus, which is not available on the AusDiagnostic panel; (ii) two BAL samples, positive for adenovirus on the AusDiagnostics Panel but negative on the PNplus. The latter samples containing adenovirus had Ct values that were much higher (32.51 and 30.28) than those identified using both methods (20.12 and 18.5).

Our comparison of routine microbiological methods used for detection of respiratory pathogens in sputum and BAL samples to the PNplus suggests that the BioFire assay offers improved detection for common respiratory bacterial pathogens (e.g., *H. influenzae*, *S. pneumoniae*, *S. aureus*). The detection of high bacterial loads appeared to correlate with the growth of the respiratory specimens on agar plates. Less agreement was observed between PNplus detection and

organisms that were cultured at only low levels on agar plates; however, the clinical relevance of the latter groups of organisms needs to be carefully considered as the low level of growth may be indicative of only colonisation and not infection. Fastidious (*H. influenzae* and *S. pneumoniae*) and antibiotic-affected organisms may also explain lack of concordance. A comparison of the PNplus to other PCR-based systems in our laboratory found similar sensitivity for the former system in detecting viruses, atypical bacteria and antibiotic resistance markers. Our study is limited by its modest sample size, the inability to confirm presence or absence of bacteria via a conventional molecular method rather than culture only and a lack of information on whether patients were treated with antibiotics, the latter potentially contributing to the lack of concordance in sputum samples with a low PNplus Ct value.

Alongside the improvement to the turnaround time for detection of respiratory pathogens that have recently reported for the PNplus,¹¹ it is anticipated that this culture-independent assay may improve the diagnosis and antimicrobial prescription practices of clinicians treating patients with serious LRTIs.

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Cobalamin (vitamin B12) testing in a tertiary neurology service: a review of current practice

Sir,

Vitamin B12 or cobalamin is a water soluble vitamin important for optimal functioning of the neurological system. Cobalamin has an essential role as a coenzyme for cytosolic methionine synthase and mitochondrial methylmalonyl-CoA mutase, crucial for biochemical processes such as myelin synthesis, nerve metabolism and neuronal regeneration.^{1,2} Mechanisms of deficiency are multifactorial and include inadequate intake (e.g., dietary/nutritional), malabsorption conditions, or defects in the cellular processing and transport

of B12.^{1,3} Neurological symptoms may range from sensory complaints through to neuropsychiatric complaints such as delirium and cognitive impairment.^{2–4}

Total serum B12 is a commonly used screening method for cobalamin deficiency; however, it lacks both sensitivity and specificity as a standalone marker.^{1,5} Around 80% of serum B12 is bound to the protein transporter haptocorrin leaving it unavailable for cellular uptake, and therefore not representative of cellular levels of the vitamin.¹ Furthermore, serum B12 levels may not correlate with the severity of clinical findings; neurological symptoms may be out of proportion to the degree of metabolic deficiency.^{1,3}

Diagnosis of vitamin B12 deficiency should ideally utilise at least two biomarkers.^{1,5} Examples include holotranscobalamin (holoTC), methylmalonic acid (MMA), and homocysteine (Hcy). HoloTC represents the complex of vitamin B12 bound to transcobalamin that is delivered to cells and therefore is considered the biologically active proportion of total B12. Low levels of holoTC result in accumulation of MMA and Hcy in the serum. Anti-intrinsic factor and anti-gastric parietal cell antibodies may be requested as follow-up testing for vitamin B12 deficiency to support the diagnosis of pernicious anaemia.

We present an audit that reviewed the clinical use of vitamin B12 levels by a tertiary neurology service. The intention was to review current ordering patterns and to determine if requests were in line with proposed testing guidelines.

A retrospective audit was performed on all vitamin B12 requests from the neurology department at Wellington Hospital between 1 November 2015 and 21 February 2017 (an 81-week period). Wellington Hospital neurology service provides tertiary level care for a population of about 500,000 in central New Zealand. Data were extracted through referrer codes on the laboratory's Triple G Ultra Laboratory information system. Both inpatient and outpatient public hospital requests were included.

Total serum B12 levels were classified as normal if between 170–800 pmol/L, borderline low if between 110–169 pmol/L, and low if less than 110 pmol/L, according to local laboratory guidelines. The Cobas Elecsys Vitamin B12 II assay (Roche, Germany) employs a competitive test principle using intrinsic factor specific for vitamin B12. Vitamin B12 in the sample competes with the added vitamin B12 labelled with biotin for the binding sites on the ruthenium labelled intrinsic factor complex [Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)]. Results are determined via a calibration curve, which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

Requests were categorised according to reported indications (neurological and haematological) for ordering B12 levels.^{1,3–5} The indications were myelopathy, peripheral neuropathy, gait ataxia, optic neuropathy or neuritis, paraesthesia, macrocytosis or anaemia, and neuropsychiatric disorders.

Electronic records including clinical letters and discharge summaries were reviewed to determine the indication for the test. Total serum B12 tests were defined as 'indicated' if they fitted into one of the seven predetermined categories. Test results identified as 'low' or 'borderline low' total B12 were further analysed to determine the presence of haematological abnormalities and whether clinicians undertook further