are negative for BCL-6, CD30 and PAX-5. Kappa/lambda restriction is seen in most cases. EMA, CD79a and BCL-2 expression is variable, and a subset of cases may be CD45 dim. Both diagnoses have a high proliferation index (reported from 80 to 90%). Furthermore, no cytogenetic abnormalities are specific for either anaplastic myeloma or PBL. MYC rearrangements are reported in up to two-thirds of PBL,^{4,5} but are also reported in up to 50% of myelomas, particularly in more aggressive phenotypes.⁶ Complex karyotype, t(4; 14), 1q amplification, chromosome 13 anomalies and TP53 mutation have all been reported in both anaplastic myeloma and PBL. Finally, while EBV and HIV are more strongly associated with PBL than anaplastic myeloma, EBV or HIV positive cases have been reported in anaplastic myeloma, and EBV or HIV negative cases have been reported in PBL.

We suggest that initial characterisation of anaplastic cells in the marrow should involve confirmation of a terminally differentiated B-cell/plasma cell immunophenotype expressing MUM1, CD38, CD138 and lacking CD20 to rule out other lymphomas with plasmablastic morphology. However, this phenotype is not absolute as evidenced by absent CD138 in this case report. The significance of CD138 negative plasma cells in myeloma is debated in the literature with proposed explanations including CD138 downregulation secondary to hypoxia either as a result of delayed processing or *in vivo* marrow hypoxia or apoptosis,^{7–9} the phenotype of an immature plasmablast with higher proliferative potential,¹⁰ and an association with poorer prognosis and reduced sensitivity to lenalidomide.¹¹ Flow cytometry analysis in this case was not delayed, with the sample being processed the same day as collection, however considering the malignant cells were unable to be found in cytogenetic media, but were present on aspirate slides for morphological and cIg FISH analysis, we suggest the malignant cells may have been undergoing apoptosis with associated loss of CD138 expression. Regardless, flow cytometry and FISH should be attempted as results provide supportive, albeit not definitive, diagnostic information. In addition, EBER and HIV tests should be performed, which if positive would be supportive for PBL over anaplastic myeloma. However, ultimately distinction between PBL and anaplastic myeloma will require correlation with serum protein electrophoresis, free light chain assessment, biochemical markers including calcium and renal function, and lytic lesions on skeletal survey. If present, a diagnosis of anaplastic myeloma is favoured, recognising that this distinction is arbitrary (i.e., lytic lesions are rarely described in PBL), and may not be possible in all cases. To our knowledge genomic profiling has not been performed on anaplastic myeloma, whereas genomic profiling shows PBL more closely resembles diffuse large B-cell lymphoma than myeloma.¹² Future studies may look at whether this provides a means for definitive distinction between these two diagnoses.

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1. Greipp PR, Raymond NM, Kyle RA, *et al.* Multiple myeloma: significance of plasmablastic subtype in morphological classification. *Blood* 1985; 65: 305–10.
2. Rao S, Kar R, Pati HP. Anaplastic myeloma: a morphologic diagnostic dilemma. *Indian J Hematol Blood Transfus* 2008; 24: 188–9.
3. Gole L, Lin A, Chua C, *et al.* Modified cIg-FISH protocol for multiple myeloma in routine cytogenetic laboratory practice. *Cancer Genet* 2014; 207: 31–4.
4. Vega F, Chang CC, Medeiros LJ, *et al.* Plasmablastic lymphomas and plasmablastic plasma cell myelomas have nearly identical immunophenotypic profiles. *Mod Pathol* 2005; 6: 806–15 [erratum in *Mod Pathol* 2005; 18: 873].
5. Castillo JJ, Bibas M, Miranda RN. The biology and treatment of plasmablastic lymphoma. *Blood* 2015; 125: 2323–30.
6. Gabrea A, Martelli ML, Qi Y, *et al.* Secondary genomic rearrangements involving immunoglobulin or MYC loci show similar prevalences in hyperdiploid and nonhyperdiploid myeloma tumors. *Genes Chromosomes Cancer* 2008; 47: 573–90.
7. Christensen JH, Jensen PV, Kristensen IB, *et al.* Characterization of potential CD138 negative myeloma “stem cells”. *Haematologica* 2012; 97: e18–20.
8. Kawano Y, Kikukawa Y, Fujiwara S, *et al.* Hypoxia reduces CD138 expression and induces an immature and stem cell-like transcriptional program in myeloma cells. *Int J Oncol* 2013; 43: 1809–16.
9. Muz B, de la Puente P, Azab F, *et al.* Hypoxia promotes stem cell-like phenotype in multiple myeloma cells. *Blood Cancer J* 2014; 4: e262.
10. Reid S, Yang S, Brown R, *et al.* Characterisation and relevance of CD138-negative plasma cells in plasma cell myeloma. *Int J Lab Hematol* 2010; 32: e190–6.
11. Kawano Y, Fujiwara S, Wada N, *et al.* Multiple myeloma cells expressing low levels of CD138 have an immature phenotype and reduced sensitivity to lenalidomide. *Int J Oncol* 2012; 41: 876–84.
12. Chang CC, Zhou X, Taylor JJ, *et al.* Genomic profiling of plasmablastic lymphoma using array comparative genomic hybridization (aCGH): revealing significant overlapping genomic lesions with diffuse large B-cell lymphoma. *J Hematol Oncol* 2009; 2: 47.

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Optimising early identification of blood culture isolates by MALDI-TOF and use of multiple target wells

Sir,

The early identification of positive blood culture (BC) isolates facilitates timely antimicrobial therapy and allows for ‘real-time’ antimicrobial stewardship. A number of matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) methods performed on positive BC broth have been reported to provide rapid (1–2 h) identification of isolates.^{1–4} The major disadvantage of these methods is their labour-intensive nature, and disruption to laboratory workflow when BCs flag positive at various times throughout the day. Recently, Bhatti *et al.* reported the use of MALDI-TOF MS to identify positive BC isolates from a short incubation of pre-warmed 5% sheep blood agar subculture.⁵ In our study, we evaluated MALDI-TOF MS identification testing, using Vitek MS (BioMerieux, France), from subcultures of positive BC on chocolate agar pre-warmed at 35°C in 5% CO₂ (pCBA) then incubated for 2, 3 and 4 h at 35°C in 5% CO₂. The identification of positive BC isolates from short incubation pCBA was compared to the

identification by MALDI-TOF from 18–24 h incubation routine cultures.

Blood culture bottles were incubated in the Bactec FX automated blood culture system (BD Diagnostics, USA). Positive BCs were examined by a Gram smear, then subcultured and incubated up to 48 h utilising the Kiestra WorkCell automation system (BD Diagnostics). In parallel with the standard protocol, positive BCs were subcultured onto pCBA, pre-warmed at 35°C, 5% CO₂ for 4–18 h. One drop of BC broth was inoculated and struck out onto pCBA, immediately incubated at 35°C, 5% CO₂, and examined after 2, 3 and 4 h incubation. If there was a visible film of bacterial growth, MALDI-TOF MS was performed as per manufacturer's instructions. Four target wells were applied for each pCBA culture. The identification by Vitek MS was

considered 'acceptable' if the confidence level was >90%. These results were compared to MALDI-TOF results of routine subcultures at 18–24 h incubation. Results were categorised as 'concordant identification' if acceptable spectra identification from pCBA matched routine cultures, either to genus or species level. There was 'no identification' if there was no acceptable spectra identification from pCBA at 4 h incubation, and 'discordant identification' if acceptable spectra identification from pCBA was discrepant (to genus level) with routine cultures.

During the study period, a total of 230 bottles from 118 patients, including 60 paired BC sets, were tested. There were 210 monomicrobial and 20 polymicrobial positive bottles, and for monomicrobial cultures, identification was concordant for 155/210 (73.8%) bottles (Fig. 1). All concordant

(A) Gram-negative bacteria

(B) Gram-positive bacteria

^a Including 1 isolate acceptable identification to genus (*Citrobacter* spp. at 2 h); 18–24 h routine subculture was *Citrobacter werkmanii*.

^b Including *Acinetobacter baumannii* (n=2 at 2 h, n=1 at 4 h), *Neisseria meningitidis* (n=1 at 4 h), *Sphingomonas paucimobilis* (n=1 no acceptable identification within 4 h).

^c Including *Micrococcus luteus/lutae* (n=3 no acceptable identification), *Bacillus* spp. (n=2 at 3 h) and *Clostridium clostridioforme* (n=2 no acceptable identification).

Fig. 1 MALDI-TOF identification of monomicrobial positive blood cultures from 2–4 h subcultures (pCBA) by organism category. (A) Gram-negative bacteria. (B) Gram-positive bacteria.

Table 1 Summary of discordant early identification MALDI-TOF results

No.	Direct Gram stain	Early identification (pCBA, 2–4 h)	Mismatch Gram	Uncommon isolate	Target wells ^a	Final identification ^b
1	Gram-negative bacilli	<i>Enterococcus faecalis</i>	Yes	No	1	<i>Pseudomonas fluorescens</i>
2	Gram-negative bacilli	<i>Vibrio fluvialis</i>	No	Yes	1	<i>Pseudomonas fluorescens</i>
3	Gram-positive cocci (resembling <i>Streptococcus</i>)	<i>Cedeeva neteri</i>	Yes	Yes	1	<i>Streptococcus intermedius</i>
4	Yeast	<i>Cryptococcus uniguttulatus</i>	No	Yes	1	<i>Candida dubliniensis</i>
5	Yeast	<i>Cryptococcus uniguttulatus</i>	No	Yes	1	<i>Candida glabrata</i> complex
6	Gram-positive cocci (resembling <i>Staphylococcus</i>)	<i>Chryseobacterium indologenes</i>	Yes	Yes	1	<i>Staphylococcus epidermidis</i> and <i>Staphylococcus hominis</i>
7	Gram-positive bacilli	<i>Escherichia coli</i>	Yes	No	2	<i>Lactobacillus</i> spp. and <i>Clostridium subterminale</i>

^a Number of target wells (out of 4 tested) with acceptable identification.
^b Routine subculture, 18–24 h.

results matched to the same level of identification (genus and/or species) except one. Monomicrobial cultures with Gram-negative bacteria (108/210), were mostly Enterobacteriaceae (n = 96), and concordant identification within 4 h incubation was obtained for 96/108 (88.9%) Gram-negative positive bottles. Gram-positive bacteria were isolated from 95/210 monomicrobial bottles, and 55 of these were *Staphylococcus* species. Concordant identification was achieved for 59/95 (62.1%) of Gram-positive bottles, and 18/21 (85.7%) *Staphylococcus aureus* positive bottles were concordant compared to 17/34 (50%) coagulase-negative *Staphylococcus* bottles. There were seven monomicrobial bottles that grew yeast, but these were poorly identified. Of these bottles, 5/7 had no yeast identified within 4 h incubation and 2/7 had discordant results compared to routine subculture (18–24 h incubation). In total, 5/210 (2.4%) monomicrobial bottles gave discordant identifications. Of the 20

polymicrobial bottles, concordant identification was achieved for at least one organism for 13/20 (65%) bottles within 4 h incubation. Organisms from 5/20 (25%) bottles were not identified by 4 h incubation and 2/20 (10%) gave discordant results. Overall, there were 7/230 (3%) discordant results (Table 1), and of these 4/7 preliminary identifications did not concur with the direct Gram smear, and an uncommon isolate was reported for 5/7. In addition, 6/7 discordant results had acceptable identification for only 1/4 target wells, and the remaining discordant sample had 2/4 target wells with acceptable identification. There were 22 bottles with 1/4 target wells with acceptable spectra, and 21 bottles with 2/4 target wells with acceptable spectra. Results for these bottles were concordant with 18–24 h cultures for 16/22 (72.7%) and 20/21 (95.2%), respectively. In contrast, there was 100% concordance for all bottles with 3/4 target

Fig. 2 MALDI-TOF results with acceptable spectra identification by number of target wells.

wells ($n = 20$) and 4/4 target wells ($n = 112$) with acceptable spectra. These findings indicate that to maximise concordance, a minimum of 3/4 target wells with acceptable spectra are required. Our rate of concordance for Gram-negatives (88.9%), within 4 h incubation, using pCBA, was higher than reported by Bhatti *et al.* (79%) where Vitek MS was also used; and comparable with two other reports that employed the Bruker MALDI-TOF typer and obtained 96.4%, 97.6% concordance.^{5–7} However, our rate for concordant identifications for Gram-positives (62.1%) differed from Bhatti *et al.* (80%),⁵ and the Bruker MALDI-TOF studies (18.6%, 37.3% concordance).^{6,7} In our study, we found a higher rate of discordant identifications (2.4% monomicrobial; 10% polymicrobial) compared to that reported by Bhatti *et al.* (0.7% monomicrobial; 0% polymicrobial) and Kohlmann *et al.* (1.2% monomicrobial; 3.0% polymicrobial).^{5,7}

This study was limited by a small sample size of 230 positive BC tested and a lack of diversity of species. In monomicrobial positive BC, five species (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis*) represented 136/210 (64.8%) isolates. There were only low numbers ($n \leq 6$) of other species including yeasts, and so limited detailed assessment outside the five most commonly isolated organisms. However, the study isolates represented the distribution of expected species locally, and were similar to those reported by Bhatti *et al.*⁵

In conclusion, the early MALDI-TOF method is a practical and relatively simple method for the rapid identification of positive BC isolates, particularly Enterobacteriaceae, for which 90/96 (93.8%) concordant identification was achieved. Notably, there was discordant identification for 7/230 (3%) positive BC, despite adequate MALDI-TOF MS spectra. MALDI-TOF protein profiles may have been modified because the culture was slow growing/immature culture, or from a polymicrobial BC. We found that misreporting could be reduced by ensuring that all MALDI-TOF results were interpreted in conjunction with the direct BC Gram smear and only reported when more than two target wells provided a congruent identification (Fig. 2). Early identification from pCBA is not suitable for anaerobic or slow growing microorganisms, such as yeasts. Where these isolates are indicated by microscopy, alternative methods such as direct MALDI-TOF from the BC broth may be required.

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1. microbiological methods for identification of bacteria from positive blood culture bottles. *J Clin Microbiol* 2012; 50: 346–52.
2. Foster AGW. Rapid identification of microbes in positive blood cultures by use of the Vitek MS matrix-assisted laser desorption/ionization–time of flight mass spectrometry system. *J Clin Microbiol* 2013; 51: 3717–9.
3. Lupetti A, Barnini S, Morici P, Ghelardi E, Nibbering PH, Campa M. Saponin promotes rapid identification and antimicrobial susceptibility profiling of Gram-positive and Gram-negative bacteria in blood cultures with the Vitek 2 system. *Eur J Clin Microbiol Infect Dis* 2013; 32: 493–502.
4. Stevenson LG, Drake SK, Murray PR. Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption/ionization–time of flight mass spectrometry. *J Clin Microbiol* 2010; 48: 444–7.
5. Bhatti MM, Boonlayangoor S, Beavis KG, Tesic V. Rapid identification of positive blood cultures by matrix-assisted laser desorption/ionization–time of flight mass spectrometry using prewarmed agar plates. *J Clin Microbiol* 2014; 52: 4334–8.
6. Idelevich EA, Schüle I, Grünastel B, Wüllenweber J, Peters G, Becker K. Rapid identification of microorganisms from positive blood cultures by MALDI-TOF mass spectrometry subsequent to very short-term incubation on solid medium. *Clin Microbiol Infect* 2014; 20: 1001–6.
7. Kohlmann R, Hoffmann A, Geis G, Gattermann S. MALDI-TOF mass spectrometry following short incubation on a solid medium is a valuable tool for rapid pathogen identification from positive blood cultures. *Int J Med Microbiol* 2015; 305: 469–79.

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Concentrating non-dilute urine before protein electrophoresis for the detection of monoclonal free light chains is unnecessary and wasteful

Sir,

Urine electrophoresis (UEP) is used for the detection of monoclonal free light chains (mFLC) in plasma cell disorders. It is also used to investigate the types of proteinuria, tubular and/or glomerular.¹ The procedure varies amongst laboratories. Some analyse neat while others concentrate the urine to varying magnitude, depending on the initial (neat) urine total protein concentration. For example, all urine samples with a total protein concentration of below 0.2 g/L require a concentrating step. The neat or concentrated urine sample is then subjected to electrophoresis and/or immunofixation and the gels are visualised and reported by a trained laboratory scientist. The whole UEP procedure, particularly the concentrating step, is laborious and time-consuming. There have been studies to revisit or reduce the role of UEP in the routine detection of mFLC.² We investigated whether concentrating the urine sample provides an additional value in the investigation of mFLC compared to using neat urine sample for UEP or serum free light chains measurement only.

Routine patient urine samples with total protein ≤ 0.5 g/L were prospectively analysed both neat and concentrated. Urine samples from healthy volunteers ($n = 13$, age range 24–61 years) were used as normal controls. The urine was concentrated by ultrafiltration with a 5 kDa cut-off membrane (Vivaspin 2; Sartorius, Germany). An initial 2 mL urine was added to the centrifugal concentrator and the centrifugation stopped when approximately 0.2 mL urine remained in the upper ‘concentrate’ pocket, giving a theoretical 10-fold concentrating magnitude. Protein electrophoresis was then

1. Buchan BW, Riebe KM, Ledeboer NA. Comparison of the MALDI Biotyper System using Sepsityper specimen processing to routine