by SNP CMA, we were able to identify that our patient had no functional copy of the *TP53* gene.

The poor prognosis in this patient was likely driven by *MYC* and *PVT1*-containing double minutes together with low-hypodiploidy and bi-allelic loss of function of *TP53*. These changes could be comprehensively delineated because of the use of multiple complementary genomic techniques. In clinical practice, identification of a comprehensive snapshot genomic changes associated with poor prognosis in multiple myeloma aids in communication of a personalised risk profile allowing the clinical team and patient to make an informed decision regarding treatment.

While IMWG does not specifically recommend SNP CMA, we suggest that subsequent editions of the guidelines include SNP CMA to replace conventional karyotyping for identification of copy number changes across the genome. SNP CMA is the only assay that can accurately detect doubling of a hypodiploid clone; however, it must be used with FISH to detect IGH rearrangements as an efficient and cost-effective way of identifying cytogenetic changes in patients with multiple myeloma. This case study also provides evidence for inclusion of additional genomic changes such as hypodiploidy and *MYC-PVT1* containing double minutes as poor prognostic markers.

Acknowledgements: The first author thanks Professor Jane Dahlstrom of ACT Pathology for mentoring in academic report writing.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

Fiona Webb¹, Dipti Talaular^{1,2,3}

¹Department of Diagnostic Genomics, ACT Pathology, Canberra Health Services, Canberra, ACT, Australia;

²Department of Haematology, ACT Pathology, Canberra Health Services, Canberra, ACT, Australia; ³Australian National University, Canberra, ACT, Australia

Contact Fiona Webb.

E-mail: fiona.webb@act.gov.au

1. Swerdlow SH, Campo E, Harris NL, *et al.* WHO: Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon: IARC, 2017; 585.
2. Sonneveld P, Avet-Loiseau H, Lonial S, *et al.* Treatment of multiple myeloma with high-risk cytogenetics: a consensus of the International Myeloma Working Group. *Blood* 2016; 127: 2955–62.
3. National Pathology Accreditation Advisory Council (NPAAC). *Requirements For Cytogenetic Testing*. Third Edition. Canberra: NPAAC, 2013.
4. Hayes M, Li J. An integrative framework for the identification of double minute chromosomes using next generation sequencing data. *BMC Genet* 2015; 16 (Suppl 2): S1.
5. Huh YO, Tang G, Talwalkar SS, *et al.* Double minute chromosomes in acute myeloid leukemia, myelodysplastic syndromes, and chronic myelomonocytic leukemia are associated with micronuclei, MYC or MLL amplification, and complex karyotype. *Cancer Genet* 2016; 209: 313–20.
6. Yamamoto K, Yakushijin K, Ito M, *et al.* MYC amplification on double minute chromosomes in plasma cell leukemia with double IGH/CCND1 fusion genes. *Cancer Genet* 2020; 242: 35–40.
7. Szabo AG, Gang AO, Pedersen MO, *et al.* Overexpression of c-myc is associated with adverse clinical features and worse overall survival in multiple myeloma. *Leuk Lymphoma* 2016; 57: 2526–34.
8. Schilling G, Hansen T, Shimoni A, *et al.* Impact of genetic abnormalities on survival after allogeneic hematopoietic stem cell transplantation in multiple myeloma. *Leukemia* 2008; 22: 1250–5.

9. Tseng YY, Bagchi A. The PVT1-MYC duet in cancer. *Mol Cell Oncol* 2015; 2: e974467.
10. Van Wier S, Braggio E, Baker A, *et al.* Hypodiploid multiple myeloma is characterized by more aggressive molecular markers than non-hyperdiploid multiple myeloma. *Haematologica* 2013; 98: 1586–92.
11. Morgan Jr RJ, Gonchoroff NJ, Katzmman JA, *et al.* Detection of hypodiploidy using multi-parameter flow cytometric analysis: a prognostic indicator in multiple myeloma. *Am J Hematol* 1989; 30: 195–200.
12. Flynt E, Bisht K, Sridharan V, *et al.* Prognosis, biology, and targeting of TP53 dysregulation in multiple myeloma. *Cells* 2020; 9: 287.

DOI: <https://doi.org/10.1016/j.pathol.2021.03.004>

Evaluation of direct antimicrobial susceptibility testing using positive blood culture broth in an Australian laboratory

Sir,

Sepsis continues to cause significant mortality worldwide, with evidence that early institution of appropriate antibiotic therapy has a mortality benefit.^{1,2} The microbiology laboratory plays an important role in providing timely identification of organisms and their susceptibility profiles to enable tailoring of antimicrobial therapy. Methods that significantly reduce the time to identification of isolates have been shown to reduce the time to effective and optimal antibiotic therapy and contribute to hospital anti-microbial stewardship programs.^{3,4} Using methods described in previous studies we investigated whether using positive blood culture bottles to directly inoculate the Vitek-2 cards to perform susceptibility testing correlated well with traditional methods and enabled significantly faster results.^{5–7}

This study was conducted at the Mater Pathology laboratory in Brisbane, Australia, from July to November 2018. Samples from the Mater public hospital, Mater mothers hospital and the Mater private hospital were included in the study. Blood specimens were incubated in the BacT/Alert 3D automated microbial detection system (bioMérieux, France). After blood culture bottles flagged positive a Gram stain was performed. All blood cultures that flagged positive between 08:00 hour Monday to 16:00 hour on Friday with Gram stains that showed Gram-positive cocci or Gram-negative bacilli were included in the study. Exclusion criteria were: any blood culture bottles that were from the same patient episode with the same organism that had already been included in the study; those that flagged positive outside of the hours stipulated above; those in which the organism identified was not validated for Vitek-2 antimicrobial susceptibility testing; those which showed Gram-positive bacilli or Gram-negative cocci on Gram stain; and those in which more than one organism was cultured (polymicrobial cultures). Identification was performed on isolates from solid growth media primarily using the matrix assisted laser desorption/ionisation mass spectrometry (MALDI-TOF MS) BioTyper (Bruker Daltonics, Germany). Routine susceptibility testing was performed pure growth of isolates on solid media using a Vitek-2 automated system (bioMérieux) according to the manufacturer's instructions for the determination of minimum inhibitory concentration (MIC) values. The values were then categorised according to European Committee for

Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoint version 7.1, 2015, as susceptible (S), intermediate (I) or resistant (R). This method was then compared with the study method ('rapid-direct susceptibility testing') where positive blood culture broth was used to directly inoculate the Vitek-2 cards (bioMérieux) as described by Sakarikou *et al.*⁵ They were then processed using a Vitek-2 automated system (bioMérieux) according to the manufacturer's instructions for the determination of minimum inhibitory concentration (MIC) values. The time taken from when the blood culture bottle flagged positive to when antimicrobial susceptibility results were obtained was recorded and was expressed in hours and minutes.

The Vitek-2 MIC values obtained for each organism-antimicrobial combination were compared between the routine and rapid-direct susceptibility testing methods to assess essential agreement. MIC values were assessed as equal if within one-doubling dilution. The results expressed as $\geq$ or $\leq$ to a value were assumed to be $>$ or $<$ the value for this comparison, so that equality between the MIC values was not over-estimated. The essential agreement between the MIC values for the two methods was expressed as a percent. Categorical agreement was also assessed by comparing the susceptibility category for each organism-antimicrobial combination for both methods. Any differences were classified into the following categories: very major error (VME; false susceptibility using the rapid-direct method), major error (ME; false resistance using the rapid-direct method), or minor error (ME; intermediate versus susceptible or resistant, for either method) and expressed as a percent. For the rapid-direct susceptibility method to be considered equivalent to the routine susceptibility method, it was determined that the essential and categorical agreement should be $\geq 90\%$, with VMEs not exceeding 3%.⁸ The mean time taken from when the blood culture flagged positive to when results were obtained were compared using Student's *t* test with a *p* value of ≤ 0.05 considered significant.

A total of 90 blood culture isolates were screened for inclusion into the study. Of these, 26 were excluded, for the following reasons: eight had no routine susceptibility performed as they were assessed as contaminants, one was a duplicate isolate, for three isolates the rapid-direct Vitek-2 susceptibility testing was terminated, 10 were organisms that were not suitable for Vitek-2 susceptibilities, one had an unavailable Vitek-2 susceptibility report, and three were part of a mixed culture. Therefore, there were 64 isolates from 61 individual patients included in the study that had both rapid-direct and routine Vitek-2 susceptibility results available: 33 Gram-negative and 31 Gram-positive isolates. *Escherichia coli* was the most common Gram-negative, and of the Gram-positive 25 were *Staphylococcus* spp. (including 14 *Staphylococcus aureus*) and six were *Enterococcus* spp. (Table 1).

The results for the Gram-negative organisms are shown in Table 2. Overall essential agreement was 95% and categorical agreement was 98%, with 0.2% VME rate. For the Gram-positive organisms there was a total of 25 antimicrobials tested (14 for *Staphylococcus* spp. isolates and 17 for *Enterococcus* spp.), resulting in 508 isolate-antimicrobial combinations. Overall essential agreement was 87% and categorical agreement was 95%, with an overall VME rate of 2.4%. Essential agreement ranged from 70% to 100% and categorical agreement from 87% to 100%. For *Staphylococcus* spp., VMEs were seen in the cefoxitin screen (4%),

Table 1 Organisms included in the study

Organism identification	Total	%
<i>Aeromonas</i> spp.	1	1.6%
<i>Citrobacter koseri</i>	1	1.6%
<i>Escherichia coli</i>	22	34.4%
<i>Klebsiella pneumoniae</i>	3	4.7%
<i>Morganella morganii</i>	1	1.6%
<i>Pseudomonas aeruginosa</i>	4	6.3%
<i>Stenotrophomonas maltophilia</i>	1	1.6%
<i>Staphylococcus aureus</i>	14	21.9%
<i>Staphylococcus epidermidis</i>	8	12.5%
<i>Staphylococcus capitis</i>	2	3.1%
<i>Staphylococcus warneri</i>	1	1.6%
<i>Enterococcus faecalis</i>	4	6.3%
<i>Enterococcus faecium</i>	2	3.1%
Total	64	

ciprofloxacin (4%), clindamycin (4%), erythromycin (3%), gentamicin (4%), oxacillin (13%) and trimethoprim-sulfamethoxazole (13%). MEs were seen in clindamycin (4%), daptomycin (4%), erythromycin (4%), fusidic-acid (8%), linezolid (4%), rifampicin (4%), teicoplanin (4%), tetracycline (4%), and vancomycin (4%). MEs were also seen with tetracycline (13%).

There were six *Enterococcus* spp. included in the study, with no vancomycin-resistant enterococci. Categorical agreement was 100% for all 17 isolate-antimicrobial combinations. Essential agreement was 100% for all combinations except for three *Enterococcus*-antimicrobial combinations (benzylpenicillin, chloramphenicol and daptomycin).

There were six Gram-negative isolates (5 *E. coli* and 1 *K. pneumoniae*) that were found to have an extended spectrum β -lactamase (ESBL). For these isolates, ceftriaxone and ceftazidime tested as resistant in both the rapid-direct and routine susceptibility testing. There were three methicillin-resistant *S. aureus* (MRSA) isolates, with one isolate demonstrating categorical agreement for the cefoxitin screen and oxacillin. The other two had VMEs. One MRSA isolate was cefoxitin screen negative and had an oxacillin MIC of ≤ 0.25 when tested by the rapid-direct susceptibility testing but had a positive cefoxitin screen and an oxacillin MIC of >4 when tested by the routine method, resulting in two VMEs. For another MRSA isolate there was categorical agreement between the cefoxitin screen (both tested as positive) but the oxacillin MIC was ≤ 0.25 when tested by the rapid-direct susceptibility method, and >4 when tested by the routine method, resulting in one VME. There were no vancomycin-resistant enterococci in the study.

The mean time in hours from when the blood culture flagged positive to when a susceptibility result was available is shown for each of the methods in Fig. 1. Time to result for rapid-direct blood culture susceptibility testing was on average 9 hours faster than routine susceptibility testing (≤ 0.001).

Therefore, the rapid-direct susceptibility method was shown to have acceptable categorical and essential agreement rates, as well as a low enough rate of VMEs in Gram-negative isolates, to be considered accurate enough to be used in the microbiological laboratory. However, categorical and essential agreement rates were found to be too low to recommend using this method on Gram-positive organisms. Of note is the poor performance of the cefoxitin screen (VME rate of 4%) and oxacillin MIC testing (VME rate of 13%) in *Staphylococcus* spp. One of the major advantages

Table 2 Performance of rapid-direct blood culture susceptibility testing compared to routine method for Gram-negative bacilli (GNB) isolates

Antimicrobial agent	Organisms, <i>n</i>	Essential agreement, %	Categorical agreement, %	Error rate (%)		
				Very major	Major	Minor
GNB (overall)	454	95	98	0.2	1	1
Ampicillin	27	96	100	0	0	0
Amoxicillin/clavulanic acid	27	93	96	0	0	4
Ticarcillin/clavulanic acid	31	97	100	0	0	0
Piperacillin/tazobactam	31	94	97	0	0	3
Cefazolin	28	93	100	0	0	0
Cefoxitin	27	96	100	0	0	0
Ceftazidime	32	97	100	0	0	0
Ceftriaxone	32	94	97	0	3	0
Cefepime	32	97	100	0	0	0
Meropenem	31	94	100	0	0	0
Amikacin	32	97	100	0	0	0
Gentamicin	32	91	97	0	3	0
Tobramycin	31	97	97	0	3	0
Ciprofloxacin	32	97	97	0	0	3
Trimethoprim/sulfamethoxazole	29	93	93	3	0	3

Fig. 1 Mean time from blood culture flagging positive to susceptibility result available with 95% confidence intervals.

of the rapid-direct susceptibility method was postulated to be early detection of resistant isolates. It successfully detected all six of the ESBL Gram-negative isolates, but as mentioned it did not perform so well when used on MRSA isolates. The rapid-direct method worked well for six isolates of *Enterococcus* spp. in our study but with such small numbers and such low resistance rates it is difficult to draw conclusions, and more research is needed. Results were available faster using the rapid-direct method compared to the routine method, allowing for more timely recommendation of appropriate antimicrobial therapy. This has the potential to improve patient outcomes as well as aid in antimicrobial stewardship.^{2,4}

There were a number of limitations with this study. Only 64 of the 90 isolates screened were included. A higher number of isolates would improve the statistical robustness of this study. Isolates were batched and were only prepared for the experimental rapid-direct susceptibility method twice a day (at 08:00 and 16:00). There were relatively few resistant isolates included in the study, with no vancomycin-resistant enterococci or carbapenemase-producing *Enterobacteriales*.

In conclusion, for Gram-negative organisms this experimental rapid-direct susceptibility method using positive

blood culture broth to directly inoculate Vitek-2 cards performed just as well as performing susceptibility testing on bacterial isolates and allowed the results to be communicated to clinicians significantly faster.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

Anna Brischetto¹, Jacqueline Schooneveldt², Sanmarie Schlebusch^{1,3}

¹Queensland Health Scientific Services: Health Support Queensland Forensic and Scientific Services, Brisbane, Qld, Australia; ²Mater Pathology, Qld, Australia; ³School of Medicine, The University of Queensland, Brisbane, Qld, Australia

Contact Dr Anna Brischetto.

E-mail: anna.brischetto@health.qld.gov.au

1. Tabah A, Koulenti D, Laupland K, *et al.* Characteristics and determinants of outcome of hospital-acquired bloodstream infections in intensive care

units: the EUROACT International Cohort Study. *Intensive Care Med* 2012; 38: 1930–45.

- Kumar A, Roberts D, Wood KE, *et al.* Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006; 34: 1589–96.
- Schuts EC, Hulscher MEJL, Mouton JW, *et al.* Current evidence on hospital antimicrobial stewardship objectives: a systematic review and meta-analysis. *Lancet Infect Dis* 2016; 16: 847–56.
- Huang AM, Newton D, Kunapuli A, *et al.* Impact of rapid organism identification via matrix-assisted laser desorption/ionization time-of-flight combined with antimicrobial stewardship team intervention in adult patients with bacteremia and candidemia. *Clin Infect Dis* 2013; 57: 1237–45.
- Sakarikou C, Altieri A, Bossa MC, *et al.* Rapid and cost-effective identification and antimicrobial susceptibility testing in patients with Gram-negative bacteremia directly from blood-culture fluid. *J Microbiol Methods* 2018; 146: 7–12.
- Ling TK, Liu ZK, Cheng AF. Evaluation of the VITEK 2 system for rapid direct identification and susceptibility testing of gram-negative bacilli from positive blood cultures. *J Clin Microbiol* 2003; 41: 4705–7.
- Bruins MJ, Bloembergen P, Ruijs GJ, Wolfhagen MJ. Identification and susceptibility testing of Enterobacteriaceae and Pseudomonas aeruginosa by direct inoculation from positive BACTEC blood culture bottles into Vitek 2. *J Clin Microbiol* 2004; 42: 7–11.
- Jorgensen JH. Selection criteria for an antimicrobial susceptibility testing system. *J Clin Microbiol* 1993; 31: 2841–4.

DOI: <https://doi.org/10.1016/j.pathol.2021.03.013>

Weak positive SARS-CoV-2 N2 gene results using the Xpress Xpert assay: the need for an alternate interpretative criteria in a low prevalence setting

Sir,

The Xpert Xpress SARS-CoV-2 (Xpert) test (Cepheid, USA) is the most common Australian rapid nucleic acid test (NAT) used for detection of SARS-CoV-2 in nasal and nasopharyngeal specimens. This rapid RT-PCR assay detects two targets: the E (envelope) and N2 (nucleocapsid) SARS-CoV-2 coding regions. The assay cartridge is a closed system that includes sample processing, probe check internal controls which ensure adequate sample processing, and checks for sample-associated inhibition and reagents functionality.¹ Following the manufacturer's instructions¹ the results are interpreted automatically by the assay software into four categories: 'positive', 'presumptive positive', 'negative' and 'error'. Specimens positive for both E and N2 targets, or single N2 target, are reported as 'positive', while a single E target positive result is reported as 'presumptive positive'.

The Xpert assay is currently performed at 37 NSW Health Pathology (NSWHP) laboratories, and by 10 May 2021, 55,411 tests had been performed on nasal or nasopharyngeal swabs. A total of 317 samples tested positive for both E and N2 targets. There were 42 notified instances where only N2 target positive results were obtained and two where only the E target was detected. All of these 44 tests detected the target at a high cycle threshold (Ct) number (range 36.8–44.6, average 42.0). Of the 42 N2 target only positive tests, 16 (38.1%) were deemed as true infection as confirmed by alternate NAT, SARS-CoV-2-specific serology, and/or consistent epidemiological exposure (Table 1). The remaining 26 (61.9 %) individuals with only N2 target positive results had neither clinical nor epidemiological clues

Table 1 Initial and repeat testing results on individuals who had only N2 target positive results (classified as true-positive results)

Serial no.	Xpert Xpress SARS-CoV-2 CT values		Repeat results on Xpert Xpress SARS-CoV-2	Repeat results on original sample on alternate platform	Retest SARS-CoV-2 assay used	Results on recollected sample (collection day in relation to original sample collected)	Retest SARS-CoV-2 assay used (on repeat collection)	COVID-19 serology results	History including epidemiological exposure
	E	N2							
1	0	42.5	NA	Pos	B	NA	NA	NA	Known Pos
2	0	40.5	NA	Pos	G	NA	NA	Pos	Known Pos
3	0	40.8	NA	Pos	C	NA	NA	Pos	Known Pos
4	0	41.6	NA	Pos	C	NA	NA	NA	Contact of Pos case
5	0	41.7	NA	Pos	E	Pos (-1 day)	G	Pos	Known Pos
6	0	42.2	NA	Neg	B;C;D	Pos (+1 day)	A	Pos	Previous Pos O/S
7	0	44.6	NA	Pos	D	NA	NA	Pos	O/S returned traveller
8	0	38.6	NA	Pos	D	NA	NA	Pos	O/S returned traveller
9	0	42.4	NA	Pos	D	NA	NA	Pos	O/S returned traveller
10	0	41.1	NA	Pos	D	NA	NA	Pos	O/S returned traveller
11	0	44.1	NA	Pos	D	NA	NA	Pos	O/S returned traveller
12	0	40.6	NA	Pos	E	NA	NA	Pos	O/S returned traveller
13	0	42.3	NA	Pos on B, Neg on C	B;C	Pos (+1 day)	A;B	NA	Contact of Pos case
14	0	39.8	Pos	Pos	B	Neg (+1 day)	B	NA	Mariner
15	0	40.4	NA	Neg	C	Pos (+1 day)	B	NA	Known Pos
16	0	36.8	NA	Pos	B	Neg (+4 days)	B	Neg	Known Pos

A, Xpert Xpress SARS-CoV-2; B, AusDiagnostics SARS-CoV-2; C, BD SARS-CoV-2 on BD Max platform; D, Allplex SARS-CoV-2; E, cobas SARS-CoV-2 on cobas 6800 platform; F, BioFire COVID-19 2.1; G, in-house polymerase chain reaction; H, Aptima SARS-CoV-2; NA, not available; Neg, negative; O/S, overseas; Pos, positive.